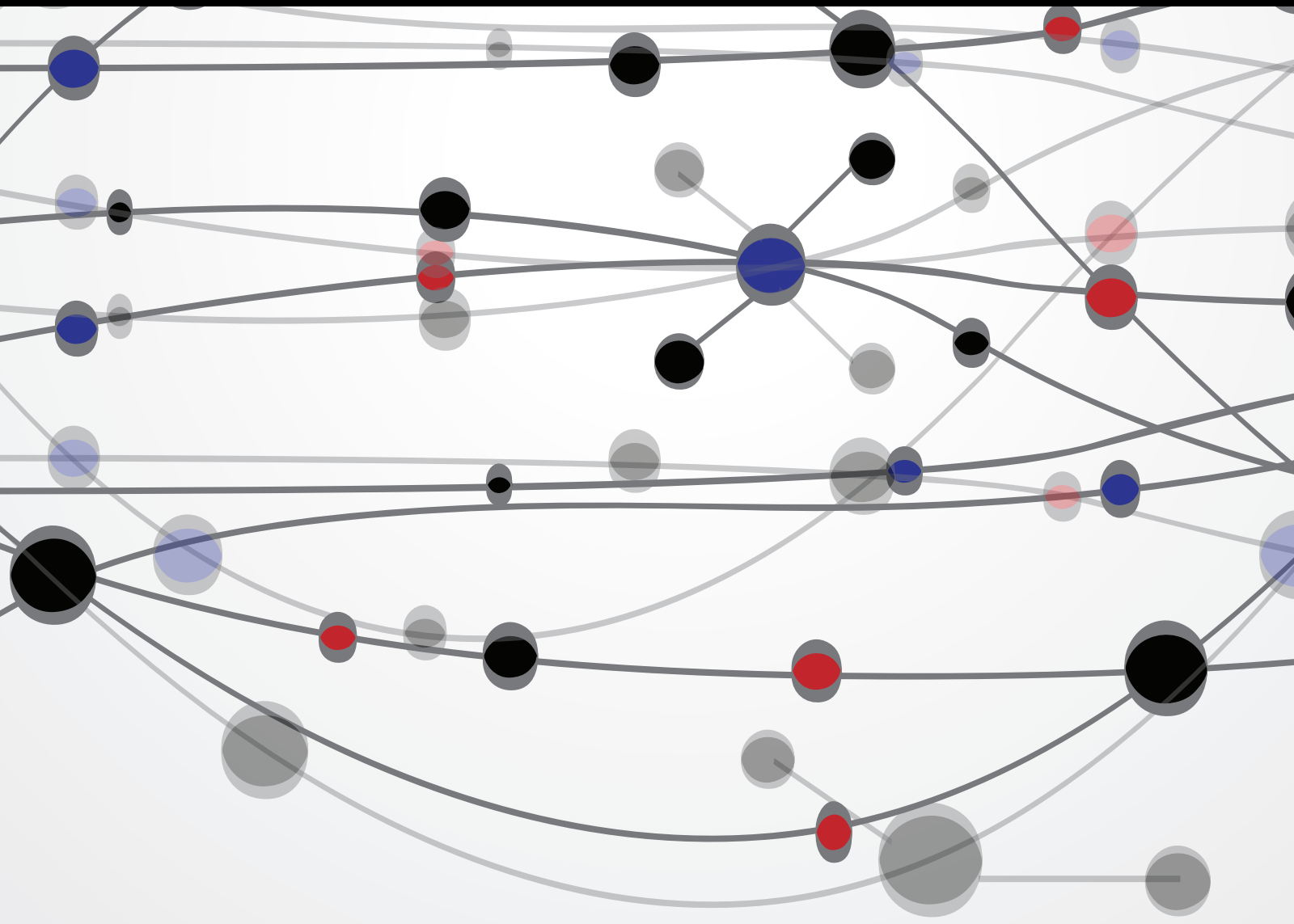
The Scientific World Journal

Oral Tissues Interactions with Lights and Matters

Guest Editors: Samir Nammour, Umberto Romeo, Carlos de Paula Eduardo, and Toni Zeinoun



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Editorial

Oral Tissues Interactions with Lights and Matters

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Oral tissues interactions with lights and matters are gaining interest in the medical field and more specifically in dentistry. The introduction of tooth colored resin composite had a significant impact in pushing dentistry into the esthetic arena based on 2 major developments: adhesion and light-cured materials. Different light curing devices were introduced based on the mode of action and wavelength properties. But probably one of the major breakthroughs of technology in clinical dentistry is the integration of laser therapy as a therapeutic option into treatment plan for clinical improvement. The introduction of different laser devices with different wavelengths (diode lasers, Nd:YAG, Er:YAG, Er, Cr:YSGG, and CO_2) allows specific and selective clinical applications on soft and/or hard dental tissues opening the door to laser therapy in diagnosis, cavity preparation, esthetic and oral surgeries.

This special issue is a compendium of different studies and fundamental and clinical researches. Some papers are focused on the effect of laser therapy in dental bleaching, its side effects on enamel and pulp, and the advised chemical components and techniques. We also included interesting studies about tissue engineering and pulp revascularization of immature permanent teeth which probably will be one of the most challenging topics in the near future. Main papers treating basic and fundamental researches reported very interesting methods and results in the application of laser therapy, sonic/rotary instruments, and light cure devices in restorative and esthetic dentistry (bonding, marginal integrity, polymerization, and dental hypersensitivity) and biological interaction (stem cells, biopsy, and histology). Some papers on clinical studies encompass the effect of laser on the periodontium, effect of mouthwash on biofilm control, and the use of analgesic combination in the management of chronic temporomandibular disorders.

We hope that the content of this special issue provides valuable insights on the interaction of oral tissues with lights and matters to clinicians and researchers.

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Research Article

Three-Dimensional Finite Element Analysis of Anterior Two-Unit Cantilever Resin-Bonded Fixed Dental Prostheses

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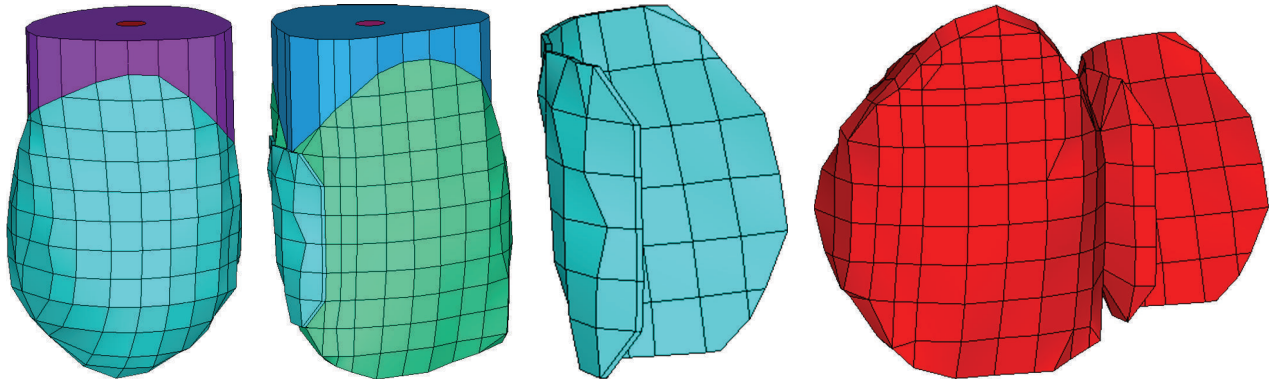
The aim of this study was to evaluate the influence of different framework materials on biomechanical behaviour of anterior two-unit cantilever resin-bonded fixed dental prostheses (RBFDPs). A three-dimensional finite element model of a two-unit cantilever RBFDP replacing a maxillary lateral incisor was created. Five framework materials were evaluated: direct fibre-reinforced composite (FRC-Z250), indirect fibre-reinforced composite (FRC-ES), gold alloy (M), glass ceramic (GC), and zirconia (ZI). Finite element analysis was performed and stress distribution was evaluated. A similar stress pattern, with stress concentrations in the connector area, was observed in RBFDPs for all materials. Maximal principal stress showed a decreasing order: ZI > M > GC > FRC-ES > FRC-Z250. The maximum displacement of RBFDPs was higher for FRC-Z250 and FRC-ES than for M, GC, and ZI. FE analysis depicted differences in location of the maximum stress at the luting cement interface between materials. For FRC-Z250 and FRC-ES, the maximum stress was located in the upper part of the proximal area of the retainer, whereas, for M, GC, and ZI, the maximum stress was located at the cervical outline of the retainer. The present study revealed differences in biomechanical behaviour between all RBFDPs. The general observation was that a RBFDP made of FRC provided a more favourable stress distribution.

1. Introduction

Resin-bonded fixed dental prostheses (RBFDPs) have proven to be a reliable treatment alternative for the replacement of missing teeth [1, 2] especially in cases where conservation of tooth tissue is needed and limited financial resources are available. According to a recent systematic review, RBFDPs exhibit an estimated survival rate of 87.7% (95% confidence interval: 81.6%–91.9%) after 5 years [3]. Notwithstanding their good clinical performance, the most frequent complication was debonding, with a reported cumulative debonding rate of 19.2% (95% CI: 13.8–26.3%) after 5 years [3].

The use of more extensive preparation of the abutment teeth, including palatal or lingual coverage with 180-degree wraparound, chamfer, cingulum rests, and proximal guide planes and grooves, is a way to improve the retention of RBFDPs [4]. Another way to minimize debonding is to design RBFDPs as a two-unit cantilever. Several clinical studies of the last decade have demonstrated that two-unit cantilever RBFDPs performed as well as or even better than their three-unit fixed-fixed counterparts [5–11]. Elimination of interfacial stresses, induced by a combination of dynamic tooth contacts and differential movements of the abutment teeth, is the most widely accepted explanation for their successful clinical performance [4, 12].



(a) (b) (c)

Figure 1: 3D FE model of a cantilever two-unit RBFDP: (a) abutment and adjacent tooth, (b) cement layer, and (c) RBFDP.

The framework of RBFDPs is traditionally made of metal alloys, but their poor aesthetics and the growing awareness towards possible adverse health effects of dental alloys, such as Ni-, Cr-, Co-, Pd-, and Au-containing alloys [13–17], stimulated the interest in metal-free restorations. Nowadays, all-ceramic [10] and fibre-reinforced composites (FRC) [18, 19] are viable alternatives for framework fabrication of RBFDPs. Some clinical cases reported promising results for all-ceramic RBFDPs [20, 21]. In addition Kern and Sasse reported 10-year survival rates for glass-infiltrated alumina-based RBFDPs of 73.9% for three-unit fixed-fixed designs and 94.4% for two-unit cantilever designs [11]. The same authors reported a survival rate of 93.3% after 5 years for single-retainer zirconia-based RBFDPs [22]. Finally, Sailer et al. evaluated the clinical performance of single-retainer lithium disilicate glass ceramic-based RBFDPs finding a 5-year survival rate of 100% [23]. A recently published systematic review reported for FRC-FDPs a survival rate of 73.4% (95% CI: 69.4–77.4%) after 4.5 years [19]. During a 5-year multicenter clinical study FRC RBFDPs exhibited a survival rate of 64% [24]. The differences in material properties, especially elastic modulus, adhesive properties, and thermal expansion coefficient, are believed to affect the mechanical and clinical performance of RBFDPs [25]. In order to better understand the failure mechanism of two-unit cantilever RBFDPs, increased knowledge on the biomechanical behaviour of these restorations is needed.

The aim of the present study was to compare, by means of three-dimensional finite element analysis (3DFEA), the biomechanical behaviour of anterior two-unit cantilever RBFDPs made of various framework materials.

2. Material and Methods

2.1. Definition of Structures, Geometric Conditions, and Materials. A FE model representing a single tooth gap in the anterior right maxilla, consisting of a central incisor, a missing lateral incisor, and a canine (Figure 1(a)), was created. The central incisor served as the abutment tooth but was not provided with a retainer preparation. The missing lateral incisor was replaced by a two-unit cantilever RBFDP (Figure 1(c)) with a retainer on the central incisor. A wing-shaped retainer design, which enwrapped the palatal and distal surface of the abutment tooth, was selected and the pontic was shaped according a modified ridge lap design. Three-dimensional FE model of the cement layer, with a uniform thickness of 100 μm, is shown in Figure 1(b). A more detailed description of the creation of the FE model was published earlier by Shinya et al. [25].

The geometry of the healthy standard tooth as abutment has been previously described [26]. Not only the natural tooth geometry but also the composition (enamel, dentine, and pulp tissue) was mimicked. Roots under the bone level, periodontal ligaments, and alveolar bone were not created.

Materials properties are derived from clinically used materials (reference brand between parentheses): hybrid particulate filler composite (PFC) for laboratory use (Estenia C&B; Kuraray medical Inc., Tokyo, Japan), hybrid PFC for chairside use (Filtek Z250; 3M ESPE, MN, USA), unidirectional FRC for laboratory use (Estenia C&B EG fiber; Kuraray medical Inc., Tokyo, Japan), unidirectional fibre-reinforced composite for direct and chairside use (EverStick C&B; StickTech Ltd., Turku, Finland), Au-Pd alloy (Olympia; J.F. Jelenko, Armork, NY, USA), lithium disilicate glass ceramic (IPS Empress 2; Ivoclar-Vivadent, Schaan, Liechtenstein), zirconia (InCeram Zirconia; Vita, Bad Säckingen, Germany), feldspathic porcelain (Creation; Klema, Meiningen, Austria), resin-based luting cement (Variolink 2; Ivoclar-Vivadent, Schaan, Liechtenstein), enamel, dentin, and pulp. The material properties, mostly obtained from existing literature, are summarised in Table 1. The materials were assumed to be isotropic, homogeneous, and linear elastic, except for FRC. The mechanical behaviour of continuous unidirectional FRC, influenced by their anisotropic (orthotropic) properties, can be described by 3 young's moduli, 3 Poisson's ratios, and 3 shear moduli [27]. Twenty-node brick element such as solid 95 in ANSYS has the anisotropic material option. The orientation of the element coordinate system was altered in such a way that it matched the fibre direction.

Five different groups with various framework materials were evaluated:

(1) FRC-Z250: a FRC-FDP made of continuous unidirectional E-glass FRC framework (Figure 2) veneered with hybrid PFC for direct and chairside use;

Table 1: Elastic properties of the materials used in the finite element model.

	E modulus (GPa)	Poisson's ratio	Shear modulus (MPa)	Reference
Enamel	80.0	0.30	—	[58]
Dentin	17.6	0.25	—	[59]
Pulp	0.002	0.45	—	[60, 61]
Resin luting cement	8.3	0.24	—	[62]
Chairside PFC	11.5	0.31	—	[63, 64]
Laboratory PFC	22.0	0.27	—	[27]
Chairside FRC				a
Longitudinal (X)	46.0	0.39	16.5	
Transverse (Y, Z)	7.0	0.29	2.7	
Laboratory FRC				[27]
Longitudinal (X)	39.0	0.35	14.0	
Transverse (Y, Z)	12.0	0.11	5.4	
Lithium disilicate glass ceramic	96.0	0.25	—	[62]
Zirconia	205	0.22	—	[62]
Au-Pd alloy	103	0.33	—	[65, 66]

a: data obtained by StickTech Ltd. (Turku, Finland).

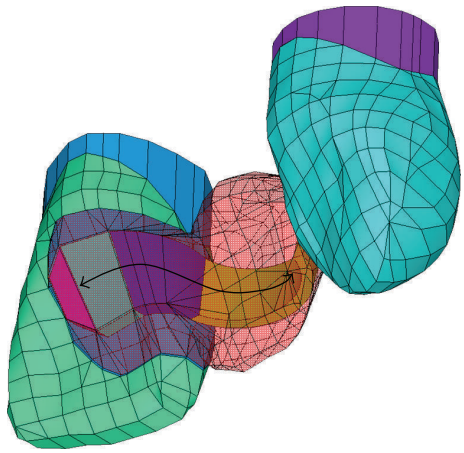


Figure 2: 3D FE model of a two-unit cantilever FRC RBFDP: position of the FRC framework in relation to the FDP and the abutment teeth is shown. Double arrowed black line represents the fibre direction.

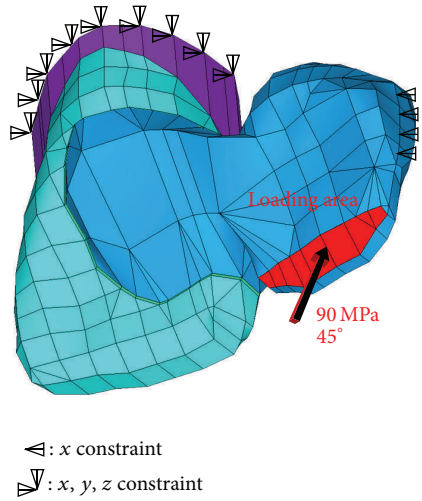


Figure 3: Loading and boundary conditions of a 3D FE model representing two-unit cantilever RBFDPs.

(2) FRC-ES: a FRC-FDP made of continuous unidirectional E-glass FRC framework veneered with hybrid PFC for laboratory use;

(3) M: a metal-ceramic FDP made of type 3 Au-Pd alloy framework veneered with feldspathic porcelain;

(4) GC: an all-ceramic FDP made of lithium disilicate glass ceramic framework veneered with feldspathic porcelain;

(5) ZI: an all-ceramic FDP made of zirconia framework and veneered with feldspathic porcelain.

A FRC framework was designed with thickness of 0.6 mm and a height of 3.0 mm [28]. The three-dimensional FE model of the FRC framework and its position in relation to the RBFDP are shown in Figure 2.

2.2. Mesh Generation, Boundary Conditions, and Data Processing. In order to avoid quantitative differences in stress value, all solid models were derived from a single mapping mesh pattern that generated 103,861 twenty-node brick element (Solid 95 in ANSYS) and 154,784 nodes. Loading and boundary conditions are depicted in Figure 3. A stress of 90 MPa was applied at a 45° angle to the incisal edge of the pontic. In the present study, the FE model was loaded by applying a stress of 90 MPa in a 45° angle to the incisal edge of the pontic tooth. An applied stress of 90 MPa to a

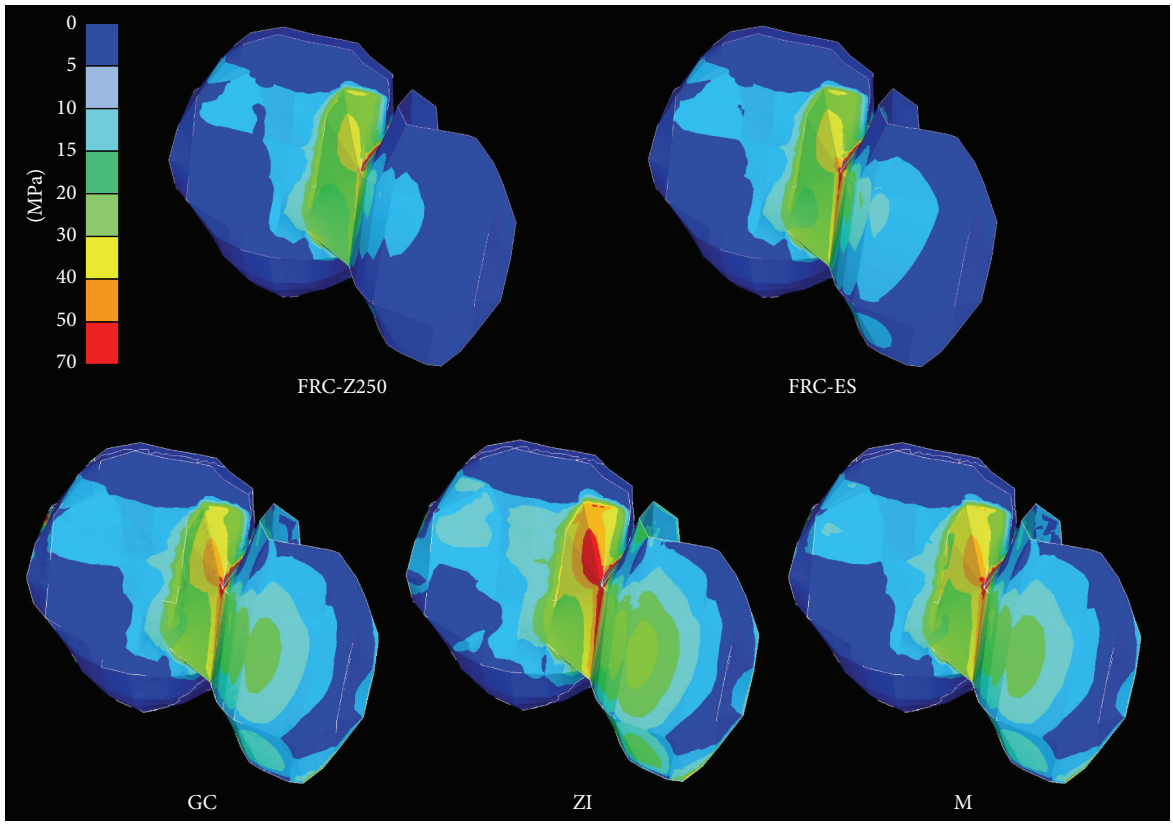


Figure 4: Principal stress distribution within two-unit cantilever RBFDPs of various framework materials.

5.5 mm^2 incisal area corresponds to a load of 495 N. The applied load is significantly higher than previously reported maximum anterior mastication loads of 108–382 N [29, 30] and therefore can be regarded as the worst-case scenario. In clinical circumstances, an anterior occlusal contact more closely resembles an area than a point; for that reason it was chosen to apply the load in an area. The terminal elements of the abutment tooth were fixed in all directions, as well as the final elements of the contact area to canine in distal direction. 3DFEA was presumed to be linear static and was performed on PC workstation (Precision Work Station M90, Dell Inc., Texas, USA) using FE analysis software ANSYS 11 (ANSYS Inc.; Houston, TX, USA). The locations and magnitudes of the principal stress (MPa) and displacement (mm) were identified and used for evaluating the biomechanical behaviour. Maximum principal stress describes the highest stress and can be regarded to be a tensile stress.

3. Results

3.1. Stresses in the FDP (Figure 4). Differences in maximum principal stress were observed (Table 2) and showed a decreasing order: ZI > M > GC > FRC-ES > FRC-Z250. Stress concentrations were located in the connector area, more precisely at the occlusal embrasure, for all framework materials. However, additional stress concentrations were observed at the contact area with the adjacent tooth for all framework materials and at the mesiocervical edge of the retainer for GC (20–30 MPa), M (30–40 MPa), and ZI (50–70 MPa). Stresses at the contact area with the adjacent tooth were lower for FRC-ES and FRC-Z250 (30–40 MPa) in comparison to GC (50–70 MPa), M, and ZI (>70 MPa).

3.2. Stresses at the Cement-Retainer Interface (Figure 5). Differences in maximal principal stress were also observed (Table 2) at the cement-retainer interface and showed a decreasing order: ZI > M > GC > FRC-ES > FRC-Z250. However, their location differed and was observed in the upper part of the proximal area for FRC-Z250 and FRC-ES, while they were located in a semicircular way around the connector and at the cervical edge of the retainer for M, GC, and ZI.

3.3. Stresses in the Cement Layer (Figure 6). FEA revealed (Table 2) only slight differences in maximal principal stress and showed a decreasing order: FRC-Z250 > ZI > FRC-ES > M > GC. They were located in a different area of the cement layer. Highest stress concentrations were located in the upper part of the proximal area for FRC-Z250 and FRC-ES, while they were located at the cervical margin for M, GC, and ZI.

3.4. Stresses on the Abutment Tooth (Figure 7). On the abutment tooth only slight differences in maximal principal stress were observed (Table 2). Highest value was 34.9 MPa for

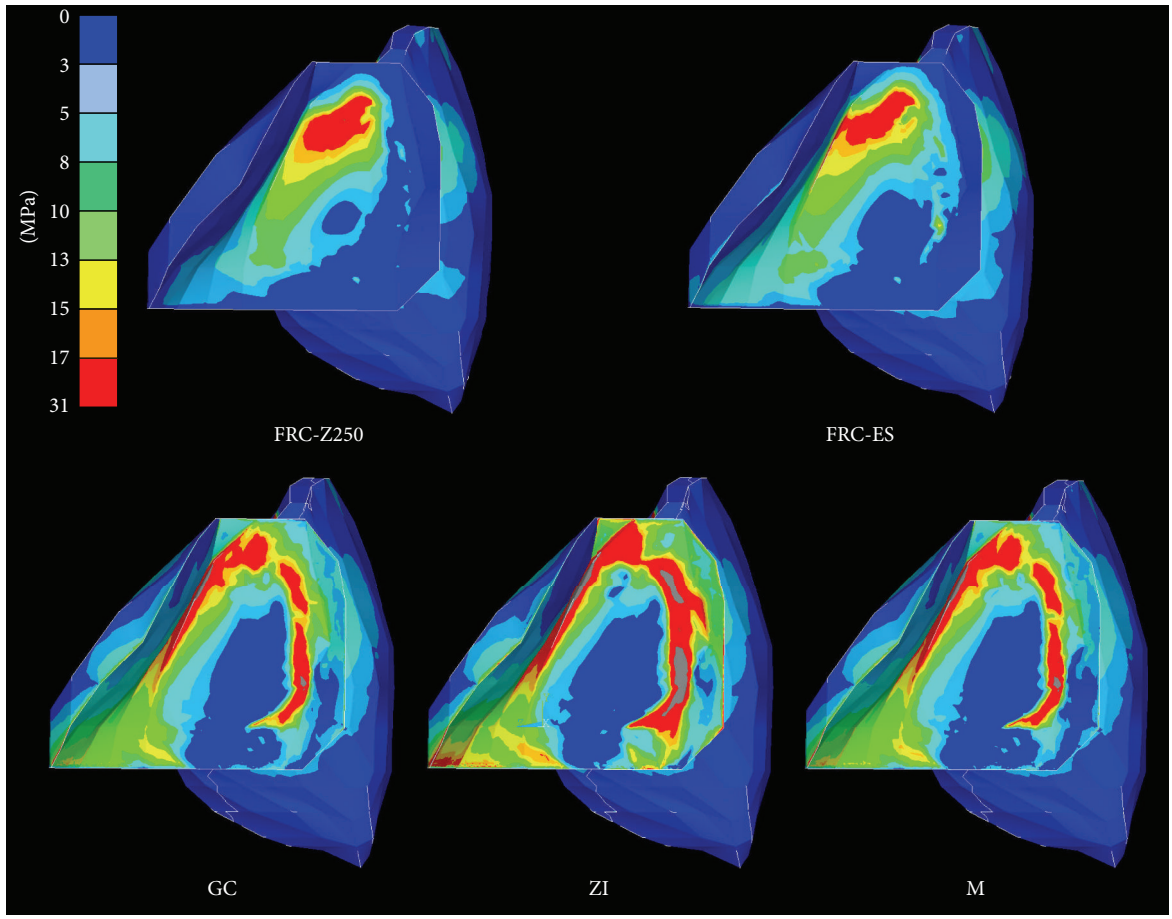


Figure 5: Principal stress distribution at the cement-retainer interface for two-unit cantilever RBFDPs of various framework materials.

Table 2: Maximum and minimum principal stress (MPa) and displacement (mm) for two-unit cantilever RBFDPs of various framework materials.

	FDP			Cement-retainer interface			Cement layer			Abutment tooth		
	Max.	Min.	Disp.	Max.	Min.	Disp.	Max.	Min.	Disp.	Max.	Min.	Disp.
FRC-Z250	156.9	−56.2	0.048	17.5	−5.3	0.010	31.3	−7.1	0.010	34.9	−7.6	0.010
FRC-ES	177.1	−67.2	0.035	23.9	−9.7	0.010	27.3	−7.1	0.010	30.9	−9.8	0.010
GC	178.4	−116.3	0.019	32.7	−42.5	0.009	23.7	−4.1	0.009	31.4	−4.8	0.009
ZI	239.6	−154.3	0.017	60.8	−75.3	0.009	27.5	−3.3	0.009	31.7	−7.2	0.009
M	197.1	−149.9	0.019	36.1	−45.8	0.009	24.5	−3.7	0.009	31.9	−5.0	0.009

FRC-Z250 and the lowest value was 30.9 MPa for FRC-ES. Once again, their location showed some differences. Highest stress concentrations for FRC-Z250 and FRC-ES were observed at the upper middle part of the proximal area and were surrounded by a large area of stress concentration (17–31 MPa) that extended towards the palatocervical area, while they were located in a small area of the palatocervical area of the abutment tooth for M, GC, and ZI.

3.5. Displacement (Table 2). Differences in maximum displacement were observed in the pontic part of the RBFDP between the different materials. Higher displacement of the RBFDP was encountered with FRC-Z250 and FRC-ES and then with M, GC, and ZI. Although, the maximum displacement at the cement-retainer interface, cement layer, and abutment tooth revealed the same trend as those for RBFDPs, a difference of 0.001 mm between highest and lowest value could not be regarded as clinically relevant.

4. Discussion

A static fracture strength test, during which a FDP is vertically loaded till failure, is the most common way to evaluate the mechanical behaviour of FDPs in laboratory conditions [31]. The drawbacks of this approach, such as the difficulty in fabricating uniform FDPs in terms of shape and dimensions, are reckoned by researchers familiar with it. Although, FEA can be regarded as a relatively easy and inexpensive way to

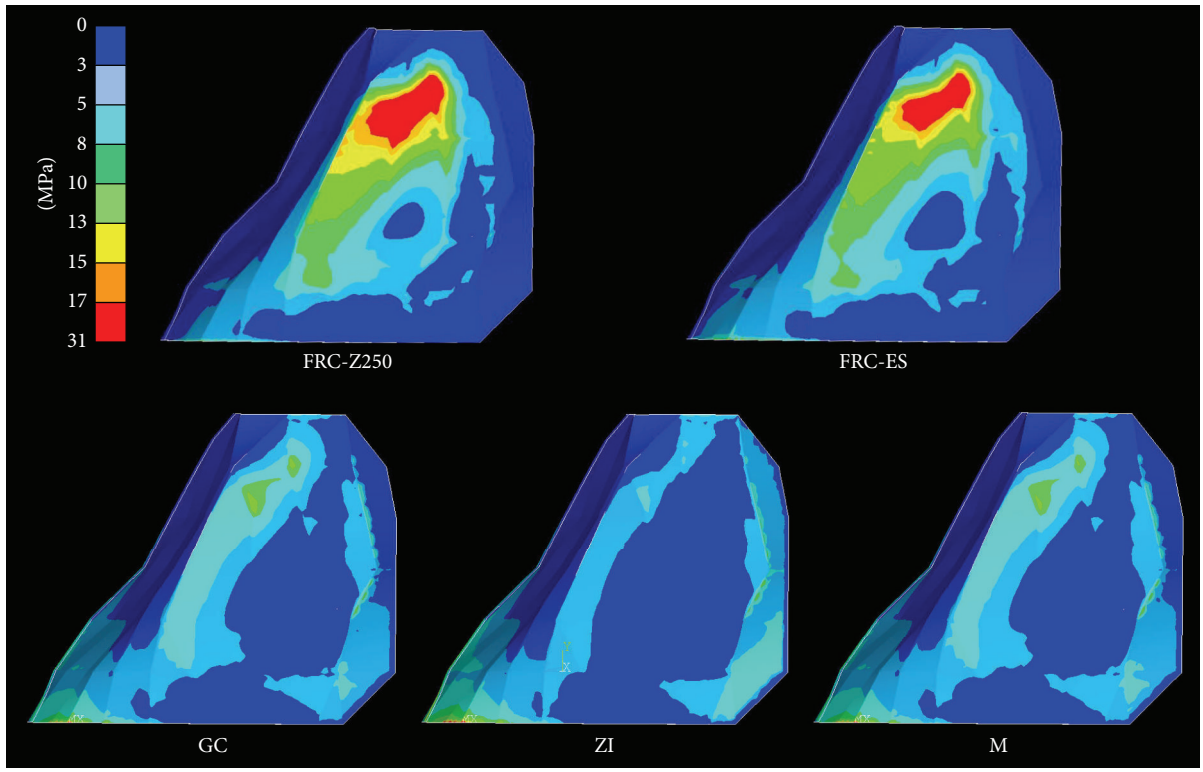


Figure 6: Principal stress distribution within the cement layer for two-unit cantilever RBFDPs of various framework materials.

evaluate the mechanical behaviour of complex structures, some limitations should be acknowledged. Some of these limitations can be drawn back to the simplifications made to the finite element models, for example, tooth model without roots, periodontal ligament [32] and, bone, and the assumptions made related to their material properties [33]. The latter were illustrated by the fact that all materials, except FRC, were assumed to be isotropic, homogenous, and linear elastic, despite the anisotropic nature of tooth tissue like dentine [34]. Therefore, one should be aware of the fact that the reported values cannot be regarded as absolute values. The main purpose of this study was to compare the biomechanical behaviour of anterior two-unit cantilever RBFDP made of different framework materials. Nevertheless, the ideal approach is to interpret the results from both FEA and mechanical testing simultaneously, which allows providing more reliable and validated data than either method alone [35]. So mechanical testing on two-unit cantilever RBFDPs in the same condition as this study could be a valuable asset.

In the present study, the FE model was loaded by applying a stress of 90 MPa in a 45° angle to the incisal edge of the pontic tooth. An applied stress of 90 MPa to a 5.5 mm^2 incisal area corresponds to a load of 495 N. The applied load is significantly higher than previously reported maximum anterior mastication loads of 108–382 N [29, 30] and therefore can be regarded as the worst-case scenario. In clinical circumstances, an anterior occlusal contact more closely resembles an area than a point, for that reason it was chosen to apply the load in an area.

Roots, periodontal ligament, and bone, which are responsible for physiologic tooth mobility, were not included in the FE model. Under clinical conditions, a part of the loading is transferred via the roots and the periodontal ligament into the bone. The lack of physiologic tooth mobility in the present FE model negatively influences the outcome of the FEA, in such a way the principal stress values are overestimated. The effect of tooth mobility was illustrated by Rosentritt et al., who found higher fracture strengths for anterior cantilever RBFDPs when luted to abutment teeth with high mobility [36]. Clinically, rationality to use cantilever design over fixed-fixed design is related to the teeth mobility. If teeth with increased mobility are involved, risk for debonding of fixed-fixed RBFPD from one end is relatively high. A debonded retainer may result in secondary caries that often is not diagnosed in time [2, 4, 7].

The present FEA revealed differences in biomechanical behaviour between RBFDPs made of different framework materials. Although the location of the stress concentration, observed at the FDP level, was identical for all framework materials, the values differed. The differences in displacement and principal stress can be explained by the differences in elastic modulus between framework materials. RBFDPs made of materials with higher stiffness suffered less displacement, but higher principal stress, than those made of less stiff materials, which can be illustrated by comparison of zirconia and chairside FRC. Zirconia exhibits an elastic modulus of 205 GPa and showed 0.017 mm displacement and 239.6 MPa maximum principal stress in comparison to 0.048 mm and

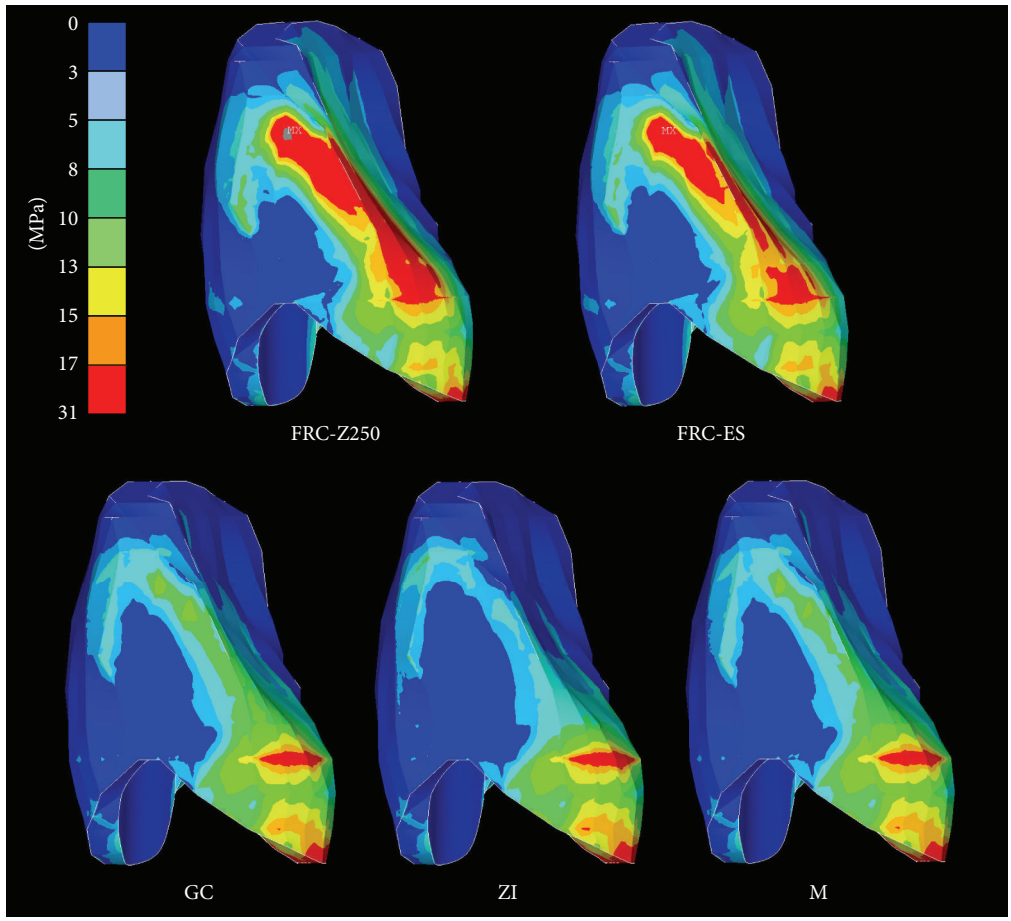


Figure 7: Principal stress distribution at the abutment tooth for two-unit cantilever RBFDPs of various framework materials.

156.9 MPa by the chairside FRC with an elastic modulus between 11 GPa (chairside hybrid composite) and 46 GPa (FRC). The highest maximum principal stress was located at the occlusal embrasure of the connector. It has to be noticed that the connector in our FE model was designed with a sharp embrasure and that stresses in this location can be significantly decreased by changing the connector design [37] and its radius of curvature [37, 38]. Recently, Plengsombut et al. confirmed this finding by revealing a significant lower fracture strength for specimens with a round connector in comparison to those with a sharp connector [39].

A similar situation with regard to stress values was found at the level of cement-retainer interface. Far more interesting were the differences in location between FRC on one hand and M, GC, and ZI on the other hand (Figure 5). A possible explanation is the difference in design between both groups of FDPs. In a FRC-FDP the stiffer fibres acts as a stress dissipater and transfers the stress from the pontic to the central part of the retainer. On the contrary, with FDPs made of a more stiff and uniform framework (M, GC and ZI) the stress is transferred more uniformly through the FDP to an area around the connector and towards the cervical margin of the retainer. Debonding of the FDPs due to early failure of the adhesive interface between retainer and cement layer is likely to be caused by such unfavourable stress location in combination with direct exposure to the oral environment. In particular zirconia, known for its weak adhesion to resin luting cements [40–42], will be prone to adhesive failure.

At the level of the cement layer there was only a slight difference in maximum principal stress values, but as expected the differences in location, as seen at the cement-retainer interface, between FRC on one hand and M, GC, and ZI on the other hand (Figure 6) became more pronounced. It is noteworthy that the cement layer, in the case of M, GC, and ZI, is able to absorb the stresses in the area surrounding the connector and to dissipate them towards the cervical outline. Stress transfer towards unfavourable locations can result in premature failure of the cement layer.

The difference in maximum principal stress value between different framework materials was even lower at the level of the abutment tooth. However, the location of the stress concentration, as depicted in Figure 7, was different. Adhesive failure at the enamel-cement interface is not very likely to occur, as enamel bonding is a reliable procedure with reported values for resin luting cements, like Variolink 2, of 49.3 MPa [43].

Based on the results of this study the predominant failure mode of two-unit cantilever RBFDPs for each framework material might be predicted. Although acceptable bond strength to resin luting cements can be achieved by glass

ceramics, their low strength could make them susceptible to connector fracture and therefore probably less suitable for the fabrication of anterior two-unit cantilever RBFDPs. On the contrary, the only clinical study published on cantilever glass ceramic RBFDPs reported 100% survival after 6-year concluding [23]. Nevertheless, in this study some minor chippings at the pontic were described [23]. There are more studies available on cantilever alumina RBFDPs [11, 44, 45]. These cantilever alumina RBFDPs exhibited a 10-year survival rate of 94.4% [11]. During their study only one cantilever RBFDP was lost due to fracture of the connector. Koutayas et al. reported connector fracture as the predominant fatigue failure of cantilever alumina RBFDPs [44, 45]. Since glass ceramics exhibit flexural strength of 252 MPa [46], which is inferior to the flexural strength of alumina (429 MPa) [47], and their bond strength to resin luting cements is superior to that of alumina [48], more fractures would be expected with cantilever glass ceramic RBFDPs. A possible explanation is the fact that alumina-based RBFDPs are made of an alumina core veneered with feldspathic porcelain, while lithium disilicate glass ceramic-based RBFDPS can be made from monolithic lithium disilicate. It is known that monolithic ceramic restorations exhibit higher fracture strength than bilayered or veneered ceramic restorations [49, 50].

Although FRC RBFDPs seem to be more promising as they exhibit good bond strength to resin luting cement, connector fracture seems to be the failure mode to be expected. Clinical [51] and *in vitro* [52, 53] findings on FRC RBFDPs also confirm this prediction. Connector fracture in all-ceramic RBFDs results in immediate loss of the pontic resulting in an acute aesthetic problem, while in case of FRC RBFDPs the glass fibres keeps the pontic in place. Nevertheless, they are at the moment only suitable as low cost temporary alternative due to the low strength of the veneering composite. Further improvements can be expected from modified framework designs [54, 55] and improved resin composites [56].

Zirconia and metal RBFDPs are suspected to fail most likely because of debonding. A multitude of clinical research on cantilever metal RBFDPs corroborates this prediction [6, 8, 9], since debonding was reported as the major reason of failure. A metal alloy exhibits plasticity, which can explain this mode of failure. Zirconia, regardless of its high strength, does not seem to be the ideal material for cantilever RBFDPs, due to the unfavourable stress distribution and low bond strength to resin luting cement leading to premature debonding. Only a limited amount of *in vitro* and *in vivo* studies on zirconia RBFDPs is available. *In vitro* studies have shown that minimal invasive cantilever zirconia-based RBFDPs subjected to fatigue loading predominantly failed due to debonding [36, 57]. However, the same studies showed a decrease in percentage of debonding in favour of retainer fractures, when a more retentive retainer design was used. Although, one should be aware that the high stress concentration at the mesiocervical edge of the retainer indicates (Figure 5) that retainer fracture in those studies is most probably the result of partial debonding. Due to partial debonding more complex torque and bending forces act on the retainer, which results in retainer fracture. The only clinical study on cantilever zirconia-based RBFDPs also reported debonding as major failure [22]. Recent improvement of the adhesive performance of zirconia by selective infiltration etching increased the bond strength to Panavia F2.0 up to 49.8 MPa [42]. The achievement of a strong and durable bond with zirconia-based materials makes it the most promising alternative to metal-based anterior two-unit cantilever RBFDPs.

5. Conclusions

Within the limitations of this study, 3DFEA revealed differences in biomechanical behaviour between RBFDPs made of different framework materials.

(1) The general observation was that a RBFDP made of FRC provided a more evenly distributed stress pattern from loading area towards abutment tooth.

(2) Maximum principal stress was located at the occlusal embrasure of the connector for all framework materials: highest value was found for ZI, while the lowest was found for FRC-Z250.

(3) Advanced stress analyses suggest a possible difference in predominant failure mode: connector fracture for FRC- and glass ceramic-based RBFDPs and debonding for metal- and zirconia-based RBFDPs.

(4) A stress concentration was found at the contact area with the adjacent tooth, indicating that the applied load is partially transferred to the adjacent tooth.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Low-Level Laser Therapy in the Treatment of Recurrent Aphthous Ulcers: A Systematic Review

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Recurrent aphthous ulcers (RAUs) are the most common lesion found in the oral cavity. There is no definitive cure for RAUs and current treatments are aimed at minimizing symptoms. Since low-level laser therapy (LLLT) modulates inflammatory responses, and promotes pain reduction and cellular biostimulation, LLLT can be suggested as an alternative treatment for RAUs. The literature concerning the potential of LLLT in the treatment of RAUs was evaluated. A systematic literature review identified 22 publications, of which only 2 studies were adopted. The eligibility criteria consisted of randomized controlled trials (RCTs). Both RCTs achieved significant results concerning LLLT and pain-level reductions and reduced healing times. Despite the variance in irradiation conditions applied in both studies, very similar wavelengths were adopted. There is accordingly strong evidence that wavelength plays an important role in RAU treatment. Taking into account the different parameters applied by selected RCTs, it is not possible to suggest that a specific protocol should be used. However, in light of the significant results found in both studies, LLLT can be suggested as an alternative for RAU treatment. Additional RCTs should be performed in order to reach a clinical protocol and better understand the application of LLLT in RAU treatment.

1. Introduction

Recurrent aphthous ulcers (RAUs), also known as canker sores or aphthae, are frequent lesions that affect the oral cavity [1]. These ulcerations affect 5–66% of the population [2]. The lesions are characterized by recurrent bouts of single or multiple rounded, flat, painful oral ulcers [2]. These ulcers result in oral epithelium wounds, which have exposed nerve endings and are associated with pain [3]. The pain inhibits patients' abilities to eat, drink, and maintain oral hygiene [4]. RAUs typically appear with grey-white pseudomembranes surrounded by thin erythematous halos [2]. These lesions typically occur in the nonkeratinized mobile oral mucosa [5, 6]. The normal progression of the lesions requires 10–14 days for healing.

Three clinical subtypes of RAUs have been established according to magnitude, number, and duration of outbreaks: minor, major, and herpetiform [7, 8]. The minor RAUs represent 70–85% of all cases and manifest themselves as small, rounded, or oval lesions, covered by a grayish-white pseudomembrane and surrounded by an erythematous halo [7, 8]. Minor RAUs episodes typically involve the appearance of 1–5 ulcers measuring less than 1 cm in diameter. These episodes are self-limiting and heal within 4–14 days without leaving scars [9–11]. Major RAUs are the most severe form of the disease and represent 10% of all cases [8–10, 12]. Major RAUs measure over 1 cm in size and tend to appear on the lips, soft palate, and pharynx. The lesions persist for over 6 weeks and can leave scars [8–10, 12]. The herpetiform subtype accounts for 1–10% of all cases and is characterized by recurrent outbreaks of small, deep, and painful ulcers. Several aphthous ulcers, measuring 2-3 mm in size, can develop simultaneously, even though they can merge and form larger ulcerations [8–10, 12].

The etiology of RAUs is not clear [13]; however a series of factors are known to predispose an individual to the disease.

The primary predisposal factors include genetic factors, alpha-hemolytic streptococcal infections [14, 15], a decreased immune system integrity, and deficiencies in folic acid, iron [15, 16], or vitamin B12 [15, 17]. Additional predisposing factors include stress, trauma [15, 16, 18, 19], allergies to certain foods [15, 16, 18], and endocrine imbalances [15, 16, 18, 19]. Immune alterations have been observed, beginning with an unknown antigenic stimulation of the keratinocytes and resulting in the activation of T lymphocytes, cytokine secretion, including tumor necrosis factor-alpha (TNF-α), and leukocyte chemotaxis. TNF-α is believed to play an important role in the development of RAUs. TNF-α has been found to be increased 2–5-fold in the saliva of affected patients [20]. Alterations in salivary enzyme defense system have been reported in affected patients [21]. An increase in lymphocyte infiltration of the epithelium is reported, which prompts ulcer formation [8–10]. On the other hand, many systemic diseases are known to be associated with RAUs, including Chron's disease, ulcerative colitis [22], Behcet's syndrome [23–25], hematological disorders, vitamin deficiencies, gastrointestinal diseases, cyclic neutropenia, Reiter syndrome, periodic fever, aphthous pharyngitis and cervical adenopathy, and immune deficiencies [26, 27].

The diagnosis of RAUs is based on patient anamnesis and clinical symptoms. There is no specific diagnostic test for RAU, though it is essential to discard possible underlying systemic causes. It is prudent to request a complete series of laboratory tests, including a complete blood count, and evaluation of iron, vitamin B12, and folic acid. A biopsy of the lesion is only recommended in the case of diagnostic uncertainty, since the findings only indicate a simple nonspecific inflammatory lesion [8–10].

Since the main etiology of RAS is still unknown, a definitive cure does not exist and the present treatments are aimed at alleviating the symptoms [28]. Some treatments have been suggested; however, such treatments are palliative, not curative [28]. Current treatment options include topical analgesic and anesthetic agents, corticosteroids, antibiotics, multivitamins, cauterization, and a variety of combined therapies [1]. Most of the treatments are associated with side effects or other disadvantages that make their usage clinically questionable [28]. A challenge to patient management is to significantly stimulate the healing process and minimize patient discomfort, without side effects [29].

Low-level laser therapy (LLLT) is also known as "soft laser therapy," "laser phototherapy" (LPT), and "cold laser therapy." Since LLLT modulates inflammatory responses with a reduction in oedema and pain and cellular biostimulation, this therapy could be considered to be an alternative treatment for RAUs [47]. This systematic literature review aimed to assess studies of LLLT used for the treatment of RAUs in terms of pain reduction and wound healing. An analysis of the quality of the studies was performed in order to gather state of art evidence about the use of LLLT for the treatment of RAUs.

2. Materials and Methods

A systematic review of the relevant literature was conducted via database research. Literature searches were conducted independently by two authors using the following databases: in the following databases until 1 June, 2014, MEDLINE, Pubmed, Embase, Cochrane Database, LILACS, and Google Scholar. Two authors independently extracted the data in duplicate. Mesh terms were used individually or combined in appropriate language forms: "aphthous," "laser therapy," "low-level laser therapy, and LLLT." Twenty-two articles were identified (Table 1). Articles in languages other than English, German, Portuguese, and Spanish were excluded; *in vitro* studies, as well as cases reports, were also excluded. Eligibility criteria consisted of randomized controlled trials (RCTs). Considering these criteria, 2 eligible studies were isolated.

Table 1: Publications found in databases up until June 1, 2014.

Authors	Year
Howell et al. [29]	1988
von Ahlften [30]	1987
Mikhaïlova et al. [31]	1992
Parkins et al. [32]	1994
Contreras et al. [33]	1994
Neiburger [34]	1995
Acuña Castro and Ovalle Castro [35]	1997
Prikuls [36]	2000
Bladowski et al. [13]	2004
Eman and Hussein [37]	2002
Kashmoola et al. [38]	2005
Monteiro and Tonani [39]	2007
Khademi et al. [40]	2009
de Souza et al. [41]	2010
van As [42]	2011
Caputo et al. [3]	2012
da Silva Marciano et al. [43]	2012
Anand et al. [44]	2013
Rozo et al. [45]	2013
Misra et al. [46]	2013
Aggarwal et al. [1]	2014
Albrektson et al. [4]	2014

3. Results and Discussion

Out of the 22 publications found in databases concerning LLLT in RAU treatment (Table 1), only two eligible studies were selected [1, 4].

Both eligible RCT studies applied LLLT for the treatment of minor RAUs [1, 4]. Albrektson et al. (2014) [4] developed a randomized, single-blinded, placebo-controlled clinical trial with predetermined inclusion and exclusion criteria. Forty patients with RAUs participated in the study (n = 20/group). The laser parameters described by the authors were GaAlAs semiconductor laser with a wavelength of 809 nm, 60 mW, 1800 Hz, a duration of 80 seconds per treatment, and a dose of 6.3 J/cm^2 [4]. In the treatment group, the laser tip was in direct contact with the ulcer, for a duration of 80 seconds; in the placebo group, the same procedure was conducted, but without any power [4]. LLLT was applied on three occasions,

each separated by 24 hours. In the study of Albrektson et al. (2014) [4], patients were asked to rate their pain on a visual analogue scale (VAS) and also discuss their subjective experience of eating, drinking, and brushing their teeth before the placebo or laser treatment and also on subsequent days [4]. In the laser group, the pain score significantly decreased on day 1 (VAS rating: 84.7 to 56.2) and on day 2 (VAS rating: 56.2 to 31.5) ($P < 0.0001$) [4]. In the placebo group, the pain score changed from 81.7 to 80.7 (days 0 to 1) and to 76.1 on day 2 [4]. All participants at the start of the trial had difficulty eating that was either moderate or severe. In the laser group, 75% of the participants had moderate or severe difficulty eating on day 1 [4]. Twenty percent of the participants had moderate or severe difficulty eating on day 2, and none of the patients had difficulty eating on day 3 [4]. In the placebo group, all participants had moderate or severe difficulty eating on days 1 and 2 [4]. On day 3, 85% of patients still had moderate or severe eating difficulty ($P < 0.0001$). The same results were obtained with drinking liquids. On day 0, 90% of patients in the laser group and 80% of the patients in the placebo group reported moderate or severe difficulties drinking [4]. On day 1, 50% of the participants in the laser group had moderate or severe difficulties drinking ($P < 0.001$) [4]. On day 2, only 5% of participants in the laser group had moderate or severe difficulties drinking; in the placebo group, 80% of patients had difficulties drinking on both days 1 and 2 ($P < 0.001$) [4]. The difference was not as stark for participants who reported severe difficulty brushing their teeth. On day 1, 50% of the patients in the laser group reported severe difficulty brushing their teeth, compared with 75% of patients in the placebo group ($P < 0.006$). On day 2, only 5% of patients in the laser group had severe difficulty brushing their teeth, compared with comparison 65% of patients in the placebo group ($P < 0.0001$) [4]. The study of Albrektson et al. (2014) revealed that LLLT promoted a highly significant analgesic effect in acute minor aphthous ulcers in comparison with the placebo group and also significantly reduced pain levels associated with eating, drinking, and brushing teeth [4].

In the second selected study, Aggarwal et al. (2014) [1] developed a sham-controlled, split-mouth study with predetermined exclusion and inclusion criteria. Thirty patients with two minor RAUs in their oral cavity participated in the study [1]. The study assessed pain reduction, lesion size, and healing time. In each patient, one of the ulcers was randomly allocated to be treated with LLLT [1]. The laser parameters described by the authors were as follows [1]: diode laser, 810 nm, and 0.5 W [1]. The treatment consisted of one appointment with four sequential sessions of LLLT applications, each lasting 45 seconds with a gap of about 30–60 seconds between each session, for a total laser application time of about 3 minutes [1]. The application of the laser was done in noncontact mode, with a distance of 2-3 mm between the laser tip and the surface of the ulcer [1]. The laser beam was applied in a continuous circular motion, covering the entire ulcer surface. For the ulcers included in the sham group, the same technique was done without activating the laser unit [1]. Immediately after LLLT application, complete pain relief was observed in 28 of the 30 patients in the LLLT group [1]. The LLLT group showed a statistically significant reduction in pain compared with the sham control group ($P < 0.001$) [1]. The complete resolution of the ulcers in the active group required 3.05 ± 1.10 days, compared with 8.90 ± 0.45 days in the sham control group. Compared with the sham group, authors found the complete healing time for the LLLT group to be highly significant, with a P value less than 0.001 [1].

Although both selected studies [1, 4] were the most well designed studies found in the literature concerning LLLT in the treatment of RAUs, both manuscripts still omit certain critical pieces of information. Both studies do not discuss the use of a power meter to check the power output before laser irradiation. It is not uncommon for laser equipment to have true power outputs that deviate from the stated power levels. Studies should always measure the power output before laser irradiation. Additionally, the method of randomization used in both studies was not described.

Both studies used different ranges of power in their treatment of minor RAUs (60 mW [4] and 0.5 W [1]). Furthermore, the studies differed in other respects as well: contact [4] and noncontact [1] of the laser tip with tissue and three irradiations in one day [1] versus one irradiation per day for three days [4]. The studies nonetheless obtained highly significant treatment results. Therefore, despite the variance in irradiation conditions, the authors used very similar wavelengths in their treatments: 809 nm [4] and 810 nm [1]. In their meta-analysis, Enwemeka et al. (2004) [48] studied the effect of low-power lasers (<500 mW) on conditions such as sores and ulcer wounds and concluded that laser therapy is effective at repairing tissue and controlling pain, although the outcomes may be influenced by the wavelength of the laser. Some studies concerning acute pain also revealed that lasers operating at infrared wavelengths led to more effective pain reduction [49–52]. Other studies in the literature that were not selected for this review once were not controlled clinical trials used other laser wavelengths, including 633 nm [29], 670 nm [41], and 904 nm [38]. These studies did not find significant differences in their results. There is accordingly strong evidence that wavelength plays an important role in the final results of RAU treatment. Future studies are encouraged to test the influence of different wavelengths on pain control and reducing the sizes of RAUs.

Clinical and laboratory evidence lends support to the use of low-level lasers to promote wound repair [53–57], as well as reduce pain [48, 58] and inflammation [59–64]. Increased blood flow and capillary vasodilatation were observed after LLLT, which are known to promote healing [65]. These effects include lymphocyte stimulation, activation of mast cells, and increased ATP production. Furthermore, the proliferation of various types of cells such as fibroblasts [63], macrophages, epithelial [66], and stem cells [67] was observed. All of these combined factors promote anti-inflammatory and biostimulatory effects, thus enhancing wound healing [56, 68, 69]. The activation of mast cells leads to the release of proinflammatory cytokines, which promotes local leukocyte infiltration of tissues. Since mast cells play a key role in leukocyte functions, the modulation of mast cell activity by LLLT can be of considerable importance in promoting

wound healing in oral cavities [65]. Increased proliferation, maturation, and locomotion of fibroblasts have been noted as side effects of LLLT. In addition, a reduced production of prostaglandin E2 (PGE2) and an increase in the production of basic fibroblast growth factor have been observed [70, 71]. These effects may improve wound healing.

One mechanism related to pain relief that has been proposed is the modulation of pain perception by modification of nerve conduction via the release of endorphins and enkephalins [47, 72]. An additional mechanism described is related to enhanced ATP synthesis in the mitochondria of the neurons [73]. When ATP synthesis is reduced, the consequence is mild depolarization, which decreases the threshold of triggering an action potential. On the contrary, an increase in ATP synthesis, which is caused by LLLT, will induce hyperpolarization and obstruction of stimuli, which would thus reduce the induction of pain stimuli [73]. The mechanism of increased ATP synthesis after LLLT is dependent upon absorption by photoreceptors in mitochondrial components, specifically in the electron transport (respiratory) chain [74]. In addition, the inhibition of prostaglandin E2 and interleukin-1 beta also help in reducing pain (PG increases pain by sensitizing the receptors by lowering their thresholds) [75]. Moreover, described study found that conduction of nerve fibers was inhibited by LLLT, due to a reversible conformational change in the voltage-gated Na-K channels [76].

Immune mechanisms appear to play an essential role in the pathogenesis of RAUs and likely involve a cell-mediated immunoresponse mechanism, with the generation of T cells and TNF-α by leucocytes (macrophages and mast cells) [77]. LLLT could affect the inflammatory process, causing a diminution in some cytokine levels, such as interleukin-1b (IL-1b), TNF-α, and interferon-g (IFN) [78]; however, their effects on RAUs should be investigated further.

Taking into account the fact that there are few eligible RCTs published in the literature concerning LLLT for the treatment of minor RAUs, it is not possible to dictate that a specific protocol should be used by clinicians. However, in light of the two significant results found in both studies, LLLT can be thought of as an encouraging alternative for RAU treatment, without side effects. Since the effect of LLLT in the treatment of other clinical subtypes of RAUs (major and herpetiform) was not assessed in the selected studies, future RCTs studies are encouraged to evaluate this effect. RCTs with larger samples and multicenter studies should also be developed in order to enrich our knowledge about the application of LLLT in all RAUs subtypes. Furthermore, long follow-ups of patients were not performed by the two selected RCTs. It is important to observe the frequency of the lesions before and after LLLT treatment. Follow-up analysis is strongly recommended.

4. Conclusions

Considering that there are just two controlled clinical trials published in the literature concerning LLLT for the treatment of minor RAUs, it is not possible to dictate that a specific protocol should be used. However, in light of the two highly significant results found in both studies, LLLT can be suggested to be a positive alternative for the treatment of RAUs. Even so, additional studies should be performed in order to support and establish a clinical protocol for all RAUs subtypes, as well as elucidate the specific mechanisms by which LLLT can promote the pain relief from and healing of RAUs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

Laser Supported Reduction of Specific Microorganisms in the Periodontal Pocket with the Aid of an Er,Cr:YSGG Laser: A Pilot Study

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Objective. The aim of this study was to evaluate the effectiveness of a radial firing tip of an Er,Cr:YSGG laser as an adjunct to a nonsurgical periodontal treatment. *Methods*. Twelve patients with chronic or aggressive periodontitis were treated by conventional periodontal treatment using ultrasonic devices and hand instruments and, additionally, in two quadrants with an Er,Cr:YSGG laser. A new radial firing tip (RFPT 14-5, Biolase) was used with 1.5 W, 30 Hz, 11% air, 20% water, and pulse duration 140 μs. Microbiological smears were taken before treatment, one day after lasing, and three and six months after lasing. Pocket depths of all periodontal sites were measured before and six months after treatment. *Results*. The total bacterial load of *Prevotella intermedia*, *Tannerella forsythia*, *Treponema denticola*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans* inside the pocket was reduced significantly throughout the whole examination time. Greater pocket depth reductions were observed in all groups. There was a slight higher reduction of pocket depth in the lased group after six months. *Conclusions*. These results support the thesis that Er,Cr:YSGG laser supported periodontal treatment leads to a significant reduction of periopathogenes and thereby helps the maintenance of periodontal health.

1. Introduction

The therapy of periodontal diseases has become one of the major fields in modern dentistry. 52.7% of the German population are infected with this disease and even 39.8% suffer from an aggressive kind of periodontitis. The British population is even more affected: 62% of the population are infected with moderate periodontal disease [1].

Gilthorpe et al. [2] described a sequential deterioration and repair that occur at the individual tooth sites over time as disease progression function. This phenomenon is individually accelerated or decelerated by different factors. One can differentiate between risk factors that are modifiable by the individual and/or the dentist and the ones that are nonmodifiable [3].

Nonmodifiable factors influence naturally the onset of periodontitis as well as the progression. They are

(i) age/gender/ethnicity,

(ii) genetical preconditions (immune system, saliva),

(iii) systemical diseases (diabetes mellitus, HIV, osteoporosis, and leukaemia). For diabetes mellitus, there is evidence that both Type-I and Type-2 diabetes have higher prevalence, extent, and severity of periodontal disease [4].

Modifiable factors are as follows.

(i) Socioeconomic Indicators. Evidence suggests that education has a greater influence than income in favourably affecting the level of periodontitis in the population [5].

(ii) Specific Microbiota. The consensus report of the 1996 World Workshop in Periodontics identified three species *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythia* as causative factors of periodontitis [3]. The authors also suggest that these three species cannot be considered the only causative pathogens but are rather the ones for which sufficient data have accumulated. In common knowledge, *Prevotella intermedia, Treponema denticola,* and *Fusobacterium nucleatum* ssp. are also mentioned as leading germs for periodontitis.

(iii) Cigarette Smoking is one of the key factors for the deterioration of the healing process, which means in the same time that the disease progression is promoted by smoking habits [6].

The only factor, which can be influenced by the dentist, besides enlightenment, is the bacterial population inside the periodontal pockets. Since this is one of the defined major risks, the dentists' work is a key factor in diagnosis, therapy, and maintenance of the periodontal condition of the patient.

This study concentrates on two types of periodontitis corresponding to the classification of periodontal diseases according to the 1999 international workshop for classification of periodontal diseases and conditions in Oak Brook (Illinois, USA): Type II: chronic periodontitis, Section B: generalized type and Type III: aggressive periodontitis, Section B: generalized type.

Just like Socransky et al. [7], Borrell and Papapanou [3] pointed out the key factor in periodontal treatment besides enlightenment of the patient; the reduction of the pathogen microorganisms is the overall aim of periodontal treatment for the dentist. These microorganisms are the reason for inflammatory chain reactions in the gingiva, which lead to the loss of gingival attachment to the tooth and in the end to the loss of the tooth.

In science, it has been proven that laser treatment has a bactericidal effect on dental tissue. Different wavelengths have been analyzed. The newest investigations since the beginning of this century show promising results concerning the Erbium, chromium doped Yttrium-Scandium-Gallium-Garnet laser (hereinafter Er,Cr:YSGG laser). This present study evaluates the capability of the Er,Cr:YSGG laser with a wavelength of 2,780 nm and a 360° firing elastic tip to be the appropriate tool to reduce pathogenous microorganisms in the oral cavity and to eliminate the biofilm on the diseased root surfaces and the infected gingiva around the tooth in addition to a nonsurgical conservative periodontal treatment by being comfortable to work with for the dentist and being nearly pain free for the patient. This special tip is able to work on the hard tissue side inside the periodontal pocket on the one hand, which is to scale the root surface as well as to treat the soft tissue side of the pocket: (1) deepithelize the gingiva from the junctional epithelium, (2) sterilize the pocket exudates and the connective tissue, and (3) carbonize the opened blood vessels.

2. Materials and Methods

2.1. Selection of Patients. In this study, 12 patients with a chronic or aggressive periodontitis have been examined and treated following the same treatment plan. In each quadrant, one could find pocket depth of at least 4–6 mm.

4 patients were females; 8 were males. Smoking was not an excluding factor for this study design. All patients had no further systemic diseases or medicamental treatment. They did not have periodontal treatment within the last 3 years and also had not taken antibiotics for the last 3 months before the start of the examination and throughout the study time. The patients were asked not to use any chlorhexidine within the treatment period.

2.2. Therapy Protocol. One week before measuring the pocket depths and taking the microbiological probe, the patients were in the office to see the dental hygienist for a professional supragingival dental cleaning. In this session, the oral hygiene index and the sulcus bleeding index were evaluated. The second appointment was the periodontal charting and the microbiology.

Two weeks after this pretreatment, all patients had a nonsurgical subgingival scaling with a sonic scaler (Kavo Sonicflex 2003 L, Biberach, Germany) and gracey curettes (Hu-friedy, Chicago, Illinois, USA) for all teeth. This scaling and root planning happened in a 24-hour time frame for optimal conditions. In addition, two quadrants were treated with a Waterlase MD Er,Cr:YSGG laser (Biolase, Irvine, California, USA). Those two quadrants were randomly distributed within the upper and lower jaw, right or left side, similar to a split mouth design, so that either the first and fourth quadrant or the second and third quadrant would get a laser treatment additionally.

The irradiation was repeated three times, each in a seven-day period for the same two quadrants. The pockets were lased in the bottom-up technique, which means the tip was placed at the bottom of the pocket and moved slowly coronal by circulating parallel to the surface of the teeth. The first postoperation microbiological smear was taken one day after the third lasing. This smear is taken only from the two quadrants which had the laser treatment.

Three months later, the patients have seen the dentist again for a microbiological smear, a hygiene session, and the oral indices. The last recall within this study was six months after treatment. In this session, another microbiological smear was taken and the pocket depth was determined again. The oral indices, that were reviewed each time the patient came to the office, were only taken to control the oral hygiene at home to exclude an error, which would be the lack of adequate oral hygiene.

2.3. Laser Settings. The MD standard handpiece was used with the new RFPT 5-14 tip. This tip has a new and special shape. It allows firing 360° to an irradiation cone as well as

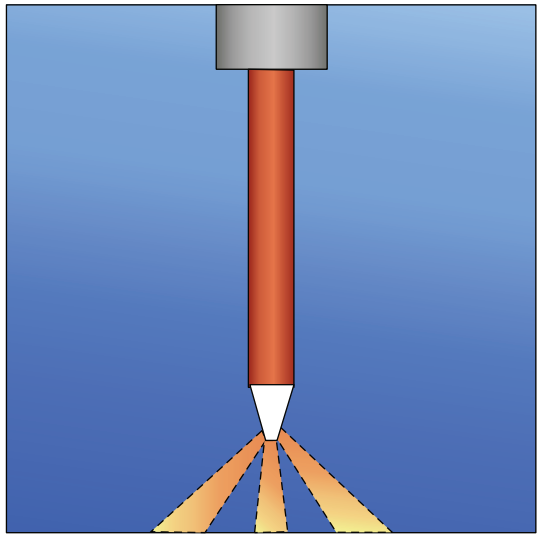

Figure 1

straight at the same time. It has a diameter of 580 μm and a length of 14 mm. It produces primarily radial emission (80%) of laser energy with a portion of straight emission (20%) as seen in Figure 1 [8]. The company also accentuates the better access to the narrow part of the periodontal pocket since the tip has no side edges and is rather flexible. Another advantage of this radiation pattern is the significantly reduced power directly in front of the tip end. That may reduce a potential risk of damaging periodontal ligament within the pocket. At the same time, the efficiency of laser power emitted radially away from the end of the tip is increased for more efficient radiation of the root surface and soft tissue of the gum.

The very end of the tip has a diameter of 100 μm. The outcoming radial radiation exits within the bottom 1/3 of the sidewall in a 52° angle, as seen in Figure 1. This tip has to be renewed after every use.

The study was done as an observational study according to the German regulations ("Medizinproduktegesetz") which are regulating the medical and ethical conditions for medical treatments. Furthermore, the used laser device and settings have also the approval of the Food and Drug Administration (FDA) for general use in dentistry.

For the laser sessions of the periodontal pockets, the settings for troughing and inner epithelium removal were used, as it is recommended and FDA-approved by the Biolase company:

1.5 Watt, 30 Hz, 11% air, 20% H_2O, H-mode (pulse duration: 140 μs)

The average power of this setting is 1.2 Watt since the used tip has a calibration factor of 0.8. The pulse energy is 40 mJ and the peak power is 285.71 W.

2.4. Microbiological Diagnostic. The pretreatment biological smear was taken from the five deepest pockets in the oral cavity but at least one site in each quadrant as a pool probe. They were gained with sterile paper points that were placed to the deepest part inside the pocket. The paper points were deposited there for five seconds, then taken out, and immediately put in a transport box. All five paper points were transported in the same box.

Carpegen Periodiagnostik in Münster, Germany, did the diagnostics. They use the real time PCR (polymerase chain reaction) to determine the six periodontal pathogens:

(i) *Aggregatibacter actinomycetemcomitans (A.a.)*

(ii) *Porphyromonas gingivalis (P.g.)*

(iii) *Tannerella forsythia (T.f.)*

(iv) *Treponema denticola (T.d.)*

(v) *Fusobacterium nucleatum* ssp. *(F.n.)*

(vi) *Prevotella intermedia (P.i.)*

With this special technique, it is possible to determine each germ with a detection limit of 100 cells. This is a very exact quantification so this technique has a very high specificity as well as sensitivity. Those cases, characterized by the number of germs being under the detection limit, were also declared with 100 cells because they are below any physiological effect.

The microbiological analyses lead to conclusions of the

(i) overall bacterial load in the periodontal pocket,

(ii) the fraction of periodontal pathogens of the bacterial load, which means the counting of the six above-named germs in percentage of the whole bacterial number,

(iii) an accurate counting of each bacterium.

2.5. Statistics. The statistical analysis for the pocket depth has been done with descriptive statistics due to the low case numbers. For the microbiological statistics, the significance level was calculated by the Wilcoxon test. P values <0.05 were accepted for statistical significance.

3. Results

The pocket depth of all sites in each patient was measured before periodontal treatment and six months after treatment. There were 580 measured sites that were treated conventionally and 588 measured sites that were treated with the laser additionally.

The pocket depths before treatment were between 2 and 12 mm. For a better analysis, it is necessary to differentiate this a little bit more. Only patient 9 had pocket depths between 10 and 12 mm. All the other measured sites were in a range of 2–8 mm.

After six months the post-op pocket depths ranged between 1 and 5 mm. All deep sites in patient 9 have been reduced to a depth of four and beneath. Only in patient 5 were there sites that measured 5–7 mm still after treatment. Those deep pockets were only found in the nonlased group.

The mean reduction of the pocket depth was almost the same in the conventionally treated quadrants and the lased quadrants. The mean reduction for the conventionally treated sites was 1.89 mm (standard deviation of 0.76). The mean reduction for the lased sites was 1.92 mm (standard deviation

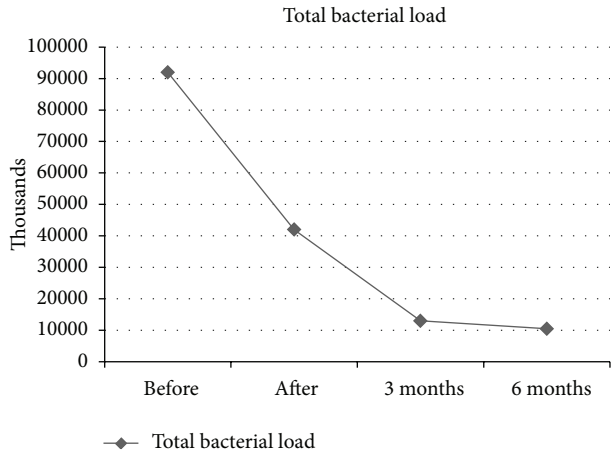

Figure 2

Table 1: Clinical Parameters at baseline and 6 months after treatment.

	Baseline	6 months	Mean reduction
Test group	5.28 ± 0.91	3.36 ± 0.72[a]	1.92 ± 0.64
Control group	5.11 ± 0.83	3.22 ± 0.52[a]	1.89 ± 0.76

[a]$P > 0.05$; P values represent statistically significant changes from baseline within each group.

of 0.64). So, there is a slight higher reduction of pocket depth after six months in the lased group (Table 1).

The total bacterial load means all bacteria that were found and counted in the biopsies, not only the six mentioned pathogens. This number, as one can see in Figure 2, was reduced throughout the whole period of examination. Directly after treatment, the bacterial number was reduced from $91.2 \cdot 10^9$ to $42.1 \cdot 10^9$ and, after three months, to $12.9 \cdot 10^9$ 12972500. Six months after treatment, the bacterial load was the lowest value with $10.3 \cdot 10^9$. In Figure 2, the mean values of the bacterial load of all patients are illustrated.

The bacterial quantity was reduced by the laser treatment significantly from the start point to 3 months after treatment ($P < 0.002$) and also to 6 months after treatment ($P < 0.004$).

The percentage decrease of all bacteria in mean for all patients from the disease to 6 months post-op is −88.72%.

Single Count of Each Pathogen. Prevotella intermedia was the most present germ in the reviewed oral sites at baseline. Its mean number at baseline was $10.2 \cdot 10^9$. Since it was not present in four patients, the mean number has a very high standard deviation ($25 \cdot 10^9$). In patients where the germ was located, it was present in a very high number, up to a maximum of $9.1 \cdot 10^7$. Therefore, this germ was reduced extensively after 3 months and after six months to three orders of magnitude smaller (mean value after six months is $8.7 \cdot 10^5$). The number continues to decrease throughout the whole examination period. Its reduction was significant after 3 months to baseline ($P = 0.013$).

Porphyromonas gingivalis, at baseline, was present in all but four patients. After three months, in all other eight patients, the number of *P.g.* was reduced for more than 92%. This is a reduction of two orders of magnitude. After six months, the quantity in two patients increased again. There was still a reduction of more than 99% in three patients; in altogether five patients the number was reduced for more than 85%. The mean number of subsistence of this germ slightly increased from 3 months after treatment to the 6-month post-op examination although there was no significance in the reduction of *P.g.*

Tannerella forsythia was reduced in all patients but one extensively directly after treatment from a mean number of $2.4 \cdot 10^6$ to $2.6 \cdot 10^5$. This reduction was even slightly clarified after three months, with a mean number of $1.4 \cdot 10^5$ germs and continuing like so after six months, with $1.3 \cdot 10^5$ in mean. The last microbiological count showed a percentage reduction to baseline of 94.67%. The reduction after three months was significant to baseline ($P = 0.012$) as well as after six months to baseline ($P = 0.028$).

Treponema denticola was present in all patients at baseline with a mean number of $3.3 \cdot 10^6$. It was reduced with a high significance from baseline to one day after treatment ($P = 0.002$) and continued to become less at 3 months after treatment with a significance level of $P = 0.006$ to baseline. This continued for the next three months. The reduction after 6 months showed a reduction to baseline with $P = 0.003$. A big reduction was proven again from $2.7 \cdot 10^5$ germs at 3 months to $1.2 \cdot 10^5$ at 6 months after treatment. Its appearance was reduced in all patients. In one patient, this germ was reduced to 100% and in four patients to more than 99% after 6 months.

Fusobacterium nucleatum also showed a response to the laser treatment. It was present at baseline in 10 patients with $5.3 \cdot 10^5$ counts in mean. The quantity stayed throughout the whole examination in the same order of magnitude. It was reduced one day after treatment to $2.5 \cdot 10^5$ with a significance level of $P = 0.001$ and was reduced even more after 3 months to $1.3 \cdot 10^5$ ($P = 0.006$). The next examination showed an increased number of $2.4 \cdot 10^7$ which is still in mean −54.64% below baseline but similar to the first post-op day and therefore still a significant reduction to baseline with $P = 0.003$.

The germ *Aggregatibacter actinomycetemcomitans* must be evaluated in a more differentiated way. It was only present in three patients in very unequal numbers. Therefore, it was not reasonable to calculate mean values but to look at them individually.

In two patients, it was reduced in the quantity from baseline. In one patient, its number even increased drastically.

Fraction of Periodontal Pathogens. The third part of diagnostics in the microbiological smear is the fraction of the mentioned pathogens from all bacteria inside the periodontal pocket. This is a number in percentage, which has been determined in every smear. The fraction was very diverse for each patient, so there is a high standard deviation (written in brackets) for the mean number. This was especially the case for the 6-month posttreatment result. The mean for all patients was decreased by almost the half from 12, 63%

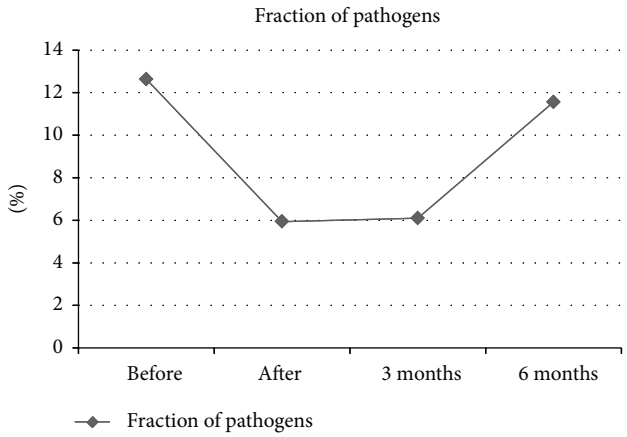

Figure 3

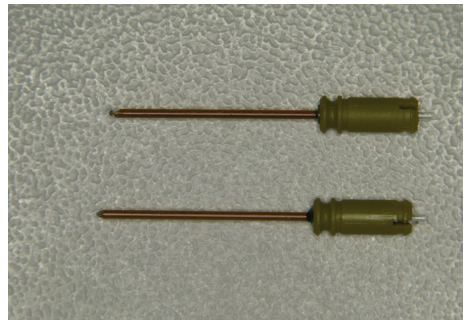

Figure 4

(0.08) to 5, 94% (0.06) directly after treatment and stayed like this for 6, 1% (0.06) 3 months after treatment (Figure 3). Its number grew within the next 3 months then to 11, 56% (0, 11).

4. Discussion

Surprisingly, there is no significant difference between both treatment methods considering the pocket depths after six months. Although the antibacterial effect of the laser treatment was very effective and enduring, the pocket depth reduction is similar to the nonlased quadrants. An interesting investigation would be a long-term control after one year or even after three years to determine the long-term role of the microbiological results. This will show whether the lased pockets succumb to less recolonization or are recolonized by only less harmful germs. Crespi et al. [9] found in their 2-year follow-up study a significant difference in the probing depth to favour Er:YAG treatment over conservative treatment with ultrasonic devices.

Schwarz et al. [10] also found a significant better attachment level on those sites that were treated with laser after two years compared with SRP. This might confirm the supposition that laser treatment has a better maintenance effect than all other kinds of conservative periodontal treatment.

Discussing this part of pocket depth reduction, there are two important issues to take into consideration.

Smoking was not an excluding factor for the participating patients. Since this is a key factor in the progression of periodontal disease, whether in proximate studies smokers should be excluded can be discussed. How far nicotine influenced the outcome of this study and inhibited reattachment cannot be considered.

Second issue is the initial pocket depth. Several studies indicate this issue by pointing out the difference in reduction of pocket depth depending on the initial pocket depth [11]. In the present study, initial pocket depths of 2–11 mm were measured and included in the study. P. A. Adriaens and L. M. Adriaens [11] make a difference between "initial pocket depths of 4–6 mm and those over 6 mm." For a more differentiated conclusion, it would be recommendable to make this parting. So the appropriate therapy could vary whether one has to deal with a chronic periodontitis (Type II) or an aggressive periodontitis (Type III).

The results of this study show a discrete reduction in the bacterial load inside a significant number of examined periodontal pockets as well as a considerable reduction of each examined bacterium itself.

A special focus is on the result of the reduction of the pathogenous bacteria. This is the crucial number for periodontal disease and destroying of periodontal tissue. Since periodontitis is an inflammatory event caused by the examined pathogens, the aim of periodontal treatment is to reduce their fraction inside the periodontal pocket. Nine out of ten examined objects showed a significant reduction of these pathogens, which leads to the assumption that the Er,Cr:YSGG laser has a large impact on the microflora inside a periodontal pocket.

Since we have modern tools, now, like 360° firing tips, the research with those instruments should progress. Maybe different settings or an advanced water-cooling system for this tip could lead to a treatment plan which maybe makes the hand instrumentation secondary or needless. Then, the treatment with laser would be the gold standard for periodontal treatment.

Application Pros and Cons. In the past, for pocket curettage, preference had been given to diode lasers and Nd:YAG lasers by clinicians because of their flexible fiber delivery system, which is suitable for all hidden areas inside the oral cavity [12]. But as the operator with this new flexible 14 mm long tip, I must say it is an alleviation to work with this kind of instrument. No danger of breaking the tip inside the pocket appeared during this study and all teeth could have been treated appropriately. It was easy to access all furcations, pocket depths, and distal regions although this tip is for one-way use, since its apex is deformed after treating 4 quadrants. This was evaluated only by visual validation (Figure 4).

During the study, most of the patients felt pain and asked for local anesthesia for the next laser session. Additionally, three of them disapproved the laser treatment for pain that stayed for 1-2 days after every treatment. This pain was described as a dull pinch on the whole gingiva of the laser treated quadrants. In two patients, transformation on the

gingival surface appeared. Those white mucosal modifications disappeared after 2 days. They were not hurting or bleeding by contact, although those patients felt this just described pain inside the whole jaw. A reason for this could be the applied energy. Perhaps this was too much energy for periodontal treatment with this tip or at least for the soft tissue side of the periodontal pocket. An explicit advantage of this tip is the decreased amount of straight energy emission, thereby reducing the potential damage to the periodontal ligament when the tip is used vertically [8]. Consequently, a lot of the applied energy penetrates to the surrounding tissue. Another explanation could be the water-cooling. Although water-cooling was used, it can happen that, in deep pockets, the water-cooling does not reach the pocket bottom and laser energy is transformed in too much thermal energy in this region. Since the tip is 14 mm long and sometimes the pocket tissue is very tight, water-cooling may not follow the way into the pocket to the end of the tip.

Further studies for this special Perio tip are necessary to clear this matter.

Special Role of Actinobacillus actinomycetemcomitans. There are still a lot of things and functions of *A.a.* that are not known yet, but what is clear is that this organism is capable of causing marked alterations in its host as a result of its powerful toxins and its ability to adhere to host cells and to enter into them and travel through them [13]. Laser due to the thermal energy that is transferred to the tissue kills this germ. This may be an accusation for human treatment. But considering that the heat that is needed for deleting *A.a.* is about 42°C, the laser energy that is needed is below any concerning level.

In the present study, only in four patients was *A.a.* present at baseline. In three cases, its quantity was reduced at six months after treatment. In one patient, it could be reduced under the detection limit. This is a very good result compared to other studies that have examined periodontal treatment with or without laser, but it demands more evidence based examinations since now in research only therapies using amoxicillin and metronidazole in combination with full-mouth scaling and root planning were able to reduce *A.a.* in all cases [12, 14].

Abuse of Antibiotics. A lot of diverse studies of conservative periodontal treatment have shown that mechanical treatment itself, even when performed with sonic or ultrasonic devices, in some, especially severe, cases of periodontal disease, does not lead to a complete healing or a satisfactory reattachment. This is due to the fact that mechanical treatment does not delete the periopathogenes adequately. Herrera et al. [15] reviewed the beneficial effect of adjunctive systemic antibiotics on the clinical outcomes of nonsurgical periodontal treatment. The gold standard for this adjunctive medication is a treatment with amoxicillin and metronidazole as a combination dose for seven days. van Winkelhoff and Winkel state in 2009 [16] that this mentioned antibiotic combination still seems to have the best clinical outcome.

However, a big issue in the application of systemic antibiotics is a multiplicity of possible side effects. Even mild secondary effects can lead to the patient not taking the medication properly as prescribed, thereby decreasing the efficacy of the medication [17].

Another problem in medication with systemic antibiotics is that many bacteria become more and more resistant to antibiotics or find ways to guard themselves against these substrates, especially those germs that are organized in a biofilm [18]. van Winkelhoff and Winkel [16] are convinced of the necessity of taking a bacterial profile before each periodontal treatment, since they found that treatment of *P. gingivalis*-negative patients with antibiotics may be considered an overtreatment. They say that the subgingival microbial profile at baseline may be one determining factor of the clinical effects of systemic antimicrobial therapy. Facing the progress of resistance of bacteria against antibiotics, it is an essential tool in periodontal treatment to take a bacterial profile before treatment and to decide in each case whether there is not an equal or even better treatment plan for each individual case. This study shows a new effective way of treating chronic periodontitis without antibiotics, excepting germ pools that contain *A.a.*

Like Herrera et al. [15], I would like to still stick to the old postulate that, due to the problems related to their indiscriminate use like systemic side effects and increase in bacterial resistance, the use of systemic antimicrobials in periodontitis should be restricted to certain patients and certain periodontal conditions. Moreover, when antibiotics are prescribed, they should be given within the context of biofilm disruption and related to the properties of the target.

If laser treatment helps to avoid loading human systems with antibiotics only in some cases, it is already a very good step for the health of the patient.

5. Conclusion

With the laser, it is possible to deepithelise the flap, respectively, the inner-epithelium lining, in order to increase the distance for the epithelial cells to travel and to allow the periodontal ligament cells to reach the radicular surface first. This will avoid a so-called long epithelial attachment.

Not only the acceptance of laser supported periodontal treatment by the patients, which helps to encourage the compliance, but also the very high bactericidal effect as it is shown in this and in other studies, makes the laser treatment an indispensable part of periodontal treatment. Furthermore, as it seems to be in the present study and as it has been reported that the bactericidal effect of laser treatment has a better maintenance effect and therefore a better wound healing effect than SRP alone [19]; the laser assisted treatment may be a definitely better treatment as the nonsurgical periodontal treatment without laser. The clinical and microbiological improvements may be a combination of a beneficial conditioning of the root surface, mechanical disorganization of the biofilm, and reduction in viable bacteria as well as inactivating bacterial toxins [20]. Therefore, it can be assumed that the repetition laser sessions may be done

with less energy, since scaling and root planning work on the hard tissue side is done and only the antimicrobial part is of importance.

The new RFPT 5-14 tip is characterized by a good handling for the operator, a low fracture risk, and, due to its flexibility, a good access to furcations, distal regions, and other difficult parts inside the oral cavity. However, more studies have to follow in order to specify the laser settings, particularly the water-cooling, to reduce the patients' pain and the soft tissue reaction.

Those side reactions should be remedied in order to find a new omnipotential tool for periodontal treatment, since the 360° firing tip offers the opportunity to efficiently treat the soft tissue site of the periodontal pocket and at the same time clean and decontaminate the hard tissue side of the tooth. This is a clear advantage of this new tip in connection with the wavelength of 2,780 nm.

6. Summary

The results in this study prove that the Er,Cr:YSGG laser with a 360° firing tip is able to reduce pathogenous microorganisms in the periodontal niche significantly. Microbiological examinations showed a strong reduction of the whole bacterial amount in the pocket as well as the number of each periodontal pathogen. This result stayed true until 6 months after treatment. The pocket depth of the treated sites showed a better reduction after using the laser compared with the nonlased sites.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Influence of Air Abrasion and Sonic Technique on Microtensile Bond Strength of One-Step Self-Etch Adhesive on Human Dentin

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The purpose of this *in vitro* study was to evaluate the microtensile bond strength of one-step self-etch adhesive to human dentin surface modified with air abrasion and sonic technique and to assess the morphological characteristics of the pretreated dentin surface. The occlusal enamel was removed to obtain a flat dentin surface for thirty-six human molar teeth. The teeth were randomly divided into three experimental groups ($n = 12$ per group), according to the pretreatment of the dentin: (1) control group, (2) air abrasion group, and (3) sonic preparation group. Microtensile bond strength test was performed on a universal testing machine. Two specimens from each experimental group were subjected to SEM examination. There was no statistically significant difference in bond strength between the three experimental groups ($P > 0.05$). Mean microtensile bond strength (MPa) values were 35.3 ± 12.8 for control group, 35.8 ± 13.5 for air abrasion group, and 37.7 ± 12.0 for sonic preparation group. The use of air abrasion and sonic preparation with one-step self-etch adhesive does not appear to enhance or impair microtensile bond strength in dentin.

1. Introduction

Achieving effective bonding to dentin is still a major challenge because of higher organic content of dentin, fluid pressure from the dentinal tubules, and the presence of the smear layer [1–3]. There are two main strategies used to create effective dentin bonding: etch-and-rinse adhesives which work by removing the smear layer with phosphoric acid, followed by the application of a primer and an adhesive and the self-etching adhesives which are composed of acidic primer, responsible for interaction with the smear layer, and an adhesive for infiltration of partially demineralized dental tissues. Acid etching of dentin, which removes the smear layer completely and demineralizes the subsurface [4], is an established and predictable clinical procedure, but features inherent to dentin conditioning can influence the bonding performance of adhesives [5]. Dentinal collagen exposed by an etch-and-rinse adhesive has been found to be highly vulnerable to hydrolytic and enzymatic degradation processes [6–8]. A promising approach to adhesion is the use of one-step self-etch adhesives that slightly demineralize the dentin surface and simultaneously provide resin infiltration [9]. When using self-etch adhesives, a hybrid layer is formed with the smear layer incorporated [4]. Self-etch adhesives can improve dentin bonding strength and provide adhesion to dentin comparable or even superior to bonds obtained with adhesive systems that advise acid-etching as a separate step of the bonding protocol [3, 4, 10]. Advantages of using self-etch adhesives include simplification of the bonding procedure, reduced technique sensitivity, since etching, priming, and

Table 1: Chemical composition and application procedure of G-bond, according to the manufacturer.

Chemical composition G-bond	Application mode G-bond
Acetone (40%), 4-META (15%), Water (20%), urethane dimethacrylate monomer (UDMA) (9%), triethylene glycol dimethacrylate (TEGDMA) (10%), phosphate monomer, 4-META: 4-methacryolxyethyl trimellitate anhydride; fumed silica filler, photoinitiators	Apply one coat of adhesive on dentin surface (dry or wet). Leave undisturbed for 10 s. Strong air-drying for 5 s. Light-cure for 10 s

bonding occur simultaneously [11], reduced risk of incomplete resin impregnation of the demineralized dentin, and reduced incidence of postoperative sensitivity [12]. Furthermore, self-etch adhesives are less sensitive to moisture control [13]. "Mild" self-etch adhesives (pH around 2) only partially dissolve the dentin surface, so that a substantial amount of hydroxyapatite remains available within a submicron hybrid layer [14], encapsulating and protecting the collagen [14, 15]. Adhesion is consequently obtained micromechanically through shallow hybridization and by additional chemical interaction of specific carboxyl/phosphate groups of functional monomers with residual hydroxyapatite [14]. Due to all their advantages, it is recommended for adhesive procedures to use a mild self-etch approach that appears to provide better long-term perspectives at dentin [16].

Different techniques are used for cavity preparation or modification of dentin surface which may result in distinct smear-layer features [17, 18]. The characteristics of a smear layer, obtained with different dentin pretreatments, influence strongly the effectiveness of self-etch adhesives and different bonding interactions could be expected [4, 19–21]. Dental adhesives were developed primarily for cavities prepared with burs. Due to newer different preparation techniques used in restorative dentistry, it is necessary to assess their effect on bonding of self-etch adhesives to dental hard tissues.

Air abrasion is a technique for cavity treatment which involves the use of aluminum oxide powder, in a fine stream of compressed air. As the particles collide with dentin, the kinetic energy of the particles is released, resulting in fracture of microscopic fragments [22]. In this way, air abrasion creates a roughened tooth surface which may make it more conducive to bonding. More recently, various types of sonic instruments were introduced for use in cavity preparation [23]. Sonic instruments might remove the smear layer from the dentin surface leaving it roughened.

The aim of this *in vitro* study was (1) to evaluate the microtensile bond strength of one-step self-etch adhesive to human dentin modified with air abrasion and sonic preparation and (2) to evaluate the morphological characteristics of the pretreated human dentin surface.

2. Materials and Methods

Thirty-six intact human molar teeth, with no restorations or caries lesions, extracted for periodontal or orthodontic reasons, were used in the experiment. After extraction, the teeth were thoroughly cleaned using brushes and curettes and stored in 1% chloramine solution at room temperature for one month until use. The teeth were randomly divided into three experimental groups ($n = 12$ per group), according to the dentin preparation: (1) control group; (2) air abrasion group; and (3) sonic preparation group.

2.1. Preparation of Specimens. The entire occlusal enamel was removed by sectioning with a circular diamond blade in an Isomet 1000 saw (Buehler, Düsseldorf, Germany), with a speed of 150–200 rpm under continuous water cooling to obtain flat dentin surface. In order to form smear layer on the bonding surface of dentin, the surface was hand polished with wet sandpapers of different grit size [24], from coarser to finer (400-, 600-, 1000-grit) for 60 seconds each. The bonding surface was washed with water and gently dried with an air syringe of a dental unit (Kavo Primus, 1058 S/TM/C/G, Biberach/Riss, Germany) prior to the pretreatment. One operator prepared all specimens with the particle abrasive instruments and sonic instruments. For the air-abrasive procedure, 50 μm particles of aluminium oxide (Rondoflex, KaVo, Biberach, Germany) were used in a perpendicular direction to the dentin surface with 80 psi pressure for 15 seconds. In third group, the entire dentin surface was treated with a sonic instrument (KaVo Sonicflex 2003 L, KaVo, Biberach, Germany) with a diamond microtip number 32 for 15 seconds.

Ten teeth from each experimental group were selected for bonding procedure and subsequent microtensile bond strength testing. The remaining two teeth from each experimental group were used for scanning electron microscopy (SEM) analysis. Following the application of the adhesive system (G-bond, GC, Tokyo, Japan) according to the manufactures instructions (Table 1), a composite resin block (Gradia Direct, GC, Tokyo, Japan) 5 mm high was built up on the bonding surface, with the application of layers of the material not thicker than 2 mm, each one cured with a Bluephase LED light (Ivoclar Vivadent, Schaan, Liechtenstein, 1200 mW/cm^2, soft start) for 20 seconds. The bonded specimens were stored in distilled water at 37°C for 24 hours. The bonded teeth were then embedded into acrylic resin (Orthocryl, Dentaurum, Ispringen, Germany). Afterwards, the embedded teeth were cross sectioned longitudinally with a diamond blade in Isomet 1000 saw (Buehler, Düsseldorf, Germany), with a speed of 150–200 rpm under continuous water cooling, to obtain multiple beam-shaped sticks, with a cross-sectional top of about 1 mm^2. Beams were stored in at room temperature in sterile gauze soaked in saline. Before testing the bond strength, each beam was checked under the stereomicroscope (Olympus SZX-12, Optical Co, Europe, GMBH, Hamburg, Germany) to verify that the adhesive interface was perpendicular to its long axis. Only the beams

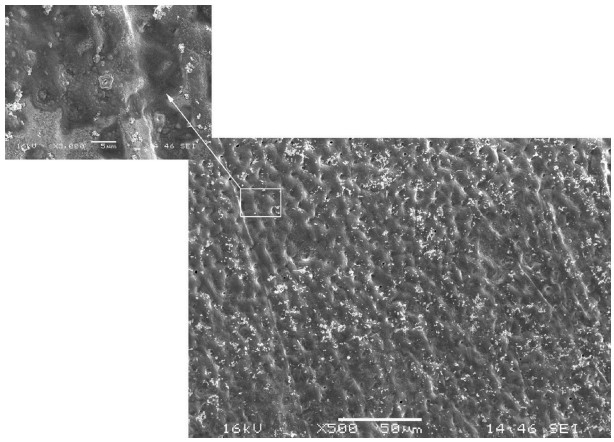

Figure 1: SEM (×500) showing dentin surface of the specimens in the control group. At higher magnification (×3000) intact smear layer can be observed.

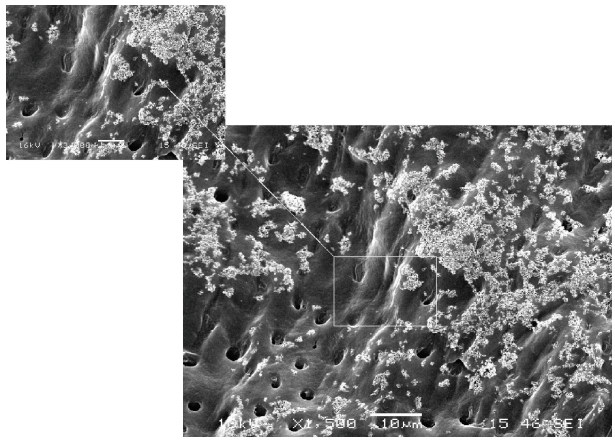

Figure 2: SEM (×1500, ×3000) showing dentin surface in air abrasion group.

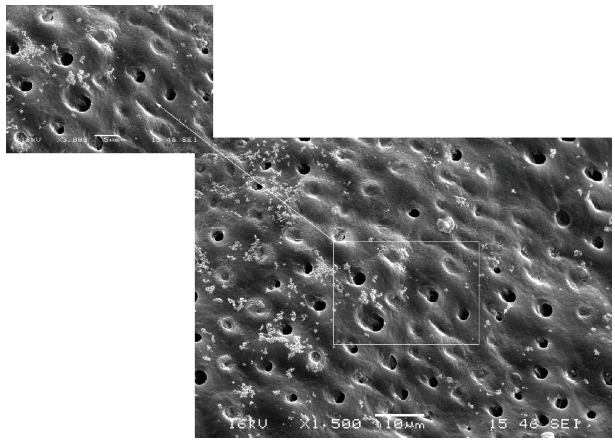

Figure 3: SEM (×1500, ×3000) showing dentin surface in sonic technique group.

with the adhesive interface perpendicular to the long axis were used in the experiment.

2.2. Testing Microtensile Bond Strength. The microtensile bond strength was tested with a universal testing machine (Triax Digital 50, Controls, Milano, Italy). Ends of each beam were glued with cyanoacrylate adhesive (Loctite gel, Henkel, Düsseldorf, Germany) to specially designed metal plates. Each beam was placed in the testing machine and the tensile load was applied at a crosshead speed of 0.5 mm/min, until the composite separated from the dentin. The load at the point of failure was recorded. Test beams were observed under a stereomicroscope to verify the failure mode (adhesive, cohesive, or both). Failures were classified as adhesive failure if the fracture site was entirely within the adhesive, mixed failure if the fracture site continued from the adhesive into either resin composite or dentin, and cohesive failure if the fracture occurred exclusively within the resin composite or dentin [25]. The cross-sectional area at the site of fracture was measured for each specimen to the nearest 0.01 mm with a digital caliper so the bond strength at failure (MPa) could be calculated.

2.3. SEM Evaluation. Two specimens from each experimental group were selected randomly after surface preparation and subjected to SEM examination, to observe the bonding surface. For the SEM analysis, specimens were cleaned in an ultrasonic bath for 5 minutes, gently decalcified with a 32% phosphoric acid (Bisco, Schaumburg, Illinois, USA) for 30 seconds, washed, and air dried. Samples were then dehydrated in an ascending ethyl alcohol series (25%, 50%, 70%, 80%, 90%, and absolute alcohol) with three baths for 5 seconds for each concentration, critical-point dried, and sputter coated with a gold layer in a vacuum apparatus (Polaron Range SC 7620, Quorum technology, Newhaven, UK). Specimens were observed under SEM (JSM-6060LV JEOL, Tokyo, Japan) operating at 16 kV and micrographs of dentin surfaces were taken at standardize magnifications.

2.4. Data Analysis. Data were statistically analyzed by a one way ANOVA, after confirming normal distribution of the results with Kolmogorov-Smirnov statistical test. Comparisons between groups were done using a Scheffe test at a 0.05 significance level. The statistical analysis was performed using Statistica 7.0 (StatSoft, Tulsa, OK, USA).

3. Results

3.1. SEM Observation of Dentin Surfaces. The control group revealed a dentin surface with a small number of exposed dentin tubules and intact peritubular and intertubular dentin (Figure 1). It was also possible to verify an intact smear layer (Figure 1).

Particle abrasion preparation procedure formed somewhat roughened dentin surface, with partially opened dentin tubules and intact peritubular and intertubular dentin (Figure 2). In the specimens prepared with the sonic technique, dentin surface was almost completely clean of smear layer with mostly open dentin tubules, but intact peritubular and intertubular dentin (Figure 3).

Table 2: Microtensile bond strength values in MPa obtained for the different experimental groups and number of adhesive and cohesive failures.

Experimental group	Mean/MPa	SD	A*-failure	C*-failure
Control	35.3	12.8	43	21
Air abrasion	35.8	13.5	66	18
Sonic	37.7	12.0	66	14

A*: adhesive; C*: cohesive.

3.2. Microtensile Bond Strength. The number of specimens which were tested in the control, air abrasion, and sonic group was 64, 84, and 80, respectively. Means and standard deviations of microtensile bond strength expressed in MPa are shown in Table 2. There was no significant difference in microtensile bond strength between the three experimental groups ($P > 0.05$). In all groups, fractures were observed mostly between resin and dentin (adhesive failure) (Table 2).

4. Discussion

In this study, microtensile bond test was used to test the dentin adhesion of mild self-etch adhesive after three different methods of dentin preparation. *In vitro* studies examining the bond strength of restorative materials are important because they can predict their clinical behavior and long-term success. The advantages of such *in vitro* tests are their speed and simplicity, measuring just one experimental parameter and testing large number of specimens. Microtensile bond test, although possessing some limitations, remains useful as screening tools for new dental materials, adhesive approaches, and investigation of different experimental variables [26]. Reliable and accurate measurements of the microtensile bond test can be achieved if only the adhesive failures are considered for the bond strength calculation, which requires microscopic evaluation to verify the failure mode [27], and these requirements were fulfilled in the present study. Furthermore, reliability of bond strength data also depends on a number of adhesively failed specimens and a minimum of 30 specimens should be available for testing [27] and this study tested 43 specimens in the control group and 66 specimens in other two experimental groups. Although the teeth which were used for this study were collected and stored for one month until use, according to study of Santana et al. [28] this storage time does not influence the results of microtensile bond test. In order to create a standard and uniform smear layer, sandpapers of different grit sizes were used in the present study. This method provides a flat surface with fewer grooves and irregularities in comparison to rotary cutting instruments [29] and a uniform smear layer created can then be used for different surface treatments.

The results of this study showed that air abrasion and sonic technique did not influence the bond strength of one-step self-etch adhesive. SEM observations in previous studies showed that aluminium oxide air abrasion is able to produce roughened surface, increasing the surface area available for wetting and bonding by the adhesive resin [30, 31] which was confirmed with the micrographs in the present study. Similar appearance of dentin surface was observed after treatment using sonic technique. However, air abrasion and sonic technique did not increase microtensile bond strength in this study, which confirms the results of other studies [32, 33]. Considering that the surface roughness obtained with the air abrasion did not increase the adhesive bond strength in the present study, this characteristic is not the only factor influencing the bonding. Other factors also influence the adhesion: the chemical composition of the dentin surface and physical parameters [34]. Another factor which should be considered regarding mild self-etch adhesives is that they have micromechanical and chemical bond to hard dental tissues. Mild self-etching adhesives, such as the one used in the present study, do not completely expose collagen for micromechanical retention but provide an additional mechanism of ionic bonding [35]. 4-Methacryloxy-ethyl trimellitate anhydride (4-META), a demineralizing monomer with carboxylic groups, also found in the adhesive used in the present study, has been reported to improve adhesion to both enamel and dentin by establishing that ionic bond to calcium in hydroxyapatite [36]. Functional monomers in self-etching adhesives have also been shown to bond chemically to both dentin apatite and collagen [35]. The use of sonic instruments did not improve the bonding to dentin as well, although the surface was clean of smear layer. Considering that self-etch adhesives incorporate the smear layer in the hybrid layer [4] and that the formation of the resin tags in open dentinal tubules does not influence the bonding strength of self-etch adhesives [37], as the adhesive used in the present study, a possible conclusion is that these factors could explain why sonic technique did not improve the bonding to dentin.

According to the Soares et al. [38], aluminum oxide sandblasting procedure decreased the bond strength to bovine dentin which is not consistent with the results of the present study. Differences in the results can be explained by different samples employed in the studies. While Soares et al. [38] used bovine teeth for bond strength testing, in this study human teeth were used. Schilke et al. [39] reported that the density of dentin tubules is significantly greater in human dentin than in bovine dentin, which could explain different results. Furthermore, differences in the relative amounts of intratubular and intertubular dentine [40], or the nature of the intertubular matrix [41], in human and bovine teeth may result in differences in adhesive bond strength measurement. The use of air abrasion and sonic technique with one-step self-etch adhesive does not enhance or impair microtensile bond strength in dentin.

5. Conclusion

Beside conventional techniques using drills and burs, different techniques are used for preparation of hard dental tissues. According to the results of this study, the use of air abrasion and sonic technique with one-step self-etch adhesive does not appear to enhance or impair microtensile bond strength in dentin. Air abrasion and sonic technique can be used in combination with one-step self-etch adhesive as an alternative to conventional techniques.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

The Influence of Polymerization Type and Reinforcement Method on Flexural Strength of Acrylic Resin

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The aim of this study was to evaluate the flexural strength of acrylic resin bars by varying the types of resin polymerization and reinforcement methods. Fourteen groups ($N = 10$) were created by the interaction of factors in study: type of resin (self-cured (SC) or heat-cured (HC)) and reinforcement method (industrialized glass fiber (Ind), unidirectional glass fiber (Uni), short glass fiber (Short), unidirectional and short glass fiber (Uni-Short), thermoplastic resin fiber (Tpl), and steel wire (SW)). Reinforced bars ($25 \times 2 \times 2$ mm) were tested in flexural strength (0.5 mm/min) and examined by scanning electron microscopy (SEM). Data (MPa) were submitted to factorial analysis, ANOVA, and Tukey and T-student tests ($a = 5\%$) showing significant interaction ($P = 0.008$), for SC: Uni (241.71 ± 67.77)^{a}, Uni-Short (221.05 ± 71.97)^{a}, Ind (215.21 ± 46.59)^{ab}, SW (190.51 ± 31.49)^{abc}, Short (156.31 ± 28.76)^{bcd}, Tpl (132.51 ± 20.21)^{cd}, Control SC (101.47 ± 19.79)^{d} and for HC: Ind (268.93 ± 105.65)^{a}, Uni (215.14 ± 67.60)^{ab}, Short (198.44 ± 95.27)^{abc}, Uni-Short (189.56 ± 92.27)^{abc}, Tpl (161.32 ± 62.51)^{cd}, SW (106.69 ± 28.70)^{cd}, and Control HC (93.39 ± 39.61)^{d}. SEM analysis showed better fiber-resin interaction for HC. Nonimpregnated fibers, irrespective of their length, tend to improve fracture strength of acrylics.

1. Introduction

Heat- or self-polymerized acrylic resins are generally composed of polymethyl methacrylate (PMMA). They are used for complete dentures, provisional restorations, or even aesthetic surgery corrections [1, 2]. PMMA has a relatively low flexural strength [3] and can undergo failure as a result of occlusal disharmonies, overload, fatigue, and impacts caused by accidents [4]. In order to strengthen PMMA, several methods have been proposed.

The use of metal and fiber reinforcements produces beneficial results [5–7]. Metal wires can be placed inside polymers, but fibers have been demonstrated to be more effective [5]. Metal and glass fiber exhibit different mechanical properties. Due to their high modulus of elasticity, lack of resilience, and poor adherence to acrylic resin matrix, metals demonstrated significantly higher interfacial stresses within resin matrix [8, 9]. Silanized glass fibers are able to adhere to acrylic resin matrix [10]. Also, their lower modulus of elasticity compared to metals guarantees a more favorable stress distribution pattern [8]. Fiber reinforcement and resin matrix together have similar mechanical performance without high stress concentration at the interface, reducing chances of failure [9]. The potential success of the interaction between glass fibers and acrylic resins occurs when a resilient and flexible material (acrylic resin matrix) and a strong reinforcement (glass fibers) are put together [8, 11–13].

Table 1: Materials used in this study.

Material	Batch number	Manufacturer
Interlig (impregnated woven glass fiber)	12443	Angelus Indústria de Produtos Odontológicos S/A, Londrina, Brazil
Pure glass fiber	**	Maxxi Rubber, São Paulo, Brazil
Silane (coupling agents)	10916	Angelus Indústria de Produtos Odontológicos S/A, Londrina, Brazil
Thermoplastic resin	2207	Sanifill, São Paulo, Brazil
Steel wire-NiCr (0.48 × 0.63 mm)	1122520	Morelli Ortodontia Ltda, Sorocaba, Brazil
Self-polymerized acrylic resin	030211	Artigos Odontológicos Clássico Ltd, São Paulo, Brazil
Heat-polymerized acrylic resin	089215	Artigos Odontológicos Clássico Ltd, São Paulo, Brazil

**Not supplied by the manufacturer.

The effectiveness of fiber reinforcement is influenced by many variables including the quantity of fibers [14, 15] and their length [14, 16], direction [16], form [17], orientation [18], position [18], adhesion to the polymer matrix [19], impregnation with the resin [20], and type of resin [16]. The greater the amount of fibers the greater the reinforcement effect if fibers are located in the prosthesis tensile stress zone [21]. During compression, stresses are compressive at occlusal contact points and tensile stresses develop at the opposite site, next to alveolar ridges. Between these two stresses a neutral surface is called the neutral stress zone [6, 22].

Unidirectional long fibers generate orthotropic mechanical properties inside composites, producing the reinforcement effect in one specific direction [23]. On the other hand, short randomly distributed fibers or multidirectional long fibers produce an isotropic reinforced material [24], where the reinforcement effect is multidirectional [23]. If the highest stress direction is known the orthotropic reinforcement is preferred to improve mechanical properties [25, 26].

The fibers adhesion to the polymer matrix and the fibers impregnation with the resin affect the degree of reinforcement [19, 20], due to effective stress transfer from the weak polymer matrix to the fibers [2, 16]. Acrylic restorations reinforced with nonimpregnated fibers show lower fracture resistance than those reinforced with impregnated fibers [27]. However, the residual monomer release in autopolymerized or heat-polymerized acrylic resins [28, 29] increases with the addition of preimpregnated fibers [27] and this could affect the strength of the reinforced material.

It is expected that, after silanization, the reinforcement effect of pure nonimpregnated glass fibers would be similar to industrialized glass fibers. Also, it is hypothesized that fibers would produce better reinforcement than metal wire. The aim of this study was to evaluate the flexural strength differences of acrylic resin bars related to different resin polymerization and reinforcement method.

2. Materials and Methods

The materials used in this study are listed in Table 1. Twelve test groups and two control groups ($n = 10$ per group) were created with the combination of studied variables: type of acrylic resin (heat- (HP) or self-polymerized (SP)) and reinforcement method (industrialized preimpregnated glass fiber (Ind), unidirectional pure glass fiber (Uni), short pure glass fiber (Short), unidirectional and short pure glass fiber (Uni-Short), thermoplastic resin fiber (Tpl), and steel wire (SW)). The number of samples per group was based on a similar previous study [7] with the exception that more specimens were included (10 instead of 6) to implement the statistical analysis significance.

2.1. Preparation of Specimens. A condensation silicon impression material (Clonage; DFL, Rio de Janeiro, RJ, Brazil) mold was constructed from a stainless steel pattern to produce standardized rectangular specimens with dimensions of 25 mm (±2.0) × 2 mm (±0.1) × 2 mm (±0.1) and 0.11 g (±0.01), according to ISO 4049/2000 [30]. All reinforcements (glass fiber, thermoplastic resin, and steel wire) were 23 mm in length with the exception of short glass fibers (3 mm). The steel wire had a rectangular cross-section with 0.48×0.63 mm. In order to standardize the amount of glass fiber for each specimen 0.01 g of fibers was employed for all fiber groups, as weighed on an analytical balance (HR-200; A&D Company Limited, Japan). Groups with association of short (3 mm) and long (23 mm) length pure glass fibers had the total weight equally divided between the 2 fiber sizes. The thermoplastic resin (dental floss) was cleaned with 70% alcohol for 30 min. A silane-coupling agent (Silano; Angelus, Londrina, PR, Brazil) was applied to all nonimpregnated fibers.

The silicon mold was filled with a thin layer of low viscosity acrylic resin and, right above this layer, the reinforcements were positioned and fully covered with a second layer of acrylic resin, following the powder/liquid ratio recommended by the manufacturer. All reinforcements were oriented in the direction of the long axis of the specimen. The mold was covered with a clean glass slab to remove excess resin and kept at room temperature (25°C) for 20 minutes under 9.8 N load until polymerization of the resin was completed. The heat-polymerized acrylic resin specimens were polymerized in a crockpot curing (VRC, São Paulo, SP, Brazil) under 380 MPa pressure, at 120°C for 15 minutes.

Specimens containing industrialized preimpregnated glass fibers were light polymerized by irradiating 3 different areas at their top surface (center, left, and right) with a LED light source (Foshan, Guangdong, China) at 850 mW/cm^2 for 40 seconds each. Control specimens were fabricated without any reinforcement (0.109 g (±0.01)). Specimens were finished with 600, 1000, and 1200 grit silicon carbide paper (Norton, São Paulo, SP, Brazil) under constant water stream. All specimens were stored in distilled water at 37°C for 24 hours before testing.

Table 2: Flexural strength means and standard deviations (MPa) for different polymerization and reinforcement methods.

Groups	Mean (SD)	
	Self-polymerized (SP)	Heat-polymerized (HP)
Unidirectional glass fiber (Uni)	241.71 (67.77)^{Aa}	215.14 (67.60)^{Aba}
Short glass fiber (Short)	156.31 (28.76)^{BCDa}	198.44 (95.27)^{ABCa}
Unidirectional and short glass fiber (Uni-Short)	221.06 (71.97)^{Aa}	189.56 (92.27)^{ABCa}
Industrialized glass fiber (Ind)	215.61 (46.59)^{Aba}	268.93 (105.65)^{Aa}
Thermoplastic resin (Tpl)	132.51 (20.21)^{CDa}	161.32 (62.51)^{CDa}
Steel wire (SW)	190.51 (31.49)^{ABCa}	106.69 (28.7)^{CDb}
Control	101.47 (19.79)^{Da}	93.39 (39.61)^{Da}

(i) Different capital letters mean significant differences within the same acrylic resin (vertical comparison only; $P < 0.05$).
(ii) Different lowercase letters mean significant differences within the same reinforcement method (horizontal comparison only; $P < 0.05$).

2.2. Flexural Strength Test. Specimens were positioned on a 3-point bending flexural strength testing apparatus (K5005 MP; Kratos, Cotia, SP, Brazil) with two supports 20 mm apart and tested at a crosshead speed of 1 mm/min. The load at fracture was recorded in Newtons and flexure strength (FS) was calculated in MPa with the following equation: $FS = PL/wb^2$, where "P" is the maximum load at fracture, "L" is the distance between the supports (20 mm), "w" is the sample thickness, and "b" is the height. The samples' thickness and height were measured with a digital caliper.

2.3. Scanning Electron Microscope (SEM) Examination. Random samples were selected from each group and analyzed with a SEM. The samples, fixed on metal stubs, were placed in an ultrasonic bath of deionized water for 10 minutes and then sputtered with gold (1 cycle of 120 s), under vacuum, in a sputtering device (MED 010; Balzers Union, Balzers, Liechtenstein). The surfaces were analyzed by SEM (LEO 435 VP; LEO Electron Microscopy Ltd., Cambridge, UK), focusing on the fracture features, integrity, and homogeneity along the interfaces between reinforcement material and acrylic resin. Samples were examined under magnification varying from ×20 to ×10,000. The unit operated at 20 kV, WD = 15–18 mm and with a spot size range of 25 pA to 100 pA.

2.4. Statistical Analysis. Statistical analysis was performed with Kolmogorov-Smirnov test of normal distribution and two-way ANOVA (2 × 6) followed by Tukey's honestly significant difference (HSD) test with a general linear model procedure in SSPS17.0 (SPSS Inc., Chicago, USA) to analyze the interaction between polymerization type and reinforcement method. One-way ANOVA followed by Tukey's HSD test was used within each acrylic resin group to compare effectiveness of different reinforcements. For pairwise comparisons of resin types within each reinforcement group Student's t-test was used. For all tests, groups were considered statistically different at $\alpha = 5\%$.

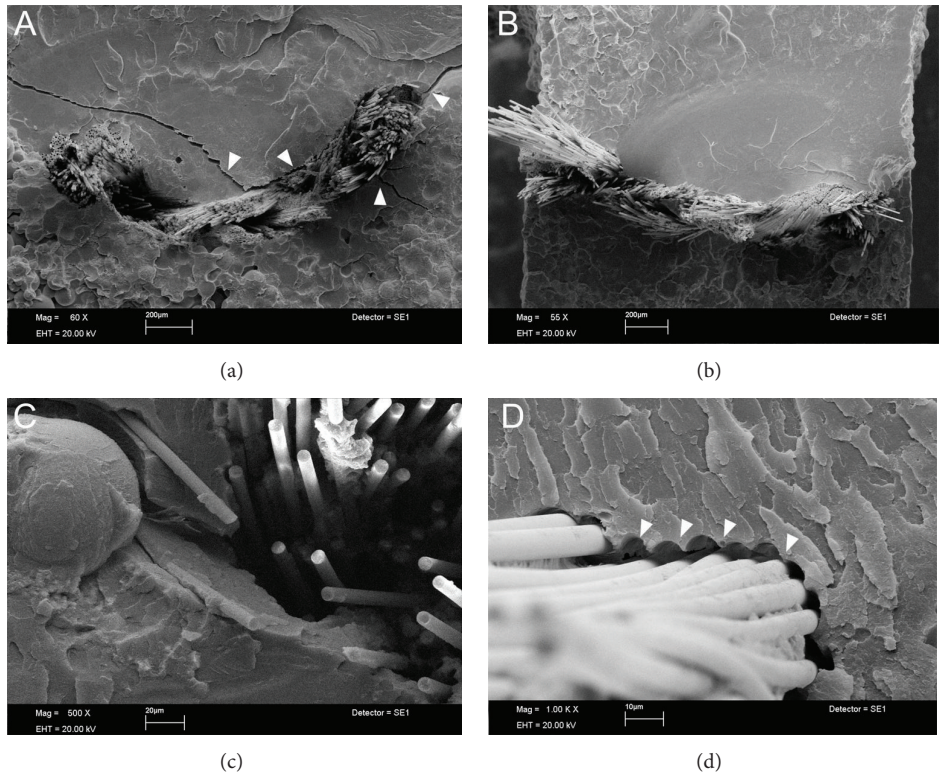
3. Results

Statistical analysis showed significant interaction between factors ($P = 0.008$) and for the type of resin ($P = 0.0001$) but not for the reinforcement method ($P = 0.728$). The results of combination of studied variables, type of acrylic resin (heat- (HP) or self-polymerized (SP)) and reinforcement method (industrialized preimpregnated glass fiber (Ind), unidirectional pure glass fiber (Uni), short pure glass fiber (Short), unidirectional and short pure glass fiber (Uni-Short), thermoplastic resin fiber (Tpl), and steel wire (SW)), are presented in Table 2. For the SP groups the control was similar to Tpl and Short, and for the HP groups the control was similar to SW and Tpl. For the SP groups the highest reinforcement effect was presented by Uni but was similar to Uni-Short, SW, and Ind. For the HP groups the highest reinforcement effect was presented by Ind but was similar to Uni, Short, and Uni-Short. Pairwise comparisons between resin polymerization types within the same reinforcement method showed differences only between SW groups, with the SP-SW presenting higher fracture strength than HP-SW.

SEM analysis showed Ind groups with areas of poor interaction between glass fiber and SP resin with the presence of empty spaces, suggesting potential sources for crack propagation (Figures 1(a) and 1(c)). In HP resin this situation was not found, showing better micromechanical interlocking (Figures 1(b) and 1(d)). Images of Uni showed partial rupture of glass fibers (Figure 2(a)). The opposite occurred in SW groups, where the metal remained intact but with poor interaction with the HP resin which resulted in the wire dislodgement (Figure 2(b)). For the SP resin, the steel wire showed a closer interaction with resin (Figure 2(c)). In short glass fiber groups it was possible to see that the reinforcement moved from the tensile to the neutral stress zone in HP (Figure 3(a)); for SP resin the fiber reinforcement kept in a more favorable stress zone (Figure 3(b)), but the micromechanical interlocking was still better in HP than in SP (Figures 3(c) and 3(d)). All Tpl groups showed complete dislodgement between reinforcement and acrylic resins (Figure 4(a)) and the presence of wax around fibers (Figure 4(b)).

4. Discussion

Fibers are known to reinforce dental polymers [16, 18, 19]. This study compared the effect of different reinforcements on the flexural strength of self-polymerized and heat-polymerized acrylic resins. It was initially hypothesized that the use of pure glass fibers would improve flexural strength similarly



(a) (b)

(c) (d)

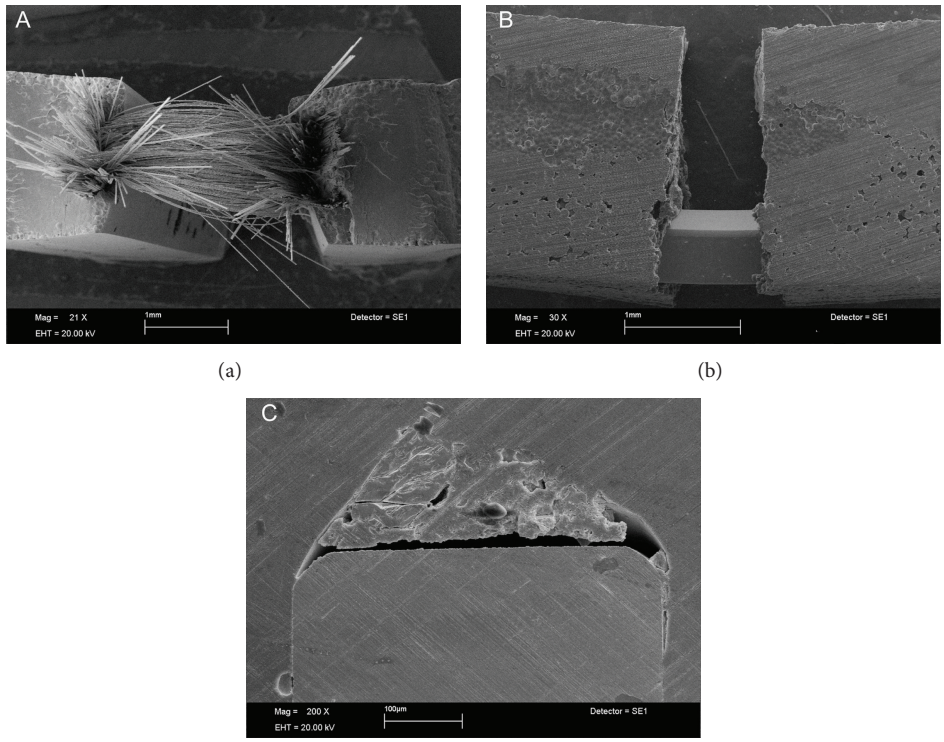
Figure 1: Woven glass preimpregnated fibers in industrialized glass fiber groups. (a) With self-polymerized resin (×60 magnification). Spaces between fibers and resin are due to failure of chemical and micromechanical interaction resulting in stress concentration regions with crack development (see arrows). (b) Micromechanical interlocking with heat-polymerized resin (×55 magnification). Note closer relationship between fibers and resin. (c) Presence of spaces between fiber and SP groups (×500 magnification). (d) Signals of spaces created after failure of micromechanical interlocking with HP (×1,000 magnification).

to preimpregnated (pre-preg) industrialized glass fiber. The results of this work showed that the use of glass fiber reinforcement significantly increased mechanical properties for both resins and different fibers had similar behavior, confirming this hypothesis. Also, it was hypothesized that fibers would enable better reinforcement than steel wire, but this could be only partially accepted. The results of this work showed that self-polymerized groups fiber reinforcement produced similar reinforcement as the steel wire and heat-polymerized groups short fiber reinforcements presented similar flexural strength as steel wire. Fiber-to-resin interaction, residual monomer attack, voids, and crack development may be the reasons for these results.

Heat-polymerized acrylics (HP) release less residual monomers than the SP ones once high temperatures promote higher degree of conversion and reduced powder (PMMA)/liquid (MMA) ratio in the mixture, affecting flexural strength [29]. Besides that, fiber reinforced resin can present voids and cracks (Figures 1(a) and 3(b)). Voids and cracks may be developed due to monomer attack at the pre-preg resin (Figure 1(a)) or even as a consequence of fiber insertion, in cases of poor impregnation of fibers by resin (Figure 3(b)) as well as a result of the polymerization shrinkage of resin [19, 31]. These defects affect the load-bearing capacity of the fiber/resin complex [19, 25]. In spite of the fact that addition of fibers increases residual monomer generation [28] the present study did not show any significant reduction in strength, even with the preimpregnation of glass fibers. However, a better micromechanical interaction between fibers and HP groups was observed (Figures 1 and 3), possibly due to applied pressure, high temperature during heat polymerization, and lower polymerization shrinkage [29].

The oxygen inside voids inhibits the polymerization of acrylic resins and the porosities can increase water sorption by polymeric matrix with a detrimental effect on mechanical properties in a long-term evaluation [25]. The residual monomers promote the degradation of the pre-preg in industrialized fibers [25], which possibly affect the interaction between fiber and resin. Since HP resins produce less residual monomers better interaction was expected with fibers than SP resin. Figure 3(c) shows fibers fracturing at the same location as the resin without dislodgment from the matrix; on the other hand, Figure 3(d) shows dislodged fibers with poor interaction with resin. The high temperature during resin polymerization for HP creates a condensed silane-coupling layer at the fiber boundaries, increasing adhesion [32].

Comparisons of flexural strength (FS) among the groups showed interaction between the factors in the study. For SP groups all reinforcements improved FS with the exception of Short and Tpl groups, and for HP groups Tpl and SW did not show improved FS. In agreement with this study, a previous report [7] found similar FS of unreinforced SP and



(a) (b)

(c)

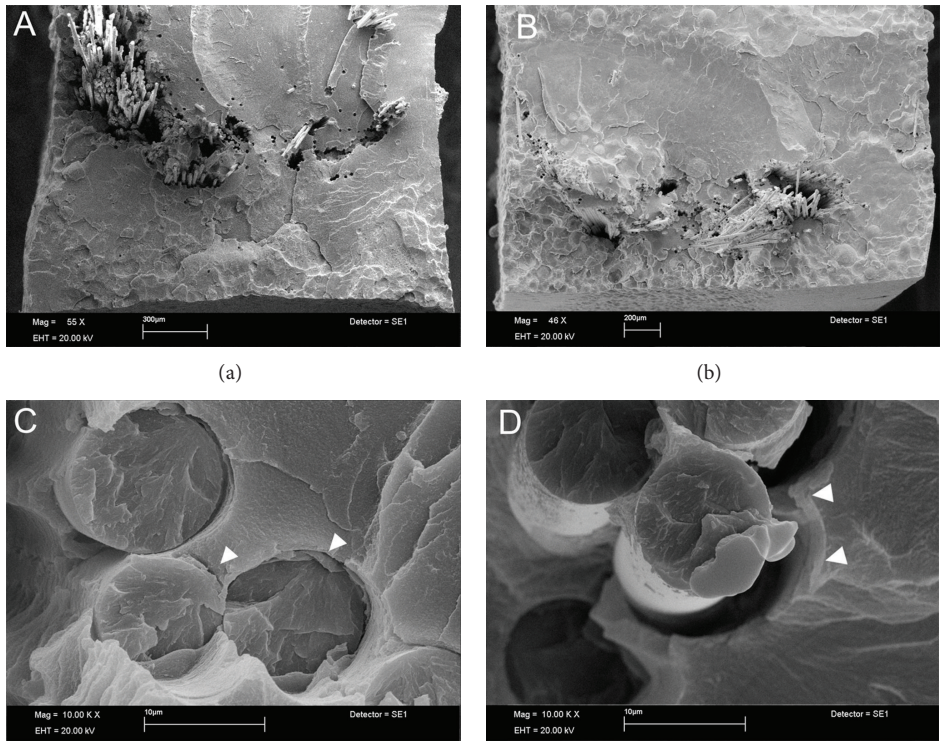
Figure 2: Fiber rupture and metal dislodgement within acrylic resins. (a) Unidirectional glass fibers in self-polymerized resin showing partial fiber rupture (×21 magnification). (b) Intact steel wire with resin fracture and separation: metal smooth surfaces did not micromechanically interlock with heat-polymerized resin (×30 magnification). (c) Tensile side of specimen with steel wire and self-polymerized resin (×200 magnification). In spite of observed spaces, wire's lateral surfaces showed close interaction with resin, providing greater reinforcement.

HP acrylics and also higher FS for fiber reinforced groups. In addition, Ind-HP showed the highest FS, according to Bertassoni et al. [7] but not different from Ind-SP. Since PMMA is a high viscosity polymer an intrinsic difficulty to wet glass fibers is expected, and pre-preg fibers would virtually enable better interaction [7]. The present results can only partially agree with this assumption because some nonimpregnated fiber groups (Uni-SP, Uni-Short-SP, Uni-HP, Short-HP, and Uni-Short-HP) had similar FS compared to pre-preg groups (Ind-SP and Ind-HP). One possibility for the observed differences could be the fiber silanization, which is responsible for higher FS, as suggested by previous studies [10]. Only the Short-SP group did not reach similar FS compared to Ind-SP, possibly due to voids within fibers (Figure 3(a)) and fiber-to-resin adherence failure. A higher FS with short glass fibers reinforcement depends on the fiber critical length [31, 33].

Fiber's critical length is a measure of minimum fiber length required for maximum stress transfer within the polymer matrix. Working with a bisGMA resin, the critical fiber length was established between 0.5 and 1.6 mm [33] and for acrylics this value increases to 6 mm [31]. If a deterioration of adhesion between fibers and resin takes place it is necessary to increase the fiber critical length in order to achieve a reliable mechanical friction at the interfaces. In the present study the FS for short fiber reinforcement on SP resin was similar to the control group due to poor adhesion with the resin matrix (Figure 3(d)). In the Short-HP group a better fiber-to-resin adhesion was observed (Figure 3(c)) but fibers moved from the tensile stress zone to the neutral zone (Figure 3(a)), which can possibly account for the relative increase of FS. It was hypothesized that even with a fiber length lower than the critical length (6 mm) [31] the reinforcement effect could be higher if fibers had kept the original position inside tested specimens.

Tpl groups showed similar FS compared to controls, irrespective of the acrylic resin. Dental floss is composed by thermoplastic resin fibers showing presence of wax around fibers, which resulted in poor adhesion (Figures 4(a) and 4(b)) and reduced FS. Stainless steel wire generally produces higher transverse strength when incorporated into polymers [5, 16], but in the present study only SP resin had an increase in FS in comparison to control group. Despite the higher values of FS, SEM images (Figure 2(c)) did not show an effective micromechanical interaction between resin matrix and reinforcement.

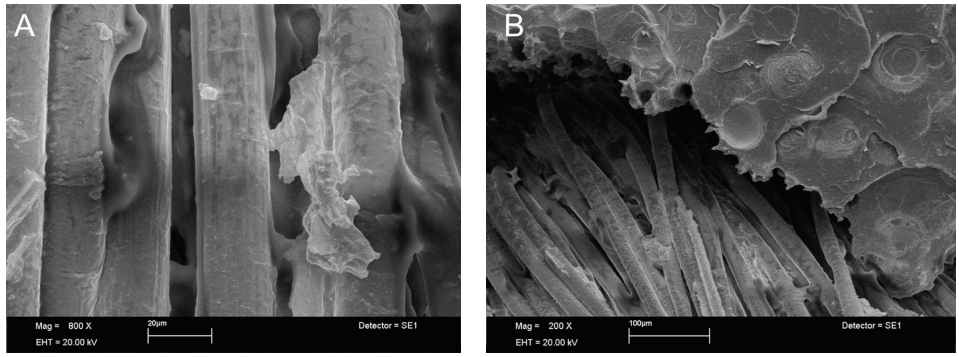
In general, acrylic resin reinforcement with glass fibers produced improved fracture strength. Provisional or even definitive prosthesis can successfully employ fiber reinforcement in order to assure better longevity and ease of repair [34]. The use of pure nonimpregnated glass fiber presents itself as a less expensive and easy handling option and can be advantageous over steel wire when considering aesthetics and reinforcement capabilities. Future research may focus on



(a) (b)

(c) (d)

Figure 3: Short glass fiber samples. (a) Heat-polymerized resin showing fiber dislodgement (×55 magnification). Fibers changed their original position (tensile stress zone) to neutral stress zone possibly due to applied pressure during heat polymerization. (b) Self-polymerized resin showing lower fiber dislodgement, which could be found at tensile stress zone (×46 magnification). (c) Higher magnification (×10,000 magnification) of heat-polymerized specimen showing fibers close to resin and with clear signals of adhesion to resin matrix (arrow). (d) Higher magnification (×10,000 magnification) of self-polymerized specimen showing space between fibers and resin as a result of the decrease of adhesive interaction (arrows).



(a) (b)

Figure 4: (a) Thermoplastic fiber showing presence of wax around fibers (×800 magnification). Interaction with resin was jeopardized. (b) Complete dislodgement between thermoplastic resin fibers and acrylic due to presence of wax around fibers (×200 magnification).

improving adhesion of fiber to different dental polymers in order to reduce the critical length and improve mechanical properties.

5. Conclusions

According to the results and limitations of the present study it is possible to conclude the following.

(1) Fiber reinforcement significantly increases fracture strength of acrylic resins and this is related to the resin polymerization method.

(2) A better interaction between fibers and resin results in higher flexural strength. Heat-polymerized resin tends to produce better wetting of fiber.

(3) Nonimpregnated fibers, irrespective of their length, tend to improve flexure strength of acrylics.

(4) Steel wire reinforcement may reinforce self-polymerized acrylics but its micromechanical interaction does not seem to be effective.

Disclosure

The authors disclose no commercial interest in products or companies mentioned in the paper.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Laser Phototherapy Enhances Mesenchymal Stem Cells Survival in Response to the Dental Adhesives

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Background. We investigated the influence of laser phototherapy (LPT) on the survival of human mesenchymal stem cells (MSCs) submitted to substances leached from dental adhesives. *Method*. MSCs were isolated and characterized. Oral mucosa fibroblasts and osteoblast-like cells were used as comparative controls. Cultured medium conditioned with two adhesive systems was applied to the cultures. Cell monolayers were exposed or not to LPT. Laser irradiations were performed using a red laser (GaAlAs, 780 nm, 0.04 cm^2, 40 mW, 1 W/cm^2, 0.4 J, 10 seconds, 1 point, 10 J/cm^2). After 24 h, cell viability was assessed by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide reduction assay. Data were statistically compared by ANOVA followed by Tukey's test ($P < 0.05$). *Results*. Different cell types showed different viabilities in response to the same materials. Substances leached from adhesives were less cytotoxic to MSCs than to other cell types. Substances leached from Clearfil SE Bond were highly cytotoxic to all cell types tested, except to the MSCs when applied polymerized and in association with LPT. LPT was unable to significantly increase the cell viability of fibroblasts and osteoblast-like cells submitted to the dental adhesives. *Conclusion*. LPT enhances mesenchymal stem cells survival in response to substances leached from dental adhesives.

1. Introduction

Laser phototherapy (LPT) is a therapeutic approach that promotes healing or repair of injured tissues. For this reason, LPT has been used as an adjuvant therapy in various clinical procedures in dentistry. In fact, LPT has been proven effective in improving dental tissue repair when applied to the dental tissues, such as after cavity preparation and restoration [1–3]. In a previous work, our group has observed that LPT was able to increase cell viability of cultures exposed to substances released from the dental bleaching gels [4]. However, this study was performed using dental pulp cells in an advanced stage of differentiation. In the dental pulp tissue, mesenchymal stem cells (MSCs) are known to play an important role during dental pulp tissue healing or repair. In fact, the dental pulp holds cells in multiple stages of commitment, which, therefore, may interplay for the tissue homeostasis [5, 6]. Unlike end-stage cells, MSCs can undergo asymmetric division, that is, one cell differentiates toward a differentiated cell, while the other replicates into another mesenchymal cell [7]. Accordingly, MSCs have the ability of self-renewal and are able to differentiate into at least two cell types [8]. LPT has already shown improvement in the MSCs proliferative rate and differentiation [9–11]. Current results stress that the association of laser and MSCs may be of particular relevance in the dentistry field.

Dental adhesives are materials commonly applied to the dental substrates and may lead to a certain degree of cytotoxicity in cell cultures. The percentage of unconverted resin monomers leads to the risk of formation of oxygen-free radicals (ROS), which, in turn, may result in inflammation and postoperative sensitivity [12–14]. Due to incomplete polymerization, uncured monomers are able to percolate dentinal tubules and reach dental pulp tissue [12]. Regarding the relevance and plasticity properties of MSCs, it is of interest to verify whether LPT could help MSCs overwhelm noxious substances derived from these materials. Bearing this in mind, the aim of this study was to test the effect of LPT on

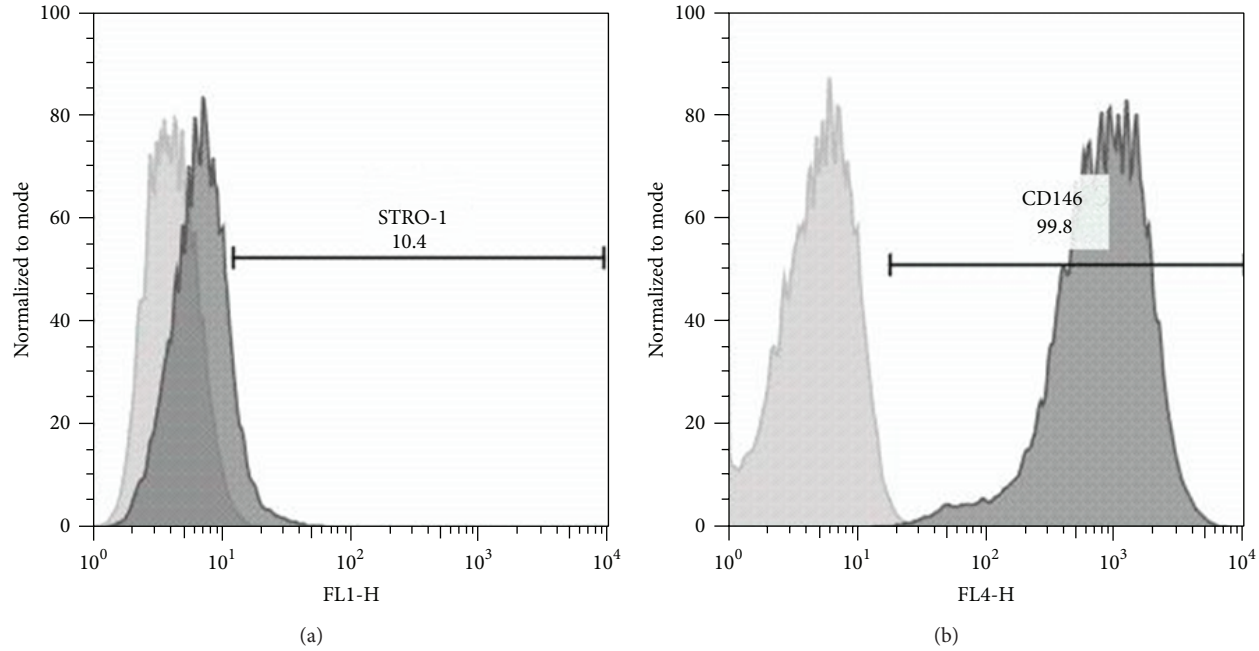

Figure 1: Characterization of the stem cells from human exfoliated deciduous teeth through the expression profile of mesenchymal stem cell markers: STRO-1 (a) and CD146 (b). Observe the positivity to both markers.

Table 1: Adhesives tested.

Name, brand (lot number)	Class	System	Composition
Adper Single Bond 2 Adhesive, 3 M ESPE (Lot N200625BR)	Etch and Rinse	1 bottle	Bisphenol A glycidyl methacrylate (Bis-GMA), 2-hydroxyethyl methacrylate (HEMA), dimethacrylates, camphorquinone (CQ), polyacrylic acid, poly (itaconic acid), ethanol, and water
Clearfil SE Bond, Kuraray (Lot 01657A/01108A)	Self-etch	2 bottles	**Primer**: 10-methacryloyloxydecyl dihydrogen phosphate (MDP), HEMA, hydrophilic dimethacrylate, dicamphorquinone, N,N-diethanol-p-toluidine, water **Bond**: MDP, Bis GMA, HEMA, hydrophobic dimethacrylate, dicamphorquinone, N,N-diethanol-p-toluidine, silanized colloidal silica

the survival of MSCs using substances leached from dental adhesives as a model of cytotoxicity.

2. Methods

This study was previously approved by the Research Ethics Committee of the School of Dentistry of the University of São Paulo (CAE: 03511012.5.0000.0075).

2.1. Cell Culture. Mesenchymal stem cells (MSCs) derived from human exfoliated deciduous teeth were isolated according to Miura et al. [15] and characterized as showing positivity for mesenchymal stem cell surface markers (STRO-1 and CD146) (Figure 1). Other cell types studied were fibroblasts of oral mucosa and osteoblast-like cells. The cell lineages were kindly provided by the Basic Research Laboratory at the School of Dentistry of the Universidade de São Paulo.

Aliquots of the cultures were thawed and grown as follows: MSCs were grown in DMEM-HAM's F12 (LGC Biotechnology, Cotia, Brazil) and supplemented with 20% fetal bovine serum (Hyclone, Logan, USA), 1% L-glutamine, 1% nonessential amino acids, and 1% penicillin/streptomycin. Fibroblasts and osteoblast-like cells were grown in high-glucose DMEM and supplemented with 15% fetal bovine serum and 1% solution of penicillin, streptomycin, and amphotericin B. All cell types were maintained in an incubator at 37°C, in a humidified atmosphere containing 5% CO_2 and 95% air.

2.2. Substances. Two types of dental adhesives were used as follows: (1) etch and rinse (Adper Single Bond 2, 3 M ESPE, St. Paul, USA) and (2) self-etch (Clearfil SE Bond, Kuraray Co., Osaka, Japan), described in detail in Table 1.

2.3. Conditioned Medium. A culture medium conditioned by the dental adhesives was used to reproduce the substances leached from the Adper Single Bond 2 or Clearfil SE Bond [16]. The conditioned medium was obtained as follows: using

Table 2: Experimental groups.

Groups	Polymerization	Nonirradiated	Irradiated
Cells with no treatment	—	Control	—
Adper Single Bond 2	No	SBNP	SBNPL
Adper Single Bond 2	Yes	SBP	SBPL
Clearfil SE Bond	No	CFNP	CFNPL
Clearfil SE Bond	Yes	CFP	CFPL

a microbrush tip, one drop of each material was dispensed to a predelineated area at the bottom of 1.5 mL microtubes. The Clearfil SE Bond comes with primer and adhesive components in separate vials. Considering this, the bond and the primer were applied with the primer on the top to simulate the clinical situation where the primer remains closer to the dentin than the bond. Next, 1 mL of culture medium was added to the microtubes containing each material. These microtubes were kept at 37°C for 1 h.

Photopolymerization, when applied, was performed using a light emitting diode (Elipar free light LED curing light, 3 M ESPE) and previously checked with a radiometer, in accordance with the manufacturer's instructions for each adhesive system.

2.4. Laser Phototherapy (LPT). Laser phototherapy was performed using a continuous wave gallium-aluminum-arsenide (GaAlAs, 780 nm) diode laser (Twin Flex II, MMOptics, São Carlos, Brazil) with a spot size of 0.04 cm^2. The irradiations were performed in contact and punctual mode, with the following parameters: output power of 40 mW, power density of 1 W/cm^2, energy of 0.4 J, and energy density of 10 J/cm^2. In order to avoid indirect light exposure wells adjacent to the test well were empty. Each well was irradiated once in a central point for 10 s. Laser parameters were chosen based on a previous study [4]. The output power was checked with a power meter (Lasercheck, Coherent Inc., Santa Clara, USA), before and after the irradiations.

2.5. Experimental Groups. The experimental groups are presented in Table 2.

2.6. Experiments. Each cell type was seeded at a cell density of 1×10^4 cells/well in quadruplicate into 96 microtitration well-plates. Twenty-four hours later, the culture medium was replaced by the conditioned medium. Next, the cultures were either submitted or not to LPT, according to the specific experimental group studied. The conditioned medium was left in contact with the cells for 1 hour and then replaced by fresh medium. The plates were incubated for another 24 hours and then subjected to the cell viability assay.

2.7. Cell Viability Assay (MTT). Analysis of cell viability was based on the measurement of mitochondrial activity using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide reduction assay (Vybrant MTT Cell Proliferation Assay Kit, Invitrogen, Carlsbad, USA), according to the manufacturer's instructions. Immediately following the end of the test procedures, the optical density was read in a spectrophotometer (Biotek II Biochrom Ltd., Eugendorf, Austria) using a 562 nm filter. The mean optical density of the positive control group was considered as 100%.

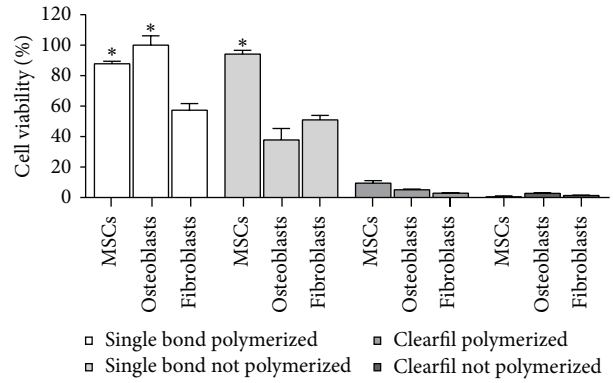

Figure 2: Graphic representation of the cell viability of all cell lineages (MSCs, fibroblasts, and osteoblastic-like cells) in response to substances leached from the dental adhesives: Adper Single Bond 2 and Clearfil SE Bond, whether or not polymerized. *Significantly higher than all other groups ($P < 0.05$).

2.8. Statistical Analysis. Each experiment with four replicates per group was repeated three times. Data were compared by ANOVA followed by Tukey's test using the GraphPad Prism 5.0 software (GraphPad Software Inc., La Jolla, USA). The level of significance was 5% ($P \leq 0.05$).

3. Results

The cell viabilities of the different cell types in response to the substances leached from the polymerized and nonpolymerized dental adhesives are graphically represented in Figure 2. Different cell types responded differently to the same material. The highest cell viabilities were observed in response to substances leached from Adper Single Bond 2, especially when polymerized.

MSCs and osteoblast-like cell cultures submitted to substances leached from polymerized Adper Single Bond 2 presented similar cell viabilities ($P > 0.05$), which were significantly higher than those of fibroblast cultures ($P < 0.05$) (Figure 2). MSCs submitted to substances leached from nonpolymerized Adper Single Bond 2 presented cell viabilities significantly higher than those of the other cell types ($P < 0.05$). Substances leached from the Clearfil SE

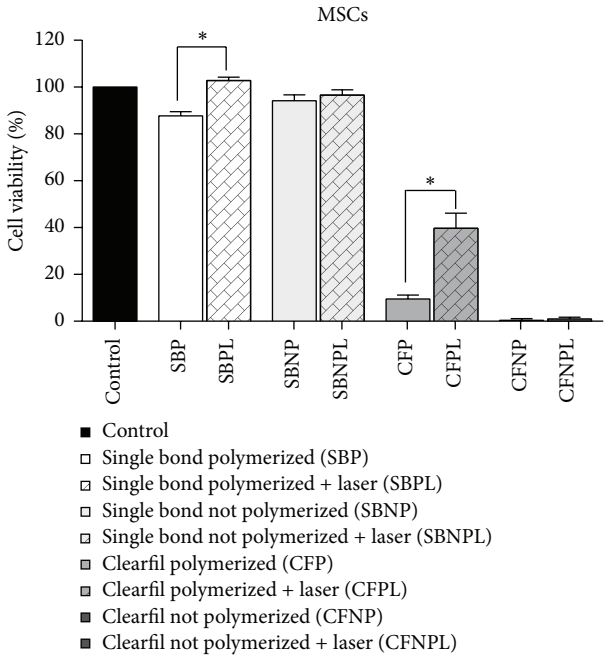

Figure 3: Graphic representation of the cell viabilities of MSCs in response to the substances leached from the dental adhesives: Adper Single Bond 2 and Clearfil SE Bond, whether or not polymerized and followed by LPT or not. *Significantly different from the nonirradiated group submitted to the same adhesive system.

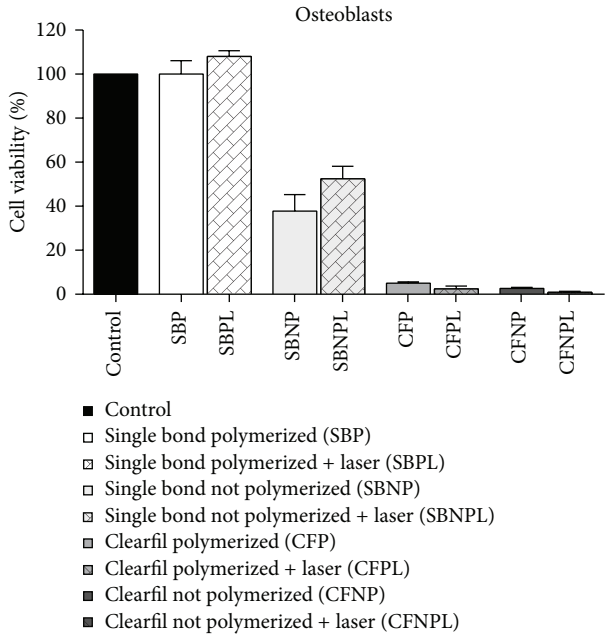

Figure 4: Graphic representation of the cell viabilities of osteoblast-like cells in response to the substances leached from the dental adhesives: Adper Single Bond 2 and Clearfil SE Bond, whether or not polymerized, followed by LPT or not. There are no differences between irradiated and nonirradiated cells submitted to the same adhesive system.

Bond, whether polymerized or nonpolymerized, caused a high percentage of cell death in all cell types tested (Figure 2).

The LPT effects on the cell lineages are graphically represented in Figures 3 to 5. The cell viabilities of MSCs submitted to the substances leached from all the materials, followed by LPT, were higher than or at least similar to those of the nonirradiated cultures submitted to the same conditioned medium. MSCs treated by LPT presented significantly higher cell viabilities when submitted to both polymerized adhesives tested ($P > 0.05$) (Figure 3). The cell viabilities of osteoblast-like cells (Figure 4) and fibroblasts (Figure 5) submitted to the substances leached from all the materials followed by LPT were similar to those of the nonirradiated cultures submitted to the same conditioned medium ($P > 0.05$).

4. Discussion

The role of MSCs in response to damaged odontoblasts due to cavity preparation [17] has drawn attention to the response of these cells facing other injuries, such as dental materials percolation through the dentinal tubules [14, 18–21]. Based on the above, our hypothesis was that LPT could improve the survival of MSCs subjected to noxious substances derived from the dental materials. To verify this hypothesis, prior to LPT, two types of dental adhesives were used to imbalance the ideal culture conditions for MSCs, fibroblasts, and osteoblasts-like cells. We found that LPT significantly improved survival of MSCs. In spite of that,

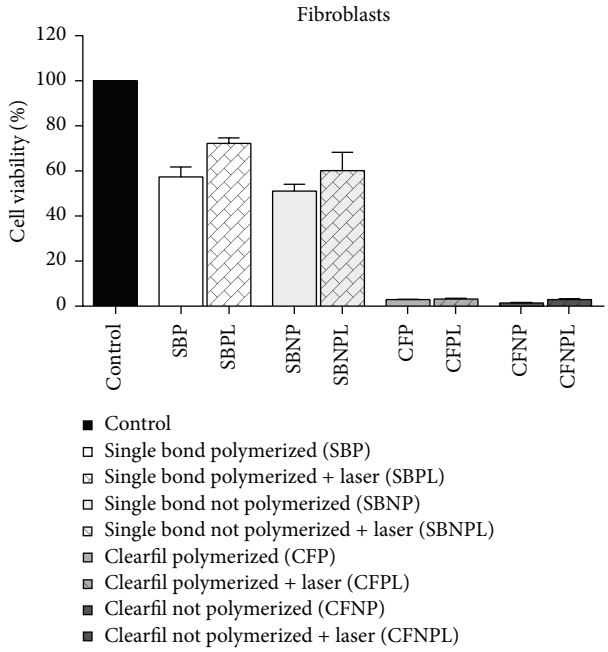

Figure 5: Graphic representation of the cell viabilities of fibroblasts in response to the substances leached from the dental adhesives: Adper Single Bond 2 and Clearfil SE Bond, whether or not polymerized and followed by LPT or not. There are no differences between irradiated and nonirradiated cells submitted to the same adhesive system.

overall, no increased cell survival was observed for fibroblast or osteoblast-like cells.

In this study, culture media conditioned by the dental adhesives were used as harm stimuli to test the LPT biostimulation. In the tested conditions, Clearfil SE Bond was highly cytotoxic to all cell lines tested, regardless of being polymerized or not. Substances leached from Adpter Single Bond, regardless of being polymerized or not, were less cytotoxic to MSCs than to the other cell types. Overall, MSCs were less sensitive to toxic substances released by the adhesives, compared to the other cell types tested. These results may be partially explained by the aforementioned properties of MSCs. Their high proliferative nature and plasticity [15] may have contributed to their better response to the noxious substances. In fact, MSCs are involved in the reparative mechanisms of the dental pulp [7] and are recruited to replenish lost specialized cells, such as odontoblasts. In contrast, oral mucosa fibroblasts and osteoblast-like cells are more demanding as end-stage cells and may not respond to stressful conditions at the same level of undifferentiated cells [22].

LPT was able to improve the survival of MSCs to the cytotoxic effect of both adhesive systems when applied after polymerization. On the other hand, for fibroblasts and osteoblast-like cell lineages, LPT was not able to significantly offset the cytotoxic effects of substances released from dental adhesives. Additionally, it was observed that when the materials promoted slight cytotoxicity in the cell lines, LPT had a minimal influence on the improvement of cell survival. In fact, LPT seems to act mainly on cells with compromised cellular functions [23–25]. This can be confirmed by the results obtained for MSCs in the Clearfil SE Bond polymerized groups, whether irradiated or not, which showed to be highly cytotoxic. The percentage of cell viability was very low when the material was applied to the cell cultures, but after irradiation, cell survival rates increased significantly. In other cell types, and in the groups that presented moderate cytotoxicity, although LPT did not significantly increase cell viability, a trend toward improved cellular response could be observed.

With wavelengths in the red or near-infrared ranges, the energy emitted by the laser is capable of being absorbed by cellular components resulting in modulatory effects on basic cellular functions, especially in tissues subjected to stress conditions [26]. Although we cannot mechanistically explain our current results, some previous studies corroborate to elucidate the positive effect of LPT observed here. Laser irradiation can upregulate levels of mRNA of Notch-1, which play an important role in MSCs self-renewal [9]. Accordingly, the modulation of channel gating by laser light may be a critical step in the upregulation of Notch-1 signaling in MSCs, thus stimulating their proliferation [9]. Another study reported that laser irradiation is able to inhibit NF-κB nuclear translocation due to LPS stimulation through an increase in the intracellular level of cyclic AMP (cAMP), suppressing, and, therefore, the release of important proinflammatory cytokines (COX-2, IL-1B, IL-6, and IL-8) [27]. As such, both studies suggest that LPT can help MSCs overwhelm biological stressful situations.

Overall, the Clearfil SE Bond showed severe cytotoxicity to all cell types, whereas Adper Single Bond 2 was reasonably well tolerated. These results are consistent with others described in the literature, although different experimental conditions were reported [14, 18, 28, 29]. Demirci et al. [14] found that Clearfil SE Bond leads to decreased cell viability in a concentration-dependent mode. In fact, dental adhesives cause an imbalance in the cellular redox state with the generation of reactive species of oxygen in cultured dental pulp cells. The ROS formation can interfere with the signal transduction regulating cell survival pathways [30, 31].

The higher cytotoxicity of Clearfil SE Bond in relation to Adper Single Bond 2 can be partially explained by the pH composition. Adper Single Bond 2 is a total etch adhesive and has no acidic monomers in its composition. On the other hand, Clearfil SE Bond is a self-etch adhesive and thus has acidic agents incorporated into the resinous materials, leading to a pH of about 2. Therefore, immediately after the adhesive system came into contact with the culture medium, there was a change in color from orange to yellow; and the yellow remained until the system was applied on the cell cultures. This means that the pH was very low and the buffering capacity of the culture medium was not enough to neutralize the acidic substances leached from the Clearfil SE Bond. Apart from it, this is an *in vitro* study conducted on cultured cells. As such, it has limitations and does not represent the *in vivo* physiology of the dental pulp tissue.

5. Conclusions

In summary, this preliminary data suggest that LPT is able to modulate cellular functions to improve MSCs viability under harm stimulus produced *in vitro*. Further studies should be conducted to verify the mechanism of action of LPT in these cells. Under the limit conditions of this study it was concluded that LPT is able to enhance the survival of mesenchymal stem cells after contact to substances leached from dental adhesives.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Toothpaste Prevents Debonded Brackets on Erosive Enamel

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This study evaluated the effect of high fluoride dentifrice on the bond strength of brackets after erosive challenge. Eighty-four enamel specimens were divided into seven groups ($n = 12$): WN (distilled water/no acid challenge), W3C (distilled water/3 cycles of acid challenge), and W6C (distilled water/6 cycles of acid challenge) were not submitted to dentifrice treatment. Groups RF3C (regular fluoride dentifrice/3 cycles of acid challenge) and RF6C (regular fluoride dentifrice/6 cycles of acid challenge) were treated with dentifrices containing 1450 μg F^-/g and HF3C (high fluoride dentifrice/3 cycles of acid challenge) and HF6C (high fluoride dentifrice/6 cycles of acid challenge) were with 5000 μg F^-/g. Acid challenges were performed for seven days. After bond strength test, there was no significant difference among groups submitted to 3 cycles of acid challenge ($P > 0.05$). Statistically significant difference was found between the regular and high fluoride dentifrices after 6 cycles of acid challenge (<0.05). Similar areas of adhesive remaining were found among control groups and among groups W6C, RF3C, RF6C, HF3C, and HF6C. The high fluoride dentifrice was able to prevent the reduction of bond strength values of brackets submitted to acid challenge. Clinical relevance: the high fluoride toothpaste prevents debonded brackets on erosive enamel.

1. Introduction

Many factors may influence the retention of brackets during orthodontic treatment with fixed appliances [1]. These include the quality of enamel, substances that alter its structural components, type of material used for bonding, and technique employed [2].

The dental enamel should be healthy to permit bonding of brackets; however, dental caries and erosion are common factors that cause loss of mineral components of teeth [2]. Dental caries involves the loss of mineral structure by chemical dissolution due to a reduction in dental biofilm pH [3]. Dental erosion is defined as the induced loss of minerals by acidic substances of nonbacterial origin in contact with the tooth structure [4].

Diets rich in carbonated beverages, fruits, and other acids are being consumed more frequently, which consequently has been increasing the dental erosion [5]. The excess ingestion of these substances is of major concern not only because of high sugar levels, but also because they present pH levels below the critical limit for enamel demineralization (pH < 5.5) [6]. Studies on acidic beverages have demonstrated that these substances cause enamel decalcification around the brackets, consequently increasing the risk of marginal leakage [2, 5].

One of the treatment options to avoid mineral loss is the use of substances with high fluoride concentration, including varnishes and dentifrices. High fluoride dentifrices (above 5000 μg F^-/g) have been developed for "high risk individuals" [7]. Its efficiency to avoid mineral loss has been confirmed in previous studies [8–10].

However, other studies have demonstrated that the use of fluoridated solutions negatively interferes with the bond strength of orthodontic brackets [11, 12].

Table 1: The groups were divided according to treatment and acid challenge.

Group	Treatment	Toothpaste	Acid challenge
WN	Distilled water		No
W3C			3 cycles
W6C			6 cycles
RF3C	Regular fluoride toothpaste	Colgate Tripla Ação dentifrice, 450 μg F^-/g, Colgate Palmolive, São Bernardo do Campo, Brazil	3 cycles
RF6C			6 cycles
HF3C	High fluoride toothpaste	Duraphat dentifrice, 5000 μg F^-/g, Colgate Palmolive, Piscataway, USA	3 cycles
HF6C			6 cycles

This study evaluated the effects of regular and high fluoride dentifrices on the bond strength of brackets to enamel submitted to acid challenge. The null hypotheses tested were as follows: (i) the bond strength of brackets is not affected by acid challenge; (ii) the type of dentifrice does not influence the bond strength of orthodontic brackets.

2. Methodology

2.1. Preparation of Specimens. Eighty-four permanent bovine incisors were collected and their crowns were separated from the roots, cleaned with periodontal curettes, and stored in distilled water for a maximum period of six months at a temperature of 5°C. The procedures were performed following the specific protocol TR 11405 established by the International Organization for Standardization (ISO) [13]. The crowns were embedded in chemically cured acrylic resin (Jet Clássico, São Paulo, Brazil) in PVC molds (20 mm diameter, PVC Amanco, Joinville, Brazil), maintaining the lingual aspects immersed.

The buccal aspects of crowns were cleaned with fluoride-free prophylactic paste (Dentsply, Konstanz, Germany) for 10 seconds and rinsed with water for the same period.

The 84 specimens were randomly assigned to seven groups ($n = 12$), as described in Table 1.

2.2. Dentifrice Treatment. The specimens were immersed in dentifrice (dilution: 3 g of dentifrice/10 mL of distilled water, adding up to 153 g of dentifrice/510 mL of distilled water) for 3 minutes at controlled temperature and pH under constant shaking, using a magnetic shaker (IKA Laboratory Equipment, Staufen im Breisgau, Germany). However, specimens of WN, W3C, and W6C groups were immersed in 600 mL of distilled water under the same conditions of dentifrice treatment.

The treatment cycles were conducted for 7 days, twice a day. After treatment, the specimens were carefully rinsed with distilled water.

2.3. Application of Brackets. Metallic brackets for maxillary central incisors (Morelli, Sorocaba, Brazil) with base area of 14 mm^2 were placed on enamel surfaces of all specimens. The buccal aspect of crowns was conditioned with 35% phosphoric acid (Ultradent, South Jordan, USA) for 20 seconds, rinsed with water, and air-dried. The primer of Transbond XT (Unitek, Landsberg, Germany) was applied following the manufacturer's instructions. Then, the Transbond XT adhesive (Unitek, Landsberg, Germany) was applied on the bracket base, the assembly was placed on the buccal aspect of the crown and a standardized force of 500 g was applied. The excess material was removed with a dental probe (Duflex, Juiz de Fora, Brazil).

A single operator performed all procedures. Each bracket was light cured at a distance of 1 mm from the bracket base to the light-curing tip for 40 seconds, being 10 seconds on each side of the bracket. The specimens were then stored in distilled water (37°C, 24 hours).

2.4. Procedures for Dental Erosion (Intervals of Acid Challenges). The specimens were suspended in 1 L beaker containing 600 mL of orange juice (Del Valle, Santa Bárbara D'Oeste, Brazil) (pH 3.5 ± 0.03) using plastic rods. The orange juice was gently shaken using a magnetic shaker for 15 minutes. The specimens were removed from the orange juice and carefully rinsed with 15 mL of distilled water, removing the acid excess from the surface. In WN group, the specimens were kept in 600 mL of water under 3 minutes of constant shaking.

The acid cycles were performed for 7 days. Twelve specimens in each group were exposed to 3 cycles per day and the other half of specimens were exposed to 6 cycles of acid challenge per day (15 minutes for each cycle). The specimens were stored in artificial saliva during rest. Among cycles, specimens were kept in artificial saliva for 2 hours.

2.5. Overnight Storage. The specimens were stored in artificial saliva at controlled temperature and pH. The artificial saliva was prepared as follows: 0.5 mmol/L $Ca(NO_3)_2$ $4H_2O$; 0.9 mmol/L Na_2HPO_4 $2H_2O$; 150 mmol/L KCl; 0.02 mol/L $H_2NC(CH_2OH)_3$ (TRIS); 0.05 μg/mL NaF, pH 7.0 [14, 15].

2.6. Bond Strength Test (Shear Bond Strength: SBS). For the bond strength test, an occlusogingival force was applied by the mechanical testing machine on the upper surface of the bracket between the upper wings and the brackets base, at a speed of 0.5 mm/min [16, 17]. The force required to displace the bracket was measured in Newton (N) and the shear bond strength (SBS) was calculated by dividing the force value by the bracket base area (1 MPa = 1 N/mm^2).

2.7. Analysis of Adhesive Bonded to the Tooth after Debonding of Brackets. After the shear bond strength, the specimens

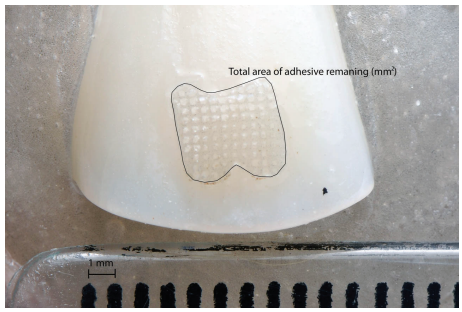

Figure 1: Photograph for analysis of the total area of adhesive bonded to the tooth after debonding of the bracket. Note that a scale was used to serve as reference for the digital scale. Thereafter, the area was calculated on the software Adobe Photoshop CS5.

were photographed with a digital camera (Nikon, Tokyo, Japan) connected to a 100 mm lens (Nikon, Tokyo, Japan). A calibrated ruler was used in the photograph to be used as proportional scale. Thereafter, the area of adhesive bonded to the tooth was calculated on the software Adobe Photoshop CS5 (Adobe Systems Incorporated, San Francisco, USA) (Figure 1). Figure 2 shows the schematic drawing of the methodology used in this study.

2.8. Statistical Analysis. The Shapiro-Wilk normality test and Levene homogeneity test were applied for bond strength tests and area of adhesive remaining data. Bond strength data showed normal distribution and they were analyzed by one-way ANOVA and post-hoc Tukey tests ($P < 0.05$). The area of adhesive remaining did not pass the normality test and was submitted to the Kruskal-Wallis and post-hoc Dunn's tests ($P < 0.05$). The Graph Prism software (Graphpad, La Jolla, USA) was used for statistical analyses.

3. Results

The means and standard deviations are presented in Figure 3. Groups W3C, RF3C, and HF3C showed no statistically significant differences ($P > 0.05$). Statistically significant difference was found between the regular and high fluoride dentifrices after 6 cycles of acid challenge ($P < 0.05$). The group WN had greater bond strength values than groups W3C, W6C, RF3C, and RF6C ($P < 0.05$). Similar areas of adhesive remaining were found among control groups (WN, W3C, and W6C) and among groups W6C, RF3C, RF6C, HF3C, and HF6C (Figure 4). Additionally, all groups, except group HF6C (6.84 mm^2), presented mean above 50% (7 mm^2) of adhesive bonded to the tooth after debonding of brackets.

4. Discussion

This study investigated the effects of regular and high fluoride dentifrices on the bond strength of brackets after acid challenge. The results of this study rejected the null hypotheses as the bond strength of brackets is not affected by acid challenge and the type of dentifrice does not influence the bond strength of orthodontic brackets.

This type of dentifrices application and acid challenge has been effective in *in vitro* studies [2, 5, 7, 18]. This investigation evidenced that group WN presented higher bond strength values after the shear bond strength testing than groups W3C and W6C. Previous studies have demonstrated similar characteristic during evaluation of brackets debonding between bovine and human enamel [19–21]. Due to the easy achievement, these teeth may be better selected, increasing the homogeneity of specimens and allowing results with lower method error [11].

The sustained force of 500 g was applied on the bracket to avoid interference in the outcome of bond strength. Studies [22–24] have shown that the no application of sustained force during the bonding process affects the adhesive layer and decreases the bond strength. It happens mainly because the sustained force reduces fluid interference from the underlying tooth. In the present study, the force gauge instrument (Correx Co, Bern, Switzerland) was positioned perpendicularly to the buccal aspect of the crown.

The treatment was performed before bonding of brackets to evaluate if the dentifrices, especially with high fluoride concentration, interfere with the bond strength of brackets in patients presenting dental erosion. Additionally, this sequence of the methodology was made to simulate the regular use of these dentifrices. If the treatment was performed after bonding of brackets, this study did not confirm the second null hypothesis. Therefore, the present results revealed that the high fluoride dentifrice did not negatively interfere with the bonding of brackets, corroborating previous studies using substances with high fluoride concentrations before bonding of brackets [12, 25–27].

Other earlier studies demonstrated that substances with high fluoride concentration might interfere negatively with bonding [28–32]. The application of topical fluoride interferes on enamel etching with phosphoric acid, making it more resistant and reducing its surface energy [29, 30, 32]. Thus, enamel demineralization occurs in a nonstandardized manner, impairing the penetration of adhesive and formation of resin tags [30, 32]. Additionally, no previous study has analyzed the bond strength of adhesive materials using previous treatment with this dentifrice. Notwithstanding the high fluoride concentration, the dentifrice was unable to change the demineralization pattern of phosphoric acid. Flury et al. concluded that fluoride mouthrinses increase the bond strength of composite resin in teeth submitted to dental erosion [33].

Previous studies have demonstrated the efficacy of high fluoride dentifrices to prevent tooth demineralization, acting by the deposition of components, especially fluoride particles, and remineralization of the affected substrate [7, 34]. The present study demonstrated that this dentifrice was able to prevent the reduction of bond strength of brackets submitted to acid challenge. This may be explained by the fact that fluoride particles avoided the enamel demineralization by replacement of calcium and phosphate around the bracket base, thus reducing the chances of premature debonding [33].

Dentifrices with 1450 $\mu g\ F^-/g$ presented similar outcomes with groups W3C and W6C. It may be inferred that regular fluoride concentration was not enough to have a significant

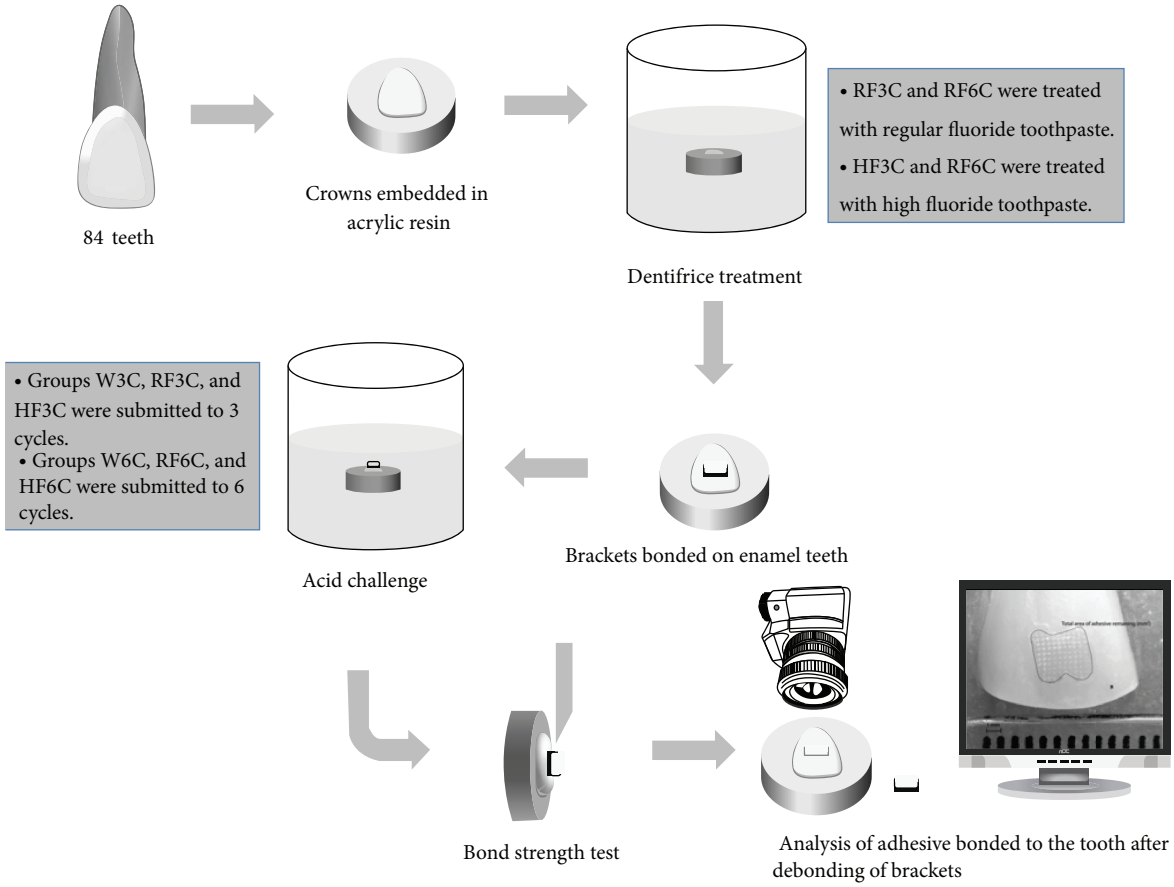

Figure 2: Schematic drawing of the methodology used in this study.

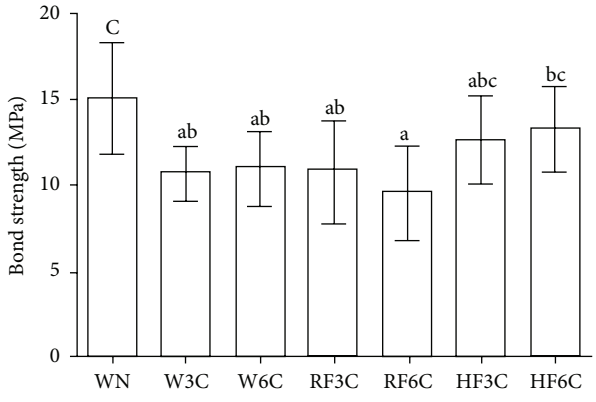

Figure 3: Bond strength values of groups submitted to the shear bond strength test. Different letters indicate statistical difference (one-way ANOVA and post-hoc Tukey tests, $P < 0.05$).

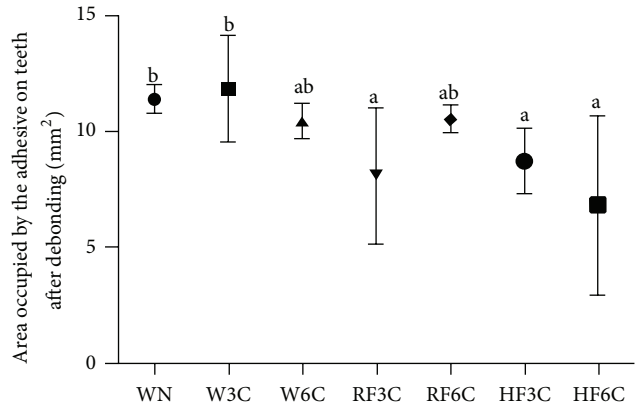

Figure 4: Values in mm^2 of adhesive material bonded to the tooth after debonding of brackets. Different letters indicate statistical difference (Kruskal-Wallis and post-hoc Dunn's tests, $P < 0.05$).

influence on reduction of bond strength of brackets. This confirms that high fluoride concentration (5000 $\mu g\, F^-/g$) was able to remineralize the enamel around the bracket, avoiding the premature debonding.

Many studies employ the Adhesive Remnant Index to evaluate the type of failure occurring after the shear bond strength testing [12, 26, 35, 36]. Even though this method is widely used, it is not able to accurately demonstrate the quantity of adhesive material bonded to the tooth. Therefore, this study used photographs of specimens after debonding of brackets and the area of adhesive material bonded to the tooth was calculated with the aid of a guide ruler on the software Adobe Photoshop CS5. These results demonstrated

that, in most specimens, the adhesive material bonded on the tooth was greater than 7 mm^2. This reveals that, even though enamel demineralization impaired the bonding of brackets, failures substantially occur at the interface between bracket and adhesive material. Also, excessive bonding of bracket is not interesting because this bracket must be removed later, and a strong bonding may impair its removal and cause enamel cracks [37].

5. Conclusion

The acid challenge provides significantly lower bond strength values compared to control group (no acid challenge). The high fluoride dentifrice was able to prevent better the reduction in bond strength values of brackets than regular fluoride dentifrices after 6 cycles of acid challenge.

Conflict of Interests

Professors Roger P. Ellwood and Ian Pretty work in Colgate Palmolive Dental Health Unit, Manchester, UK.

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Research Article

Assessment of Root Canal Enlargement Using Mtwo and BioRace Rotary Files

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Objective. To evaluate root canal enlargement following mechanical shaping using 2 nickel titanium rotary systems. *Material and Methods*. Forty single-rooted teeth were immersed in resin and sectioned perpendicular to the long axis at 4, 8, and 12 mm from the apex. Digital capture of sections was performed before and after canal instrumentation using Mtwo and BioRace instruments. The area increase of endodontic space was calculated by subtraction. *Results*. The use of both instruments has allowed the removal of great amounts of dentin from the canal walls, even when the endodontic morphology is characterized by awkwardness to reach recesses. *Conclusions*. Both procedures seem to be valid and no differences were found between Mtwo and BioRaCe considering the amount of dentin removed at different distances from the apex.

1. Introduction

In the last decade, the introduction of new technologies such as nickel-titanium (NiTi) instruments and new canal filling systems have allowed the dentist, together with the use of microscopes, to set even more effective therapeutic protocols [1, 2]. The introduction of these instruments has enabled root canal instrumentation to be faster while remaining respectful of the original root canal anatomy [3].

This can be further possible thanks to the file design [4, 5] and the crown-down approach [2, 6]. Several NiTi instruments are available on the market. Besides the metal type, instrument geometry is the major factor that influences the behavior of instruments towards torque stresses, fracture strength, rotation speed, and operator sensitivity [7].

The first generation NiTi instruments had poor shaping ability (neutral cutting angle) and nowadays a lot of instruments with great shaping ability (positive cutting angle) are available on the market. This characteristic has inevitably modified their use; in fact, while the first generation instruments, not very sharp, required longer appointments for the patients the latest instruments, with greater shaping ability, allow the dentist to perform faster procedures and shorter appointments [8–11].

The aim of this study is to compare dentin removal during shaping with 2 different nickel-titanium systems by measuring the cross-sectional area of the root canals before and after instrumentation.

2. Materials and Methods

Forty human teeth single-rooted, without restorations, with intact crowns, and fully formed apices, extracted for orthodontic and/or periodontal reasons, were selected. All teeth were cleaned in 5% NaOCl solution for 24 h, carefully cleaned of periodontal tissue and calculus, washed under running water, dried, and stored in 10% formalin solution. All specimens had a root canal with a curvature angle lower than

20° that was evaluated using the Schneider technique [8, 12] by two radiographs (mesial-distal and buccal-lingual). Roots with resorptions, fractures, and open apices were excluded and every tooth had its crown removed at the cement-enamel junction (mesial side). The working length of the canals was determined by observing a file number 10 protruding through the apical foramen and subtracting 0.5 mm from the recorded length. An experimental model was made, capable to standardize the position of every sample, using the Bramante modified technique [13].

All specimens were immersed in self-curing transparent resin (Viapal uP 0004/64; Vianova Resin, Hamburg, Germany), in order to create resin blocks which were cut to obtain sections containing resin and a root portion. For each specimen, 3 sections perpendicular to the longer axis were created at 4, 8, and 12 mm from the apical foramen. Digital captures of all sections (coronal view) were recorded before reassembling them using a repositioning device to file the canals [14] (Figure 1). All the resin blocks were randomly divided into 2 groups, A and B, of 20 samples each.

Group A was instrumented with Mtwo (Sweden & Martina, Padova, Italy); group B was instrumented with BioRace (FKG Dentaire, La Chaux de Fonds, Switzerland).

For both groups, every NiTi instrument was used just for 8 seconds (which is less than the suggested working time from the manufactures) and only for 5 canals so any instrument was used only for 40 seconds in order to preserve the sharpening; irrigation was carried out with EDTA-based Glyde chelating solution (Maillefer, Ballaigues, Switzerland), alternating it with NaClO Niclor 5 (Ogna, Muggio, Italy) to facilitate the progression of the instrument inside the canal, to reduce the torsional stress and minimize the usury on the blades [3].

For any digital capture, a stand (Figure 2) was created to maintain a digital camera (Coolpix 5400, Nikon, Japan) and the sections in a repeatable position, to permit pre- and postpreparation image comparison through superimposition. Every image was digitalized before reaming in order to store the original morphology of the endodontic section (coronal view). Root canal shaping was always performed by the same operator. The clinical protocols were carried out using the following sequences.

Group A. Mtwo: 10/.04, 15/.05, 20/.06, 25/.06. The simultaneous technique without any early coronal enlargement (total 32 seconds).

Group B. BioRace: 25/.08, 15/.05, 25/.04, 25/.06 (total 32 seconds) (crown-down technique) [6, 15–17].

It is remarkable that, in this study, any instrument (except BR0) was taken at the working length, with light apical pressure, and used just for 8 seconds; all the tested instruments were used with lateral pressure (brushing mode) to obtain a circumferential cut.

After shaping, all specimens were disassembled and the sections were repositioned on the stand for another capture. Digital image analysis was carried out with AutoCAD graphic software (Autodesk Inc, USA) [4], which permitted the highlighting of endodontic space profiles before and after reaming; red color (before shaping) and blue color (after shaping) were used.

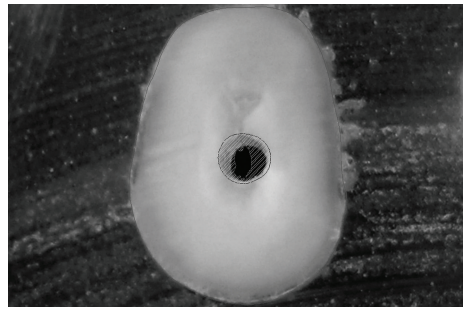

Figure 1: Example of area before and after reaming.

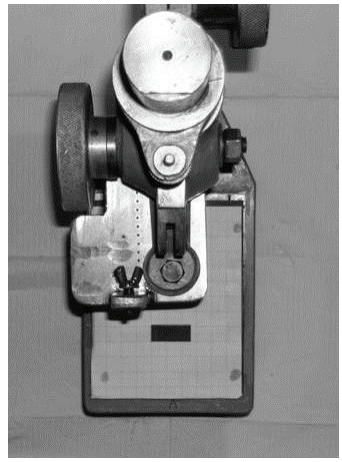

Figure 2: Stand to maintain a digital camera and the sections in a repeatable position.

Statistical data analysis was carried out with Statistica software v.6.1 (StatSoft Italia s.r.l). The significance of the differences in pre- and postshaping areas at the 3 sections was evaluated in both groups with the Wilcoxon test; the significance level was fixed at $P < 0.05$.

3. Results

During simulated clinical use no instrument had intracanal fracture, while some files of both groups showed small visible signs of plastic deformation, especially close to the tip; no transportation of the root canal or strip perforation occurred.

The operator, in the evaluation stage, was blinded to the type of file and all results are summarized in Table 1.

The increase in the postpreparation endodontic space area in group A (Mtwo) was statistically significant in all 3 sections (coronal: $P = 0.000089$, middle: $P = 0.000022$, and apical: $P = 0.000022$).

In group B (BioRace) pre- and postpreparation differences were statistically significant in all 3 sections (coronal: $P = 0.00002$, middle: $P = 0.000022$, and apical: $P = 0.000089$).

Table 1: Results.

Sections	Group A		Group B	
	Area increase mm^2	Mean mm^2	Area increase mm^2	Mean mm^2
Coronal	0.15 to 0.8480	0.4964 ± 0.2072	0.1901 to 1.1242	0.6571 ± 0.2323
Middle	0.0864 to 0.6223	0.3233 ± 0.1536	0.0532 to 0.6012	0.3272 ± 0.1432
Apical	0.0078 to 0.5499	0.1930 ± 0.1565	0.0052 to 0.4832	0.1431 ± 0.1235

The comparison between the two groups of samples at the coronal, middle, and apical sections, carried out with the Mann-Whitney U test, did not show statistically significant differences between the two different types of rotary instruments (coronal sections: $P = 0.7643$, middle sections: $P = 0.1202$, and apical sections: $P = 0.1460$).

4. Discussion

Within the limits of an "in vitro" study, the Bramante technique [13] (modified by Kuttler et al. [14]) offers a method that is relatively simple and economical and provides useful information about the action of instruments in the canal space. An alternative method of assessing root canal instrumentation techniques is the microcomputer tomography that is more expensive and requires well-trained operators in order to obtain valid results.

This study evaluated two different procedures based on NiTi rotary instruments that were used for just 32 seconds inside the canal and without preflaring with Gates-Glidden or Largo burs.

In ideal conditions, both files seem to create rapidly a round shape regardless of the initial root canal's morphology. The analysis of the results showed the shaping ability for both types of instruments that permitted proper dentin removal from the canal walls; and pre- and postpreparation differences were statistically significant in all 3 sections.

The Mtwo removed smaller amounts of dentine compared to BioRace at the coronal sections. NiTi Mtwo instruments (simultaneous technique) do not remove indiscriminately coronal root dentine with an early coronal enlargement, but rather progressively eliminate dentine at the orifice through a selective coronal enlargement [17].

Given the cutting ability of the rotary instruments tested, few seconds were sufficient to ensure a proper shaping reducing, at the same time, stresses in the NiTi alloy. Even if the canal preparation shape became dictated more by anatomy than by differences in instrumentation method [18] both types of instruments tested showed a similar tendency to modify the canal walls. Even though the use of Mtwo files compared with canal preparation with K3 or RaCe instruments showed a less production of debris, as described by Schäfer et al. [19], within the parameters of this study, the statistical analysis did not reveal a significant difference between the Mtwo group and the BioRace group considering the quantity of dentin removed at all different levels. The lack of significance between the two groups may be a consequence of the high degree of similarity between them; a variety of root canal anatomy within the groups may have produced a relatively high dispersion of the data.

Both rotary instruments tested were also used with a brushing motion which may have influenced the final shape of all canals more than the differences (shaping ability) between Mtwo and BioRace.

Considering our data, simple procedures and sharp rotary instruments, such as Mtwo and BioRace tested in this study, may allow the dentist, in few minutes, to obtain an efficient enlargement of the root canals.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

Analgesia Evaluation of 2 NSAID Drugs as Adjuvant in Management of Chronic Temporomandibular Disorders

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The aim of this triple-blind full-randomized clinical trial was to quantify analgesia in masticatory muscles and temporomandibular joints after occlusal splint therapy associated with the adjuvant administration of nonsteroidal anti-inflammatory drugs (NSAID) isolated or associated with other therapeutic agents. Pain relief was also recorded. Eighteen volunteers who had been suffering from chronic pain in masticatory muscles due to temporomandibular disorders were selected after anamnesis and assessment using RDC/TMD translated to Portuguese. The 3 proposed treatments were NSAID (sodium diclofenac), panacea (sodium diclofenac + carisoprodol + acetaminophen + caffeine), and a placebo. The total treatment duration was 10 days, preceded and succeeded by patients' pain assessment. A washout interval of 11 days was established between each therapy. All participants received all treatments in different moments, in a full randomized crossover methodology. The assessment of drug therapies was performed using visual analogue scale for pain on palpation followed by 11-point numerical scale to quantify pain during treatment. Statistical analysis has shown that, after 10 days of treatment, all therapies were effective for pain relief. NSAID therapy promoted analgesia on the third day, while placebo only promoted analgesia in the eighth day. It has been concluded that sodium diclofenac used as splint adjuvant therapy, promotes significant analgesia in a shorter time.

1. Introduction

Temporomandibular disorders (TMD) result from musculoskeletal dysfunction of the orofacial region affecting masticatory muscles, temporomandibular joints (TMJ), and other associated structures. The main characteristics of these problems are facial and TMJ pain, headache, earache, dizziness, masticatory muscle hypertrophy, limited mouth opening, locked jaw, abnormal teeth wear, joint sounds, and others [1]. Besides the wide variety of signs and symptoms, pain is the main reason leading the patient to search for treatment [2].

Dentist must be aware on the proper diagnosis and treatment of temporomandibular disorders, because they represent the second most frequent patients complaints (only less frequent than dental pain) [3]. The origin of these disorders is probably multifactor, resulting from a dynamic adaptive and destructive process [4]. Temporomandibular disorders pain may result from an inflammatory cause, mainly due to microlesions on the masticatory muscles and TMJ. About 44% to 79% of individuals report traumatic events affecting the orofacial region, including macro- and microtraumas from masticatory muscles overloaded, or repetitive loading and muscular fatigue [4]. These events can cause microlesions at masticatory muscle fibers leading to a local inflammatory mediators' release, such as prostaglandins, bradykinin, histamine, and substance P, which may induce or facilitate nociceptive afferent impulse transmission to superior nervous center, developing peripheral and central sensitization [5–7].

In the management of temporomandibular disorders, it is primordial to relief the pain, preventing relevant alterations

in neuronal circuits and secondary hyperalgesia caused by persistent afferent signs [8] which is substantially modulated by psychological, behavioral, and psychosocial factors [9]. When peripheral events are not controlled, acute pain may become chronic, mainly due to patient vulnerability including genetic predisposition, hormonal factors, behavioral habits, and previous unsuccessful therapies. General disorder can present negative symptoms as depression, social isolation, indifference, inactivity, excessive health care use, and affliction, with reduced biomedical treatment response [4].

Structural and neurochemical alterations on the central nervous system associated with the presence of psychosocial factors increase and perpetuate painful sensation, making chronic pain a great challenge for clinicians [10]. In such case, nonsteroidal anti-inflammatory drugs could act inhibiting inflammatory mediators released in muscle tissues, reducing signs and symptoms, including pain.

Thus, the aim of this full randomized triple-blind crossover study was to quantify analgesia obtained by NSAID, associated or not with other therapeutic agents, in patients diagnosed with chronic muscle pain. The null hypothesis is that NSAID may reduce or eliminate pain resulted that from TMD.

2. Material and Methods

2.1. Participant's Selection. All the clinical and laboratorial steps were performed by only one researcher, to avoid interexaminer differences, standardizing procedures, and reducing result bias.

Eighteen adult volunteers, both men and women, between 35 and 70 years of age (mean age 50 years), who were included in a maintenance-care program at the Faculty of Dentistry of Ribeirão Preto, University of São Paulo, were enrolled in this study. The inclusion criteria were masticatory muscle pain. The exclusion criteria included pregnancy, lactation, anti-inflammatory, or antibiotic therapy within the previous 3 months, current smokers, or individuals with any systemic condition that could influence pain management. The local ethics committee approved this study, and all of the experiments were undertaken with the informed written consent of each participant in accordance with ethical principles (Lawsuit n. 2006.1.558.58.0, CAAE n. 0022.0.138.000-06).

A complete patient trial was carried out by using an anamnesis with questions regarding general health. There were also excluded participants on this study with (1) under 18 years old; (2) who worn removable dental prosthesis; (3) who were taking any other medicament; (4) whose health did not allow intake of those studied drugs; and (5) alcohol addict.

The Brazilian Portuguese version of Research Diagnostic Criteria of Temporomandibular Disorders (RDC/TMD) [11] was used in trial.

2.2. Experimental Design (Treatment Protocol). All participant enrolled in this investigation received a flat, full-covered, and rigid occlusal splint adjusted in centric relation contact position, with canine guides and simultaneous occlusal contacts. Patients were advised to use the occlusal splint during all the 10 days of investigation. The provoked pain was performed by finger pressure on both sides of masseter, temporalis, sternocleidomastoideus, trapezius, and TMJ lateral pole. Data from intensity of pain was assessed on the first and last day of study. Patients were instructed to mark on 10 mm visual analogue scales (VAS) the pain intensity of each examined site. In addition, during treatment period, patients were instructed to mark at an 11-point numeric scale, from 0 (absence of pain) up to 10 (the worst pain ever), once a day during nocturnal period, the value related pain intensity of that day.

During treatment, patients were instructed to use the 3 treatment protocols proposed in this investigation: (a) NSAID (sodium diclofenac), (b) panacea (sodium diclofenac + carisoprodol + acetaminophen + caffeine), and (c) Placebo. A full randomized crossover methodology was adopted and, in this manner, all patients were submitted to all treatments in different moments. Therefore, the sequence of treatments did not follow a standardized sequence, avoiding bias. A washout interval of 11 days was respected between each proposed protocol.

The drugs used in this study are displayed in Table 1, including composition, active ingredients, concentration, manufacturer, and lot number. Medicaments A and B were acquired in pharmacy stores. Medicament C was manufactured in the Faculty of Pharmaceutical Sciences of Ribeirão Preto (University of São Paulo, Brazil) using a capsule shape.

During treatment, individuals were informed to take two daily medicament dosages, according to the manufacturer's recommendation, during 10 days, followed by an 11-day washout interval, started after the last dosage of each drug. All patients were instructed not to wear occlusal splint and not take any medicament during washout period.

2.3. Data Analysis. Statistical analysis was performed by an investigator not informed about medicaments compositions. Normality tests (Kolmogorov-Smirnov) were performed for the provoked pain data obtained by VAS. Most data did not present Gaussian distribution, and then a logarithm transformation was done. A 0.5 constant was added to all pain scores, to allow logarithm be used on zero scores [12]. After that, data normalization was obtained, allowing repeated measures by ANOVA.

For daily pain scores, statistical analysis was performed using nonparametric statistic methods. Friedman's test was applied to investigate pain value differences among each day of treatment. When differences were found, Wilcoxon's test was performed to compare the initial pain score to each subsequent treatment day. For both tests, significant differences were considered when $P < 0.05$.

3. Results

Data from VAS analysis are displayed in Figures 1, 2, 3, and 4. First and final values among evaluated groups did not show significant differences ($P > 0.05$), except for right masseter

Table 1: Composition, active ingredients, concentration, manufacturer, and lot number of drugs used in the study.

Medicament	Active ingredient	Concentration	Manufacturer	Lot number
A Register number 1.0370.0150.003-6	Acethaminophen Sodium diclofenac Carisoprodol Caffeine	300 mg 50 mg 125 mg 30 mg	Teuto Laboratory (Anápolis, GO, Brazil)	183567
B Register number 1.0370.0070.003-7	Sodium diclofenac	50 mg	Teuto Laboratory (Anápolis, GO, Brazil)	40284
C Placebo (control)	Cornstarch	110 mg	Faculty of Pharmaceutical Sciences of Ribeirão Preto of USP	

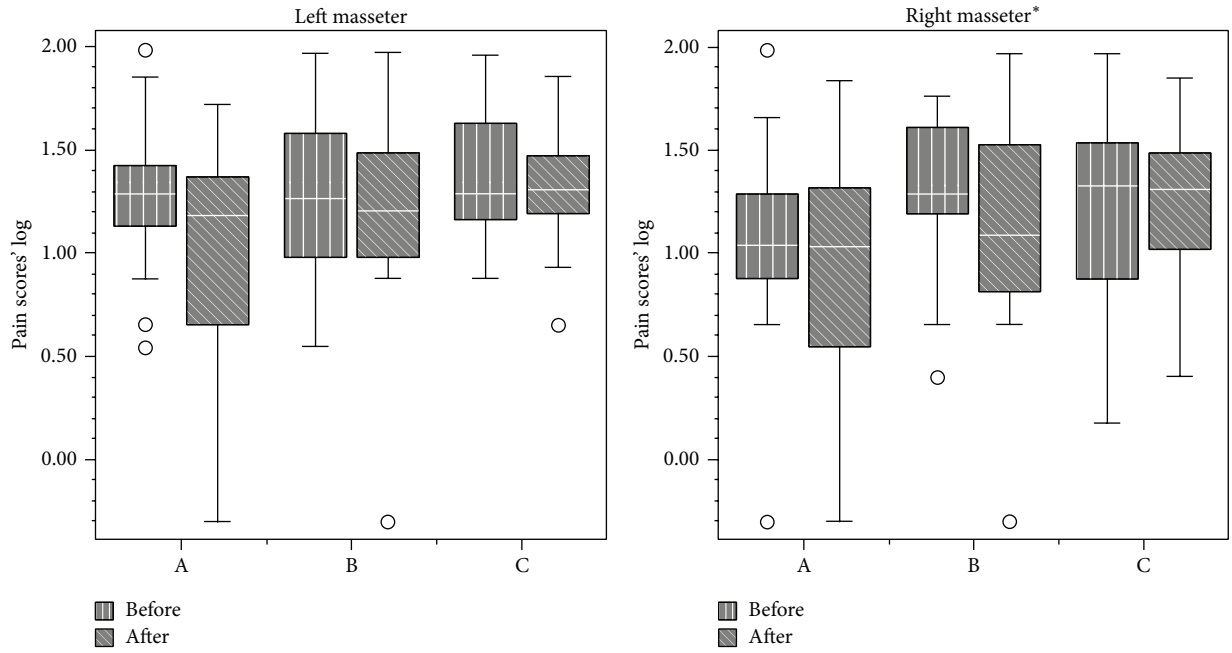

Figure 1: Pain scores (median and interquartile intervals) before and after treatment in the masseter muscle from groups A (NSAID—sodium diclofenac), B (panacea—sodium diclofenac + carisoprodol + acetaminophen + caffeine), and C (placebo). (*Significant differences; $P < 0.05$).

muscle, in which differences were observed between groups A and C ($P < 0.05$).

When data from 11-point numeric scale was evaluated (daily pain scores), it significant differences were observed between all sites after treatment protocols ($P < 0.05$), except for right TMJ and right/left temporalis muscle ($P > 0.05$).

For A treatment (NSAID—sodium diclofenac), Friedman's test showed significant differences among daily pain scores ($P = 0.00002$). Wilcoxon's test results revealed differences between days 1×3 ($P = 0.019$), 1×4 ($P = 0.019$), 1×6 ($P = 0.026$), 1×8 ($P = 0.006$), 1×9 ($P = 0.021$), and 1×10 ($P = 0.022$).

Regarding B treatment (panacea—sodium diclofenac + carisoprodol + acetaminophen + caffeine), Friedman's test showed significant differences among daily pain scores ($P = 0.00016$). Wilcoxon's test results showed significant differences between days 1×3 ($P = 0.018$), 1×4 ($P = 0.027$), 1×5 ($P = 0.002$), 1×7 ($P = 0.012$), 1×9 ($P = 0.004$), and 1×10 ($P = 0.011$).

About C treatment (placebo), Friedman's test indicated significant differences among daily pain scores ($P = 0.012$). Wilcoxon's test results showed significant differences only in days 1×8 ($P = 0.025$).

4. Discussion

Analgesic therapies, mainly those based on drugs administration, have been used extensively for the control and treatment of pain, but it is still lacking or scarce in the current literature data on the effects of NSAID associated with occlusal split and other therapeutic agents on the pain relief of patients with chronic muscle pain. In this investigation, we evaluated the efficacy of NSAID, associated or not with therapeutic agents,

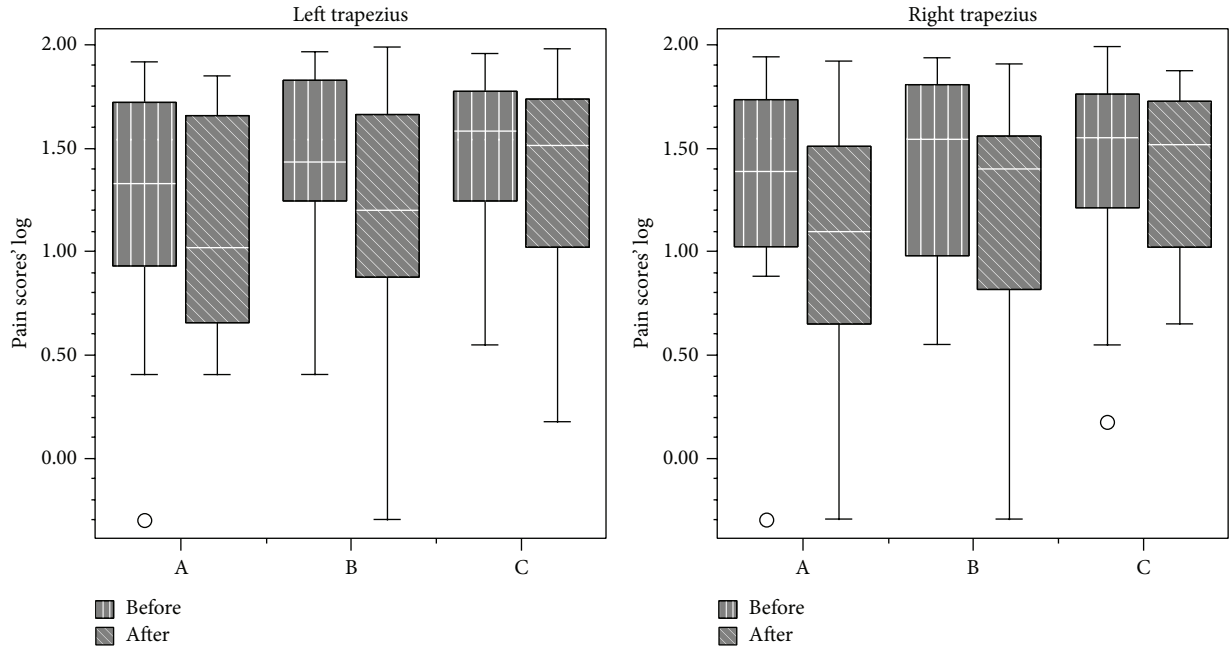

Figure 2: Pain scores (median and interquartile intervals) before and after treatment in the trapezius muscle from groups A (NSAID—sodium diclofenac), B (panacea—sodium diclofenac + carisoprodol + acetaminophen + caffeine), and C (placebo).

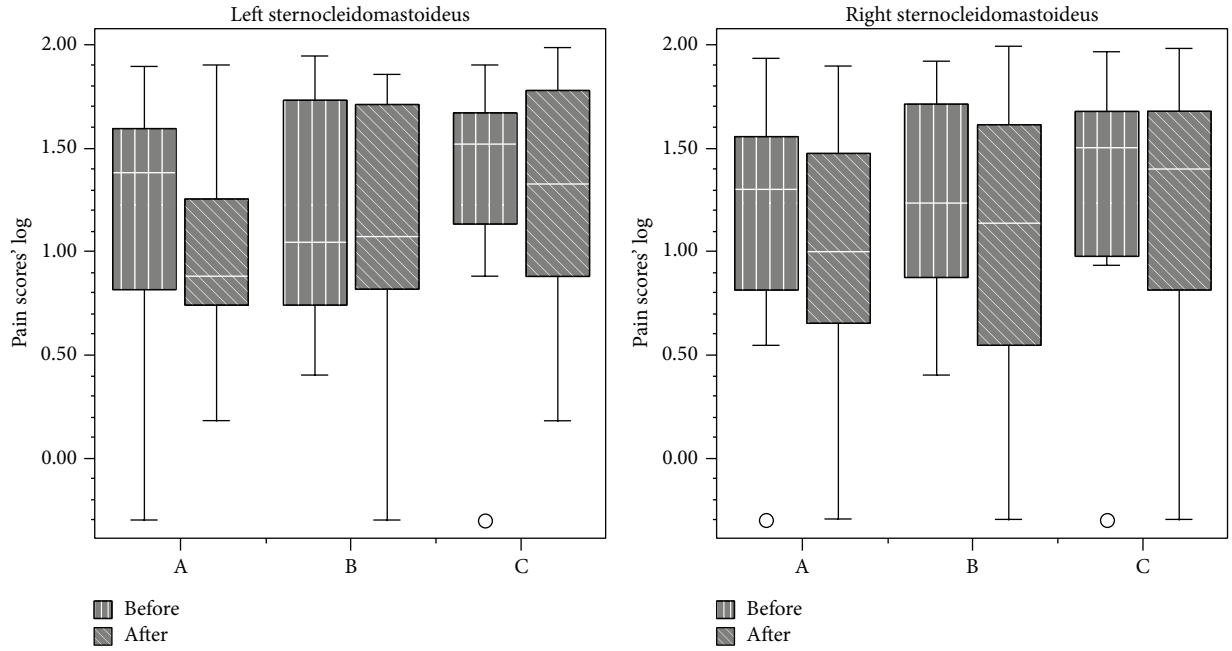

Figure 3: Pain scores (median and interquartile intervals) before and after treatment in the sternocleidomastoideus muscle from groups A (NSAID—sodium diclofenac), B (panacea—sodium diclofenac + carisoprodol + acetaminophen + caffeine), and C (placebo).

in the adjuvant treatment of chronic muscle pain. A placebo was used as negative control.

Overall, our findings demonstrated that all the therapies were effective in pain relief. NSAID therapy promoted analgesia on the third day, while placebo promoted analgesia only in the eighth day. No significant differences between medicaments were observed for VAS analysis, except for the right masseter. Daily analysis by 11-point scale showed significant differences between all groups during the 10 days for all sites, except for the right TMJ and right/left temporalis muscle.

The diagnosis method using RDC/TMD and the well-controlled experimental design with inclusion/exclusion criteria associated with the triple-blind randomized crossover

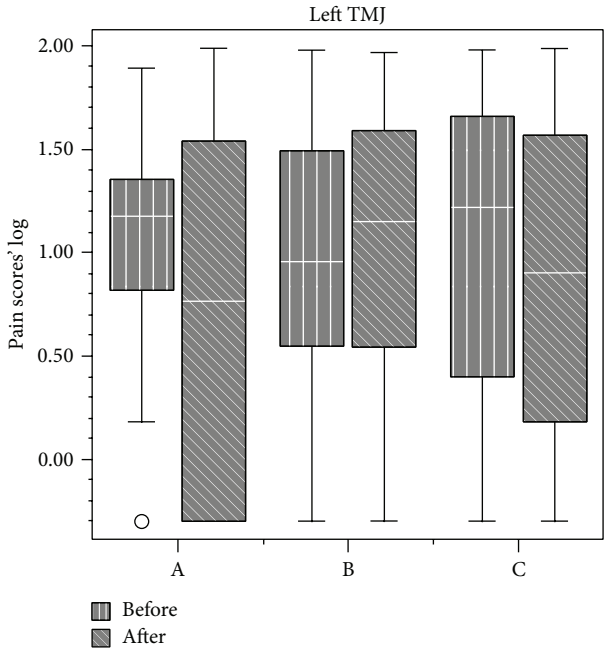

Figure 4: Pain scores (median and interquartile intervals) before and after treatment in the temporomandibular joint from groups A (NSAID—sodium diclofenac), B (panacea—sodium diclofenac + carisoprodol + acetaminophen + caffeine), and C (placebo).

clinical methodology were essential to minimize clinical research bias [13–16]. The placebo group, used as control, was fundamental to compare pain relief induced by tested medicaments. Occlusal splints were important for this study to become ethically practicable. Thus, even patients in placebo treatment group received a widely accepted therapy [13, 17, 18].

Our data are in accordance with other similar studies. In a systematic review [19], scientific evidences about splint therapy benefits in TMD symptoms remission after treatment with stabilizing splints were observed. In a study with electromyography [17], immediate decreasing in masseter and temporalis muscle activity after occlusal splints installation was observed, reducing TMJ overload, balancing masticatory muscles, and reducing TMD symptoms. In another study, in which patients were submitted to splint therapy during 1 year, they have reported a relevant decrease in the TMD symptoms, including TMJ sounds [18].

Chronic musculoskeletal pain treatment is complex, and its condition includes inflammatory and noninflammatory characteristics. Some events as repetitive overloading and trauma are clearly associated with peripheral tissue damage and muscle inflammation [7, 9]. Specifically, in masticatory muscles, pain can be a result of occlusal dysfunction and parafunctional habits. Prolonged and repetitive muscular contractions can be aggressive, causing microscopic tissue lesions, sensibility, and pain [20]. In this case, the NSAID could be helpful do reduce or eliminate local inflammation and pain.

The initial hypothesis of this study was that a NSAID (sodium diclofenac) could minimize or inhibit the local releasing of inflammatory substances in many of the masticatory muscles [5] and TMJ pain conditions [21]. In addition, when associated with other substances, such as muscle relaxant (carisoprodol), analgesic (acetaminophen), and central analgesic and adjuvant (caffeine), the effects could be improved.

Provoked pain analysis showed pain reduction on the right and left masseter, sternocleidomastoideus, trapezius, and left TMJ for all treatments. Thus, they were significantly effective in reducing pain sensation, but medicaments A and B were not more effective than placebo after 10-day treatment.

Pain relief could be related to reduction of peripheral sensitization by decreasing of overload, fatigue, and inflammatory components release in masticatory muscles and TMJ [22, 23]. Therefore, allodynia and hyperalgesia could be decreased, as well as painful sensation. The symptom remission could be attributed to splint therapy because, after treatment, results were similar even when using medicaments as adjuvant.

However, occlusal splint associated with medicaments A or B reduced pain in an earlier moment (third day) when compared to control group (eighth day); it has been evident after patients' daily pain scores analysis. Thus, sodium diclofenac, whether associated or not, was shown to be more effective in reducing inflammation process from masticatory muscles and tissue microlesions of TMJ, when compared to placebo [24]. Its action, based on cyclooxygenase inhibition, has prevented development of inflammatory substances from arachidonic acid [23].

Apparently, the use of occlusal splint reduced functional overload on masticatory muscles and TMJ, promoted bilateral balancing, and diminished muscle and joint activity [17] thus, preventing new tissue microlesions and inflammatory mediators release. Concomitantly, the anti-inflammatory acted as inhibiting inflammatory mediators release and reducing peripheral sensitization and, consequently, painful sensation in a short time.

Standardized volunteers' selection based on their TMD signs and symptoms using RDC/TMD was possible, although psychosocial factors could have great variation among them [4]. It means that similar dysfunctions can present different responses, according to patients' mental and social health. Those factors have great influence on pain behavior and should always be considered in chronic pain management. Treatment based on biomedical model, ignoring psychosocial characteristics, may lead to failure and patient's frustration [25, 26]. Nevertheless, an adequate initial approach is essential for treatment success.

Even in chronic pain patient who, according to biopsychosocial pain model, usually has no notable sensorial component when compared to amount of psychosocial factors, the inflammation management using medicaments showed in our investigation significant results in pain relief.

Although participants in this study were chronic TMD patients, with painful complaints strongly influenced by psychological and social status, treatments were based on noxious input decreasing, by drug intake and use of occlusal splint, but analgesia was obtained in a short time and could have a positive effect on patients expectations related to

treatment, making them believe in good results, changing their behavioral in regard to their problems, and improving the psychosocial pain components [27].

Management of those drugs in a short period is important to avoid gastric, intestinal, renal, and hepatic problems, as described in the literature [9]. All NSAIDs have gastrointestinal risks of gastritis and possible bleeding and its chronic use increases the risk of renal insufficiency, especially patients with diabetes [22].

Acetaminophen is usually well tolerated at therapeutic doses but may have chronic renal or hepatic adverse effects [27]. Muscle relaxant side effects include high incidence of dizziness, drowsiness, headache, blurred vision, nausea, and vomiting and must be used with caution [28].

Carisoprodol abuse is reported in the literature [29], and its excessive use can cause physical and psychological dependence [28]. Nevertheless, no additional benefit was observed with sodium diclofenac associated with carisoprodol, caffeine, and acetaminophen. Conversely, the literature reports on carisoprodol abuse and dependence and presence of side effects as sleepiness and others related to central nervous system, such as drowsiness, dizziness, palpitation, irritation, and headaches [30]. Clinicians must be alerted to the medicament risks and benefits before prescribing it to patients.

Management of chronic pain is not frequently limited to pharmacotherapy and splint therapy. It should be multidisciplinary, focusing all pain aspects according to biopsychosocial model. The correct and effective intervention of patients suffering of pain could significantly increase their confidence on the proposed therapies. In such case, it seems that starting a treatment with occlusal splint associated to an adjuvant nonsteroidal anti-inflammatory for a short period could be very adequate.

5. Conclusion

Within the limitations of this investigation, we conclude that (1) all proposed therapies evaluated reduced TMD pain after 10 days of treatment and (2) NSAID (sodium diclofenac) and panacea (sodium diclofenac + carisoprodol + acetaminophen + caffeine) were more effective than placebo, promoting significant pain relief on the third day, while placebo group promotes only on the eighth day.

Conflict of Interests

The authors declare that they have no conflict of interests. There is no relationship between the manufacturer's drugs and the University of São Paulo (the institution in which the study was held).

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Review Article

Laser Teeth Bleaching: Evaluation of Eventual Side Effects on Enamel and the Pulp and the Efficiency In Vitro and In Vivo

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Light and heat increase the reactivity of hydrogen peroxide. There is no evidence that light activation (power bleaching with high-intensity light) results in a more effective bleaching with a longer lasting effect with high concentrated hydrogen peroxide bleaching gels. Laser light differs from conventional light as it requires a laser-target interaction. The interaction takes place in the first instance in the bleaching gel. The second interaction has to be induced in the tooth, more specifically in the dentine. There is evidence that interaction exists with the bleaching gel: photothermal, photocatalytical, and photochemical interactions are described. The reactivity of the gel is increased by adding photocatalyst of photosensitizers. Direct and effective photobleaching, that is, a direct interaction with the colour molecules in the dentine, however, is only possible with the argon (488 and 415 nm) and KTP laser (532 nm). A number of risks have been described such as heat generation. Nd:YAG and especially high power diode lasers present a risk with intrapulpal temperature elevation up to 22°C. Hypersensitivity is regularly encountered, being it of temporary occurrence except for a number of diode wavelengths and the Nd:YAG. The tooth surface remains intact after laser bleaching. At present, KTP laser is the most efficient dental bleaching wavelength.

1. Introduction

Heating hydrogen peroxide (HP) results in an acceleration of its decomposition and oxidant-free radical formation [1]. Therefore, the dental bleaching process can be accelerated by additional heat activation. One of the activation methods resulting in an increase of the temperature in the bleaching gel is power bleaching with high-intensity light [2].

The effectiveness of this method for vital tooth bleaching has been demonstrated in animal studies, clinical studies and reports, and a number of reviews [3–6]. Side effects for the tooth, that is, alteration of the enamel surface, posttreatment, and pulp sensitivity, have been suggested and investigated [4, 5, 7, 8].

Potential adverse effects on enamel were primarily investigated in vitro using extracted human and bovine teeth. Reports on the effects of light-activated systems were divergent, which was also the case for conventional in-office bleaching techniques. On the one hand, changes in microhardness, the presence of porosities, changes in surface roughness, a reduction in fracture toughness, alteration of the calcium/phosphate ratio, erosion, decrease in abrasion resistance, and the formation of depressions were reported. The enamel surface changes varied mostly with the bleaching products used, especially high concentrations of hydrogen peroxide; that is, 30–35% (w/w) and 35% (w/w) carbamide peroxide (CPO) (11-12% HP) could have a damaging effect, whereas low concentrations 10% or 16% CPO (w/w) (3–5% HP) had no effect [8]. On the other hand, rehardening of porous enamel as a result of saliva ion reprecipitation has been described. Although remineralisation due to the saliva

may be responsible for a gradual mineral rebuild-up, full repair of the enamel is not established due to a degradation of the organic matrix [8]. To date, nevertheless, no clinical adverse effects of power bleaching on enamel have been reported.

Sensitivity after bleaching is higher when HP is combined with thermal activation [4–9]. Diverging results once again have been published regarding the effect of power bleaching on the pulp [4–9]. Also for this topic there is a lack of in vivo studies and there are no studies evaluating long-term effects of HP exposure on dental pulp.

An intrapulpal temperature increase of 5.5°C is nowadays regarded as the threshold value, which should not be exceeded to avoid irreversible pulp damage [10]. It appears that temperature during light-activated bleaching is in general under control, especially due to the presence of a bleaching [4, 10].

2. Aim

At present, there is no review on the efficiency of laser activated bleaching and its effect on the tooth (enamel and pulp). The aim of this review is therefore to evaluate the influence of the temperature rise during laser bleaching on the pulp, the postoperative sensitivity, and eventual enamel alterations. The efficiency is evaluated on the basis of the colour change in vitro and in vivo.

3. Methods and Materials

The electronic literature search included the databases PubMed and Web of Science for manuscripts published with full journal reference from January 1950 to November 2014. All languages were accepted provided there was an abstract in English. The following MeSH terms and key words were used: "lasers" AND "tooth bleaching," "lasers" AND "tooth discoloration," "tooth bleaching" OR "teeth bleaching" AND (argon laser OR diode laser OR KTP laser OR Nd:YAG laser OR Er:YAG laser OR Er, Cr:YSGG laser OR carbon dioxide laser). Two reviewers (AD and BV) independently assessed abstracts and full-text articles. First the reviewers considered the abstracts as potentially relevant. Abstracts dealing with this topic but without access to full journal article were not taken into consideration. Case reports were included only when they exclusively reported observations which were not described in other publications. Then full articles were read. Both reviewers selected independently the same 71 full-text articles; that is, Cohen's kappa = 1.0.

4. Results

4.1. Temperature Rise in the Pulp. Taking into account the subject of the present review both power intensity and wavelength of the light used during the bleaching procedure must be taken into consideration [11]. An overview of the changes in temperature in the pulp during laser dental bleaching is given in Table 1.

4.1.1. CO_2 Laser (10,600 nm). Luk et al. [12] reported that the use of a CO_2 laser (10,600 nm) on teeth for bleaching purposes led to a temperature increase of 13.1 to 22.3°C with gel at the enamel surface and 6.9 to 16.6°C at the pulpal side of the dentine. Due to a lack of controlled clinical studies this wavelength was not approved for bleaching by the ADA [30]. At present this wavelength is no longer used for dental bleaching.

4.1.2. Nd:YAG (1,064 nm). Next to the CO_2 laser, the highest temperature elevations in the pulp were registered with the Nd:YAG laser (1,064 nm) irrespective of the use of coloured bleaching gels (blue, red, and transparent) [13, 14].

4.1.3. Diode Lasers. High power *diode lasers* (784–980 nm) are also known to be able to rise the pulpal temperature and should be used in combination with a bleaching gel. An overview of the reported data is given in Table 1.

Laser activation with a 830 nm *diode laser* (30 s, 3 W) without bleaching gel may result in a temperature increase of 16°C in the pulp chamber; when applying the gel during laser activation only 8.7°C temperature increase was recorded [10].

With a 915 nm *diode laser*, there was an increase of temperature with 26.7°C at 3 W-20 s and 12.2°C at 1.5 W-20 s [16]. In the same study temperature rise was lower after application of a bleaching gel; the decrease was product related: By White gel (By Dental, Pistoia, Italy) at 3 W resulted in a rise of 17.0°C and at 1.5 W of 7.6°C with Whiteness HP (FGM Produtos Odontológicos, Joinville, Brazil) it was +25.6°C at 3 W and +6.0°C at 1.5 W. The bleaching gel thus acts as a selective absorber near the dental surface, preventing light penetration into the internal tooth structure. Apparently the composition of the gel is also important as the gel layer was 2 mm thick in both investigations.

An increase of 2.61°C with a *diode laser* (810 nm) (4 W, 20 s) and 1.86°C with an Er:YAG (2,940 nm) (40 mJ, 10 Hz, 20 s) was registered by Sari et al. [17]. The temperature in the gel, however, was 6.21°C for the diode and 20.11°C for Er:YAG.

The increase in the pulp chamber temperature with a *diode laser* (830 nm) used at 1 W-30 s is below the critical temperature increase of 5.5°C that is nowadays regarded as the threshold value and which should not be exceeded to prevent irreversible pulp damage [18]. In the same study the diode laser at 2 W-30 s resulted in a temperature increase up to 6.8°C and 8.7°C with 3 W-30 s; the importance of use of the gel with appropriate thickness was emphasized by measurements of the temperature at the surface: 1 W resulted in 37°C, 2 W in 64.1°C, and 3 W in 86.3°C.

Similar findings were registered by Fornaini et al. [19] where an 808 nm *diode* at 2 W during 3 × 30 s resulted in heating of the gel up to 43.1°C and at 4 W-3 × 30 s up to 74.9°C.

A temperature increase of 2–8°C and 4–12°C was observed when a 960 nm *diode* laser was used to activate Opalescence Xtra (Ultradent Products, South Jordan, UT, USA) and Opus White (Opus Dent, London, UK) for 0.9 W-60 s and 2 W-30 s [20].

A mean increase of 11.75°C in the pulp was seen with an 810 nm *diode* used at 10 W-15 s [22].

With a hydrogen peroxide bleaching agent, the mean maximum pulpal temperature rise was 2.95°C for a LED,

Table 1: Increase in temperature (°C) in the pulp (without or with gel application) after exposure to laser light.

Authors	Wavelength	Settings	Bleaching gel	Result/temperature
Luk et al., 2004 [12]	10,600 nm (CO_2)	600 mW 6 × 30 sec/180 sec interval GT: 2 mm *D*: 1 to 2 mm	Opalescence Extra Quick White Star Brite Nypro Gold	Pulp without and with gel +13.34 +9.75 +10.73 +6.93 +13.08 +7.98 +22.26 +16.55
Michida et al., 2009 [13]	1,064 nm (Nd:YAG)	600 mJ, 10 Hz (0.6 W) 20 sec GT: 0.5 to 1 mm *D*: 5 mm	Whiteness HP	Pulp with gel +4.33
Dominguez et al., 2011 [14]	1,064 nm (Nd:YAG)	75 mJ, 10 Hz 3 × 20 sec GT: 2 mm *D*: 2 mm	Ena White Power Opalescence Endo Q White	Pulp with gel +3.1 +2.5 +2.9
	2,940 nm (Er:YAG)	40 mJ, 10 Hz 3 × 20 sec GT: 2 mm *D*: 2 mm	Ena White Power Opalescence Endo Q White	Pulp with gel +0.2 0 0
	532 nm (diode)	1.6 mJ, 15 Hz 3 × 20 sec GT: 2 mm *D*: 2 mm	Ena White Power Opalescence Endo Q White	Pulp with gel +0.25 0 0
	675 nm (diode)	200 mW 3 × 20 sec GT: 2 mm *D*: 2 mm	Ena White Power Opalescence Endo Q White	Pulp with gel +2.6 +1.3 +1.3
Klaric et al., 2013 [15]	770 nm (femtosecond diode)	800 mW, 15 min Unfocused GT: ? *D*: ?	Without gel ZOOM 2 Boost Vivastyle 30 Vivastyle 16 Vivastyle 10	Enamel surface Pulp +3.2 +2.7 +2.1 +1.4 +2.0 +1.4 +2.1 +1.4 +2.1 +1.4 +2.2 +1.4
		800 mW, 15 min Focused GT: ? *D*: ?	Without gel ZOOM 2 Boost Vivastyle 30 Vivastyle 16 Vivastyle 10	Enamel surface Pulp +16.8 +15.7 +9.4 +8.7 +9.6 +8.8 +9.5 +8.7 +9.7 +8.8 +9.4 +8.6
Sulieman et al., 2005a [10]	830 nm (diode)	3 W, 30 sec GT: 2 mm *D*: just above the surface of the gel	Opus Mix bleaching powder + 35% HP liquid	Pulp without and with gel +16 +8.7
Kivanç et al., 2012 [16]	915 nm (diode)	3 W, 20 sec GT: 2 mm *D*: 10 mm	By White (BW) Whiteness HP (WHP)	Pulp without and with gel +26.7 BW +17 WPH +25.6
		1.5 W, 20 sec GT: 2 mm *D*: 10 mm		Pulp without and with gel +12.2 BW +7.6 WPH +6
Sari et al. [17], Epub 2013	810 nm (diode)	4 W, 20 sec GT: *D*:	Whiteness HP	Pulp with gel +2.61
	2940 nm (Nd:YAG)	40 mJ, 10 Hz, 20 sec GT: *D*:		Pulp with gel +1.86

Table 1: Continued.

Authors	Wavelength	Settings	Bleaching gel	Result/temperature
Sulieman et al., 2006 [18]	830 nm (diode)	30 sec 3 W 2 W 1 W GT: 2 mm *D*: just above the surface of the gel	Opus Mix bleaching powder + 35% HP liquid	Surface / Pulp without and with gel 86.3 / +11.6 / +8.7 64.1 / +7.7 / +6.8 37 / +5.23 / +5.5
Fornaini et al., 2013 [19]	808 nm (diode) 532 nm (KTP)	3 × 30 sec—rest time 2 min 2 W 4 W GT: *D*:	48 g HP solution + 29 g carbopol (HP concentration 27 to 35%)	Peak gel temperature 43.1 74.9 Peak gel temperature 32 45.1
Wetter et al., 2004 [20, 21]	960 nm (diode)	0.9 W, 60 sec GT: ? *D*: 2 mm 2 W, 30 sec GT: ? *D*: 2 mm	Opalescence Xtra (OPX) Opus White (OW)	Pulp with gel OPX +8 OW comparable (8) Pulp with gel OPX +12 OW comparable (8)
Eldeniz et al., 2005 [22]	810 nm (diode)	10 W, 15 sec GT: OPX?—QS: 1 mm *D*: ?	Opalescence Xtra (OPX) Quasar Brite (QS)	Pulp with gel +11.75
Zhang et al., 2007 [23]	980 nm (diode) 532 nm (KTP)	0.8 W, 30 sec GT: 1 mm *D*: 1 mm 1 W, 30 sec GT: 1 mm *D*: 1 mm	Hi Lite	Pulp with gel +7.72 Pulp with gel 3.76
Verheyen et al., 2006 [24]	810 nm (diode)	1 W, 30 sec GT: ? *D*: ?	Opus White (OW) Ti-O2 gel (TO)	Pulp with gel OW 3 TO 0
Goharkhay et al., 2009 [25]	810 nm (diode)	1 W, 60 sec 2 W, 60 sec GT: ? *D*: 6 mm	Opalescence Xtra Boost	Pulp without and with gel +5 / 0 +9.6 / +2.8
Pleffken et al., 2012 [26]	660 nm (diode)	50 mW, 3 × 160 sec GT: ? *D*: 2 mm	35% experimental HP gel	In the gel / Pulp with gel +2.29 / +1.8
Carrasco et al., 2008 [27]	LED-laser 470/790 nm	40 mW, 3 × 30 sec GT: 1 mm *D*: 5 mm	Whiteness HP	Pulp without and with gel +0.35 / +0.33
Torres et al., 2008 [28]	LED-laser 470/784 nm	120 mW 5 × 40 secc GT: 2 mm *D*: 5 mm	Whiteform Perox Red	Critical temperature rise of 5.5°C not reached
Coutinho et al., 2009 [29]	LED/laser 470/795 nm LED/laser 530/795 nm	120 mW, 3 × 60 sec 20 mW, 3 × 60 sec	Whiteness HP	Pulp with gel Incisor +3.1 Canine +2.5 Premolar +1.9 Incisor +0.6 Canine +0.2 Premolar +0.3

GT: gel thickness; *D*: distance between light source and the bleaching gel.

3.76°C for a KTP laser (1 W-30 s), and 7.72°C for a 980 nm *diode laser* (0.8 W-30 s) [23].

With an output power of 1 W-30 s of an 810 nm *diode* laser, pulpal temperature increase was shown to be approximately 3°C with the Opus White gel (Opus Dent), whereas a TiO_2 emulsion showed almost no temperature changes in the pulp [24].

A treatment protocol with intermittent irradiation of six times for 5 s, with 5 breaks in between, at a power setting of less than 1 W, with a 810 nm *diode laser* excluded thermal damage to the pulp, whereas the temperature at the surface was 5°C. Irradiation at 2 W with the same protocol resulted in a temperature elevation of 9.6°C at the surface and 2.8°C in the pulp chamber [25].

The *addition of colorants* may help to provide a better absorption of high power diode laser light in the bleaching gel and less transmission towards the pulp chamber. Pleffken et al. [26] demonstrated that a low-intensity red *diode laser* (660 nm) (50 mW-3 × 180 s) with a green-coloured bleach gel resulted in not more than a 2.3°C temperature elevation in the pulp chamber.

It is clear that power and type (wavelength) of the light source influence temperature variation. Studies have shown that near-infrared lasers could improve the inflammatory response of the pulpal tissue, reducing pulp damage and relieving pain after the bleaching process [31]. Its use would diminish patients' sensitivity complaints after the procedure. In this respect *LED devices* were associated with *diode lasers* emitting in the near-infrared. According to Carrasco et al. [27] 470/790 nm (40 mW-3 × 30 s) temperature is under control. Other studies also demonstrated negligible temperature changes: 470/784 nm (120 mW-5 × 40 s) [28], 470/795 nm (120 mW-3 × 60 s), and 530/795 nm (20 mW/3 × 60 s [29]). Moreover it appears that these combination types of light sources with low power density are not powerful enough to provide a better bleaching efficacy as compared to other light sources [20, 23, 32].

A comparison between different laser wavelengths by Dominguez et al. [14] used as follows: that is, three bleaching gels (transparent, with blue dye, with red dye, composition of dye mentioned) exposed during 3 times for 20 sec to the light source with a 9 min interval and an overall contact time of the gel with the tooth surface during 30 min, demonstrated the following temperature effects in decreasing order: *Nd:YAG* (1064 nm) (rise of 3.1°C in the pulp chamber) > halogen lamp (120 nm) > *low power diode* (675 nm) > low power LED (380–530 nm) > *2ωNd:YAG* (532 nm) > *Er:YAG* (2940 nm). These findings coincided with the findings of Torres et al. [28] (halogen versus diode 470/784 nm) and Carrasco et al. [27] (halogen versus diode 470/790 nm versus LED).

In a study of Klaric et al. [15] a comparison was made between ZOOM2 (350–400 nm) during 15 min, LED (405 nm) during 30 min, OLED (organic light emitting diode) (400–760 nm) during 30 min, and a *femtosecond laser* (770 nm) (Millenia, Spectra Physics, USA) during 30 min: ZOOM2 resulted in high temperature elevations (+15.4°C) in the pulp whereas elevations were +21.1°C without use of bleaching gel; the femtosecond laser focused: +15.7°C without gel and +8.7°C with gel; the femtosecond laser unfocused: +2.7°C without gel and +1.4°C unfocused. In this respect it has also to be mentioned that the mechanism of heat conversion depends directly on the tissue constituents and the irradiation wavelength used. It is known that the tooth absorption coefficient is lower for the wavelength range $400 < \lambda < 500$ nm; thus scattering predominates over absorption at these wavelengths.

Er:YAG (2,940 nm). In a study of Kivanç et al. [16] temperature increase in the pulp was neglectable. A very low temperature rise of 1.86°C was registered by Sari et al. [17].

KTP (532 nm). Using the green light of the KTP to irradiate a red coloured bleaching gel resulted in a temperature of 32°C at 2 W during 30 sec and 45.1°C at 4 W during 30 sec [19].

With a hydrogen peroxide bleaching agent, the mean maximum pulpal temperature rise was 2.95°C for a LED, 3.76°C for a KTP laser, and 7.72°C for a diode laser [23].

4.2. Influence on the Characteristics and Material Properties of the Teeth. The aim of a bleaching procedure is to bleach the tooth without morphological and chemical changes. However, side effects after power bleaching in the enamel such as changes in microhardness, the presence of porosities, changes in surface roughness, a reduction in fracture toughness, alteration of the calcium/phosphate ratio, erosion, decrease in abrasion resistance, and the formation of depressions were reported. Weakening of enamel structure by oxidation of organic or inorganic elements is considered to be the main cause [33].

4.2.1. Morphological Analysis. Morphological analysis showed slight changes with the diode laser (970 nm) and the LED/laser (467 nm/790 nm) [34]. Surface effects were unrelated to the pH of the high concentration HP bleaching gels with laser activation and referred more to a better or lesser absorption of the laser light by the bleaching gel. Chromophores and the use of TiO_2 appeared to be favourable for the maintenance of an intact tooth surface [25]. No significant effects on the morphology of the enamel surface after laser bleaching with diode laser, KTP, Nd:YAG, and Er:YAG were observed by Dominguez et al. [14].

4.2.2. Mineral Content. FT-RS results showed a decreased mineral content after bleaching procedures with and without light activation and with 35% HP-based bleaching agents. The use of a LED/laser (470 nm/810–830 nm) resulted in comparable calcium loss as compared to the non-light-activated bleach gels for 2 of 3 brands. Exposing Pola Office (Southern Dental Industries, Sao Paulo, SP, Brazil) to the LED/laser did not result in a significant calcium loss [35]. An explanation for this difference was not given.

No significant differences in levels of calcium and phosphorus were seen after 470/830 nm LED/laser bleaching [36].

In a study of Cesar et al. [37] with 35% HP-based bleaching agents activated with a LED/laser (465.5 nm/790 nm), FT-Raman spectroscopy data showed no significant chemical changes in the inorganic components for the tested

groups. Carbonate and phosphate area peaks were not significantly changed. Whiteness HP Maxx (FGM Produtos Odontológicos Ltda., Santa Catarina, Brazil) and Opalescence Xtra (Ultradent Products) were also tested in the study of Berger et al. [35]. There was a significant reduction of the dental organics associated with type I collagen vibration only in the group of Whiteform-Perox Red gel (Formula & Acao, Sao Paulo, SP, Brazil). This means that there is a difference between both studies. Total contact time of the gels was identical, that is, 3 consecutive gel applications for 10 min. Irradiation protocols, however, differed: in Berger et al. [35], there was a bleaching gel left on third molars undisturbed for 1 min and then irradiated for 2 min; the light irradiation was repeated 3 times with a 1 min interval between radiations; in Cesar et al. [37] there was photoactivation of the gel for 30 sec for a total of 10 min of application on bovine teeth. Moreover, similar differences in findings were also observed with non-light-activated high concentration HP bleaching gels. Whether the differences found for the present two studies are related just to the bleaching protocol is not clear yet; in fact differences in oxidizing potential (stronger), stronger concentrations, longer treatment times, and lower pH of bleaching gels could be responsible for the changes found in the studies [37].

In a study with bovine teeth using a low power diode laser (740 nm, 300 mW power) it was seen that the enamel crystallinity was dramatically decreased by a bleaching treatment without laser irradiation. However, crystallinity increased as laser irradiation time increased. It was concluded that professional bleaching treatment with HP combined with a diode laser irradiation not only improves the bleaching effect but also protects against the change of enamel structure compared with the bleaching treatment without laser irradiation [38].

TEM analysis showed the formation of a new phase 2 μm thick layer. The assumption was also made that the chemical property of the bleaching gel could have been changed through exposure to laser irradiation. It can also be that this phenomenon accounts for bovine teeth where the enamel contains significantly more interprismatic organic material compared to human enamel even though its structure and compositions are very similar to those of human enamel.

4.2.3. Microhardness. The microhardness test is suitable for determining small changes in surface that demonstrated the effect of bleaching products on enamel [39].

A comparison between argon laser (488 nm, 200 mW, 30 sec irradiation and 4-minute intervals during 40 min) and halogen lamp-based photopolymerizer (2 min and 240 mW, and 4-minute intervals during 40 min) did not result in differences with the control group using 35% and 37% CP [37].

Zhang et al. [23] showed no differences between the control (35% HP) and KTP (1 W, 30 sec, energy density (ED) 13.33 J/cm^2), diode 980 nm (0.8 W, 30 sec, ED: 13.33 J/cm^2), and blue LED composite curing lamp (470 nm, 30 s, ED: 12.6 J/cm^2) experimental groups. Diode laser (830 nm) irradiation (3 times, 30 sec irradiation at 1.4 W of newly placed) of the 35% HP gel associated or not with ACP did not interfere with microhardness [40].

Reduction in microhardness was found after bleaching with a LED/laser (470/830 nm, light intensity of 200 mW, 1 min laser activation of the gel, followed by 2-minute rest; this procedure was repeated 3 times), which recovered to baseline values after 1 week of immersion in artificial saliva [36].

4.2.4. Enamel Permeability. Higher permeability of the enamel surface after a bleaching procedure with a LED/laser (470–790 nm) and QTH light as compared to a control (35% HP) was reported; there were no significant differences between the two bleach protocols [41].

Bleaching with a 470/830 nm LED/laser did not show any statistical difference with baseline with regard to dye penetration [36].

4.2.5. Caries Susceptibility of Bleached Enamel. In-office laser bleaching with a LED/laser (830 nm) does not result in a higher susceptibility for caries lesions [42].

4.2.6. Fracture Strength. Araujo et al. [43] showed that a LED/laser (465.5 nm/790 nm) did not influence the fracture strength of enamel after light-activated bleaching.

4.2.7. Bonding to Bleached Enamel. Bonding to intracoronally light-activated bleached dentine should be performed at least 10 days after a bleaching procedure with a LED/laser (465.5 nm/790 nm) [44]. A time interval of 2 to 3 weeks was advocated for applying silorane-based composite restorations of methacrylate based composites after bleaching with an 815 nm diode laser [45]. A week interval after bleaching with an 815 nm diode and 430–490 nm blue LED showed statistically significantly lower shear bond values as compared to the control and bleaching with QTH light (380–520 nm) [44]. The failure mode in this latter study was adhesive for the diode laser (80%) and the LED (70%); for both the control group and the QTH lamp the failure mode was mixed (adhesive and cohesive) (70%).

4.3. Hypersensitivity

4.3.1. Diodes and LED/Lasers (Diodes). Sensitivity is described by some as common with the diode laser. Bleaching with a diode laser (810 nm, 35% HP) just reached the level that can be tolerated by the patient [46]. Comparing a diode laser (810 nm, 37% HP) with PAC activation (400–490 nm, 35% HP), LED activation (400–500 nm, 38% HP) and no light activation (38% HP) resulted in the lowest sensitivity for the diode laser [47].

In the study of Kossatz et al. [48] 53.3% of the participants had sensitivity even 24 hours after laser bleaching with a LED/laser unit (470 nm/830 nm, 35% HP) with a protocol of gel activation during 1 min, leaving the gel undisturbed during 2 min and repeating this protocol 3 times and the in-office bleaching agent was refreshed every 15 minutes during a 45-minute application period. Immediate sensitivity was also scored in the study of Mondelli et al. [47] with a LED/laser (470 nm/810 nm, 35% HP). Sensitivity decreased

after 24 hours to return to normal after 7 days. There were no differences between in-office gels (light- and non-light-activated).

An increased expression of substance P was seen when a LED laser (470 nm, 35% HP) was used [49].

More recent studies demonstrated that sensitivity was generated independently of the light sources used: Almeida et al. [50] with a LED/laser at 425–480/810 nm, Martin et al. [51] with a LED/laser at 450/808 nm, and Moncada et al. [52] with a LED/laser at 425–480/808–830 nm. The latter two studies demonstrated a higher impact of the increase in concentration of bleaching agents on tooth sensitivity; treatment with carbamide peroxide generated also lower sensitivity than treatment with HP independently of the light sources.

A comparison between all these studies is difficult and impossible because each investigation is different, that is, different protocols. Moreover, complete basic information, that is, power settings, gel thickness, distance between gel, and light source, is not provided in the listed studies.

4.3.2. Nd:YAG. When using the Nd:YAG laser (1,064 nm, 35% HP) for laser bleaching at 4 W, 10 Hz, 320 μs pulse duration, and an energy density of 1.4 J/cm^2 in association with a red coloured gel, no enhancement of the bleaching success was found [52]. There was no reduction in hypersensitivity, as can be seen when Nd:YAG is used for the treatment of dentinal hypersensitivity. Only 20% of the patients in this study had a pain-free treatment with the Nd:YAG laser. The remaining patients felt the development of warmth to the point of pain, even a few days after laser treatment. The authors concluded to query the appliance of Nd:YAG laser irradiation for in-office bleaching.

4.4. Laser Bleaching: Tooth Colour Change and Efficiency

4.4.1. Colour Change In Vitro. A comparison based on analysis of photoreflectance spectra between the use of an argon laser (488 nm) and halogen lamp with 35% and 37% CPO gave better results for the 35% CPO gel. Halogen was as effective as argon laser with 35% CPO; argon was more effective than halogen for the 37% CPO [32]. A comparison between LED/diode laser (450–500 nm/830 nm), argon (488 nm), PAC (440–550 nm), and halogen (350–500 nm) showed better results for a 35% HP than for 37% CBP. A decrease in reflectance values was seen after 30 days; no difference was observed in bleaching efficiency between activated and nonactivated bleaching gels with high HP concentrations [53].

In a study comparing KTP (532 nm, 35% HP) with a diode laser (810 nm, 38% HP, 37% HP, and 35% HP) [25] improved changes in brightness of up to ten steps on the Vitapan classical shade guide were detected. Prerequisites, however, were a perfect match of the chosen wavelength and the bleaching gel. A neutral and basic pH of the bleaching gel is also advantageous. The higher bleaching power of KTP as compared to an 808 nm diode laser was confirmed by Fornaini et al. [19].

Diode laser activation (808 nm, 35% HP) of the bleaching agent was not more effective than the halogen lamp for bleaching root canal treated primary molars [54], safer for T° development. Activation of a 35% HP bleaching gel by diode laser (830 nm, 35% HP) as well as a xenon halogen light, a plasma arc lamp, and halogen light did not differ in result from the use of the same gel without light activation [55]. A comparison between a 980 nm diode laser and a xenon arc lamp (430–500 nm) used with a 35% HP bleaching gel showed that there was an increase in colour saturation (ΔC^*) of 3–32% and a change in whiteness (ΔL^*) of 0–8% [14]. The highest efficacy was achieved with the diode laser at 2 W, the lowest with the diode laser at 0.9 W. However, due to the risk of higher temperature development, the authors considered the xenon lamp as the safest. A comparison between a diode laser (808 nm) and LED (471 nm) demonstrated significant comparable change in chroma for the two 35% HP bleach gels investigated and the light sources. There was also a significant change in lightness for all test conditions, but the diode scored significantly best with the Whiteness HP bleaching agents (FGM Produtos Odontológicos, Joinville, Brazil) than with Opalescence Xtra (Ultradent Products) [21].

A comparison between different laser wavelengths by Dominguez et al. [14] demonstrated that the source of irradiation was more relevant than the bleaching agent for efficient tooth bleaching. They exposed three 35% HP bleaching gels (transparent, with blue dye, and with red dye, composition of dye mentioned) during 3 times for 20 sec to the light source with a 9 min interval; contact time of the gel with the tooth surface was 30 min. LED (380–530 nm, low power), halogen lamp (120 nm), and diode (675 nm, low power) produced greater colour changes than the rest of the light sources: Nd:YAG (1064 nm), Er:YAG (2940 nm), and 2ωNd:YAG (532 nm). The mean improvement in tooth whiteness with the latter three wavelengths is in the same order as without photoactivation. It is thus the question if these wavelengths are really suited for bleaching gel activation. These findings differ from other studies where the effect of an Nd:YAG was comparable with a halogen light [56], and the effect of KTP was better as compared to diode (980 nm) and blue LED (470 nm) [23], but these chromophores were chosen as a function of the wavelength used (Nd:YAG: Q-switch dye with maximum absorption at 1051 nm) (KTP: sulphorhodamine B with maximum absorption at 542.8 nm).

4.4.2. Clinical Efficacy. When the Nd:YAG laser (35% HP) was used for bleaching, Strobl et al. [57] found no supportive influence of the laser radiation on the bleaching. The authors registered a change in the colour of the bleach gel after laser activation, being a result of the increased formation of chemicals radicals, but could not explain why this does not translate in improved clinical result.

The use of a low-intensity red diode laser (660 nm, 35% HP) with a green-coloured bleach gel resulted in a change of colour (ΔE was increased from 5.4 to 7.2 after 1 week) [26].

Overall shade change values recorded by spectrophotometer reading expressed as ΔL, Δa, Δb, and ΔE were significantly higher for diode laser (810 nm, 37% HP) bleaching than PAC activation (400–490 nm, 35% HP), LED activation

(400–500 nm, 38% HP), and no light activation (38% HP), although shade guide evaluations did not exhibit any differences [58]. One session of 20 min of in-office bleaching with or LED (470 nm, 35% HP) (3 min irradiation to each group of 3 teeth) or diode laser (808 nm, 35% HP) (30 sec irradiation per tooth) as initiator followed by 10% CP home-bleaching during 7 days was not more effective than 10% CP home-bleaching alone during 14 days [59]. Bleaching with an 841 nm diode laser and 35% HP showed greater shade improvement for teeth with hue A shade than those with hue C and D. The bleaching process is better in younger patients and gender is not a factor that affects the bleaching process [46].

LED/laser at 470 nm (one wavelength mentioned) did not show any improvement in bleaching result for the treatment of vital teeth as compared to halogen light, LED, and non-light-activated 35% HP. All treatments resulted in an increase of ΔE (best score for the non-light-activated protocol), which was maintained for 1 month and then dropped at 6 months (from on average 8 at 1 month to 7.7 at 6 months for the light-activated systems and from 9.8 to 8.8 for the non-light-activated group) [60] but also meaning that there was only a slight colour rebound. LED/laser (470 nm/808 nm) did not improve the in-office bleaching results with 35% HP as compared to QTH (quartz-tungsten-halogen) light and at home bleaching with 10% CPO [50]. A change in colour was registered for all protocols, which was maintained over a 6-month period [50]. In another investigation LED/laser (450–500 nm/830 nm, 35% HP) did not improve the bleaching effectiveness during any phase of the study [27] as compared to two different LEDs (450–500 nm at 164 mW and 430–490 nm at 88 mW, with 35% HP) and a halogen lamp (350–500 nm at 470 mW, 35% HP) and additional sessions did not improve the results obtained in the first session. Change of color was registered for all systems. In a study by Mondelli et al. [47] in-office bleaching (35% and 38% HP) with and without activation with a LED/laser (470 nm/810 nm) was compared with home bleaching (15% CPO). All techniques and bleaching agents were effective. There was no difference in ΔE between non-light- and light-activated in-office treatment. The initial increase in ΔE decreased over a time period of 24 months (from on average 7.8 to 2 for the high concentration HP, from 9.8 to 3.3 for the home bleaching procedure with 15% CPO).

Visible green light KTP laser (532 nm, 35% HP) combined with sulphorhodamine B-photosensitizer bleaching gel activated for 30 sec at 1 W provided a clinically useful improvement in tooth shade in teeth with tetracycline discolorations [61, 62]. KTP was more efficient than a 810 nm diode laser for the removal of discolourations due to red fruits, tea, and coffee [63]. KTP is more efficient for tetracycline discolouration than a high powered green LED for the bleaching of tetracycline-stained dentine [64].

5. Discussion

Light sources are marketed with the idea that light plays a significant role in tooth bleaching as catalyst for the ionization of HP in the bleaching gel and increasing the bleaching effect. Studies on light sources with incoherent light sources have produced contradictory results, but the following conclusions were drawn on the basis of a systematic review: (1) both light-activated and non-light-activated systems showed similar immediate and short-term bleaching effects when high concentrations of HP (25–35%) were used as bleaching gel; (2) there is limited evidence that a light-activated system produced better immediate bleaching efficacy than when non-light-activated systems with a lower concentration of HP (15–20%) were used [9].

Two key factors determining overall tooth bleaching efficacy from peroxide containing gels are the concentration of the HP and the duration of application.

For as far as the specific topic of laser activated bleaching is concerned, contradictory results are found as was also seen with conventional bleaching procedure using high hydrogen peroxide concentrations. In addition, the number of laser activated bleaching studies is limited as compared to the literature on light-activated bleaching. Comparisons between the effects of different wavelengths are difficult to make: (1) for laser bleaching absorption in the bleaching gel is aimed to drive the ionization of the HP; this depends on the specific wavelength needed to directly photolyze or photooxidate the chromophores in the dentine; (2) the chosen wavelength has to coincide with the absorption peak of chromophores or photocatalysers in the bleaching gel (if present) in order to catalyse the ionisation of the hydrogen peroxide and to drive the photolysis; (3) there is the heterogeneity of the heating temperature of the gel, that is, the photothermal effect which is even so influenced not only by the wavelength, but also by the specific power settings; (4) because of the previously mentioned heterogeneity of the laser settings, seen when a specific laser wavelength is considered, the bleaching gel must be developed taking into account the specific laser wavelength; (5) in addition, power density or energy density (fluence) of the laser beam is important; temporal characteristics of the laser beam are to be considered such as continuous versus pulsed delivery and consequently the pulse rate and the pulse duration; other variables that relate to differences in the method of energy transfer such as contact versus noncontact delivery mode, focused versus unfocused, and beam diameter have also to be considered. Last but not least there are the differences in the exposure time of the gel to the laser light and the specific bleaching protocol (e.g., one exposure or consecutive exposures of a fresh bleaching gel do also contribute to the heterogeneous data).

In general, laser bleaching is performed with a hand piece or a fibre in noncontact mode, unfocused, and with continuous emission. Regarding the power or energy, high power lasers are generally used, except when bleaching is performed with the argon laser (488 or 514.5 nm) or with a 660, 675, or 740 nm diode laser.

All studies selected for this survey on laser bleaching have in common the fact that a high HP concentrated bleaching gel is used (35 to 38% HP and 35 or 37% CP, i.e., 12 to 13% HP). None of the clinical studies used low concentrations of HP. For HP concentrations of 6%, it is known that light activation produced better immediate bleaching effects [9]. The EU Council Directive 2011/84/EU of September 20, 2011 [65], restricts the use of bleaching and bleaching products:

only dentists may use products for tooth bleaching and only bleaching products that contain or release between 0.1% and 6% HP and products for tooth bleaching and bleaching that contain or release up to 0.1% HP are available as over-the-counter products. Products with HP concentrations over 6% are prohibited as cosmetics. This clearly means (1) that products containing or releasing more than 6% are prohibited for dental bleaching and (2) that dental bleaching is not considered as a medical action but only as cosmetical and hence nonhealing procedure. Information on laser activation of bleaching products up to 6% with lasers has not been published.

A number of wavelengths can be considered as not recommended for laser bleaching: Nd:YAG (1,064 nm), Er:YAG (2,940 nm), and CO_2 (10,600 nm). The effect of these laser wavelengths is purely based on heating of the bleaching gel (Nd:YAG) or should only be restricted to heating of the bleaching gel (Er:YAG and CO_2: care has to be taken not to remove tooth substance with Er:YAG and CO_2 because both wavelengths are well absorbed by water and hydroxylapatite which might result in superficial ablation of tooth substance). Although the CO_2-laser received an FDA approval for bleaching, the ADA soon after recommended not to use this wavelength for bleaching.

From all bleaching wavelengths the diode wavelengths have been most extensively investigated. A large range of diode wavelengths are used as laser bleaching wavelengths. These near-infrared lasers are used at low power or at high power. Both low power and high power diode lasers do not result in an enhanced bleaching efficacy when compared to non-light-activated bleaching with high HP concentrations. The question is even if low power diodes aid in the activation of the bleaching gel. Care, however, has to be taken with the high power diodes so as not to heat the bleaching gel at a level at which thermal damage of the pulp might occur.

Another key factor to increase the rate of the chemical reaction is to increase the temperature, where a rise of 10°C can double the reaction rate. On the one hand the thickness of the bleaching gel layer is important to ensure that the laser light can pass through this layer. The distance between the handpiece or fibre end and the gel is important when the energy is considered. Laser interaction is not limited to the gel alone and laser light has also to interact with the discolouration in the tooth.

Adding chromophores, chosen in accordance to the absorption peak of the gel, acts as a selective absorber near the dental surface, preventing light penetration into the internal tooth structure. The colour of the gel is important as it influences the final temperature, since different light sources have different emission wavelengths and the absorption peak changes following gel colour. Also here the question is if the dyes added for photoactivation of the gel with diode lasers are helpful in activating the bleaching gel. With high power diode lasers, irrespective of the thickness of the bleaching gel, care has to be taken still so as not to extensively dehydrate the enamel due to the temperature effect.

Recently LED devices were associated with diode lasers emitting in the near-infrared, which, with appropriate energy density, are being used to desensitize the teeth under bleaching [24, 66]. These studies demonstrated that near-infrared lasers could reduce the inflammatory response of the pulpal tissue, reducing pulp damage and relieving pain after the bleaching process. The use of these devices (so-called LED lasers), however, did not result in any increased bleaching efficacy. Thus the question is to what extent these low power diodes are of help in the bleaching process.

Light-activated systems were found to increase the occurrence of severity of tooth sensitivity [9]. The light source itself can increase pulpal temperature leading to increased tooth sensitivity [66]. The latter was also encountered with diode lasers [46, 48] and Nd:YAG [13, 14, 52]. For both wavelengths laser light is transmitted through the bleaching gel in combination with a heating of the gel, irrespective of the thickness of the gel leading to tooth sensitivity [10, 14, 16, 19]. An additional explanation is also that laser activated bleaching may increase the expression of substance P in the human dental pulp [58, 67].

Taking into account all different wavelengths used for laser activated bleaching, the KTP laser when used at appropriate settings and combined with the red coloured bleaching gels (Smart Bleach, SBI) has been shown to be one of the best options for photoactivated dental bleaching. Walsh [68] demonstrated a higher bleaching effect with KTP than with a diode laser based on DOTCAM analysis, a result which was also confirmed in other studies [19, 23, 69]. Its efficacy was also demonstrated for the bleaching of tetracycline discoloured teeth [61]. Temperature elevation in the pulp chamber was also under control when appropriate settings were used in conjunction with a red coloured gel (containing sulphorhodamine B as a chromophore) [19, 24]. The safety of the procedure was demonstrated by an unaltered enamel surface after KTP laser bleaching [25]; no significant differences in the enamel microhardness pre- and posttreatment [68] and no changes in the compositional structure of dentin surfaces were found [69]. Occasional mild postoperative sensitivity was seen during the 12 h following the procedure as radicals are neutralized by catalase and other pulpal enzymes [1, 68]. Catalase had been found to protect the dental pulp during vital bleaching procedures [70]. A catalase application was demonstrated to eliminate residual hydrogen peroxide during non-vital bleaching procedures [71].

6. Conclusions

(1) It is difficult to draw conclusions for laser bleaching on efficiency and efficacy from the present-day literature because of the difference in concentrations in hydrogen peroxide used, the difference in wavelengths of lasers (especially the diodes) used, the difference in laser settings and protocols used, and differences in bleaching gels used with or without photocatalyst.

(2) Comparative studies evaluating bleaching techniques with high concentrations of hydrogen peroxide and with or without the use of light activation resulted in enhanced lightening. Most often comparable results were found irrespective of light exposure.

(3) No long-term evaluations for laser enhanced bleaching procedures are available.

(4) Based on the limited number of investigations, at present, only one particular wavelength appears to be able to perform direct photobleaching (or photooxidation), that is, KTP (532 nm). When KTP is used in combination with a bleaching gel containing a chromophore (sulphorhodamine) allowing the absorption of the laser light, photodynamic reactions can be induced (photochemical activation of the gel with limited photothermal activation). This combination of wavelength and specifically dyed bleaching gel also allows for safe bleaching (no damage of the enamel, no heating of the pulp) when the guidelines of the manufacturer are followed.

(5) At present a number of wavelengths are not recommended for laser bleaching: Nd:YAG, Er:YAG, and CO_2. Combination devices consisting of LED-diode laser do not result in enhanced lightening and are in fact not effective. When using high power diode lasers for bleaching care has to be taken so as not to overheat the pulp. Also diode lasers are not really advocated for laser bleaching except when the wavelength is used in combination with a bleaching gel containing wavelength specific absorbers.

(6) With the exception of KTP used in combination with a gel with a specifically red coloured light absorber (sulphorhodamine B) for the green light (532 nm), laser activated bleaching is solely based on heating of the bleaching gel.

(7) All studies have been conducted with high concentrated hydrogen peroxide gels. This means that the soft tissues have to be thoroughly protected during the in-office power bleaching procedure. No studies were conducted to investigate the safety of laser bleaching procedures on the soft tissues adjacent to the laser activated bleaching gel.

7. Recommendations for Future Investigations

Three factors are to be considered when using a light source and should be mentioned in the studies: light intensity, spectral distribution, and irradiation time. Since the total energy depends on light intensity and irradiation time, light curing units with high intensity may allow a reduction in irradiation time. Second generation LEDs present higher power than first generation LEDs. Further research is needed to evaluate if high power (narrow band) LEDs can be used for light-activated bleaching. With the price of a number of laser devices in mind, this technology might be of interest for the activation of bleaching gels.

An important relationship exists among gel colour, laser wavelength, thermal transmission, and clinical efficacy, but not between gel temperature, shade change, and HP concentration. In this respect the use of absorbing substances to increase the radiation absorption (and the temperature in the gel) is known. With the use of TiO_2 it has been demonstrated that there is another way to improve dental bleaching without the risk of damaging the pulp. Hence the composition of the gel with the absorbers and additional compounds (agents enabling to catalyse the redox reaction) should also be given in detail.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Insight in the Chemistry of Laser-Activated Dental Bleaching

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The use of optical radiation for the activation of bleaching products has not yet been completely elucidated. Laser light is suggested to enhance the oxidizing effect of hydrogen peroxide. Different methods of enhancing hydrogen peroxide based bleaching are possible. They can be classified into six groups: alkaline pH environment, thermal enhancement and photothermal effect, photooxidation effect and direct photobleaching, photolysis effect and photodissociation, Fenton reaction and photocatalysis, and photodynamic effect.

1. Definition

The terms "whitening" and "bleaching" are often used interchangeably, which can lead to confusion when interpreting the literature. According to the US Food and Drug Administration (FDA), whitening restores teeth to their natural tooth colour, whereas bleaching makes teeth lighter than their natural colour. In other words, whitening refers to the removal or decolourizing of external stains on the surface of teeth, for example, by means of polishing agents in dentifrices, whereas bleaching is concerned with changing coloured substances within tooth structure (internal or intrinsic stains), for example, using reactive oxygen species (ROS).

2. Light Activated Bleaching

The first description of professional bleaching of discoloured teeth was provided by M'Quillen in 1867 [1]. This led in 1895 to the first commercial bleaching product, pyrozone, which was a mixture of five parts of 25% hydrogen peroxide (HP) and one part of diethyl ether [2].

Electromagnetic radiation sources were used for the first time to increase the effectiveness of bleaching in 1937 by Ames, who heated 35% HP [3]. The same approach has been used since the early 1980s, typically in the form of infrared lamps as a heat source to accelerate bleaching using concentrated HP. This approach was effective, but it was also associated with thermal stress, leading to pulp irritation. There were also difficulties in controlling caustic effects from the 35% HP liquid [4].

In 1989, dental bleaching was revolutionized with the advent of nightguard vital bleaching, a technique using a custom-made mouthguard worn at home with a low strength product such as 10% carbamide peroxide, allowing for sustained release of HP [5]. Dental bleaching was no longer reserved for the single discoloured tooth, as now multiple teeth could be whitened.

At the present time, the two most commonly used bleaching procedures are home-based bleaching methods

which rely on bleaching gels with low HP concentrations (either present as HP or released from carbamide peroxide) and in-office bleaching using high HP concentrations. In order to reduce the in-office bleaching time, various methods of accelerating the decomposition of HP are used, including chemical (e.g., alkaline pH), physicochemical (e.g., photooxidation), and physical (e.g., heat) techniques. The latter is often described by the term "power bleaching" [6, 7]. Coherent and incoherent radiation sources used to catalyse the bleaching process have included quartz tungsten halogen lamps, plasma arc lamps, mercury vapour lamps, light emitting diodes (LEDs), and lasers of various wavelengths [8–10]. Some proponents of "power bleaching" with intense light sources have claimed that adding various colourants to bleaching gels results in improved absorption of light in the gel and consequent reduced heating of the dental pulp. As well as warming the gel (photothermal effect), colourants can also trigger other photochemical reactions such as photodynamic reactions [11].

3. Laser Target Interactions

Laser light can have four possible interactions with the target tissue, depending on its optical properties [12, 13].

(1) *Absorption* of laser energy by the intended target: the amount of energy absorbed depends on characteristics such as pigmentation and the presence of light absorbing agents (i.e., chromophores), the laser wavelength used, and the laser emission mode. Light absorption can result in a range of photochemical processes, including photothermal effects (heat emission), fluorescence (light emission), photooxidation (photobleaching), or photodynamic effects.

(2) *Transmission* of laser energy through the target: this happens because the laser energy is not absorbed by the target but instead passes harmlessly through it. This effect is highly dependent on the wavelength of the laser used and the target tissue's optical characteristics.

(3) *Reflection* of the beam from the surface of the target, with no effect on the target: there are two patterns of reflection. *Specular reflection* is the perfect mirror-like reflection of light from a surface. This is in contrast to *diffuse reflection*, where incoming light is reflected in a broad range of directions. A familiar example of the distinction between specular and diffuse reflection is the finish of gloss paint versus matte paint. Whether the surface is microscopically rough or smooth in proportion to the laser wavelength used has a tremendous impact upon the pattern of reflection.

(4) *Scattering* spreads the light into the target across multiple directions, which weakens the intensity of the laser energy at any one point.

For as far as the process of bleaching is concerned, absorption of laser light in the bleaching gel is needed. HP is optically clear which means that its ability to absorb visible light wavelengths is extremely low; however it can absorb ultraviolet, middle infrared, and far infrared light, leading to its breakdown. Without the addition of a colouring agent, one cannot expect HP to absorb visible or near infrared laser light to any great extent. By choosing appropriate chromophores, a range of processes can be triggered. Absorbing energy will produce some localized heating, which increases the production of reactive oxygen species (ROS), in proportion to the elevation of temperature. Caution is needed so that the heat generated in the gel by the absorption of light does not cause general heating of the adjacent tooth to occur, since this would lead to thermal stress at the level of the dental pulp. By choosing different absorbing agents, other photochemical processes such as photodynamic effects can be achieved, leading to even greater levels of ROS. The absorption of light in the gel also reduces the amount of light that can pass through the tooth to reach the dental pulp and in the same light path both the enamel and the dentine [14].

The light absorbing properties of dentine, enamel, and dental pulp all differ from one another, with ultraviolet light and infrared light being well absorbed in the hard tissues, creating a risk of thermal stress to the dental pulp. The least absorbed of the various types of visible light is green light. This optical effect can be exploited in methods which use the effective transmission of visible green light through enamel to target complexes of tetracycline within tooth structure, without heating and/or damage to adjacent tooth structure. The effect of green light on tetracycline can be described as photooxidation or direct photobleaching. Such effects can provide an added value for laser assisted bleaching [14].

4. Mechanisms of Tooth Bleaching

Bleaching using ROS (such as HP or derived from HP) is a chemical process that occurs by oxidative decomposition of chromophores, that is, breakdown of these coloured light absorbing organic molecules. Photons (i.e., light particles) are absorbed by the target molecules (chromophores) because of their extended π-π conjugated system containing delocalized electrons. Conjugation is the overlap of one p-orbital with another across a sigma bond, typically by having alternating single and double chemical bonds. These overlapping p-orbitals allow for a delocalization of π-electrons across all the adjacent aligned p-orbitals. The π-electrons do not belong to a single bond or atom, but rather to a group of atoms, hence "delocalized." This leads to the concept of "molecular orbitals" (MOs). The energy difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) is the "band gap." This band gap is the amount of energy that is needed to bring a delocalized π-electron from HOMO (ground state) to LUMO (excited state). Light with the same energy (i.e., wavelength) as the band gap will be absorbed by the molecule and excite the π-electron. Altogether, the MOs that are formed by π-π conjugated systems lead the absorption spectrum of that molecule, which depends on the chemical structure [15]. Bleaching results from disruption of these π-π conjugated systems by oxidation (i.e., the loss of electrons) or other

chemical reactions. Typically, molecules with ring structures have these opened up into linear forms. This leads to loss of light absorbing properties and consequent discolouration of these molecules.

While HP is used widely in dental bleaching, it should be recognised that many other oxidizing agents (i.e., oxidants) exist which can cause bleaching reactions, with ozone being a common example. All oxidants have a different "bleaching potential" based on their redox potential (E°, given in Volts). The stronger this potential is, the more effective the agent will bleach chromophores [16–18]. Listed in increasing order, the values for redox potential are as follows: oxygen (O_2: +1.229 V); hydroperoxyl radical ($HO_2^\bullet$: +1.510 V); sodium hypochlorite (NaClO: +1.630 V); hydrogen peroxide (H_2O_2: +1.780 V); ozone (O_3: +2.075); and the hydroxyl radical ($HO^\bullet$: +2.800 V). Due to its high redox potential, hydroxyl radicals are the strongest ROS. Another very potent ROS is singlet oxygen ($^1O_2$); however this cannot be classified as a standard oxidizing ROS because it is able to decompose organic materials in many ways other than oxidation (e.g., *ene*-type and Diels-Alder reactions). Because of the very high reactivity of hydroxyl radicals and singlet oxygen, these cannot be stored and can only be formed *in situ* as required [16, 19–22].

In terms of the targets for oxidation, as already outlined, in discoloured teeth the coloured stains (chromophores) can be present above the enamel surface or inside the tooth structure itself. Stains on the surface can in most cases be removed easily by professional cleaning, for example, by a prophylaxis using a mild abrasive paste or fine flour of pumice. This is done as a clinical step immediately prior to an in-office bleaching procedure. As well as improving the appearance of teeth by increasing the spectral reflection of light, this external cleaning process removes both saliva and polyphenols (such as tannins) on the tooth surface which can inactivate ROS. Surface stains can also be decolourized, rather than removed, and this mechanism explains much of the cosmetic benefit gained by very low strength (0.5%) HP rinses, paint-on gels, and various oxygen releasing products such as denture cleansers.

For chromophores which reside inside tooth structure, a penetrating action is needed, in the form of either light which is transmitted internally and causes photooxidation (as discussed above for green light) and/or the production of ROS, leading to radicals which penetrate readily through the crystallized structure of enamel and dentine. When considering the selection of agents used for tooth bleaching, they should (i) be strong oxidizers (i.e., have a high redox potential), (ii) generate the types of ROS which are able to diffuse most readily into the dentine, (iii) be noncorrosive, and (iv) be nontoxic. Taking all this into consideration it has been found and repeatedly confirmed that ROS are both potent and safe tooth bleaching agents [18, 19, 23].

5. Bleaching with Lasers

The essential elements of laser light that determine its reaction with the target are the wavelength of the radiant energy (nm) emitted by the laser, the power density of the beam (measured in a square centimetre as the unit area—W/cm^2), and the temporal characteristics of the beam energy, such as continuous versus pulsed delivery, pulse rate (Hz), and pulse duration. With a pulsed laser, it is more practical to talk about the amount of energy per pulse in Joules (1 J = 1 W/s), rather than the average output power in watts. Other useful measures are energy density (J/cm^2) and the amount of energy per unit area (fluence). In addition to these factors, there are several other variables that relate to differences in how the laser energy is delivered, such as contact versus noncontact delivery mode, focused versus unfocused beams, and beam diameter.

Next to the wavelength of the laser, the mechanism of action of the laser is affected greatly by the power of the radiation delivered and the mode of operation. The type of mode influences the dynamics of heating of the target; that is, accumulated heating with continuous wave mode is higher than that with pulsed mode. At low irradiances and/or energies, laser-tissue interactions are either purely optical or a combination of optical effects, photochemical effects, and photobiostimulation. When laser power or pulse energy is increased, photothermal interactions begin to dominate. Pulsed lasers can create very high power densities within a very short time, leading to photoablation [24–26]. Such effects are not desirable in a bleaching gel.

Combining these various points, when choosing a laser wavelength to enhance bleaching efficacy it is of the utmost importance to consider the extent to which light "absorption" (which is both wavelength and target dependant) is needed and when it occurs how much of the laser energy will be converted into heat. The absorption of photons will influence the temperature rise which occurs within the bleaching product, the dental hard tissues, and/or pulp tissues. There must be a careful alignment between the laser and the characteristics of the gel, that is, the presence of additives which affect the absorption spectrum and colour. The thickness of the gel and its pH also have to be taken into account; the latter because the pH influences the patterns of radicals which are generated.

6. Enhancing Hydrogen Peroxide Based Bleaching

In general, lasers can enhance bleaching by photooxidation of coloured molecules in the teeth or by interaction with components of the bleaching gel, through photochemical reactions. Such effects will be affected by pH, temperature, light, and the presence of catalysts. The different methods of enhancing hydrogen peroxide based bleaching can be classified into six groups.

6.1. Alkaline pH Environment. Hydrogen peroxide is always stored at low/acidic pH. At pH values above 7 (alkaline), HP becomes much more reactive and has a very short shelf life. This is due to the loss of a hydrogen atom (H^+), yielding hydroperoxyl anion (HO_2^-), not to be confused with the hydroperoxyl radical ($HO_2^\bullet$). In this state not only does HP

become a safer tooth bleaching product (since acidic products demineralise and damage enamel), but also it will enhance the bleaching efficiency because of the higher reactivity of hydroperoxyl anions. This higher reactivity can be understood by (i) closer association of the anion with positively charged chromophores and (ii) additional reactions (e.g., Dakin reaction and addition to quinone) whereby the anion can disrupt chromophores. HP is most effective for bleaching at pH values between 9.5 and 10.8. Because of its inherent instability at elevated pH, HP is typically mixed with an alkalinizing agent immediately prior to its application in the mouth, in order to obtain this type of bleaching enhancement [16, 20, 27].

6.2. Photochemistry. When a molecule is irradiated by photons whose energies just correspond to the difference in energy between the ground state and some higher or excited state of the molecule, the photons are absorbed and the molecule is raised to a higher energy level. Spectra arise when molecules absorb photons of specific energy. When a molecule absorbs light/photons, it will enter a singlet excited state (electron in a higher energy state). The molecule can take up more light/photons, forcing the molecule in a triplet excited state (change in electron spin of the excited electron by intersystem crossing). The excess of energy is lost by heat emission (vibration) or by photon emission (fluorescence for singlet excited molecules and phosphorescence for triplet excited molecules). Furthermore, when in singlet or triplet excited state some molecules are sensitive to certain chemical reactions (disruptions or creation of certain chemical bonds, both intra- and intermolecular) that were not possible in their ground state. All this together is the topic of photochemistry. The following photochemical reactions can be beneficial for enhancement of hydrogen peroxide based bleaching [28, 29].

6.2.1. Thermal Enhancement and Photothermal Effect. Electric heating devices, special designed lamps, and lasers can be used to heat the bleaching gel. This will enhance the bleaching efficiency simply because chemical reactions happen faster at elevated temperature. A 10°C temperature rise can speed hydrogen peroxide decomposition by a factor of 2.2 [26]. Additionally, an increase in heat will allow better peroxide penetration into dental structures [30]. Lamps can produce heat because of inefficient conversion of electric current to heat (e.g., tungsten wires in lamps produce heat). A more direct approach is when "cold" lamps and lasers induce a temperature rise in the target substrate by the photothermal effect. Light will be absorbed by molecules inside the bleaching gel or tooth structures, leading to molecular vibration and consequent heating [30]. The condition however is that the light is absorbed by the molecules, which depends on the absorption spectrum of the molecule and the emission spectrum of the lamp or laser. Problematic with this type of enhancement is that heat can lead to dehydration of the enamel and to irreversible damage of the pulp [20, 26, 31].

6.2.2. Photooxidation Effect and Direct Photobleaching. When certain molecules enter their triplet excited state, they are prone to lose an electron; that is, they become oxidized. This process is called photooxidation. When this happens to chromophores, their π-π conjugated systems are disrupted and they are consequently bleached, that is, direct photobleaching. In case of tooth bleaching the light has to be able to penetrate the tooth structures (transmission). UV has very good photobleaching properties but does not penetrate well through teeth and causes soft tissue damage, burns, and pulp heating. Green light is optimal for photobleaching since it is not absorbed by water or hydroxylapatite. It will thus nicely penetrate the tooth structure where it can remove chromophores that absorb green light. Because of the high light density, laser light is more efficient in photobleaching than nonlaser light [26, 29, 32–35].

6.2.3. Photolysis Effect. The process of photolysis or photodissociation is one where light is absorbed by a molecule trigging the disruption of a chemical bond and the breakdown of that molecule. In this regard, HP can absorb UV light causing it to break up into two hydroxyl radicals ($HO^{\bullet}$). Because $HO^{\bullet}$ is a much more potent bleaching agent, this will enhance the bleaching efficiency. However, as mentioned before, caution has to be taken when using UV light [29, 36, 37].

6.2.4. Fenton Reaction and Photocatalysis. Iron ions ($Fe^{2+/3+}$) can catalyze the production of hydroxyl radicals ($HO^{\bullet}$) and hydroperoxyl radicals ($HO_2^{\bullet}$) out of HP by continuous oxidation and reduction. This reaction is well known as the Fenton reaction. The formed $HO^{\bullet}$ will boost the tooth bleaching. The downside however is that equimolar amounts of $HO_2^{\bullet}$ (weaker than HP) are also formed and that the breakdown of $HO^{\bullet}$ is also catalyzed by the iron ions. Photocatalysis can circumvent this problem. Fe^{3+} activated with UV light will catalyze the formation of more $HO^{\bullet}$ out of water. This process is called the photo-Fenton reaction. Of course, in this case $HO^{\bullet}$ will also be formed due to photolysis of HP. Again, using UV light is not advisable in oral applications [29, 36, 37].

6.2.5. Photodynamic Effect. This technique is based on the photodynamic therapy (PDT) where photosensitive dyes are excited by light. In their triplet excited state, they can donate highly energetic electrons and reduce O_2 and H_2O_2 forming H_2O_2, $HO_2^{\bullet}$ and $HO^{\bullet}$. Furthermore, during intersystem crossing (from singlet to triplet excitement) the dyes will form singlet oxygen ($^1O_2$) by spin-orbit coupling with triplet oxygen ($^3O_2$), the common form of molecular oxygen. Altogether, photodynamic bleaching enhancement enables the formation of the two most potent bleaching agents, that is, hydroxyl radical and singlet oxygen, and is thus a very powerful enhancement [16, 21, 22, 29, 32, 38].

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

CO_2 Laser and Topical Fluoride Therapy in the Control of Caries Lesions on Demineralized Primary Enamel

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This study evaluated the effect of CO_2 laser irradiation and topical fluoride therapy in the control of caries progression on primary teeth enamel. 30 fragments ($3 \times 3 \times 2$ mm) from primary canines were submitted to an initial cariogenic challenge that consisted of immersion on demineralizing solution for 3 hours and remineralizing solution for 21 hours for 5 days. Fragments were randomly assigned into three groups ($n = 10$): L: CO_2 laser ($\lambda = 10.6\ \mu$m), APF: 1.23% acidulated phosphate fluoride, and C: no treatment (control). CO_2 laser was applied with 0.5 W power and 0.44 J/cm^2 energy density. Fluoride application was performed with 0.1 g for 1 minute. Cariogenic challenge was conducted for 5 days following protocol previously described. Subsurface Knoop microhardness was measured at 30 μm from the edge. Obtained data were subjected to analysis the variance (ANOVA) and Duncan test with significance of 5%. It was found that the L group showed greater control of deciduous enamel demineralization and were similar to those of APF group, while being statistically different from C group ($P \leq 0.05$) that showed the lowest microhardness values. It was concluded that CO_2 laser can be an additional resource in caries control progression on primary teeth enamel.

1. Introduction

Application of fluoride compounds has been used to control dental caries in primary teeth under different forms [1, 2] and different concentrations [3]. The mechanism of fluoride interferes in the process of mineral loss, promoting inhibition of demineralization, and enhancement dental substrate remineralization [4]. The ability of acidulated phosphate fluoride (APF) to become the primary teeth and more acid-resistant when exposed to cariogenic challenge was evidenced by Castellan et al. 2007 [1]. However, for an effective fluoride action controlling demineralization, it must be constantly in the oral cavity [5].

Higher incidence of dental caries in primary teeth associated with rapid progression of these lesions due to lower mineral content [6] leads to early loss of these teeth [7], factors that encourage more studies to improve existing preventive treatments and to evaluate innovative techniques such as CO_2 laser irradiation [8, 9].

CO_2 laser irradiation is more appropriate to dental enamel because it produces radiation in the infrared region (9.3, 9.6, 10.3, and 10.6 μm) that coincides closely with some of apatite absorption bands, mainly phosphate and carbonate group absorption [10]. Therefore, higher effectiveness in caries prevention could be achieved with lower occurrence of harmful effects to dental tissues [10]. Using this laser, energy is absorbed in few micrometers of the external enamel surface and converted into heat, causing loss of carbonate from mineral and fusion of hydroxyapatite crystals, reducing the interprismatic spaces [11]. Furthermore, it increases its acid resistance, decreasing the mineral reactivity and promoting caries-preventive effect [9].

The CO_2 laser may control caries progression in permanent [12] and bovine enamel [13] when compared to fluoride

compounds [14]. The efficacy of this laser in caries control on demineralized primary enamel was also previously evaluated by Tagliaferro et al. 2006 [8] and da Silva Tagliaferro et al. 2009 [9]. However, in these studies, laser was applied on sound enamel. There are no studies in the literature evaluating the effect of CO_2 laser in previously demineralized primary enamel, simulating a patient with high cariogenic challenge and high caries risk.

As creation of an acid-resistant surface seems to be a promise in the control of caries lesions, the aim of this study was to evaluate *in vitro* the effect of CO_2 laser irradiation and topical fluoride therapy in control of caries progression on enamel of primary teeth by subsurface microhardness analysis.

2. Material and Methods

2.1. Experimental Design. The factor under investigation was surface treatment at 3 levels: L: CO_2 laser irradiation; APF: 1.23% acidulated phosphate fluoride; C: no treatment (control). The sample consisted of 30 fragments of human primary enamel distributed among three surface treatments ($n = 10$), according to a randomized and complete block design. The quantitative response variable was the subsurface Knoop microhardness (KHN) of the substrate subjected to the chemical demineralization *in vitro*.

2.2. Ethical Aspects. This research was approved by the Ethics in Research Committee of the School of Dentistry of Ribeirão Preto, University of São Paulo (Process number 2010.1.1373.58.9). Freshly extracted sound primary canines were obtained from Human Tooth Bank of the same institution.

2.3. Selection and Preparation of Samples. Primary teeth were hand scaled and cleaned with water/pumice slurry, in rotating bristle brushes at low speed (N270, Dabi Atlante, Ribeirão Preto, SP, Brazil) to remove calculus and surface-adhered debris and stored in 0.1% thymol solution. The absence of cracks, hypomineralization, and hypoplasia was confirmed under an ×20 magnifier (Leica S6 D Stereozoom, Mycrosystems Leica AG, Switzerland) and teeth with structural defects were discarded. Afterwards, the selected teeth were sectioned in the cement-enamel junction in precision cutter water-cooled (Isomet 1000, Buehler, Lake Bluff, IL, USA), to separate the root and coronal portions. The buccal surface of each tooth was sectioned to obtain a fragment of enamel measuring 3 × 3 × 2 mm.

The fragments were fixed in acrylic resin blocks using melted wax (Wax Sculpture Fixed Prosthodontics, Aspheric Chemical Industry Ltda., São Caetano do Sul, SP, Brazil) with the subsurfaces facing the external environment. The subsurfaces were then flattened with #1200-grift silicon carbide paper in a water-cooled polishing machine (Politriz, DP-9U2, Struers A/S, Copenhagen, Denmark) (Hermes Abrasives Ltd., VA, USA) and polished with 0.3 μm alumina paste (Arotec S/A Ind. Com, SP, Brazil) by felt polisher (ATM, Altenkirchen, Germany) [15]. In order to obtain a sample of patterned fragments, three readings were performed on the side of the fragments (subsurface) 30 μm from the edge and 100 μm of each other through a microhardness tester HMV-2000 (Shimadzu Corporation, Kyoto, Japan) with a diamond indenter for Knoop hardness (KHN) under 25 g load for 5 seconds [11]. The three readings were averaged and used as the microhardness value of each fragment. Specimens with microhardness values 20% above or below the mean value of all fragments were discarded [16]. Thirty fragments of primary enamel were selected based on initials Knoop hardness values of its fragments lateral side.

2.4. Initial Cariogenic Challenge. For obtaining initial microscopic lesions of standardized white spot lesion, simulating patients with high caries activity, an artificial caries challenge was performed in all fragments. The specimens were repositioned with the buccal surface facing the external environment in resin blocks and fixed with wax. All surfaces except the buccal were covered with melted wax and stored individually in plastic containers. The initial cariogenic challenge was performed during 5 days according to the protocol proposed by Argenta et al. 2003 [17]. Artificial caries lesions were produced by immersion of the fragments in demineralizing solution (pH 4.6) for 3 hours and remineralizing solution (pH 7.0) for 21 hours at 37°C. After the artificial carious lesions formation, the specimens were kept in humidity for 2 days at 4°C.

2.5. Surface Treatment. According to a complete block design and randomized, the specimens were divided according to treatment in three groups ($n = 10$): L: CO_2 laser, APF: 1.23% acidulated phosphate fluoride, and C: no treatment (control).

The CO_2 laser with $\lambda = 10.6\,\mu$m (PC 015-D CO_2 Laser System, Shanghai JueHua Laser Tech. Development Co., Ltd., Shanghai, China) was applied in ultrapulsed mode, 0.5 W average power, 0.44 J/cm^2 energy density measured with Power Meter (FieldMax II-TOP, Coherent Inc., Santa Clara, USA), 100 μs pulse duration, 0.001 sec interval between pulses, 0.4 mm beam diameter on the substrate surface, where the operator kept the laser tip perpendicularly to the substrate with distance tip/substrate of 4 mm [18] for 20 sec. Parameters used in the present study were able to produce only chemical and structural modification on primary enamel, without causing surface damage or tissue removal. After irradiation, the samples were kept in artificial saliva at 37°C for 24 hours. These were the components of artificial saliva, the reagent (213 mg of $CaCl_2{\cdot}H_2O$, 738 mg of KH_2PO_4, 1.114 mg of KCl, 381 mg of NaCl, 12 g of Tris, 2.2 g of gastric mucin, and qsp 1 liter) weighed on an analytical balance (AB204-S/FACT, Mettler Toledo, Columbus, OH, USA) and subjected to agitation, adjusting the pH to 7.0.

0.1 g of 1.23% acidulated phosphate fluoride gel (DFL Industry, Rio de Janeiro, RJ, Brazil, pH 3.6) was weighed on analytical balance (AUW220D, SPLABOR, Presidente Prudente, SP, Brazil) and applied to the dry surface deciduous enamel using microbrush (KG Sorensen, Cotia, SP, Brazil). After 1 minute [19], the specimens were washed with deionized water for 10 seconds, dried with absorbent paper, and after stored in artificial saliva at 37°C for 24 hours.

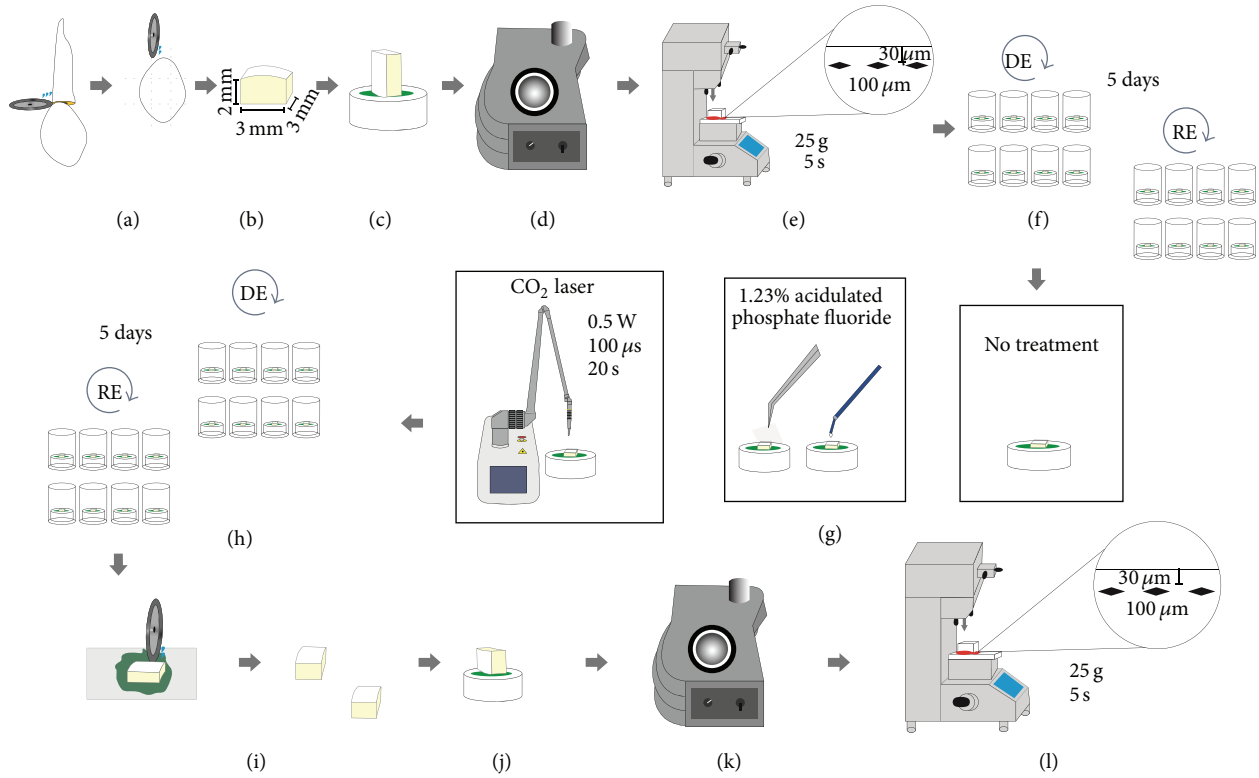

Figure 1: Schematic design of the methodology presented. (a) Section of the teeth. (b) Obtaining fragments. (c) Fixation of specimens in resin blocks. (d) Planning and polishing the enamel surface. (e) Selection of specimens. (f) Initial cariogenic challenge. (g) Surface treatments. (h) Cariogenic challenge after surface treatment. (i) Section of the fragments. (j) Fixing the fragments into blocks of acrylic resin. (k) Polishing the enamel surface. (l) Microhardness evaluation.

The control group did not receive any treatment, being kept in artificial saliva at 37°C for 24 hours.

2.6. Cariogenic Challenge Postsuperficial Treatment. The samples were replaced in plastic containers and all surfaces, except for the treated surface, and were covered with melted wax. The same pH cycling that was applied before the laser or the fluoride treatment was repeated 5 times, at a rhythm of one per day, in order to simulate the conditions of cariogenic severe challenge.

2.7. Microhardness Test. After cariogenic challenge period, specimens were sectioned longitudinally and fixed with melted wax and their internal side (sectional) was left exposed and polished in a polishing machine (DP-9U2; Struers S/A, Copenhagen, Denmark). After polishing, specimens were observed under an optical microscope to verify the superficial smoothness and were subjected to ultrasonic cleaning (Dabi Atlante, Ribeirão Preto, SP, Brazil) for two minutes to remove the debris. Then, impressions were made in one of the hemisections, keeping the long axis of the diamond indenter parallel to the external surface of the enamel using a static load of 25 g for 5 sec [1]. Three measurements were performed at the center of the fragment, with 100 μm in distance from one another, 30 μm from the edge, totalizing 3 indentations per specimen. The readings were averaged and used as the microhardness value of each slab, using a microhardness tester HMV-2000 (Shimadzu Corporation, Kyoto, Japan).

The protocol used in this study is shown in Figure 1.

2.8. Statistical Analysis. The mean values of microhardness of each specimen were analyzed and showed a normal distribution and homogeneity of variance. Thus, analysis of variance (ANOVA) was employed. The Duncan test was used to investigate differences between the mean of surface treatment factor using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA) with a significant level of 5%.

3. Results

The results showed that microhardness of subsurface treatments performed on primary teeth enamel was statistically different ($P \leq 0.05$), as shown in Table 1.

Duncan test showed that surface treatment with CO_2 laser showed the highest microhardness values (KNH) on primary teeth enamel, but it was not statistically different from 1.23% acidulated phosphate fluoride application. However, a statistically significant difference from the control group that presented the lowest microhardness values was found.

Table 1: Microhardness values (mean and standard deviation) according to the superficial treatments in different experimental groups ($P = 0.03$).

Treatment	Mean	Standard deviation
CO_2 laser	324.99[a]	33.78
1.23% acidulated phosphate fluoride	309.30[a]	68.42
No treatment (control)	209.86[b]	67.03

Similar letters indicate statistical similarity.

4. Discussion

Acidulated phosphate fluoride [1, 2] and CO_2 laser radiation [8, 9] have been used to prevent caries in primary teeth in order to interfere the balance of deremineralization. The effects of laser irradiation on the tissue are closely related to wavelength, absorption of laser light by the irradiated tissue, laser power, emission mode, energy density, and frequency [20, 21].

CO_2 laser is responsible for increasing acid resistance on irradiated enamel [1, 8, 9]. On the other hand, fluoride is able to incorporate on dental substrate, preventing the development of carious lesions, inhibiting enamel demineralization, and enhancing remineralization through minerals gain [3].

In this study, surface treatment with CO_2 laser in primary enamel was statistically similar to 1.23% acidulated phosphate fluoride. The probable reason for the increased acid resistance of the primary enamel after CO_2 laser treatment is a consequence of thermal effect [22, 23]. Heating of tooth surface results in structural and chemical alterations in the irradiated dental substrates with melting point of hydroxyapatite [24], regarding calcium [25, 26] and phosphorus loss [27], calcium and phosphorus concentration on the surfaces [28], and alterations in organic matrix [29].

Thermal variations produced by using the CO_2 laser on enamel promote reduction of water and carbonate content [22] which is converted into phosphate followed by protein decomposition at temperatures of 100–650°C, thermal recrystallization (650 and 1.100°C), and destructive phenomena such as melting of hydroxyapatite (>1.100°C) [30]. CO_2 laser may decrease dental permeability and hinder diffusion of acids, due to the surface sealing [31], reducing the demineralization of dental structure [10]. The enamel irradiated using high energy densities revealed nonhydroxyapatite phases, apparently similar to tri- and tetracalcium phosphates [32].

The thermal effects are responsible for changes in the irradiated tooth surfaces while they may differ from the temperature observed at pulp chamber, due to the support structures present around the teeth and the blood flow of the pulp tissue; this heat could be dissipated [33, 34]. The pulp temperature increase, related to the use of high power lasers, is based on the amount of energy applied and therefore, the exposure time is fundamental. High energy densities in short periods of time cause less pulp damage [35], since the thermal relaxation is inversely proportional to the square of the irradiated volume [33].

The low thermal conductivity of the enamel and the rapid decrease in temperature in the lower layer of spent glaze can also contribute to the lack of pulp damage, due to high absorption of this substrate by the appropriate wavelength of 10.6 μm CO_2 laser [36]. The low energy density, used in this study, promoted thermal relaxation time of the deciduous enamel ranging between 1 and 60 μs, and the pulse duration of the laser CO_2 was 100 μs. Esteves-Oliveira et al. 2009 [37] using energy density 0.3 J/cm^2, similar to this study, were able to decrease enamel caries progression without causing surface and subsurface thermal damage.

CO_2 laser action on primary and permanent enamel can be distinct, due to the differences between these substrates. The mineralization, calcium, and phosphorus percentage is lower in primary teeth than in permanent teeth [6]. The thickness of primary enamel is almost half of the permanent enamel that may have an influence on the demineralization [6] and may provide greater temperature rise when compared to permanent teeth, since thicker structures of enamel and dentin promote smaller temperature change [35, 38–41].

Carbonate content reduction on permanent enamel, promoted by CO_2 laser irradiation [11], results in lower hydroxyapatite solubility. The increasing in crystals size [42], melting [23, 42], and fusion [11] of irradiated enamel have also reduced the enamel dissolution on permanent teeth against acid challenge, although melting of enamel tissues is not a necessity for laser radiation to inhibit caries formation in enamel [24]. In primary teeth, CO_2 laser is also able to reduce carbonate content of enamel [9], which may have led to increased resistance to demineralization in this study.

It has been reported that, after a professional fluoride application, calcium fluoride (CaF_2) is formed on enamel surface and fluoride is released to fluid phase. This effect promotes a consequent reduction of enamel demineralization. Also, a dose-response effect is observed between the concentration of CaF_2, reservoirs on enamel and fluoride released, to "plaque fluid" and the subsequent inhibition of enamel demineralization [5]. The findings of this study have shown that topical application of APF in primary teeth is effective in the demineralization process and caries control [1, 2].

The amount of fluoride formed in the enamel depends on the concentration and the pH of the product applied and how long it remains in contact with the enamel [19]. Tenuta et al. 2008 [5] stated that the constant presence of fluoride in the oral cavity is more important than its concentration for the final enamel absorption. Thus, topical application of more acidic and concentrated fluoride compounds could provide effective protection against demineralization of tooth enamel or caries lesion formation [43], with higher incorporation of fluoride on enamel [19], however, no difference in fluoride uptake by enamel [19] was observed when fluoride was applied by one minute compared to four minutes.

In the present study, as CO_2 laser was applied on previously demineralized primary enamel simulating a patient with high cariogenic challenge and high caries risk, it is difficult to make a direct comparison with these results to previous literary studies. Until now, there is no research that performed previously cariogenic challenge on primary

teeth enamel, targeting the demineralization controlling and not preventing demineralization, having sound as substrate. Besides, the higher the demineralization is, the more difficult caries control becomes.

5. Conclusion

CO_2 laser with $\lambda = 10.6\,\mu$m was effective in the control of demineralization on previously demineralized primary enamel, presenting some advantages on being a quick, comfortable, and simple method of applying, especially in children, considering the difficulty of using a fluoride. In this way, CO_2 laser can be a resource in the control of caries lesions progression on primary teeth enamel.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Evaluation of Marginal Integrity of Four Bulk-Fill Dental Composite Materials: *In Vitro* Study

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Objective. The aim of the study was to compare under *in vitro* conditions marginal sealing of 4 different bulk-fill materials composite restorations of class II. *Methods*. Comparative evaluation concerned 4 composites of a bulk-fill type: SonicFill, Tetric EvoCeram Bulk Fill, Filtek Bulk Fill, and SDR. The study used 30 third molars without caries. In each tooth 4 cavities of class II were prepared. The prepared tooth samples were placed in a 1% methylene blue solution for 24 h, and after that in each restoration the depth of dye penetration along the side walls was evaluated. *Results*. The highest rating (score 0, no dye penetration) was achieved by 93.33% of the restorations made of the SDR material, 90% of restorations of SonicFill system, 86.66% of restorations of the composite Filtek Bulk Fill, and 73.33% of restorations of the Tetric EvoCeram Bulk Fill. *Conclusion*. The performed study showed that bulk-fill flowable or sonic-activated flowable composite restorations have better marginal sealing (lack of discoloration) in comparison with bulk-fill paste-like composite.

1. Introduction

The most essential factors determining preservation of restoration placed in a cavity are the marginal seal and absence of leakage [1, 2]. A marginal microleakage first defined by Kidd in 1976 is a process consisting in clinically undetectable penetration of bacteria, their metabolites, enzymes, toxins, ions, and other cariogenic factors between the filling and the cavity wall [3, 4]. Clinical consequences of microleakage are secondary caries, pulp inflammation, marginal discoloration, postoperative sensitivity, and the reduction of longevity of filling [5, 6]. It is believed that the existing occlusive load of the oral cavity and the thermal changes favor the formation of a marginal gap at the contact surface between the tooth and material [6, 7]. Rising expectations of patients regarding the aesthetics of fillings have recently made the composite resins the most commonly used nowadays restorative materials of lost tooth tissues. This applies to aesthetic dental restorations not only in the anterior teeth but also in the posterior teeth, so that in many countries composites do have almost totally replaced amalgam as restorative in posterior teeth [8]. Dentists expect from modern technology a composite material with high aesthetic value, less polymerization shrinkage, perfect marginal integrity, and relevant physicomechanical properties. If the material provides ease and short time of placement these are extremely desirable characteristics [7, 9, 10] but significant advances in composite technologies are not so frequent.

Embedding a composite restoration in posterior teeth is generally a time-consuming activity. The techniques of layers and thin 2 mm polymerization increments of the composites are widely recommended [11–13]. When extensive cavities are filled in posterior teeth, such a treatment can imply the risk of incorporating air bubbles or contaminants between the increments [14]. Manufacturers of composite materials, with a view to simplify the procedure of introducing the material into the cavity and its polymerization, now offer bulk-fill type composite resins. Simplification of procedures and shortening the time of embedding bulk-fill type restorations are due to possibility of applying a single up to 4 mm composite increment and it makes the work quicker by reducing the number of clinical steps. Thanks to high color translucency of these materials it is possible for the light to reach deeper but if the cavity is deeper than the maximum depth of cure 4 mm,

it is necessary to apply another layer. The innovative system of polymerization initiation determines shortening of light-curing time and increasing the depth of cure. Low shrinkage of these materials and high filler content cause shrinkage stresses to be very low and this allows for application of thicker layers. The time of color matching process is shorter because of universal color of materials and shorter time of finishing and polishing of the restoration was noticed [6, 12]. Nevertheless an ideal bulk-fill composite would be one that could be placed into a preparation having a high C-factor design and still exhibited very little polymerization shrinkage stress, while maintaining a high degree of cure throughout [15].

The newly developed bulk-fill resins offer composites including low-viscosity (flowable) and high-viscosity (sculptable) material types. SDR (Smart Dentin Replacement) Posterior Bulk Fill Flowable Base is a single component, fluoride containing, and visibly light cured radiopaque resin composite restorative material. The composition is as follows: barium aluminofluoroborosilicate glass, strontium aluminofluorosilicate glass, modified urethane dimethacrylate resin, ethoxylated bisphenol A dimethacrylate (EBPADMA), triethylene glycol dimethacrylate (TEGDMA), camphorquinone photoinitiator, butylated hydroxytoluene (BHT), UV stabilizer, titanium dioxide, and iron oxide pigments. It has handling characteristics typical of a flowable composite but can be placed in 4 mm increments with minimal polymerization stress. SDR has a self-leveling feature that allows intimate adaptation to the prepared cavity walls. Available in one universal shade, it is designed to be overlaid with a methacrylate based universal/posterior composite for replacing missing occlusal/facial enamel. SonicFill system consists of a KaVo tip providing sonic application of a bulk-fill type composite by Kerr. Shrinkage stress compensation mechanism in SonicFill system was obtained using a resin having low shrinkage properties and high around 84% filler content. Other components are glass, oxide, chemicals (10–30%), 3-trimethoxysilylpropyl methacrylate (10–30%), silicon dioxide (5–10%), ethoxylated bisphenol A dimethacrylate (1–5%), bisphenol A bis(2-hydroxy-3-methacryloxypropyl) ether (1–5%), and triethylene glycol dimethacrylate (1–5%). Tetric EvoCeram Bulk Fill (Ivoclar Vivadent) is a nanohybrid composite with a monomer matrix containing dimethacrylates (20-21% weight). The fillers contain barium glass, ytterbium trifluoride, mixed oxide, and prepolymer (78%–81% by weight). Additional contents are additives, catalysts, stabilizers, and pigments (<1.0% weight). The total content of inorganic fillers is 76-77% weight or 53-54% volume. The particle size of the inorganic fillers is between 40 nm and 3,000 nm with a mean particle size of 550 nm. Tetric EvoCeram Bulk Fill contains in its composition an inhibitor of sensitivity to light and thus provides prolonged time for modeling of filling, an inhibitor of shrinkage stress in order to achieve optimal marginal seal, and Ivocerin, polymerization photoinitiator allowing curing of 4 mm layers of material. Filtek Bulk Fill (3M ESPE), a low-viscosity, visible-light activated flowable material for filling with bulk-fill technique, is manufactured in four shades (each of which may be polymerized in 4 mm increments according to international ISO standards) and two kinds of packaging, capsules and syringes. It contains Bis-GMA, UDMA, Bis-EMA, and Procrylat resins. Fillers are a combination of zirconia and silica having a particle size of 0.01–4.5 microns and ytterbium trifluoride filler having a particle size of 0.1–5.0 microns. The inorganic filler loading is approximately 64.5% by weight (42.5% by volume), Table 1.

A clinical evaluation of the new bulk-filling technique is important to observe the anatomical shape and marginal adaptation and margins discoloration. The occurrence of annual failure rates is also meaningful. Amongst many parameters defining the quality of materials that restore lost tooth tissues, marginal integrity seems to take part as the most important. During *in vitro* studies, various methods are used to detect the presence and assess the microleakage between the tooth tissues and filling material. Although a perfect marginal seal is not achievable clinically, a good marginal quality should be the main aim for clinicians. Marginal integrity has been evaluated using high magnification and penetrating dyes to reveal marginal gaps, both externally and internally [15]. The method is fast and easy to perform, which validates the choice of this method in studies [16]. The range of dye penetrations was assessed differently in millimeters or depending on cavity/tooth anatomy. The criteria are different and can be as follows: crossing dentin-enamel junction, width of the wall, width of the enamel/dentin layer, and number of walls penetrated by dye [17–19].

The aim of the study was to compare under *in vitro* conditions marginal sealing of composite restorations of class II cavities made of 4 different bulk-fill materials. The null hypothesis tested is that bulk-fill flowable composite resins do not lead to better marginal seal in comparison with bulk-fill paste-like composites.

2. Materials and Methods

2.1. Sample Preparations. In total 30 sound third molars, with neither carious lesions nor restorations, recently extracted for orthodontic reasons with the written agreement of every patient were selected for this *in vitro* study. After extraction, the teeth were cleaned from the remaining connective tissue and debris. Then, the teeth were rinsed with distilled water and stored at room temperature.

Using a calibrated diamond bur under air-water cooling high speed handpiece, an experienced operator prepared in every tooth 4 cavities of class II to a depth of 4 mm (measured along the lateral wall), a width of 2 mm (pulpal wall), and length of 3 mm (approximal wall) (Figure 1). The margins of the cavities were finished with fine diamond bur.

2.2. Restorative Procedures. For all samples the adhesive used was an etch-and-rinse system, applied following the manufacturer's instructions. All cavities were etched with total etch technique for 30 s, using 37% phosphoric acid, and rinsed with water. Then, the adhesive system was applied for all samples according to the restoration material used and polymerized. In every tooth 4 restorations of different bulk-fill materials were placed: SonicFill (Kerr and KaVo), Tetric EvoCeram Bulk Fill (Ivoclar Vivadent), Filtek Bulk Fill

Table 1: Bulk-fill composite materials used in the study.

Resin composite	Type	Manufacturer	Bonding agent	Maximum increment thickness recommended by manufacturer	Composition according to manufacturer's information	Number of restorations
Filtek Bulk Fill	Flowable	3M ESPE	Adper Single Bond 2	4 mm	Bis-GMA, UDMA, Bis-EMA, and Procrylat resins. Fillers are a combination of zirconia and silica having a particle size of 0.01–4.5 microns and ytterbium trifluoride filler having a particle size of 0.1–5.0 microns	30
SDR	Flowable	Dentsply DeTrey	XP Bond	4 mm	Barium aluminofluoroborosilicate glass, strontium aluminofluorosilicate glass, modified urethane dimethacrylate resin, ethoxylated bisphenol A dimethacrylate (EBPADMA), triethylene glycol dimethacrylate (TEGDMA), camphorquinone photoinitiator, butylated hydroxytoluene (BHT), UV stabilizer, titanium dioxide, and iron oxide pigments	30
SonicFill	Sonic flowable	Kerr	OptiBond Solo Plus	5 mm	Glass, oxide, chemicals (10–30%), 3-trimethoxysilylpropyl methacrylate (10–30%), silicon dioxide (5–10%), ethoxylated bisphenol A dimethacrylate (1–5%), bisphenol A bis(2-hydroxy-3-methacryloxypropyl) ether (1–5%), and triethylene glycol dimethacrylate (1–5%)	30
Tetric EvoCeram Bulk Fill	Packable	Ivoclar Vivadent	ExciTE F	4 mm	Monomer matrix containing dimethacrylates (20-21% weight). The fillers contain barium glass, ytterbium trifluoride, mixed oxide, and prepolymer (78%–81% by weight). Additional contents are additives, catalysts, stabilizers, and pigments (<1.0% weight)	30

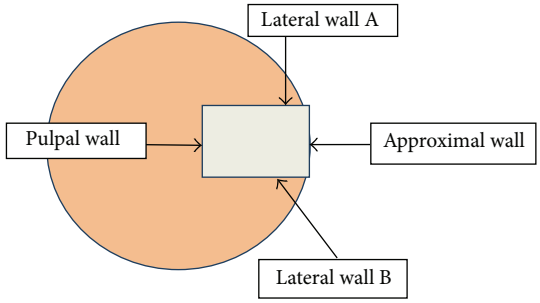

Figure 1: Graphic model of microleakage assessment on the transverse cross section.

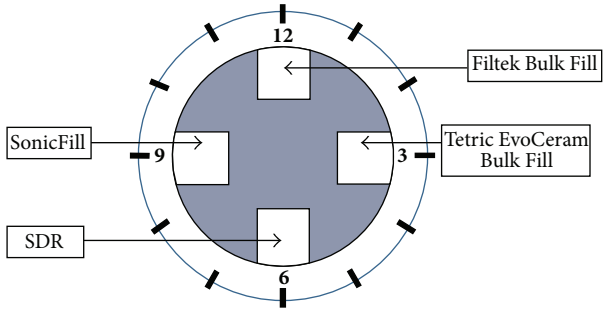

Figure 2: Order of restorations in every tooth sample.

(3M ESPE), and SDR (Dentsply DeTrey). The application of all tested bulk-fill materials was performed in accordance with the manufacturer's instructions. SonicFill composite was inserted by sonic-activation using SonicFill handpiece, Filtek Bulk Fill, from a special syringe with the application dispenser, SDR from Compula tip using a dispensing device, and Tetric EvoCeram Bulk Fill with manual filling instruments and burnishers. Polymerization of materials took place with the use of LED lamps (Advanced TPCD, USA) spectrum 440–490 nm, power 900 mW/cm^2. The restorations were finished with fine-grit diamond bur, mounted in a turbine with a water spray, and polished with graded abrasive discs and rubbers together with polishing paste (KerrHawe). Totally there were 120 restorations of 4 different types of composite bulk-fill materials placed. The order of restorations in every sample was always the same and based on a clockwise order: on 12 h, Filtek Bulk Fill, on 3 h, Tetric EvoCeram Bulk Fill, on 6 h, SDR, and on 9 h, SonicFill (Figure 2).

2.3. Microleakage Analysis. The teeth were dried and their tops were protected with pink wax and the smooth surfaces of the teeth (leaving a margin of 1 mm around the filling) were coated with nail varnish based on acetone (Inglot, Poland). The teeth were then placed for 24 hours in physiological saline to hydrate the teeth desiccated tissues. The prepared samples were placed in a 1% methylene blue solution for 24 h, after which the tooth surfaces were purified of the dye by means of rubbers and brushes with polishing compound.

For samples sections thus prepared teeth were cut with a diamond disc (0.5 mm Motyl, Poland) in the middle of the height of restorations parallel to the occlusal surface. In each restoration the depth of dye penetration was evaluated along the side walls. For the evaluation of dye penetration the Seliga optical microscope was used with a 10x magnification, pictures of the restoration interface were taken, and images were analyzed using a modified scale for bulk-fill materials with five-grade scale based on previous ones used in dental research studies [1, 7, 16–19]:

(0) no dye penetration into the filling material or along the filling-tooth interface,

(1) dye penetration into the filling material or along the filling-tooth interface up to half of the lateral wall A or B,

(2) dye penetration into the filling material or along the filling-tooth interface along all lateral wall A or B (till bottom of the cavity, pulpal wall),

(3) dye penetration into the filling material or along the filling-tooth interface up to half of both lateral walls A and B,

(4) dye penetration into the filling material or along the filling-tooth interface along both lateral walls A and B (till bottom of the cavity, pulpal wall).

The obtained results were statistically analyzed. Differences were considered statistically significant for $P < 0.05$.

3. Results

The condition of restorations made of bulk-fill composites expressed, as dye penetration, ranged from 0 till 4 and a detailed dye leakage analysis revealed differences in discoloration around the tested restorations.

Dye penetration rating using the grade scale was as follows (Table 2): the highest rating (score 0, no dye penetration) was achieved by 93.33% of the restorations made of the SDR material; 90% of restorations of SonicFill system; 86.66% of restorations of the composite Filtek Bulk Fill; and 73.33% of restorations of the Tetric EvoCeram Bulk Fill. The chosen tooth's sample without discoloration is presented in Figure 3. Score 1 (penetration of dye into half-depth of one wall) was achieved by 23.33% of restorations made of composite Tetric EvoCeram Bulk Fill and 3.33% of restorations from all other tested materials. Dye penetration along the entire length of one wall (score 2) was not found in the fillings made of the materials SonicFill and Tetric EvoCeram Bulk Fill and was observed in 6.66% of restorations made of Filtek Bulk Fill and 3.33% of the restorations made of SDR. The penetration of dye into half-depth of the two walls (score 3) of studied restorations was found only in the case of 6.66% of restorations of SonicFill and 3.33% of Filtek Bulk Fill restorations. Score 4 was achieved only by 3.33% restorations of the composite Tetric EvoCeram Bulk Fill. The chosen tooth's sample is presented in Figure 4. In these fillings, complete discoloration was seen on both walls. In the remaining materials tested, there was no discoloration of the two walls. All rates are graphically presented in Figure 5.

Due to the low percentage of negative samples and the test result being smaller than predetermined critical value,

Table 2: Dye leakage around examined restorations.

State of the restoration	SonicFill		Filtek Bulk Fill		Tetric EvoCeram Bulk Fill		SDR	
	n	%	*n*	%	*n*	%	*n*	%
No dye penetration	27	90	26	86,66	22	73,33	28	93,33
Dye penetration to half-depth of one wall	1	3,33	1	3,33	7	23,33	1	3,33
Dye penetration along one full wall	0	0	2	6,66	0	0	1	3,33
Dye penetration to half-depth of two walls	2	6,66	1	3,33	0	0	0	0
Dye penetration along two full walls	0	0	0	0	1	3,33	0	0
N/%	30	100	30	100	30	100	30	100

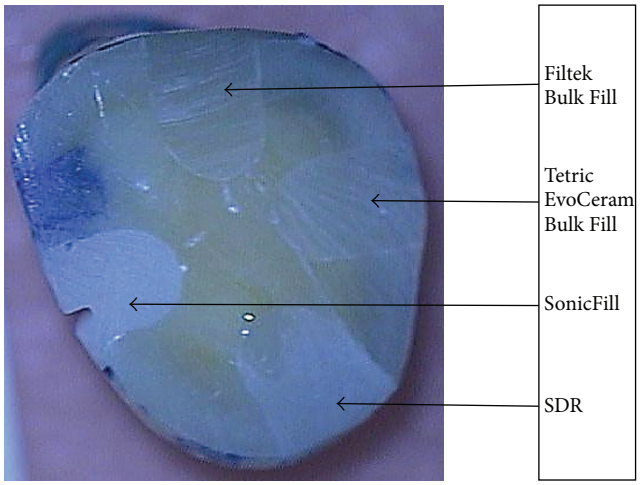

Figure 3: All restorations without microleakage (discoloration).

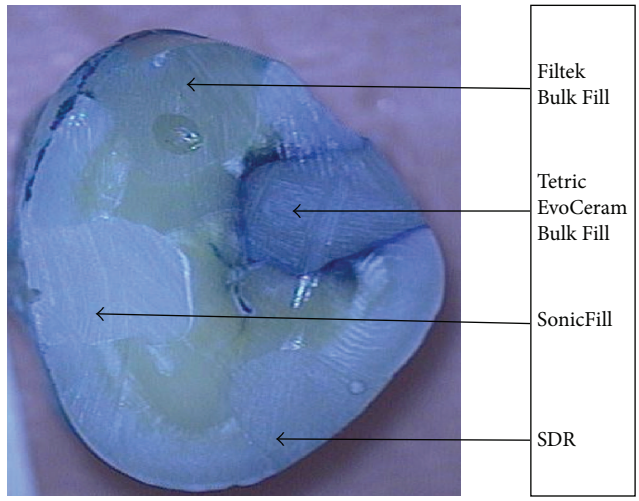

Figure 4: Discoloration of one of the restorations along the walls.

Table 3: Comparison of significant differences between pairs of composites.

	SonicFill	Filtek Bulk Fill	Tetric EvoCeram Bulk Fill	SDR
SonicFill	X	NS	NS	NS
Filtek Bulk Fill	NS	X	NS	NS
Tetric EvoCeram Bulk Fill	NS	NS	X	$P <$ **0.04**
SDR	NS	NS	$P <$ **0.04**	X

χ^2 test did not satisfy the condition of applicability. Thus, Fisher's exact test was used using a detailed comparison of the parameters. According to this test statistically significant differences were observed only between SDR and Tetric EvoCeram Bulk Fill restorations ($P < 0.04$), Table 3.

4. Discussion

Obtaining marginal integrity during filling cavities with composite materials determines tooth tissues protection against microleakage [1, 2, 20]. The biggest drawbacks of composite materials are polymerization shrinkage and thermal expansion greater than the expansion of the tooth. Polymerization shrinkage is responsible for the formation of internal stresses in the material and leakage between the filling and the walls of the cavity and the formation of posttreatment sensitivity [5, 6]. In order to reduce the risk of microleakage, the appropriate techniques should be applied that reduce the polymerization shrinkage [2, 12, 13, 20–23]. An important element in attempts to reduce the effects of the formation of internal stresses caused by polymerization shrinkage is an increase in the elasticity of the filler material and bonding system [6, 11, 20]. The increasingly common method of compensating stress is using a thin adhesive layer, the flowable composites [6, 24–26]. They have a lower modulus of elasticity so they are effective in reducing microleakage. It is generally believed that the conventional composite materials should be polymerized in increments not thicker than 2 mm [1, 27, 28]. During the polymerization of a thicker increment, the material can pass through the gel point at different times at different depths. When the superficial material layers are already in postgel phase, the deeper layers have not yet reached the gel point. The superficial part of the material becomes firm, and the deeper part is still liquid. Application of large increments of material triggers a shrinkage stress rise, and therefore the reduction of this phenomenon is a particular challenge. The recommended alternative to layered techniques, the bulk-fill techniques, has taken up this challenge. The single-increment application and polymerization method (the bulk-fill technique) proposed by the manufacturers of these composites did not compromise marginal adaptation of restorations. In

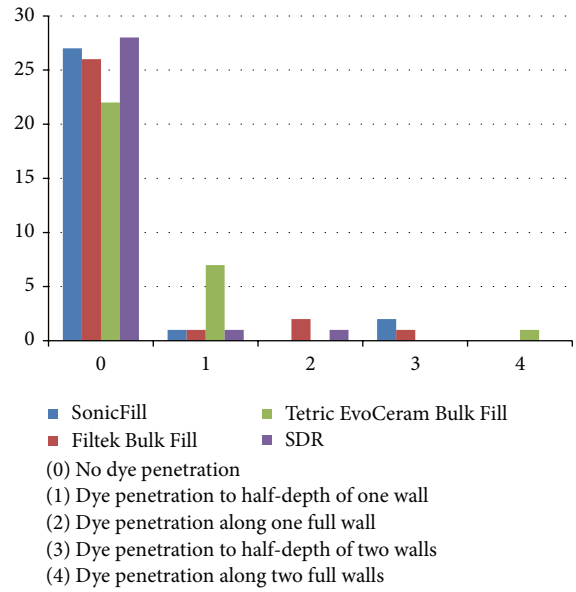

Figure 5: Dye penetration along examined walls.

assessing the integrity of the interphase tooth-filling, authors show differences resulting from the application technique [23, 27–29]. Abbas et al. and Federlin et al. obtained a lower degree of dye penetration in fillings made with layering technique than with one increment technique [17, 29]. The above quoted studies relate to restorations of the conventional composite materials. Bulk-fill composite materials evaluated in the present study seem to meet satisfactorily the requirements of this type of materials in terms of marginal adaptation. The dye penetration test showed no microleakage for high percentage (73.33–93.33%) of tested restorations. Bulk-fill composites are more translucent than other restorations, which allow the light to get to much deeper layers. The content of photoinitiators of polymerization and stress inhibitors determines the optimal marginal seal of these composites. The relationship between the method of filling cavities and marginal seal of composite fillings was also the subject of Skałecka-Sądel and Grzebieluch research [7, 20]. In the *in vitro* studies, they demonstrated that marginal integrity of class II fillings (preparation margin in enamel) of composite materials was higher when filling single increment of the material and lower with restorations of a layered material. Many factors affect the integrity of the bond between the tissues of the tooth and the material filling the prepared cavity. In addition to polymerization shrinkage, the C-factor, application method, and the polymerization of the composite resin play a significant role [1, 7, 20, 30–32]. In the present study the most favorable results were obtained when the application of the material took place using a SDR dispenser and activating sonic handpiece. It is in agreement with Ben-Amar et al. research conducted on the effect of the composite application and condensation on marginal seal [21]. Additionally, a higher marginal integrity and lower penetration of dye in fillings inserted using a sonic-activation condensing device were shown when compared with manual condensation. Statistically significant better marginal integrity of flowable tested materials, SDR, SonicFill, and Filtek Bulk Fill (compared to the composite Tetric EvoCeram Bulk Fill), may be due to their flow consistency during application. Peutzfeldt and Asmussen showed that the degree of fluidity when applying the composite material influences the marginal adaptation; increased fluidity of the composite makes it adhere better to the walls of the cavity [6]. The research by Ilie and Hickel implies that the flow composite materials based on SDR technology show a lower polymerization shrinkage compared with other flowable materials such as Filtek Supreme Flow and Esthet X Flow and also as compared to the nano- and microhybrid composites and based on silorans [33].

Report of Van Ende et al. seems to present interesting results of comparison studying three composites: conventional, liquid, and bulk-fill placed in the posterior teeth cavities of different cavity configurations coefficient (C-factor). The analyzed hypothesis was that the adaptation of the material to the cavity walls is not affected by C-factor, the type of the composite, and its application technique [31]. Verification of this hypothesis allowed the conclusion that the most satisfactory bonds with the tooth tissues were obtained when placing layered restorations in cavities with low C-factor, irrespective of the nature of the composite. On the other hand, in the cases where the C-factor was high, the choice of the composite proved important for the adaptation of the material [34]. Highly significant statistical difference was observed in the bond strength with tooth tissues between the flow-type composites and the conventional and the bulk-fill SDR resin. Markedly decreased bond strength was obtained in the case of flowable and conventional materials, in combination with a composite bulk-fill SDR material.

To interpret our results it should be also recognized that each bonding agent used, although specific for each material, may have influenced the marginal gap and this relationship between the bonding agent and the bulk-fill composite needs to be studied in the future. Additionally, bulk-fill materials may have different types of photoinitiators and thus require curing lights that activate them adequately [35]. That is why to avoid this kind of complication and to test these products as they are offered by manufacturer it was decided to study the bulk-fill materials together with a compatible bonding agent as integrated systems. It is also important to underline that the present results were obtained in the *in vitro* conditions such as a very good capability of light-curing device as well as direct access to the prepared tooth-composite samples. The achieved distance between the tip end of the light-curing device and the irradiated surface can hardly ever be obtained in working conditions in oral cavity of the patient where curing is less effective, which has been lately noticed [36].

5. Conclusions

Within the limits of this *in vitro* study, it can be concluded that bulk-fill flowable or sonic-activated flowable composite restorations have better marginal sealing in comparison with bulk-fill paste-like composites.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Evaluation of the Esthetic Properties of Developmental Defects of Enamel: A Spectrophotometric Clinical Study

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Objectives. Detailed clinical quantification of optical properties of developmental defect of enamel is possible with spectrophotometric evaluation. Developmental defects of enamel (DDE) are daily encountered in clinical practice. DDE are an alteration in quality and quantity of the enamel, caused by disruption and/or damage to the enamel organ during amelogenesis. *Methods*. Several clinical indices have been developed to categorize enamel defects based on their nature, appearance, microscopic features, or cause. A sample of 39 permanent teeth presenting DDE on labial surface was examined using the DDE Modified Index and SpectroShade evaluation. The spectrophotometric approach quantifies L^* (luminosity), a^* (quantity of green-red), and b^* (quantity of blue-yellow) of different DDE. *Conclusions*. SpectroShade evaluation of the optical properties of the enamel defect enhances clinical understanding of severity and extent of the defect and characterizes the enamel alteration in terms of color discrepancy and surface characterization.

1. Introduction

Developmental defects of enamel (DDE) are daily encountered in clinical practice. DDE are alteration in quality and quantity of the enamel, caused by disruption and/or damage to the enamel organ during the amelogenesis process. The clinical aspect of the defect depends on the stage of development during which the insult occurs as well as the extent and duration of the insult. Enamel hypoplasia (HE) is a quantitative defect and presents a scarce enamel thickness, while enamel hypomineralization (EO) is a qualitative enamel deficiency presenting alterations in enamel translucency and opacity which may be diffuse (DIO) or demarcated (DEO) with white, yellow, or brown colour [1, 2]. DDE can have a significant impact on oral health and esthetics, tooth sensitivity, and altered occlusal functions [3, 4]. Enamel defects are also risk conditions for dental caries and erosion in children [5, 6]. Epidemiologic data (DDE prevalence in permanent dentition ranges from 10% to 49%) reflect an increasing trend of this condition, which should be considered as a public health problem and a challenge for dental practitioners. Several clinical indices have been developed to categorize enamel defects and they can be divided into (a) specific fluorosis indices (Dean/WHO, Thylstrup and Fejerskov, and TSIF indices), which identify and categorize only dental fluorosis, and (b) descriptive indices (the Al-Alousi and the Developmental Defects of Enamel Index, the Modified DDE Index), with no etiological assumption [2].

The Modified DDE Index [7] is a descriptive index derived from the Developmental Defects of Enamel Index [8]. It is more practical and comparable index for epidemiological studies and it allows efficient recording of prevalence and severity of enamel defects. The criteria for classification are related with histopathological changes [9]. The DDE Modified Index divides defects into three types: demarcated (Figures 1, 2, and 3), diffuse (Figure 5), and hypoplastic (Figure 4). The diffuse opacity category probably contains most of the fluoride-related opacities. However, this group contains some nonfluoride opacities as well, and no attempt is made to differentiate between these types. The extent of the defect should be recorded in thirds of the tooth surface area and a limit in size of greater than 1 mm in diameter should

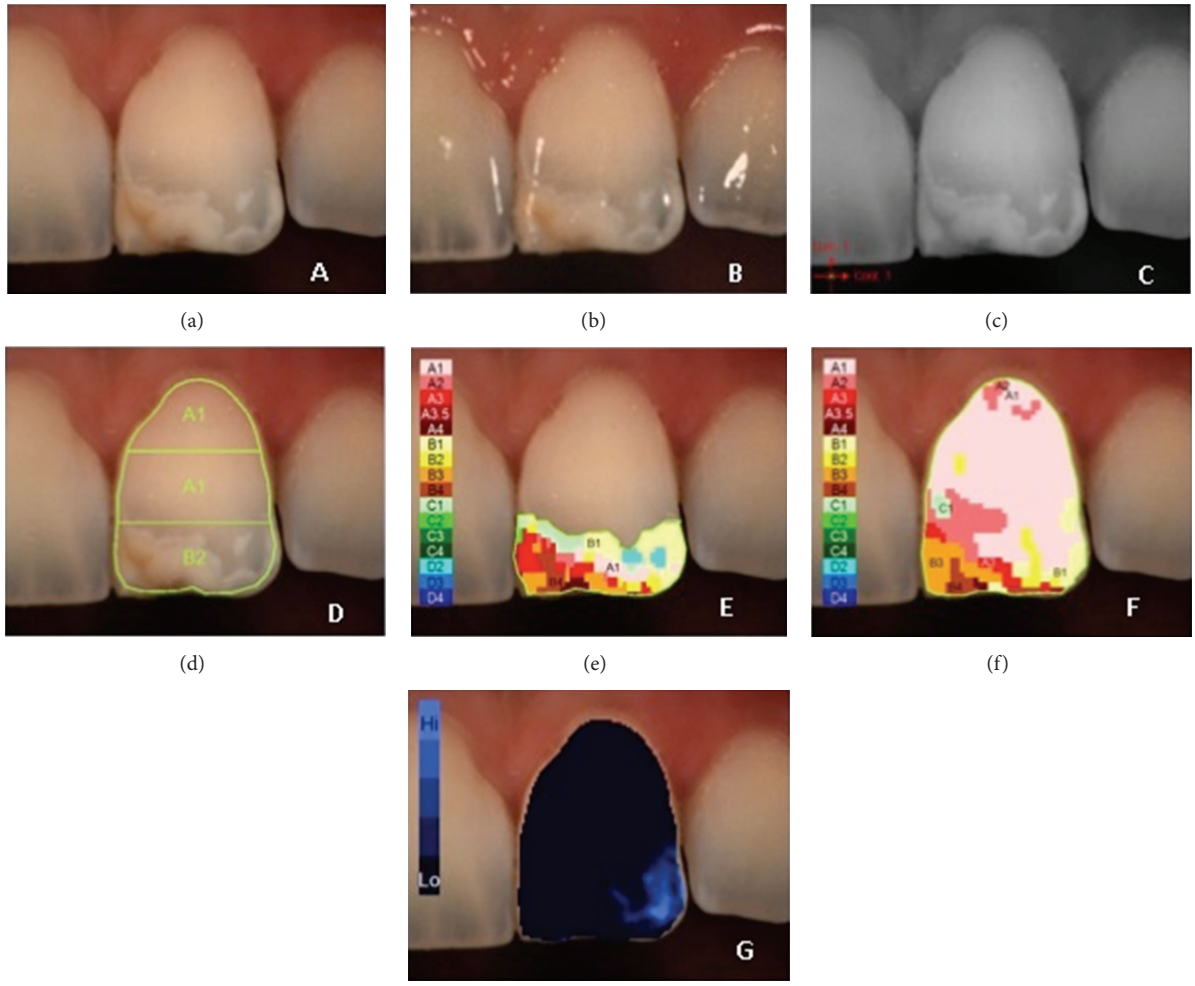

Figure 1: Example of $L^*a^*b^*$ measurements of demarcated opacity: (a) polarized image; (b) gloss mode allowing the identification of "pure enamel zones"; (c) contrast image; (d) evaluation of the three equal zones along the median axis: gingival, central, and incisal; (e) colour distribution and detailed mapping of the defect surface; (f) overall detailed mapping; (g) translucency.

Table 1

Basic type of DDE	Subtype of DDE
Demarcated opacities (DEO)	Demarcated opacities (white/cream)
	Demarcated opacities (yellow/brown)
Diffuse opacities (DIO)	Diffuse opacities lines/patchy
	Diffuse opacities confluent
	Confluent/patchy stain gloss of enamel
Hypoplasia (HE)	Hypoplasia pits
	Hypoplasia missing enamel
Discolouration	

be used to distinguish between normal and abnormal enamel defects (Table 1).

The aetiology of DDE is not completely clear. Genetic and hereditary factors such as in amelogenesis imperfecta are involved, along with acquired, systemic, and environmental factors such as fluoride intake and medications, nutritional deficiencies, prenatal infections, or chicken pox or other early childhood diseases [3, 10–12].

Clinical detecting of DDE occurs in children, adolescents, and young adults. DDE on vestibular surface of upper and lower arch may cause the patient distress, while parents are concerned about esthetic problem and ask for solution. Clinical evaluation of defect severity and extent is part of the esthetic management of these lesions and may influence the treatment option choice. The aim of this paper is to develop a clinical approach based on spectrophotometric measurements. Subjective human perception of colour is susceptible to bias. It is possible to exclude this bias by using spectrophotometer that allows an objective, quantitative colorimetric method and can be used under routine clinical conditions [13, 14]. Spectrophotometric measurement

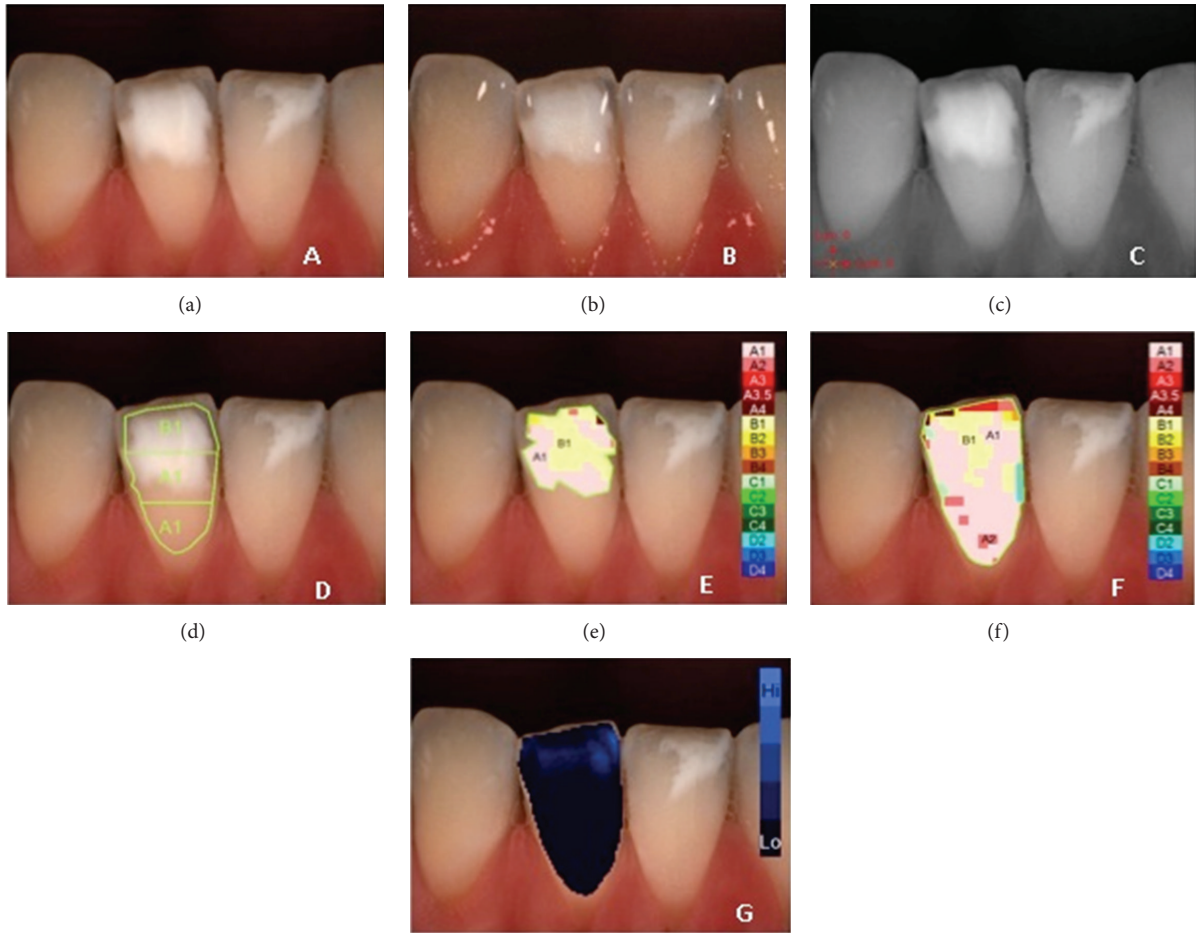

Figure 2: Example of $L^*a^*b^*$ measurements of demarcated opacity on lower central right incisor: (a) polarized image; (b) gloss mode allowing the identification of "pure enamel zones"; (c) contrast image; (d) evaluation of the three equal zones along the median axis: gingival, central, and incisal; (e) colour distribution and detailed mapping of the defect surface; (f) overall detailed mapping; (g) translucency.

generates quantitative data not only of the defects area, but also of the surrounding sound enamel surface. These data can be used for the quantification of esthetic properties of the DDE defects classified by DDE Modified Index.

2. Material and Methods

The study design was not reviewed by the Dental School's Ethics Committee, because instrumental and observational analyses are usual clinical educational activities at the Sapienza University. Prior to each measurement, teeth were cleaned with a prophylaxis paste and rinsed with water spray. Teeth dehydration was controlled to avoid shade changes due to humidity loss. The study population was of 30 subjects, in the age range of 13–19 years (mean age: 15,3); 39 teeth were analyzed. Of the enrolled patients 51,5% were girls and 48,5% were boys. There were no gender related differences between the three DDE modified index groups distributions (demarcated, diffuse, and hypoplastic).

A digital camera (Nikon D90) with a macro lens (105 mm Macro lens, Nikon) and a macro flash (R1C1 Macro flash, Nikon) documented the developmental defects of enamel on the labial surface of central and lateral, upper and lower incisors and upper and lower canines. The digital photo was used to classify the enamel defect consistently with DDE Modified Index.

In this study a calibrated reflectance spectrophotometer (SpectroShade, MICRO, Serial N HDL1407, MHT, Arbizzano di Negrar, Verona, Italy) was used. The position of the device is perpendicular to the labial surface of the clinical crown and it is reproducible in order to obtain always equal measurement conditions. The D65 light source (6500°K) illuminates each tooth simultaneously from two sides at 45° angle. The system has two detector areas (both 18 mm × 13 mm) where the reflected light is directed at 0°. One detector (color CCD chip) generates the color video image; the other (b/w CCD detector) records the spectrophotometric data. The stored data are used to create detailed CIE $L^*a^*b^*$ data of the tooth surface.

MHT spectrophotometer analyzes the dental surface every 8 nm and allows a large number of different data representations on specific tooth area. All the clinical factors

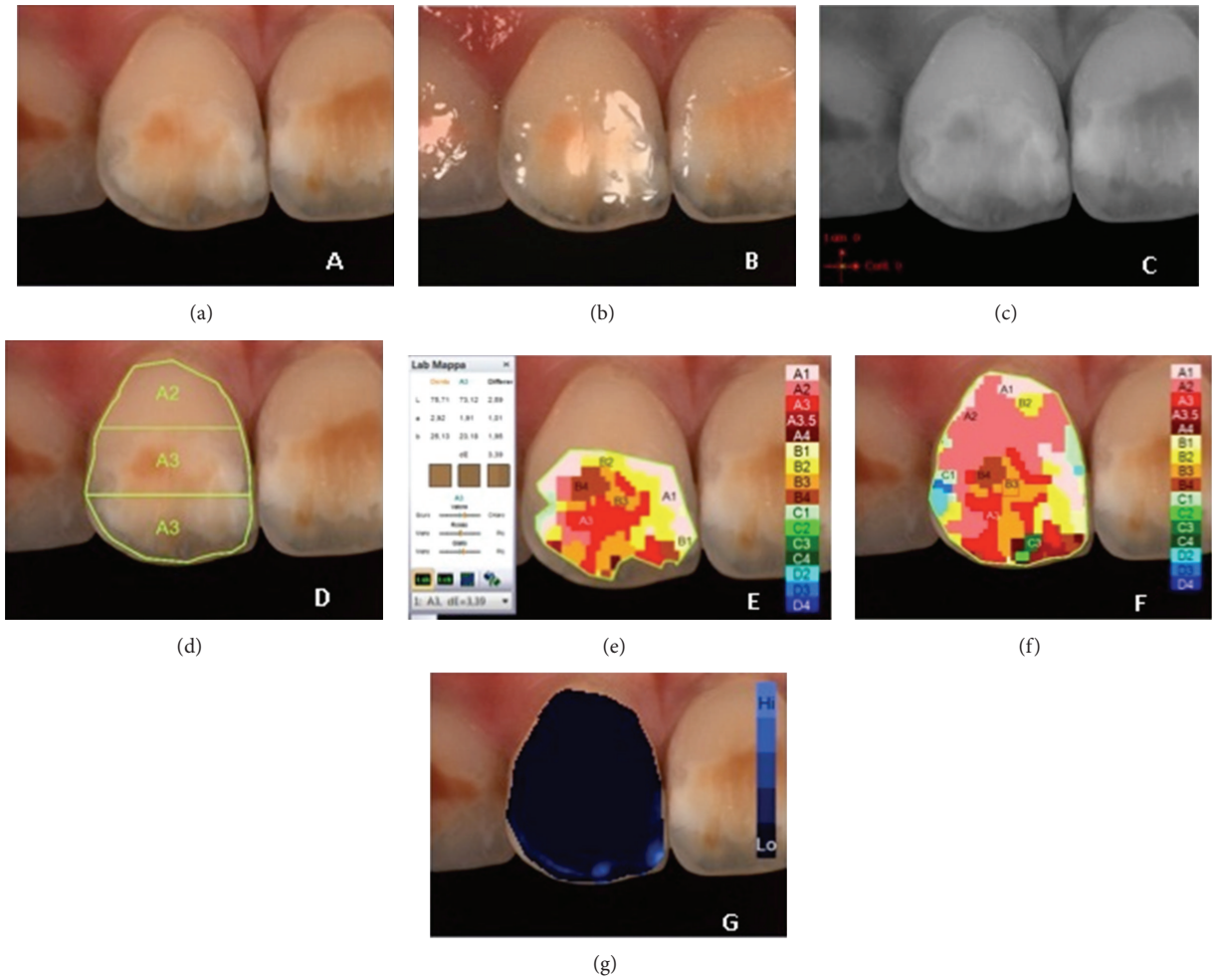

FIGURE 3: Example of $L^*a^*b^*$ measurements of demarcated opacity on upper central right incisor: (a) polarized image; (b) gloss mode allowing the identification of "pure enamel zones"; (c) contrast image; (d) evaluation of the three equal zones along the median axis: gingival, central, and incisal; (e) colour distribution and detailed mapping of the defect surface; (f) overall detailed mapping; (g) translucency.

influencing esthetic appearance of the teeth are taken into consideration with this method [15]. The MHT software divides the vestibular tooth area into three equal zones along the median axis: the same method is described by DDE Modified Index during tooth area examination. These well-defined enamel areas have different optical properties in sound tooth and the spectrophotometric evaluations show how CIE $L^*a^*b^*$ coordinates vary along the median axis from higher gingival point to the incisal edge [16]. Enamel is more translucent and, in respect to tooth color, it plays only a minor role through scattering at wave lengths in the blue range, while dentin is more opaque and, according to ten Bosch and Coops [17], determinates mainly the color of the tooth. All SpectroShade assessments were performed by one trained operator.

Consistently with DDE Modified Index, a more meaningful way to present data was to group the defects into three broad categories, that is, demarcated opacities (DEO), diffuse opacities (DIO), and hypoplastic defects (HE), with provision to record discolouration and any other defects. The demarcated opacities can present white/cream and yellow/brown shades. The diffuse opacities can be like fine delicate lines, or patchy, or confluent opacities. The diffuse opacities tend to fade into the surrounding enamel. These defects can be present on tooth surface singularly or in combination.

To define the defect in relation to the extent of the tooth surface covered by the defect, the vertical length of the measured teeth was divided into three equal zones along the median axis. In each zone all the area was detected and defined by using the device software. The L^* value (y-axis) is a measure of the lightness of an object on a scale ranging from 0 (black) to 100 (white). The a^* value is a measure of redness (positive a^*) or greenness (negative a^*). The b^* value is a measure of yellowness (positive b^*) or blueness (negative b^*). The a^* and b^* coordinates approach zero for neutral colours (white, greys) and increase in magnitude for more saturated or intense colour [18–21].

3. Results

3.1. Descriptive Statistics. The defect distribution was 61,5% DEO (demarcated opacities), 20,5% DIO (diffuse opacities),

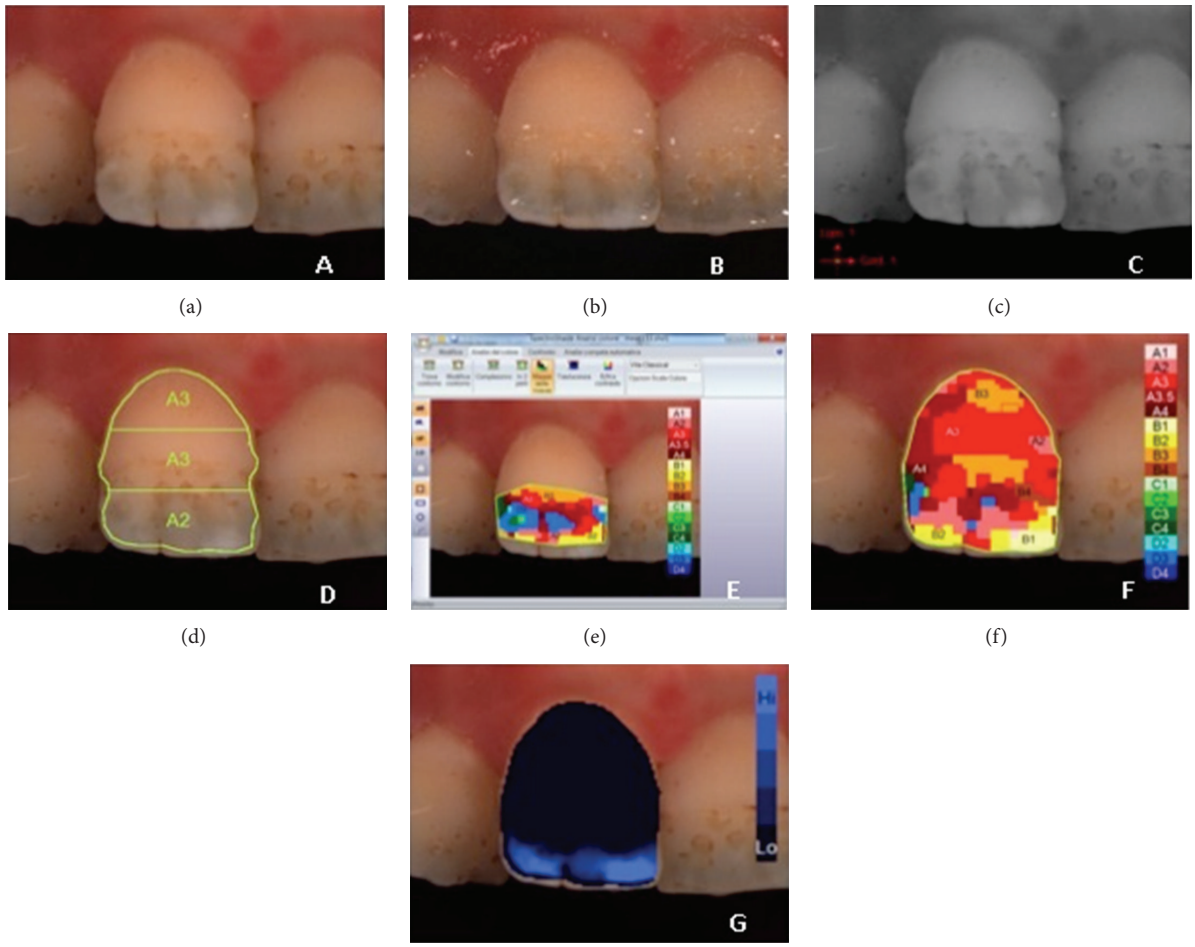

Figure 4: Example of $L^*a^*b^*$ measurements of hypoplastic defects on upper central incisor: (a) polarized image; (b) gloss mode allowing the identification of "pure enamel zones"; (c) contrast image; (d) evaluation of the three equal zones along the median axis: gingival, central, and incisal; (e) colour distribution and detailed mapping of the defect surface; (f) overall detailed mapping; (g) translucency.

and 18% HE (enamel hypoplasia). The more frequent localization of the developmental defects of enamel on the tooth surface was on the incisal area (58,9%), then on the incisal and central areas (33,4%), on all the clinical crown surface (5,1%), and in few cases (2,6%) on the central tooth area. No one defect was present exclusively on the gingival area.

The mean value of L^* of sound enamel was 72,44; the mean value of a^* was 6,46; the mean value of b^* was 19,47, respectively.

The results of the spectrophotometric measurements show the following.

(i) In the DEO (Demarcated Opacities). The mean value of L^* of the defect was 69,71. The mean value of a^* was 2,80. The mean value of b^* was 15,58.

(ii) In the DIO (Diffuse Opacities). The mean value of L^* of the defect was 69,87. The mean value of a^* was 3,33. The mean value of b^* was 11,46.

(iii) In the HE (Hypoplasia). The mean value of L^* of the defect was 65,28. The mean value of a^* was 10,65. The mean value of b^* was 21,42.

The descriptive statistics for mean $L^*a^*b^*$ values is illustrated in Table 2.

Table 3 shows the enamel defect type (DEO, DIO, and HE), localization along the median axis (incisal, central, or gingival), and the Vita 3D Master shade selection measured by MHT spectrophotometer software, respectively, of sound and defect enamel surface.

4. Discussion

Little is known about the optical properties of developmental defects of enamel in a young population. Clinical detecting of DDE is more frequent everyday but there is a lack of information about the optical properties of the defect surface and the surrounding sound enamel surface.

The tooth optical properties describe a complex phenomenon, which can be even more complex if a developmental defect of enamel is present on labial tooth surface. Value, hue, and chroma describe tooth colour, but there are more subtle secondary optical properties that affect the overall tooth appearance: translucency, opalescence, opacity,

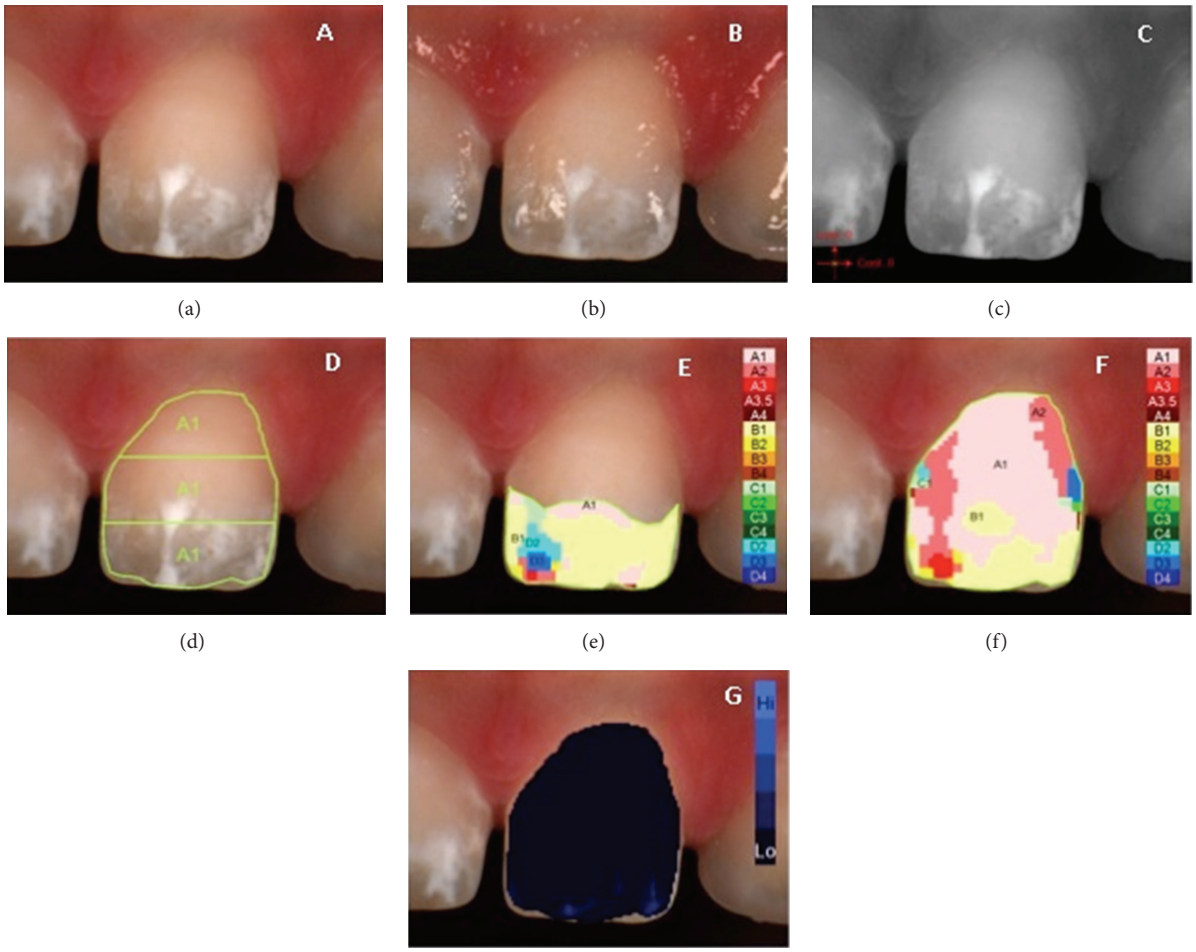

Figure 5: Example of $L^*a^*b^*$ measurements of diffused opacity on upper central incisor: (a) polarized image; (b) gloss mode allowing the identification of "pure enamel zones"; (c) contrast image; (d) evaluation of the three equal zones along the median axis: gingival, central, and incisal; (e) colour distribution and detailed mapping of the defect surface; (f) overall detailed mapping; (g) translucency.

iridescence, surface gloss, and fluorescence [20, 21]. Translucency, opacity, and opalescence have been viewed as the most important indicators of the quality and quantity of light reflection [22].

In this study the tooth labial surface was divided into three equal zones (incisal, central, and gingival) along the median axis and the aspects we consider most important for tooth perception, that is, L^*, a^*, and b^* (resp., the amount of luminosity, green/red, and blue/yellow), were analyzed. Sound enamel surface and affected surface were analyzed using the same spectrophotometric method.

The developmental defect of enamel can be localized at the cervical, middle, or incisal area of the tooth. In a sound tooth these areas have different optical properties, because of the structure complexity and variability of enamel thickness and the below dentine. Some considerations can be illustrated about the optical properties of a sound tooth. Hasewaga has measured $L^*a^*b^*$ in 5 locations along the median axis and found significant variation. L^* is highest in the center zone ($L^* = 73$) and becomes lower in the gingival zone ($L^* = 69$) and more lower towards the incisal edge ($L^* = 64$); the highest a^* value is in the gingival area ($a^* = 8{,}5$) and it gradually becomes lower towards the incisal edge ($a^* = 2{,}0$); b^* is highest in gingival region ($b^* = 20$) and gradually decreases towards the incisal edge ($b^* = 13$) [23]. The existing differences between these zones are statistically and clinically significant also for detecting and describing enamel colour alteration when developmental defects are present. Translucency indicates the quality and quantity of light reflection and it decreases from incisal towards central tooth area.

In our opinion, the less the defect is integrated within the surrounding sound enamel surface, the more the DDE is clinically evident, this condition increasing from gingival to incisal tooth area. Loss of surface gloss affects the tooth vitality appearance. When a demarcated opacity is present on the incisal zone with white, yellow, and/or brown characterization, the surface gloss is completely altered. Developmental defects of enamel interest also tertiary anatomy. The tertiary anatomy is defined by vertical, horizontal, and

Table 2

Sample	Type	L	a	b
1	DEO	69,35	2,26	19,84
2	DEO	68,22	2,89	21,26
3	DEO	71,40	4,40	24,68
4	DEO	70,91	1,47	12,17
5	DEO	65,42	3,95	16,39
6	DEO	62,50	9,34	24,35
7	DEO	79,82	1,21	9,30
8	DEO	74,94	1,51	9,29
9	DEO	68,65	7,70	28,42
10	DEO	67,36	3,03	16,04
11	DEO	65,67	3,21	17,38
12	DEO	74,13	1,54	12,95
13	DEO	66,99	1,45	10,58
14	DEO	69,77	0,82	10,78
15	DEO	64,77	8,43	16,33
16	DEO	63,55	8,61	24,74
17	DEO	75,90	1,02	14,27
18	DEO	67,28	1,18	11,46
19	DEO	65,12	0,54	12,15
20	DEO	72,30	0,85	21,05
21	DEO	74,18	1,36	7,37
22	DEO	69,26	1,17	15,26
23	DEO	69,21	0,12	8,81
24	DEO	76,29	−0,73	9,13
	Mean	69,71	2,80	15,58
25	DIO	73,18	3,24	10,34
26	DIO	72,17	0,91	8,41
27	DIO	67,57	2,12	10,81
28	DIO	66,80	2,74	11,68
29	DIO	69,39	3,13	9,21
30	DIO	66,59	6,60	14,97
31	DIO	68,75	4,58	16,10
32	DIO	74,49	3,35	10,18
	Mean	69,87	3,33	11,46
33	HE	70,34	10,74	18,98
34	HE	70,14	10,02	18,64
35	HE	62,64	5,20	21,84
36	HE	63,44	3,79	18,61
37	HE	65,01	9,84	18,34
38	HE	58,67	25,56	37,50
39	HE	66,69	9,40	16,01
	Mean	65,28	10,65	21,42

varied textures. Marginal ridges and developmental lobes define the vertical texture, while fine, transverse, and delicate wavelike grooves define the horizontal characterization. The horizontal grooves are called perikymata. Tertiary anatomy of a sound maxillary central incisor is well pronounced in young population, while with age it is lost due to horizontal and vertical wear. The tertiary anatomy is completely erased when demarcated opacities are present on the tooth surface. Enamel hypoplasia such as pitted enamel hypoplasia often can affect also vertical tertiary anatomy, determining in the meanwhile a complete aberration of tertiary characterizations.

Spectrophotometric analysis presents a lot of advantages: it excludes bias due to subjective evaluation and analyses every 1–10 nm of the visible spectrum and the collected data are accurate. Incisal, central, and gingival zones have really different CIE L^*, a^*, and b^*, because of changing thickness in enamel and dentine layers. Moving from the incisal zone to the gingival, the tooth thickness increases and opacity and a^* values increase too, while luminosity (L^* values) decreases. The gingival zone has been shown to have the lowest translucency [23] and significantly higher a^* values; b^* values slightly increase with thickness, in a constant and linear way [19]. According to Modified DDE Index, a defect can be considered greater if the covered surface is more than 1 mm of diameter. In our opinion the defect localization on the three previously described zones (incisal, central, and gingival) is an important factor for the defect characterization. Optical properties of the defect have to be confronted with the optical properties of the corresponding sound enamel zone and the more they diverge in a^* and b^* values, the more is the perception of the existing contrast. For example, as gingival zone has the lowest translucency, higher a^* values, and increased b^* values but decreased luminosity (L^* values), a defect with similar optical properties will be more accepted in this zone, while the same defect in the incisal zone will appear more evident due to higher differences in optical properties with the interested zone.

A recent study presented a correlation between the colour of enamel and the severity of hypomineralization, where yellow and brown colour of the hypomineralized enamel was at a higher risk for PEB (posteruptive breakdown) compared with white defects [24].

5. Conclusions

The analysis of the interaction of light with dental structures is really important. A novel quantitative in vivo approach for characterization of developmental defects of enamel optical parameters according to DDE Modified Index was developed during this study and it proved its feasibility on a limited number of patients. The spectrophotometric evaluation in this study required 5 minutes for each patient. The application of this method on a larger number of subjects may allow for a clinical correlation between colorimetric features and clinical severity and between diagnosis and therapeutic decision making process.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Table 3

Sample	Enamel defect		Shade (Vita 3D Master)	
	Type	Localization	Defect	Sound enamel
1	DEO	Incisal	1M1	3M1
2	DEO	Incisal/central	2R2,5	1M1
3	DEO	Incisal/central	3M3	2M1
4	DEO	Incisal	1M1	1M2
5	DEO	Incisal	5M1	3M2
6	DEO	Incisal/central	5M3	3M1
7	DEO	Incisal/central	0M1	1M2
8	DEO	Incisal	0M3	1M2
9	DEO	Incisal/central	3M3	3M3
10	DEO	Incisal	1M1	3R1,5
11	DEO	Incisal	2R1,5	3L1,5
12	DEO	Incisal	2M1	4M1
13	DEO	Incisal	1M1	2M1
14	DEO	Incisal	1M1	3M1
15	DEO	Incisal/central/gingival	3R1,5	4M1
16	DEO	Incisal/central	5M2	4M1
17	DEO	Incisal	2M1	3M1
18	DEO	Incisal	1M1	3M1
19	DEO	Incisal	2M1	3M1
20	DEO	Incisal	2R2,5	2M1
21	DEO	Incisal	1M1	2M1
22	DEO	Incisal	1M2	2M2
23	DEO	Incisal	2M1	2M1
24	DEO	Incisal	0M1	3M1
25	DIO	Incisal/central	1M1	2M1
26	DIO	Incisal	5M1	2R1,5
27	DIO	Incisal	0M1	3M1
28	DIO	Incisal	2M1	4M1
29	DIO	Incisal	2M1	4M1
30	DIO	Incisal/gingival	3M1	3M1
31	DIO	Incisal/central	3M1	3R1,5
32	DIO	Incisal/central	1M1	2M1
33	HE	Incisal/central	3M2	3M1
34	HE	Incisal/central	5M1	3M1
35	HE	Incisal	5M2	4M1
36	HE	Incisal	4M1	4R2,5
37	HE	Incisal/central	5M1	4M1
38	HE	Central	5M3	3M2
39	HE	Incisal/central	3R1,5	3M1

Acknowledgments

Fabrizio Guerra designed the study. Marta Mazur and Debora Pasqualotto collaborated in the collection of data and preparation of the paper. Gianna Maria Nardi and Denise Corridore collaborated in writing and preparation of the paper. Livia Ottolenghi coordinated the study and revised and provided final approval of the paper.

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Clinical Study

Herbal Mouthwash Containing Extracts of *Baccharis dracunculifolia* as Agent for the Control of Biofilm: Clinical Evaluation in Humans

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Baccharis dracunculifolia DC (Asteraceae), popularly known as "alecrim-do-campo," is largely distributed in South America, is shown to exhibit protective actions against gastric ulcers, has anti-inflammatory properties, and is hepatoprotective. Several essential oils obtained from *Baccharis* species possess biological activities, such as antimicrobial and antivirus activities. This randomized controlled trial evaluated the efficacy of *B. dracunculifolia* in the reduction of dental biofilm, comparing this natural product with other mouthwashes already known in the dental market. In measuring the time after use of mouthwash ($t = 1$), there was no difference between products ($P = 0.602$); that is, subjects in the study had a similar PI after the first use. After one week ($t = 2$), there was no difference between the four products evaluated ($P = 0.674$), so, all research individuals completed the study with a similar reduction in dental biofilm between themselves but it was different from initial state (Friedman test). It is possible to conclude that *B. dracunculifolia* had the same efficiency of the materials used to oral hygiene in reduction of dental plaque and, consequently, prevention of dental caries. Thus, we can consider *B. dracunculifolia* as a good candidate for new material to be implemented in dental care.

1. Introduction

Dental plaque is a complex biofilm that accumulates on the surface of the teeth, containing more than 500 bacterial species. The dental plaque is produced by initial colonizing bacteria in the salivary film of the enamel, followed by secondary colonization through the interbacterial adhesion [1]. Oral infectious diseases, such as periodontal inflammation, caries, and gingivitis, can be developed by dental biofilm formation [2]. These kinds of dental problems can cause other serious health diseases and, because of this, the study in the development of referred pathologies has been a theme of growing interest [3]. In this way, recent research suggests that periodontal diseases may contribute to the development of heart diseases [4] and stroke [5] and they can compromise the health of patients with diabetes, respiratory diseases, or osteoporosis [6]. Among oral infectious diseases, caries has been the most persistent infection in the history of humanity [3], also being the leading cause of tooth loss in children and young adults [1]. Caries disease is an irreversible microbial pathology of the calcified tissues of the teeth and it is a multifactorial disorder, greatly influenced by the diet of the individual, which often leads to cavitation, since it is characterized by destruction of the organic substance of the tooth and demineralization of the inorganic portion [7, 8]. Previous literature has shown data that the mutans group

Table 1: Summary of the products tested for efficacy in controlling biofilm.

Group	Product	Fabricant	Active ingredient
A	Basic formulation without active component	FCFRP-USP	Control
B	Plax	Colgate Palmolive, a company	Triclosan + Gantrez + NaF + alcohol (7.5%)
C	Formulation and testing with active component	FCFRP-USP	Extract and essential oil of *B. dracunculifolia*
D	Listerine	Johnson & Johnson	Essential oil + alcohol (23.6%)

FCFRP-USP: Faculty of Pharmaceutical Sciences of Ribeirão Preto of University of São Paulo, Brazil.

of Gram-positive bacteria, particularly *Streptococcus mutans*, occupies a substantial proportion of the microbiota that integrates the cariogenic biofilm, and their participation in the etiology of dental caries is very important [9]. Its virulence is directly related to the ability to produce acids for metabolizing carbohydrates. These acids reduce the pH of the biofilm and cause demineralization of tooth structure [10].

The ability of *S. mutans* to initiate a decay depends on the association of several virulence factors, including initial adhesion to the tooth surface by adhesion glycoproteins with the ability to synthesize extracellular polysaccharides insoluble (PEC). PEC is mainly produced by means of enzymes glucosyltransferases (GTFs), which promotes the accumulation and retention of microorganisms on tooth surfaces, with high capacity to catabolize carbohydrates and produce acids that soften tooth enamel and also with ability to grow and to continue metabolism of carbohydrates at low pH [11].

Biofilm bacteria have increased antimicrobial resistance [12] and, for this reason, the increase in demand for new antimicrobials has led to several investigations toward the search for antimicrobial effects of phytochemicals extracted from a range of species of botanical origin [13, 14]. In fact, recently, much attention has been given to natural products with health-promoting benefits [15, 16].

In the process of developing new pharmacologically active compounds from natural products for using in dentistry, the Southeastern Brazilian propolis may prevent dental caries [17, 18]. *B. dracunculifolia* is the most important botanical source of Southeastern Brazilian propolis, which due to its colour is called green propolis, whose healthy benefits, including the hepatoprotective effect, are well described in the literature [19]. Propolis is the generic name for the resinous substance produced by honeybees (*Apis mellifera*) and is commonly used to improve health and to prevent several diseases [20]. It has been used for medicinal purposes since ancient times, and its antimicrobial, antitumoural, and immunomodulatory activities have been reported [21].

Approximately 500 species of *Baccharis* are known, which are distributed specifically in America [22]. Among others, their potential antirheumatic, antifungal [23], antioxidant [22], and insecticide properties are known. *Baccharis* species have been extensively used in folk medicine for the treatment and prevention of anaemia, inflammation, diabetes, and stomach, liver, and prostate diseases [24]. *Baccharis dracunculifolia* DC (Asteraceae), popularly known as "alecrim-do-campo," is largely distributed in South America and is shown to exhibit protective actions against gastric ulcers [25], has anti-inflammatory properties [26], and is hepatoprotective [27]. Several essential oils obtained from *Baccharis* species possess biological activities, such as antimicrobial [28] and antivirus [29] activities. *B. dracunculifolia* and Brazilian green propolis have been reported to show similar anticariogenic [30], antiulcer [31], and immunomodulatory activities [32].

Microemulsions are systems with good thermodynamic stability and high solubilization capacity of hydrophilic and lipophilic drugs, are easy to use, and give greater stability to the most active embodied components. A microemulsion was used for extract of *B. dracunculifolia* in the present work, and its toxicity has been evaluated in our laboratory, with results of no toxicity at concentrations of use. However, there are relatively few studies reporting topical administration of microemulsions [33–35].

Considering the search for new therapeutic mouthwashes and the benefits of natural care products for general and oral health, the aim of this study was to evaluate the efficacy of *B. dracunculifolia* in the reduction of dental biofilm. In this sense, there is the possibility of finding a new type of material for use in oral hygiene that may contribute to prevention of dental caries. Thus, this study used a clinical evaluation of a pharmaceutical oral formulation based on microemulsion containing extract and essential oil of *B. dracunculifolia*, in order to propose the application of this plant material in formulations for dental use.

2. Materials and Methods

The present work was randomized and controlled, crossover type, in order to establish a triple-blind study (the handler of the formula, the packager/labeller, and clinical evaluation). Twelve healthy subjects, aged between 18 and 30 years, were recruited and chosen according to the following inclusion/exclusion criteria: good general health; no sign of destructive periodontal disease; minimum of 24 teeth, six in each quadrant; absence of antibiotic therapy for a period of three months before the study; no smoking; no regular use of mouthwashes.

All patients received verbal and written statement about the study in question and signed a consent form (Human Research Ethics Committee 2008.1.1061.58.4; CAAE number 0065.0.138.000-08). Twelve individuals were divided into four groups that received rinses solutions each (Table 1), at different times. Table 2 contains the formulation of the mouthwash test. In the present study, a Quigley-Hein plaque index was used. The patients were submitted to a (preclinical) period of 24 hours without the use of oral care products and their plaque index (PI) was measured. For this analysis, basic fuchsin was used in the form of two tablets that were chewed

Table 2: Composition of **C** formulation—mouthwash with *B. dracunculifolia*.

Ingredients	Function	(% *p*/*p*)
Sodium fluoride	Active	0.05
Sodium benzoate	Conservative	0.10
Sodium saccharin	Sweetener	0.15
Xylitol	Sweetener	2.50
Menthol	Freshness	0.20
Methylparaben	Conservative	0.10
Mint flavor	Corrective	0.20
Essential oil of *B. dracunculifolia*	Active/oil phase	0.04
Hydroethanolic extract of *B. dracunculifolia*	Main active component	0.16
PEG 40	Surfactant	6.59
Sorbitol	Cosurfactant	6.59
Glycerol	Cosurfactant	6.59
Purified water	Vehicle	100.00

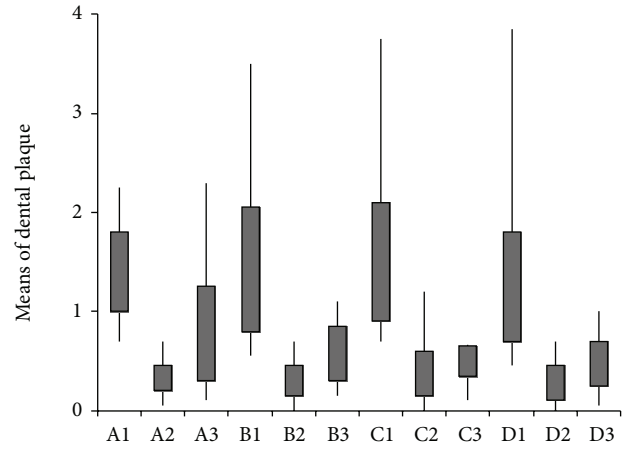

Figure 1: Mean values of biofilm for four solutions (A: Control; B: Plax; C: *B. dracunculifolia* extract and essential oil; D: Listerine) in three different experimental times (1: preliminary: basal level; 2: immediately after: measured after one-time use of mouthwash; 3: one week: a full week of mouthwash use).

and spread over all dental surfaces in order to establish the baseline index where the stained surfaces were marked in an appropriate dental chart [35].

Conventional brushes and toothpaste (soft brush and low abrasive dentifrice and no active ingredient) were provided to the twelve individuals who brushed their teeth and rinsed with the rinses formulations for 1 minute. New plaque disclosing and new dental chart with marked plaque index were performed.

Brushing and mouthwash procedures mentioned were made four times a day for a period of a week when new disclosure and new scores were marked. Before the replacement of mouthwash with another, there was another washout period in which the patients remained for a similar period of 24 hours without taking any kind of oral hygiene; to establish a new database and a new study of a formulation by a period of one week up to 4 groups used 4 formulations rinses. Thus, each experimental mouthwash was tested for a week in each subject. The total elapsed time during the full clinical study was 4 months.

A computer program generated randomization groups. The four products were compared between themselves. Also, the three different times for each product were compared too. A nonparametric Friedman test was used for these comparisons. When the Friedman test showed significant differences, Wilcoxon test for disclosure of different pairs to each other was performed. The program used for statistical analysis was SPSS for Windows, version 17 (SPSS, Chicago, IL, USA). For all tests, those differences were considered significant, where $P < 0.05$.

3. Results

Figure 1 illustrates the results of the plaque index (biofilm) recorded at each time for each mouthwash solution. The PI was measured by scores (ordinal categorical variable). Three rinses and one control Three rinses and one control (Listerine, Plax, formulation/testing with active component and basic formulation without active component) in 3 days (basal level, immediately after first use, and after one week of use) were tested to verify the modification of supragingival biofilm found in these times, with each of these solutions.

The comparison between the products in each time with the preliminary time (washout 24 hours, $t = 0$) does not present difference in plaque index (PI) of individuals for all products evaluated ($P = 0.510$), so individuals started the study with a similar index.

In measuring the time after use of mouthwash ($t = 1$), there was no difference between products ($P = 0.602$); that is, subjects in the study had a similar PI after the first use. After one week ($t = 2$), there was no difference between the four evaluated products ($P = 0.674$); that is, all research individuals completed the study with a similar reduction in plaque between themselves and different from initial state. To check which rinses differed during the different times ($P < 0.05$ in the Friedman test), each mouthwash was compared using the Wilcoxon test. Significant differences were observed ($P < 0.05$) in pairs, as we have shown: (A) there was significant difference between times (Friedman, $P < 0.001$); (B) there was no significant difference between the times (Friedman, $P < 0.001$); (C) there was no significant difference between the times (Friedman, $P < 0.001$); (D) there was significant difference between times (Friedman, $P < 0.001$).

4. Discussion

Propolis is a honeybee product that has been studied worldwide. It has been considered as food and nutraceutical products because of its medicinal characteristics [22]. Propolis contains a wide range of biological attributes, including antioxidant, antibacterial, antifungal, antiviral, antitumor, anti-inflammatory, and hepatoprotective activities [36, 37].

Such effects have been attributed to the presence of polyphenols, such as flavonoids and phenolic acids, in its composition [38].

B. dracunculifolia is used by bees to produce Brazilian green propolis. Besides, *B. dracunculifolia* is a medicinal plant used to prevent and treat diseases [39]. In the present study, the capability of *B. dracunculifolia* that decreases dental biofilm to protect against dental caries was investigated.

Our results revealed that the test formulation with active *B. dracunculifolia* reduced the rate of plaque (biofilm) after one week of use, Our results revealed that the test formulation with active *B. dracunculifolia* reduced the rate of plaque (biofilm) after one week of use, in the same level as chloride triclosan, Gantrez, and essential oils, that are established products in market used as agents of control plaque and halitosis of oral origin [40].

About the relation between *B. dracunculifolia* and oral diseases, there are only a few studies in the literature discussing a possible therapeutic application of these extracts in maintaining oral health. Leitão et al. [30] determined and compared the effects of green propolis and *B. dracunculifolia* extracts on the acid production and synthesis of glucans by glucosyltransferases from *S. mutans*. They investigated biological activities of secondary metabolites of *B. dracunculifolia* on cariogenic factors of *S. mutans* and corroborated the present study, since both suggest that *B. dracunculifolia* extracts could be successfully incorporated into pharmaceutical products employed in dental care. Also, we compared this plant with market products already used as agents for control of plaque and halitosis.

The inhibition of glucosyltransferase (GTF) activity is one of the mechanisms proposed to explain the action of *B. dracunculifolia* extract for caries prevention [30]. GTFs are important factors in the inhibition of bacterial cellular adherence in caries prevention. The glucans synthesized by GTFs promote the accumulation of cariogenic *Streptococci* on the tooth surface and contribute significantly to the production and development of dental plaque [41]. In this way, experimental data suggests that extract of *B. dracunculifolia* may inhibit bacterial metabolic reactions that precede cell doubling or may trigger precocious bacterial cell multiplication as an answer to the aggressive agent [30].

The production of acids from fermentable carbohydrates is one of the cariogenic factors of *S. mutans* that receive attention during the investigation of new medicines to be applied in dental care. In this way, the reduction effect of extract of *B. dracunculifolia* on the acidogenic potential of *S. mutans* is known, since it decreases bacterial acid production [30].

Essential oil of *Baccharis dracunculifolia* has been studied because of its exotic, strong, and long lasting aroma, which is highly prized by the perfume industry [42]. In the present clinical study, this remarkable feature was clearly evidenced by the reports of the patients who referred to the pleasant flavor and striking long-lasting taste during use and even the after taste. Preliminary study of sensory perception was performed in Preliminary study of sensory perception was performed in patients by a pharmaceutical industry. This work was conducted prior to this trial, so we can choose from four final formulations to produce the best market features. This excellent acceptance of volunteers for taste, quality foam, pH balance, color, and volume is a strong indicator of the attractiveness of the product to market rinses, which tends towards acceptance of natural products.

This study sought to maintain the liquid phase, color stability, aroma, and taste during the study period (4 months) with suitable foaming and no undesirable side effects during treatment. Among various systems used as carriers and with the release of active substances systems, the microemulsions can be highlighted, that is, emulsions formed by droplets smaller than 1 μm, which are presented as net, transparent, and thermodynamically stable isotropic dispersions consisting of water, oil, and surfactants [43]. Commonly, they exhibit various biological and pharmaceutical interesting properties, including biodegradability, biocompatibility, physical stability, and the ease of obtaining. Literature data demonstrate that the emulsions have long been used as carriers for lipophilic drugs [44], in stabilizing substances susceptible to hydrolysis, and in reduction of irritation or toxicity of some drugs [45]. These characteristics can explain the great acceptance of emulsion in present work.

B. dracunculifolia mouthwash is alcohol-free and, unlike those of chlorhexidine base, shows no incompatibility with previous use of toothpastes. There were no complaints of burning sensation after using this herbal product, unlike essential oils, whose assets are responsible for the sensation of burning mouth after use. It is known that the use of chlorhexidine before 30 seconds after toothbrushing is incompatible with toothpaste containing amphoteric detergents and sodium monofluorophosphate [46]. On the other hand, the chlorhexidine effectiveness has been higher than the use of triclosan [47]. However, due to chlorhexidine disadvantages, it is necessary to search for ideal antiplaque agent, which is not available [48]. In order to solve this problem, the demand for natural products has been considered. Medicinal plants have been widely used to treat a variety of infectious and noninfectious diseases, and 25% of the commonly used medicines contain compounds isolated from plants [49].

Despite the limitations of this study as sample size, study period, and the subjects' health conditions, this is the first work to report the comparison between a natural product and established commercial products as mouth rinses application. Based on the result that there is the same efficiency of the *B. dracunculifolia* and already marketed mouthwashes, we suggest the use of this natural substrate for prevention and reduction of dental biofilm, as well as caries disease, and more investigations are needed in order to explore the potential characteristics of *B. dracunculifolia* as a specific oral antimicrobial.

5. Conclusions

Collectively, our findings demonstrate there was no significant difference between different products used in this study, proving the effectiveness of hygiene complemented by mouth rinses in reducing the biofilm and the good effect of *B. dracunculifolia* in oral treatment. The importance of

this study is due to the natural characteristics of the new material that leads to advantages not found in existing products; however, this study should be completed with more investigations and studies, to explore the product in long term follow-up and laboratory tests to improve all the effects and side effects of the new product, since it will be used as medical product.

Conflict of Interests

The present authors clarify that there is no conflict of interests involved in this study.

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Research Article

Influence of the Nd:YAG Laser Pulse Duration on the Temperature of Primary Enamel

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The aim of this study is to evaluate the temperature change on specimens of primary enamel irradiated with different pulse duration of Nd:YAG laser. Fifteen sound primary molars were sectioned mesiodistally, resulting in 30 specimens (3.5 × 3.5 × 2.0 mm). Two small holes were made on the dentin surface in which K-type thermocouples were installed to evaluate thermal changes. Specimens were randomly assigned in 3 groups ($n = 10$): A = EL (extra long pulse, 10.000 μs), B = LP (long pulse, 700 μs), and C = SP (short pulse, 350 μs). Nd:YAG laser ($\lambda = 1.064\,\mu$m) was applied at contact mode (10 Hz, 0.8 W, 80 mJ) and energy density of 0.637 mJ/mm^2. Analysis of variance (ANOVA) was performed for the statistical analysis ($P = 0.46$). Nd:YAG laser pulse duration provided no difference on the temperature changes on primary enamel, in which the following means were observed: A = EL (23.15°C ± 7.75), B = LP (27.33°C ± 11.32), and C = SP (26.91°C ± 12.85). It can be concluded that the duration of the laser pulse Nd:YAG increased the temperature of the primary enamel but was not influenced by different pulse durations used in the irradiation.

1. Introduction

Developed by Johnson in 1961 [1], the Nd:YAG (neodymium doped with yttrium-aluminum-garnet, $\lambda = 1.064\,\mu$m) can be employed at continuous or pulsed mode emitting light with a wavelength located in the infrared range of the electromagnetic spectrum. This high-intensity laser usually has the diode laser as light guide and its absorption is diffuse and transmitted to the tissue through the optical fiber, favoring its application on the buccal cavity [2].

The Nd:YAG laser can be recommended in pediatric dentistry, since its use promotes increasing on the acid resistance of primary enamel [3, 4], sealing of pits and fissures [5] and effectiveness on the prevention of carious lesions [6, 7]. The parameters used during irradiation of dental structures must comply with the characteristics of the tissues, since the variation in surface temperature of the irradiated enamel can lead to higher heat conduction and hence spread to the pulp tissue causing irreversible damage. The temperature inside the pulp chamber should not exceed 5.5°C [8], since such heating may lead to tooth loss of vitality.

The thermal effect produced by Nd:YAG irradiation induces the formation of $TCPCa_3(PO_4)^2$ on enamel [9] and promotes changes in the organic matrix [9, 10] and alterations in the morphological [11], chemical [12, 13] and crystallographic aspects [14]. In dentin, the thermal effect promotes melting, formation of cracks and debris on the surface, and the modification of tubular dentin structure [15]. These alterations on dentin can occur due to the lower thermal conductivity of this substrate when compared to the primary enamel [16–18].

Thermal variations after employing Nd:YAG laser in permanent teeth have been reported [19–21]. The thermal

changes on the pulp chamber of primary teeth, caused by the Nd:YAG laser (picosecond-pulsed), were verified by Lizarelli et al., 2006 [18], using ablative parameters. The Nd:YAG laser (picosecond-pulsed) was considered by Lizarelli et al., 2006 [18], as a safe tool for primary teeth ablation, after checking the temperature response in the pulpal chamber and the anatomical constitution of teeth, once different topography of teeth results in profound differences in remaining dentin, with consequences for heat exchanges rate.

The pulse duration refers to the time that the tooth substrate is exposed to laser irradiation. The irradiation of primary teeth substrate promotes different times of thermal declines, which are related to the anatomical features [18]. The surface cooling can also be performed reducing the pulse duration [22], whereas the emission of shorter pulses can cause no thermal damage [23] to the irradiated surfaces. The pulse repetition rate has been described as being an important parameter with respect to heat deposition on lased-irradiated tissue. The higher the pulse repetition rate, the lower the cooling of the tissue between each pulse [24].

Due to the increased research, related to the use of laser technology in pediatric dentistry, and regarding the heat generation produced on dental substrates during irradiation, more studies evaluating the thermal changes in specimens of primary teeth irradiated with Nd:YAG laser using different pulse duration, extra long pulse (10.000 μs), long pulse (700 μs), and short pulse (350 μs), become necessary.

2. Material and Method

2.1. Experimental Design. The factor studied was the Nd:YAG laser pulse duration employed during the irradiation of enamel specimens of primary molars at 3 levels: A = EL (extra long pulse, 10.000 μs), B = LP (long pulse, 700 μs), and C = SP (short pulse, 350 μs). The experimental sample was composed by 30 specimens of primary human enamel, which were randomly assigned ($n = 10$), according to the design in randomized complete blocks. The quantitative response variable was the temperature change, in Celsius degrees, of the primary tooth substrate subjected to the Nd:YAG laser irradiation.

2.2. Teeth Selection and Preparation of Specimens. First and second upper primary human molars newly exfoliated were examined with an explorer probe #5 (Duflex, SSWhite, Rio de Janeiro, RJ, Brazil), using a stereomicroscope (Leica S6 D Stereozoom, Mycrosystems Leica AG, Switzerland), with increase of 20x. Those which presented cracks or hypoplasia were discarded. Fifteen teeth were selected and cleaned with periodontal curettes, being polished with Robinson brushes mounted in low speed turbine (Dabi Atlante, Ribeirão Preto, São Paulo, SP, Brazil) embedded in pumice and water, washed, and kept in 0.9% saline solution containing 0.4% sodium azide at 4°C [25].

The teeth were individually fixed by the coronary portion with thermoplastic wax (Wax Sculpture Fixed Prosthodontics, Aspheric Chemical Industry Ltda., São Caetano do Sul, São Paulo, SP, Brazil) in acrylic plates and taken to the section machine (Minitom, Struers A/S, Copenhagen, Denmark) in which the root portion, if it is present, was sectioned 1 mm below the cement-enamel junction. Then, the coronary portions were sectioned mesiodistally and from the buccal and lingual surfaces of each tooth specimens of 3.5 × 3.5 × 2.0 mm of thickness of enamel and dentin were obtained. The dimensions of the specimens were determined using a digital caliper (Myamoto, Tokyo, Japan), and the thickness was established with a specimeter (BioArt, São Carlos, São Paulo, SP, Brazil).

To standardize the thickness of enamel/dentin, specimens were taken to the polisher (Politriz DP-9U2, Struers A/S, Copenhagen, Denmark) and subjected to wear with sanding discs of silicon carbide #600 (Norton/Saint-Gobain Abrasivos Ltda., Guarulhos, São Paulo, SP, Brazil), aiming at planning and regularizing the surfaces. Using drill #1/4 (KG Sorensen, Barueri, São Paulo, SP, Brazil), mounted in high speed turbine (Roll Air 3, Kavo do Brasil S.A, Joinville, Santa Catarina, SC, Brazil), two holes were made manually by the same operator, at dentin surface (medium depth 0.1 mm) corresponding to the roof of the pulp chamber, to accommodate the thermal-sensors during the specimens irradiation. After these procedures, specimens were individually fixed with thermoplastic wax at cylindrical Plexiglass abutment (5.0 mm diameter) using a parallelometer to ensure that the enamel surface was kept parallel to the horizontal plane, aiming at sealing any space between the specimen and the plaque. The specimens were randomly assigned ($n = 10$) and kept in distilled water at 4°C until 2 hours before the experiment start [26].

The irradiated area was delimited by insulating type (3M do Brasil Ltda., Campinas, São Paulo, SP, Brazil), with a 4.0 mm^2 central window. Each group was irradiated with Nd:YAG laser (λ 1.064 μm) (Smartfile, Deka M.E.L.A, Calenzano, Firenze, Italy), at contact mode by means of 0.3 mm quartz fiber, which was positioned perpendicularly to the specimen. The parameters (10 Hz, 0.8 W, 80 mJ), energy density of 0.637 mJ/mm^2, and different pulse duration: A = EL (extra long pulse, 10.000 μs), B = LP (long pulse, 700 μs), and C = SP (short pulse, 350 μs), were applied to the specimens of primary enamel, for 30 seconds.

The laser parameters of Nd:YAG (10 Hz, 0.8 W, 80 mJ) used in this study were based on the favorable results obtained by [4, 6, 27, 28], to increase the acidic resistance to demineralization.

To evaluate the temperature change, a device consisting of a data acquisition card HI-Speed USB Carrier NI USB-9162 (National Instruments Corporation, Austin, Tx, USA) was used. The thermal filaments sensors (K-type thermocouples, Omega Engineering Inc., USA) were used to check the temperature. The board has 4 input channels with full resolution of 24 bits and 12 Hz maximum total rate of data acquisition.

The thermocouples were made with the aid of a spot welding of carbon and presented 120 μm of diameter and 40 cm in length. The system was connected to a computer and the Measurement and Automation Software and VI Logger Lite (National Instruments Corporation, Austin, Tx, USA), supplied by the manufacturer of data acquisition card, was employed for data collection. Two thermal-sensors strands

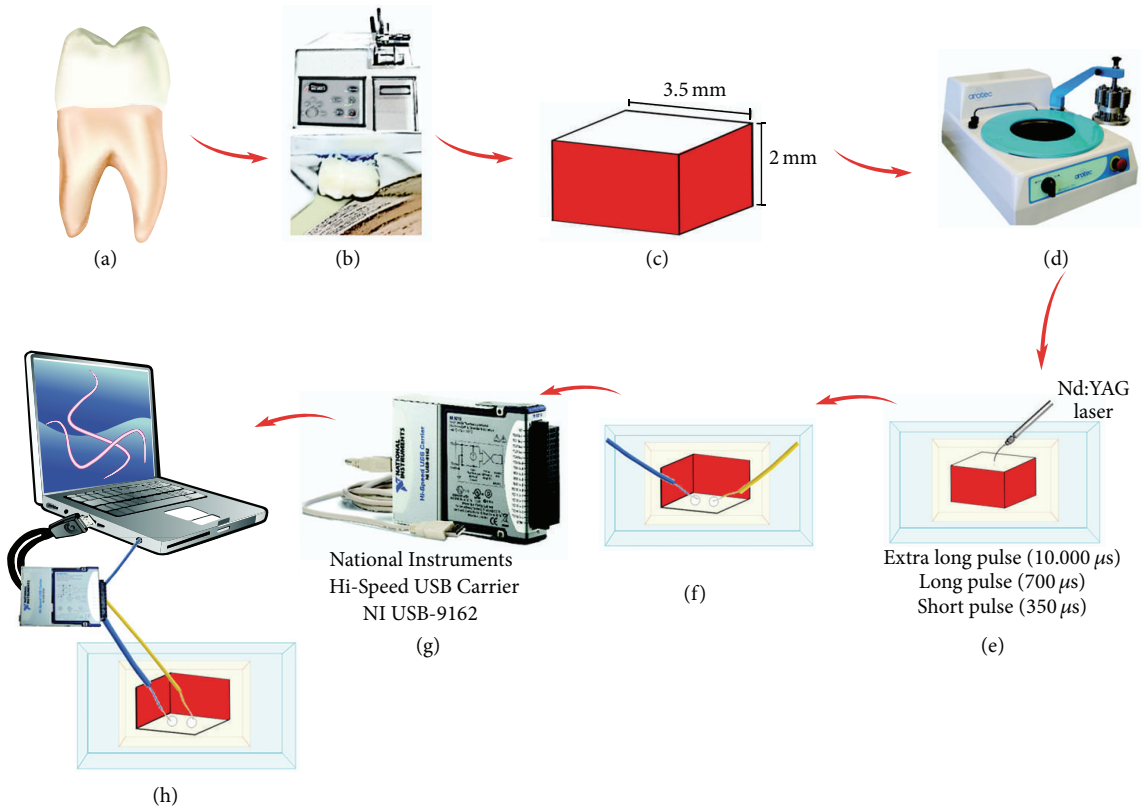

Figure 1: Schematic design of the employed methodology. (a) Primary molar; (b) section machine and sectioned tooth; (c) specimens (3.5 × 3.5 × 2.0 mm); (d) polisher; (e) fixation of specimens at acrylic plates, delimitation of irradiated area with insulating tape (4.0 mm^2), and irradiation with Nd:YAG laser; (f) holes to accommodate thermal-sensors (0.1 mm depth); (g) acquisition plate; (h) system connected to the computer.

were placed in the niches at the dentin surface of each specimen, providing better thermal contact between the thermal-sensors and the specimens; a thermal paste based on water was employed (Implastec, Votorantim, São Paulo, SP, Brazil).

For each specimen, the temperature (°C) was registered from the first laser pulse emitted by the Nd:YAG laser and repeated every 0.3 seconds, for 30 seconds using the K-type thermocouples adapted to the dentin surface 1 mm, under irradiated surface, and all measurements were performed in a temperature/humidity-controlled room. Figure 1 represents the schematic design of the employed methodology.

2.3. Statistical Analysis. The temperature mean value of each specimen was analyzed using the Kolmogorov-Smirnov test and presenting normal distribution and homogeneity of variance. Thus, analysis of variance (ANOVA) was used. Statistical analysis was performed with SPSS software for Windows, version 12.0 (SPSS Inc., Chicago, IL, USA).

3. Results

Results showed that temperature change during Nd:YAG laser irradiation using different pulse duration in specimens of primary enamel was statistically similar among themselves ($P = 0.46$), as shown in Table 1.

Table 1: Temperature changes (°C) in specimens of primary enamel, during Nd:YAG laser irradiation.

Types of pulse of Nd:YAG laser	Average ± SD
Short pulse (350 μs)	26.9 ± 12.8
Long pulse (700 μs)	27.3 ± 11.3
Extra long pulse (10.000 μs)	23.1 ± 7.7

The initial temperature and thermal changes during Nd:YAG laser irradiation at specimens of primary enamel are shown in Figure 2.

4. Discussion

The utilization of laser light to irradiate dental tissues has aroused great interest in the scientific community. The effects of laser irradiation on the tissue depend on the parameters used, such as wavelength, power, power density, exposure time, pulse duration, emission mode, energy density used per pulse, repetition rate, frequency, diameter, and beam characteristics [29, 30]. The use of reliable parameters for irradiation on dental structures may prevent possible thermal damage which could lead to irreversible pulp damage.

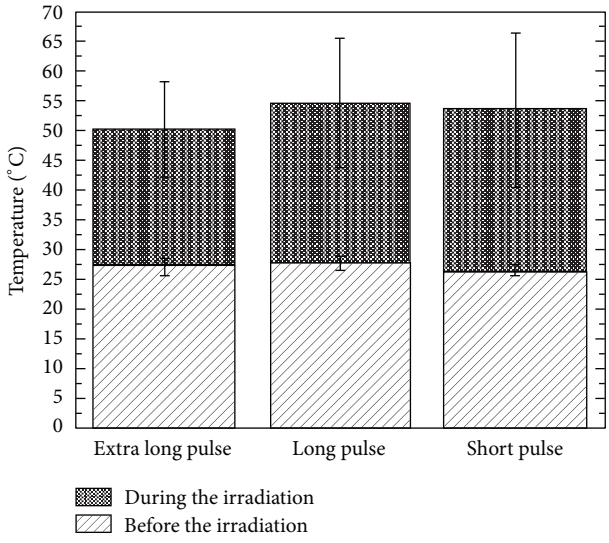

Figure 2: Initial temperature analyzed before irradiation and thermal changes (°C) measured at specimens of primary enamel during the Nd:YAG laser irradiation.

The increasing in temperature on dental tissues, produced during irradiation, is responsible for the morphological and structural changes of the irradiated surface [31, 32], thus making the tooth enamel acid resistant [29, 30].

According to Fowler and Kuroda, 1986 [12], increases in dental enamel temperatures result in structural and chemical alterations, such as loss of water and reduction in carbonate content. Besides, acid phosphate (HPO_4^{2-}) ions condensed to form pyrophosphate ($P_2O_7^{4}$) with thermal recrystallization and crystal size growth and the formation of tricalcium phosphate was observed, concomitant to the reduction of $P_2O_7^{4}$ ions. The lower amount of carbonate provides less solubility to hydroxyapatite, since carbonate causes crystal defects and does not fit so well in the lattice, generating unstable and more acid-soluble apatite phases. Pyrophosphate is able to inhibit the dissolution of hydroxyapatite crystals, whereas tri- and tetracalcium phosphates are potentially more susceptible to acid dissolution than hydroxyapatite.

The decomposition of the organic matrix is also responsible for increased acid resistance of tooth enamel. The temperature increase in the irradiated surfaces is responsible for the decreased permeability and reducing enamel solubility, causing proteins decomposition. The products of the organic material can obstruct the pores of the tooth enamel, preventing the acid ions penetration [33, 34].

On the other hand, it has been shown that irradiation with laser associated with a topical application of fluoride could increase the acid resistance by increasing the incorporation of fluoride [35] or for the greater transformation of hydroxyapatite in fluorapatite [36]. The synergism between the Nd:YAG laser and fluoride in reducing enamel solubility was verified by Zezell et al. 2009 [6] although Phan et al. 1999 [36] disagree stating that the laser irradiation can induce the formation of fluorapatite by the incorporation of fluoride in the enamel surface layer melted by the temperature increasing.

The temperature rise at pulp chamber in monkeys did not exceed 5.5°C, which would result in permanent/irreversible damage to the pulp [8, 37]. On the other hand, Baldissara et al., 1997 [38], reported that average temperature increases of 11.2°C, seen clinically and histologically, promoted no inflammation in the pulp tissue and thus may have not caused damage to the structure.

The present study compared temperature changes during irradiation with different pulse duration (extra long pulse (10.000 μs), long pulse (700 μs), and short pulse (350 μs)), on specimens of primary enamel using Nd:YAG laser, and no statistically significant difference was observed.

The results of this study showed that the temperature rise on specimens of primary enamel using Nd:YAG laser exceeded 5.5°C. A factor that may have contributed to this thermal change is based on the fact that irradiation was performed at contact mode, without air and water, by means of optical fiber. Similar results were found by Strakas et al., 2103 [39], who verified temperature changes higher than 5.5°C; however, they found significant difference between pulse duration of 180 μs and 320 μs in root canals irradiated with Nd:YAG laser.

Studies proved that when refrigeration is employed during irradiation of dental surfaces, there is a reduction on the temperature increase [40, 41], avoiding, thus, pulp necrosis [42] and carbonization of dentin [41]. The same relation of using water flow to reduce temperature was observed by [26], although they employed Er:YAG laser to irradiate specimens of primary enamel.

The photothermal interaction of Nd:YAG laser with dental tissues is obtained by means of its optical fiber, which, when irradiating dental surface, converts the absorbed laser energy in heat [43], causing higher temperature increase in teeth with less remaining dentin [19, 20, 44, 45], and the Nd:YAG laser wavelength (λ 1.064 μm) is poorly absorbed by dental structures [2].

The temperature found in specimens of primary enamel in the present study may differ from the temperature observed at pulp chamber, since, due to the support structures present around the teeth and the blood flow of the pulp tissue, this heat could be dissipated [46, 47]. The pulp temperature increase, related to the use of high power lasers, is based on the amount of energy applied and therefore the exposure time is crucial. High energy densities in short periods of time cause less pulp damage [44], since it is desirable to minimize the heat flux to reduce thermal injuries, providing enough power in less time than the diffusion of heat by conduction through the tissues, considering that the thermal relaxation is inversely proportional to the square of the irradiated volume [46].

The use of specimens of primary enamel with smaller thicknesses in the present study could also provide increased heat generation. This same relation was observed by von Fraunhofer and Allen, 1993 [48], and White et al., 1994 [20], who observed lower temperature changes in structures with greater thicknesses of enamel and dentin.

Thus, the temperature increase found in the present study can be based on the primary teeth mineralization, which is smaller, along with calcium and phosphorus percentage,

when compared to permanent teeth [49]; in addition, the thickness of primary enamel is almost half of the permanent tooth enamel [50].

Another factor that may have contributed on the temperature increase in the present study is the thermal conductivity, which is higher in dental enamel than in dentin [16–18]; however, this thermal conductivity exceeds the dentin tissue before reaching the pulp tissue [51]. It has also been stated that anterior primary teeth have the ability to cool more quickly at the beginning of irradiation, followed by a deceleration period. At posterior teeth, the cooling follows a linear decline, due to increased volume in the dental crown and amount of remaining dentin [18].

Comparison of these results with those found in literature is difficult, since the only study published in primary teeth [18] employed different methodology and ablative parameters to evaluate the thermal changes, and in the present study, the method validated in literature was chosen, following the protocols proposed by Contente et al., 2012 [26], and Brandão et al., 2012 [52]. The parameters of irradiation used in this study are recommended in primary enamel and responsible for increased acid resistance to demineralization [4, 6, 27, 28].

5. Conclusion

Considering the experimental conditions of this study, it can be concluded that the duration of the laser pulse Nd:YAG increased the temperature of the primary enamel but was not influenced by different pulse durations (extra long pulse (10.000 μs), long pulse (700 μs), and short pulse (350 μs)) used in the irradiation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Biopsy of Different Oral Soft Tissues Lesions by KTP and Diode Laser: Histological Evaluation

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Introduction. Oral biopsy aims to obtain clear and safe diagnosis; it can be performed by scalpel or laser. The controversy in this latter application is the thermal alteration due to tissue heating. The aim of this study is the histological evaluation of margins of "*in vivo*" biopsies collected by diode and KTP lasers. *Material and Methods*. 17 oral benign lesions biopsies were made by diode 808 nm (SOL, DenMatItalia, Italy) and KTP 532 nm (SmartLite, DEKA, Italy). Samples were observed at OM LEICA DM 2000; margin alterations were evaluated through Leica Application Suite 3.4. *Results*. Epithelial and connective damages were assessed for each pathology with an average of 0.245 mm and a standard deviation of ±0.162 mm in mucoceles, 0.382 mm ± 0.149 mm in fibromas, 0.336 mm ± 0.106 mm in hyperkeratosis, 0.473 mm ± 0.105 mm in squamous hyperplasia, 0.182 mm in giant cell granuloma, and 0.149 mm in melanotic macula. *Discussion*. The histologic aspect of lesions influenced the response to laser, whereas the greater inflammation and cellularity were linked with the higher thermal signs. Many artifacts were also associated to histologic procedures. *Conclusion*. Both tested lasers permitted sure histologic diagnosis. However, it is suggested to enlarge biopsies of about 0.5 mm, to avoid thermal alterations, especially in inflammatory lesions like oral lichen planus.

1. Introduction

A biopsy is a diagnostic procedure which consists in taking a tissue fragment to subject it to a histological examination and, therefore, to obtain a diagnosis of certainty that can or cannot confirm the suspicion clinical diagnostic [1].

Biopsies can be classified according to the used material, the clinician timing, the lesion site, and the used technique that can be distinguished in incisional and excisional biopsies. The incisional biopsy involves the removal of a representative portion of the lesion and a portion of healthy tissue adjacent to it [2–4]; while the excisional biopsy consists in the removal of the whole lesion allowing, at the same time, carrying out both a diagnostic and therapeutic procedure [4, 5].

The biopsy is generally indicated for the following:

(i) recognizing neoplastic, preneoplastic, and other soft tissue diseases;

(ii) identifying the origin of ulcers that do not heal within two weeks;

(iii) defining the nature of lesions that do not regress after therapy;

(iv) removing lesions of the right dimensions and verifying their nature.

Nowadays it is possible to perform oral biopsies using two different tools, the scalpel and the laser.

The scalpel allows obtaining a tissue fragment characterized by the presence of well-defined peri-incisional margins with no structural alterations. However, this surgery always requires anesthesia and sutures, and the operative field is not bloodless.

The laser devices most commonly used in oral soft tissues surgery are the diode (600–980 nm), the potassium titanyl phosphate (KTP, 532 nm), the carbon dioxide laser (CO_2, 10600 nm), the neodymium-doped yttrium aluminum

garnet (Nd:YAG, 1064 nm), and the erbium-doped yttrium aluminum garnet (Er:YAG 2940 nm).

Lasers, used for biopsies execution, have several advantages than the scalpel. In fact, they consent to obtain a good hemostasis, bloodless field and a faster healing, above all during the initial phases [6].

However, due to the thermal effects of the laser, incisional margins of tissue samples can be altered, creating doubts about the effectiveness of this method in the diagnosis of systemic disease [7]. If the use of this tool has many advantages over the cold blade, the risk of jeopardizing the outcome of histological analysis, due to laser thermal effects on peri-incisional area, still raises doubts. Actual scientific literature does not reveal *in vivo* studies concerning the evaluation of peri-incisional biopsy taken with the laser. Pathological tissues "*in vivo,*" compared to those "*ex vivo,*" are characterized by a higher concentration of liquid, lower cell cohesion, and normal or pathological amounts of blood (e.g., in inflammatory or autoimmune diseases).

This consideration could lead to an improvement in cutting ability of the laser that could permit the parameters applied to be reduced with less damage to cut margins but on the other hand to higher local heat buildup with larger thermal artefacts.

The aim of this "*in vivo*" study is to analyze the tissue fragments removed by laser surgery, to assess the epithelial and connective tissue damage caused by its thermal effects.

2. Materials and Methods

Seventeen patients (8F/9M), affected by oral benign pathologies, have been subjected to oral excisional biopsy. In some cases, lesions have been treated using an incisional biopsy because of their site or their size. All tissue samples have been removed by the same operator in order to execute a proper biopsy thanks to his experience and knowledge in laser tools and biological tissue characteristics.

Biopsies have been performed using two different wavelengths with the following parameters: diode laser 808 nm (SOL, DenMatItalia, Italy), power: 2 W in CW, fluence: 2400 J/cm^2, fiber spot: 320 μm; KTP laser 532 nm (SmartLite, DEKA, Italy), power: 1.5 W in PW, fluence: 212 J/cm^2, fiber spot: 300 μm. Parameters have been selected considering the right execution of the surgical intervention and the patient compliance never exceeding 5 minutes.

Local anesthesia with 1.8 mL of mepivacaine solution (Mepivacaina Pierrel, 30 mg/mL, injection solution 1.8 mL, Pierrel Spa, Milan, Italy) without vasoconstrictor was performed around the area of the lesion before the beginning of each surgical intervention, injecting the solution at a distance of 0,5 cm from lesions margins. The excised lesions size was between 0,5 and 1 cm of diameter. Two mucoceles were taken out by diode laser and one by KTP laser; 5 fibromas were excised by diode; 3 hyperkeratosis lesions were removed by diode and 1 by KTP; the 3 oral lichen planus, the melanotic macula, and the oral giant cell granuloma were removed by diode laser. After surgery, the samples were sent to the pathologist, for the histological evaluation and diagnosis, in a single-blind mode. No suture or medication was applied and the wound was left to heal by secondary intention. All biopsy samples were fixed in a 10% neutral-buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin-eosin for conventional histopathological evaluation.

Tissue fragments were again observed through the use of an optical microscope LEICA DM 2000, 5x and 10x magnification, and thanks to an appropriate software (Leica Application Suite version 3.4) quantitatively and qualitatively marginal alterations, due to the thermal action of lasers, have been evaluated. Quantitative evaluation carried out a measurement in millimeters and statistical analysis was carried out by calculating the arithmetic mean and standard deviation (a measure of the dispersion of data around the expected value) of the different values, while the involvement of epithelial and connective tissue in thermal alterations has been evaluated in the qualitative aspect. In every oral pathology, connective and epithelial damage have been evaluated in terms of charring and coarctation, since in many cases it was impossible to evaluate them separately.

3. Results

Follow-up at 7 and 21 days showed a complete recovery of the wound, without any complications or pain.

The presence of peripheral alterations has not influenced histological analysis: for all samples, it was possible to obtain a certainty diagnosis. Histological examination showed three mucoceles, five fibromas, four hyperkeratotic lesions, three oral lichen planus, one giant cells granuloma, and one melanotic macula.

Peripheral damage has been individually evaluated for each disease, considering that the morphological and structural characteristics of the various lesions could strongly influence the tissues response to the laser action.

Graphs have been realized to show the trend of the measures, and statistical analysis has been carried out calculating the mean and standard deviation of the different values. Moreover, in each histological group the same parameters have been evaluated.

The histological evaluation of peri-incisional margins in which the microscopic analysis was compatible with the diagnosis of mucocele showed a damage average of 0.245 mm with a standard deviation of ±0.162 mm (Figures 1 and 2; Table 1). Only in one case the epithelium was not visible because the damage was exclusively assessed to the connective tissue.

In the clinical cases in which the histological evaluation leads to diagnosis of fibromas (Figures 3 and 4; Table 2) the damage average was of 0.382 mm and the standard deviation was of 0.149 mm. Also in one of these cases, it was not possible to consider the epithelium of a sample because the damage has been measured only in the connective layer.

Histological evaluation of the peri-incisional margins of hyperkeratotic lesions (Figures 5 and 6; Table 3), compatible with the diagnosis of squamous hyperplasia, showed a damage average of 0.336 mm with a standard deviation of ±0.106 mm.

Furthermore, the histological evaluation of the peri-incisional margins of clinical cases whose diagnosis was oral

Table 1: Mucocele biopsies.

Patient	Sex	Lesion site	Laser	Damage (in mm)
D.C.	F	Inferior lip	Diode 808	0,442
N.F.	F	Upper left lip	KTP	0,213
S.S.	M	Inferior lip	Diode 808	0,102

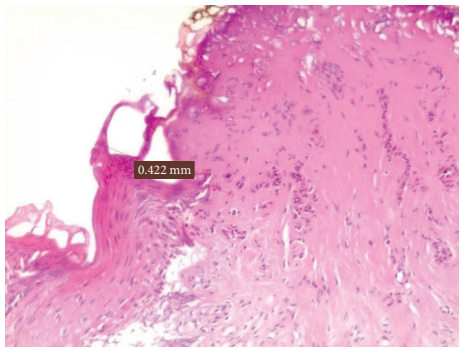

Figure 1: Damage measurement in a mucocele.

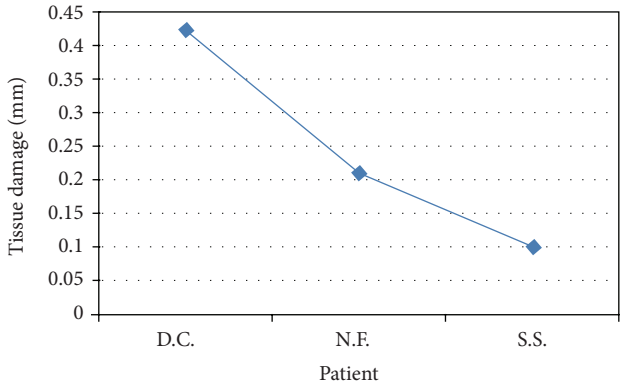

Figure 2: Peri-incisional marginal damage in mucocele.

lichen planus demonstrated a damage average of 0.473 mm with a standard deviation of ±0.105 mm (Figures 7 and 8; Table 4).

The damage average in the microscopic examination of the giant cell granuloma was 0.182 mm (Figure 9).

Finally, in the melanotic macula, removed by the diode laser 808 nm, the damage average was equal to 0.149 mm (Figure 10).

4. Discussion

Several studies are present in the literature about [8–11] the use of laser in oral soft tissue biopsy, but only few of them focus on the damage caused by this device at peri-incisional margins of tissue fragments. Every type of laser can create thermal damage to the target tissues because of the photothermal effect. While lasers work, they heat tissues, causing a temperature increase, at the point of incidence, of more than 100 degrees. Surrounding tissues can be involved in the increase of temperature and so they are permanently

Table 2: Fibroma biopsies.

Patient	Sex	Lesion site	Laser	Damage (in mm)
B.M.L.	F	Left cheek	Diode 808	0,411
D.G.	M	Left cheek	Diode 808	0,357
L.R.	F	Left tongue margin	Diode 808	0,267
O.G.	F	Left cheek	Diode 808	0,623
O.M.	M	Right cheek	Diode 808	0,252

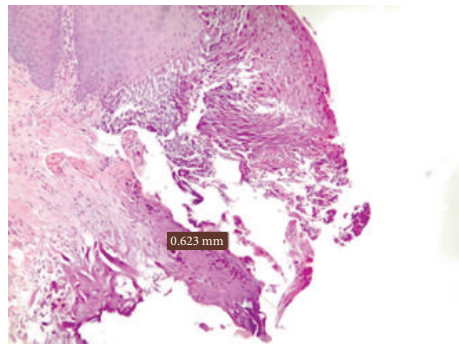

Figure 3: Damage measurement in a fibroma.

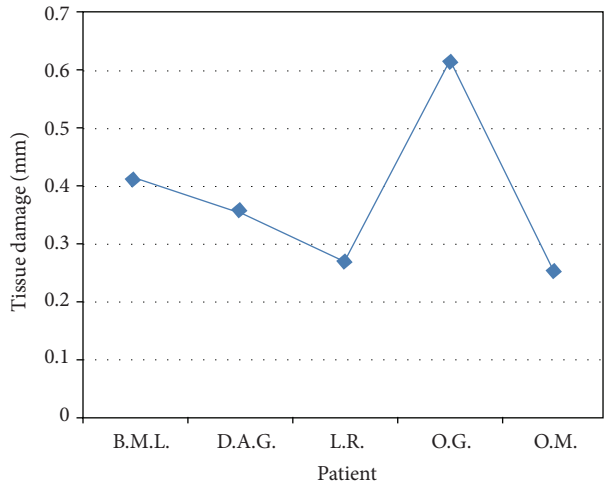

Figure 4: Representation of peri-incisional marginal damage in fibroma.

or reversibly damaged. Furthermore, the histologic exam is linked to the integrity of peri-incisional margins, and this is a basic requirement for a tool employed in biopsies.

A study carried out on rabbits by Rizoiu et al. [11] showed no differences in the histology among peri-incisional margins of samples excised by laser and by scalpel. In a Dalrymple and Russell's study [12], about the evaluation of peri-incisional margins of incisional and excisional biopsies performed by CO_2 laser on cervix lesions, it appeared that marginal alterations were on average 0.3 mm. However, due to thermal damage in 12% of cases the histological examination gave an uncertain outcome.

In a study carried out by Romeo et al. [13], the effects of Er:YAG, Nd:YAG, Er-Cr:YSGG, and two diode lasers (resp.,

Table 3: Hyperkeratotic lesion biopsies.

Patient	Sex	Lesion site	Laser	Damage (in mm)
M.B.	M	Right tongue margin	Diode 808	0,319
M.M.	M	Lower lip	Diode 808	0,446
Z.R.	F	Lower left lip	KTP	0,196
N.C.	M	Right cheek	Diode 808	0,383

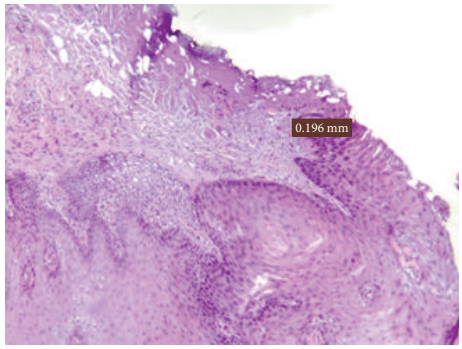

Figure 5: Damage measurement in a hyperkeratotic lesion.

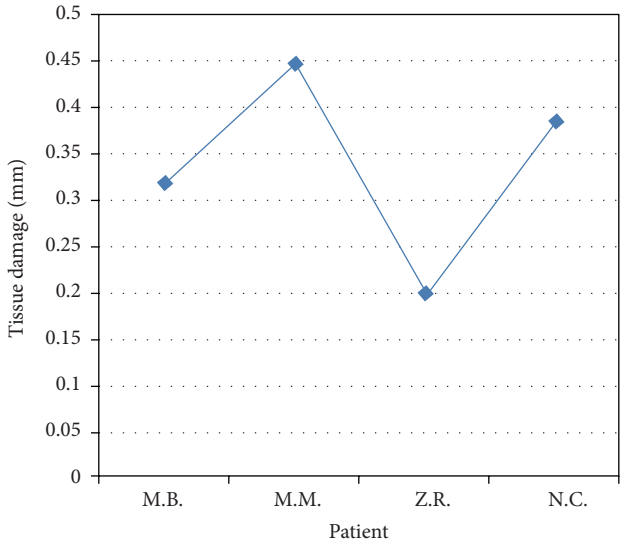

Figure 6: Peri-incisional marginal damage in hyperkeratotic lesion.

Table 4: Oral lichen planus biopsies.

Patient	Sex	Lesion site	Laser	Damage (in mm)
D.G.C.	F	Right cheek	Diode 808	0,504
L.L.	F	Right cheek	Diode 808	0,356
M.M.	M	Right cheek	Diode 808	0,561

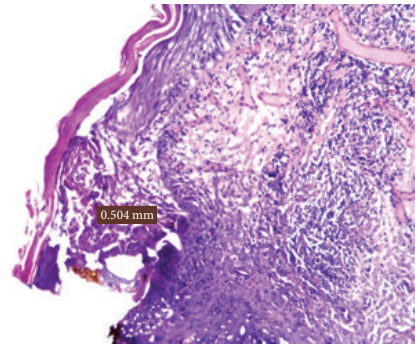

Figure 7: Damage measurement in an oral lichen planus.

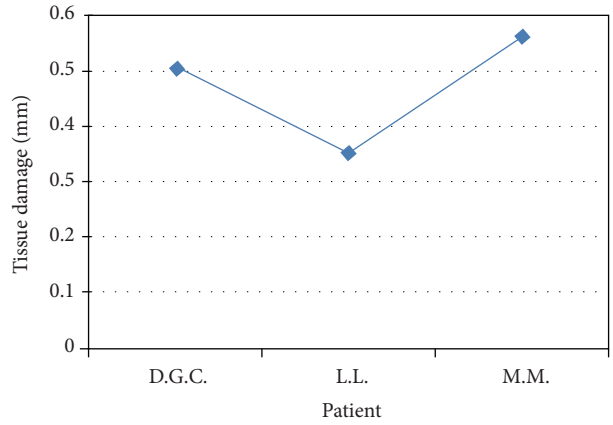

Figure 8: Representation of peri-incisional marginal damage in oral lichen planus.

808 nm and 980 nm) have been evaluated on pig tongue. It resulted in the fact that each kind of laser device could be used to perform biopsy. Even if they caused slight alterations in the taken tissue margins, no one of them compromised the histological evaluation. In particular, the best results have been obtained with the 808 nm diode laser device in pulsed wave mode and with Er-Cr:YSGG laser at higher power, which created peripheral damage less than 1 mm.

Another study carried out by Romeo et al. [14] about the histological evaluation of Er:YAG laser effect on oral soft tissues showed that, using this device with intermediate power (80–100 mJ), the thermal damage was always under the millimeter involving only the epithelium layer. So, authors concluded that thermal damages was negligible and the readability of the perioincisional margins was always possible.

Moreover, a study about the effects of the KTP [15] on oral soft tissue demonstrated that it allowed the execution of precise cut provoking a minimum cellular damage in the epithelium and in the chorion. The precision of the obtained margins make them similar to those ones obtained through the use of a scalpel. In addition to this, specimens of all tested groups were free from thermal artefacts above all when lowest fluence settings have been used.

Furthermore, in a study carried out by Merigo et al. [16] concerning the use of different wavelengths in laser-assisted surgery, it was shown that positive results have been obtained for the evaluation of laser-excised samples in terms of their readability and diagnostic reliability.

Vescovi et al. [17] performed a preliminary histological analysis of specimens from the human oral mucosa comparing Nd:YAG laser versus traditional scalpel. Epithelial

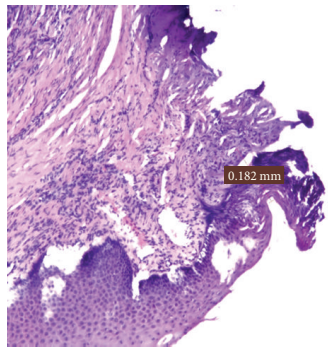

Figure 9: Damage measurement in a giant cells granuloma.

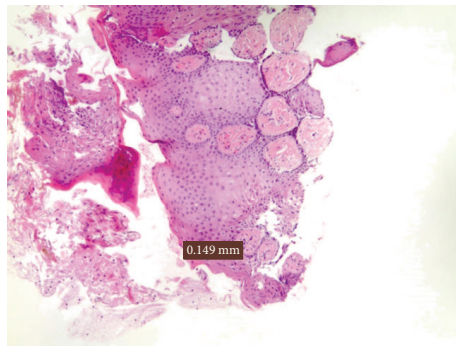

Figure 10: Damage measurement in a melanotic macula.

changes, connective tissue modifications, presence of vascular modifications, incision morphology, and the overall width of tissue modification were evaluated. Differences between specimens removed with two different parameters of Nd:YAG (3.5 W, 60 Hz and 5 W, 30 Hz) laser were not significant with regard to stromal changes and vascular stasis. The quality of incision was better and the width of overall tissue injuries was less in the specimens obtained with higher frequency and lower power (group 1: Nd:YAG laser at 3.5 W and 60 Hz).

In a retrospective study, Angiero et al. [18], 608 cases of soft tissue lesions localized in the oral cavity (cheek, gingiva, buccal mucosa, tongue, and lips) were examined. Specimens were excised with an 808 nm diode laser, output 1.6–2.7 W, in continuous-wave mode with fibers of 320 μm. The data for specimens larger than 3 mm excised with the diode laser were not significant in terms of stromal changes or vascular stasis, while epithelial and stromal changes were significantly more frequent in specimens with a mean size below 3 mm. Authors suggest that the specimens taken have "*in vivo*" a diameter of at least 5 mm in order to have a reliable reading of the histological sample, but this recommendation is valid even for a scalpel biopsy.

According to several studies the possibility to evaluate "*in vivo*" the marginal alterations of samples excised by laser is not clear. For this reason, there was a necessity to begin an "*in vivo*" study concerning the histological exam of peri-incisional margins after laser biopsy.

This study showed that the biopsy of oral soft tissues, performed by diode or KTP laser, did not create any significant marginal alterations that could compromise the histological diagnosis. Moreover, it shows, as explained in the previous "*ex vivo*" study [13], that the laser device that causes less thermal damage is the KTP. In general, in fact, the laser tissue interaction is due to the operator-dependent factors (modality of use, application time, and choice of the cutting distance from the lesion margins) and the operator-independent factors related to the wavelength and to the optical properties of the tissue.

The bioptic samples of this study showed that carbonization and coarctation were more limited in specific lesions (mucocele) than in others (oral lichen planus), demonstrating in this way how the increased cellularity and inflammation, typical of some lesions, can cause an increase of the peri-incisional damage.

Moreover, it is important to consider that many of the artifacts, found on the samples, were not due to the action of the laser but due to problems which occurred during the process of fixing, cutting, and staining of the tissue fragment.

Finally, the use of laser devices is not advisable to perform biopsies of suspicious lesions. In this case, the analysis of cellular infiltration in the adjacent tissues is fundamental and the thermal effects of the laser may affect the possibility to realize a proper analysis of the lesion margins and to establish the real cancer size [8, 19, 20].

5. Conclusions

Laser devices, used by a skilled operator, allow obtaining histological tissue fragments with important advantages both for the operator and for the patient. In fact, thanks to the laser-haemoglobin interaction, the surgical field is bloodless, permitting having greater visibility and also performing surgery in patients affected by coagulation disorders. Furthermore, it is possible to reduce the amount of local anesthesia and to achieve a faster postoperative healing, especially in the early stages.

In this study, it was always possible to obtain a sure histological diagnosis for each sample.

So, laser devices, because of their excellent surgical properties, can be used successfully to perform oral soft tissues biopsies, but a clinical preliminary analysis of the lesion is fundamental, in order to predict whether the peri-incisional thermal damage will be more or less extended. However, the peri-lesional damage did not compromise the morphological and structural characteristics of the specimens.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Er:YAG Laser for Brackets Bonding: A SEM Study after Debonding

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Background. The introduction of Er:YAG laser in dentistry for ablation of hard tissues advocated an alternative method of enamel etching for orthodontics purpose. *Materials and Methods*. 55 extracted human third molars were inserted in acrylic resin blocks and divided into five groups of 11 teeth. Group 1 was treated with 37% orthophosphoric acid for 30 seconds. Group 2 was treated with laser irradiation (Er:YAG Fidelius III, Fotona, Slovenia) at 80 mJ and 4 Hz. Group 3 underwent laser treatment (80 mJ, 4 Hz), followed by 37% orthophosphoric acid for 30 seconds. The teeth in Group 4 were treated with laser at 40 mJ and 10 Hz. The teeth in Group 5 were treated with laser (40 mJ, 10 Hz), followed by 37% orthophosphoric acid for 30 seconds. The adhesive remnant index was determined after debonding. *Results*. Kruskas-Wallis test showed that location parameters (median and mean) are significantly different between Groups 2 and 4 when compared with control group; on the contrary no significant difference was detected between Groups 3 and 5 with the controls. *Conclusion*. The use of Er:YAG laser alone, as in Groups 2 and 4, showed no significant advantages over phosphoric acid in the bonding procedure for orthodontics brackets.

1. Introduction

Phosphoric acid etching is the gold standard method of enamel preparation before application of bonding resins for orthodontic brackets [1]. Enamel etching changes the tooth surface from being of low-energy and hydrophobic to being of high-energy and hydrophilic, increasing the surface area for bonding [2]. Studies have demonstrated that this kind of attachment can have disadvantages, such as enamel decalcification, which leaves the enamel surface susceptible to acid attack (cavity formation) under orthodontic brackets [3–6]. One of the most important challenges in orthodontic treatment, however, is the frequent debonding of brackets, with the consequent lengthening of treatment duration.

With the recent introduction of erbium-doped yttrium aluminum garnet (Er:YAG) laser in dentistry for the ablation of hard tissues, including enamel and dentin, laser enamel preparation has been proposed as an alternative to phosphoric acid etching [7–9]. The Er:YAG laser can effectively modify enamel and dentin surfaces because of its 2.94 mm wavelength emission, which coincides with the main absorption band of water and OH− groups in hydroxyapatite [10].

In dentistry, the Er:YAG laser is primarily used to ablate hard tissues (enamel, dentin, and bone), but also to treat soft tissues [11–14]. Many papers [15–17] have reported that Er:YAG laser ablation of enamel and dentin is effective and efficient without causing heat damage to the pulp and without carbonization or cracks in the irradiated enamel and dentin. Moreover, use of the Er:YAG laser for dental hard tissue treatment, such as caries removal, cavity preparation, and enamel etching within certain parameters, is both safe and effective [18–21]. Additionally, the surface created by laser etching is reportedly resistant to carious attacks [22]. One study reported that the ultrastructural morphological

changes in the surface enamel of permanent teeth after irradiation with Er:YAG laser were similar to lava flow, with an opened prism core and modification of the prism form [23]. To evaluate the advantages of the Er:YAG laser for enamel surface preparation before orthodontic bracket bonding, this study compared the adhesive remnant index (ARI) scores of teeth treated with different bonding procedures.

2. Materials and Methods

Our study included 55 intact human third mandibular and maxillary molars, extracted for orthodontic reasons. The inclusion criteria were noncarious lesions or enamel defects. The teeth were stored in saline solution at 4°C for no more than 28 days before insertion into acrylic resin blocks. The teeth were then divided in five groups of 11 teeth each. The first group (control group) was treated with 37% orthophosphoric acid (etching solution, ORMCO, USA) for 30 seconds. The second group was treated with laser irradiation (Er:YAG Fidelius III, Fotona, Slovenia) at 80 mJ and 4 Hz. The third group underwent laser treatment (80 mJ and 4 Hz), followed by 37% orthophosphoric acid for 30 seconds. The teeth in the fourth group were treated with laser at 40 mJ and 10 Hz. The fifth group underwent laser treatment (40 mJ and 10 Hz), followed by 37% orthophosphoric acid for 30 seconds.

To limit the area of enamel treated, a ceramic window was prepared with the exact dimensions of an orthodontic bracket. The ceramic window was held on the tooth surface by one operator while a second one applied the acid or laser light treatment only to the area within the window (Figure 1). The Er:YAG laser was used with the following parameters: VSP mode (pulse length, 100 μs) with the noncontact handpiece (mirror) in a focus mode (theoretical distance from the tooth surface, 10 mm) using water/air spray in a continuous movement on a theoretical spot 0.8 mm in diameter (one spot next to another). The same operator (R. Kornblit) performed all *laser* enamel conditioning under 2.5 × 350 magnification (Univet medical eyewear). Immediately after enamel surface preparation, a bracket (Damon MX3-UR3, ORMCO) was attached by an experienced orthodontist (G. Ierardo) to each tooth following the different procedure for each single group as explained above. All teeth were dried before bonding placement. The bonding was performed using the same bonding adhesive (ORTHOSOLO, ORMCO) and a composite material (GRENGLOO, ORMCO) (Figures 2, 3, and 4). A microbrush was used to apply adhesive for 10 seconds on each surface, followed immediately by a thin layer of composite resin and a bracket. Teeth were cured for 30 seconds with a Coolbeam Orthodontic Curing Light (ORMCO). The bonded teeth were then kept in saline solution in five different plastic boxes at room temperature for 48 hours to allow complete polymerization. After 48 hours, all brackets were manually removed from the 55 teeth by the same experienced orthodontist (G. Ierardo), using a debonding plier (AEZ 8664008, ORMCO) designed for this procedure and exerting continuous rotational force toward the cervical part of the tooth (Figure 5). All 55 teeth were then sectioned in vertical (mesiodistal) and horizontal (cervical) directions with an abrasive disc (COD: Yellow-Flex 220) by the same operator who performed the laser preparation (R. Kornblit).

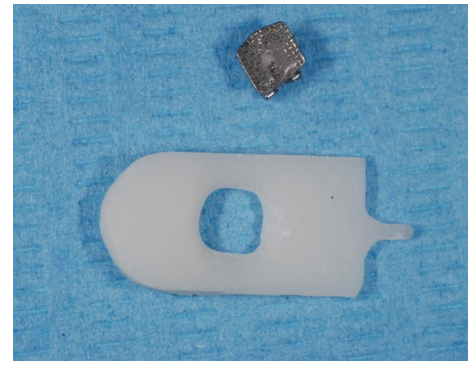

Figure 1: Ceramic mask equipped with the central hole of the size corresponding to the bracket base.

Figure 2: Phosphoric acid, bonding and composite resin.

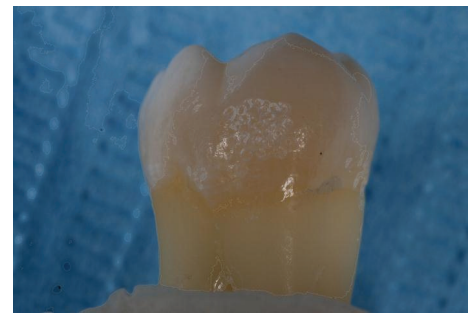

Figure 3: Enamel surface after conditioning with Er:YAG laser.

All 55 samples were dipped for 30 seconds in an ultrasonic bath at 30°C to remove any residual powder left after sectioning. The samples were then kept in an oven at 40°C for 24 hours to remove all moisture, which can interfere with the vacuum needed for metallization. All samples were then conventionally metallized (Gold sputtering JEOL JFC 1100E) and observed under scanning electron microscope (SEM) (JEOL, JSM 5310 LV).

The ARI score was recorded by a senior student who was not informed regarding the different procedure applied for each tooth using a stereoscope (Nikon, Tokyo, Japan) at 10x magnification to determine the amount of residual adhesive

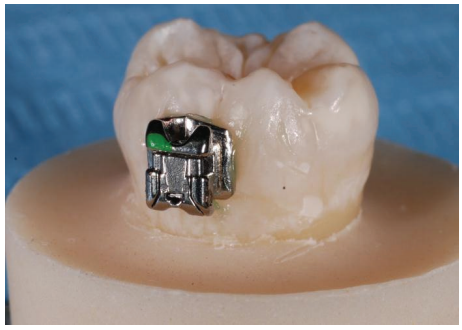

Figure 4: Bracket bonded on the enamel.

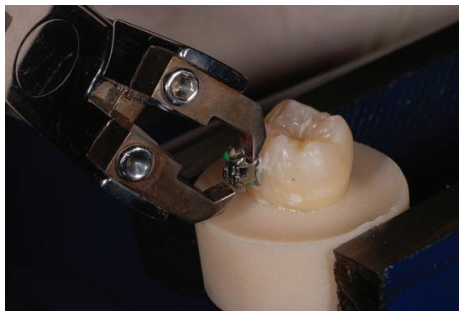

Figure 5: The sample stabilized by a vice, during the debonding.

remaining on each tooth, as described by Contreras-Bulnes et al. [24]. ARI scores were recorded using the 5-point scale described by Bishara and Trulove [25, 26]: 1 = no composite adhering to the bracket base, 2 = adhered composite on less than 10% of the bracket, 3 = adhered composite on more than 10% but less than 90% of the bracket, 4 = adhered composite on more than 90% of the bracket, and 5 = composite adhering to the entire bracket base.

2.1. Statistical Analysis. The statistical analysis aims at testing whether location parameters for variable "ARI score" are statistically different between each treatment and the control group.

ARI score is an ordered categorical variable, so non-parametric statistics are used. Thus, median, interquartile difference, and Kruskal-Wallis rank sum test [27, 28] are used in place of mean, standard error, and ANOVA, which are suitable for numeric and normally distributed variables.

At first a graphical analysis is performed, drawing box-plots of "ARI score" in control group and in each treatment.

Secondly a single group analysis is performed, computing descriptive statistics (median, 1st and 3rd quartile) in each group (Table 1).

Thirdly the null hypothesis that the medians are the same in each group is tested against the alternative that they differ in at least one group by the Kruskal-Wallis rank sum test. As the null hypothesis is rejected, the after Kruskal-Wallis multiple comparison test between treatments versus control is performed.

Table 1: ARI score in whole sample and within groups—descriptive statistics.

Group	*n*. obs.	Min.	1st Q.	Median	3rd Q.	Max.
Control group	11	1	1	1	1,5	2
Group 2	11	2	2	4	4	4
Group 3	11	1	2	2	3	4
Group 4	11	1	1	3	3,5	5
Group 5	11	1	1	3	3	5
Whole sample	55	1	1	2	3	5

3. Results

The macroscopic observation of the composition material on the tooth surface was as follows.

(i) Group 1: 6 samples presented all the composite that remained on the tooth; in 5 samples part of the composite remained on the tooth and a part on the bracket.

(ii) Group 2: 4 samples presented all the composite that remained on the tooth; in 7 samples part of the composite remained on the tooth and a part on the bracket.

(iii) Group 3: 2 samples presented all the composite that remained on the tooth; in 8 samples part of the composite remained on the tooth and a part on the bracket and 1 sample presented all the composite that remained on the brackets.

(iv) Group 4: 4 samples presented all the composite that remained on the tooth; in 2 samples part of the composite remained on the tooth and a part on the bracket and 5 samples presented all the composite that remained on the brackets.

(v) Group 5: 5 samples presented all the composite that remained on the tooth; in 5 samples part of the composite remained on the tooth and a part on the bracket and 1 sample presented all the composite that remained on the brackets.

The descriptive statistics regarding ARI score for each single group is presented in Table 1. No cracks were observed under SEM in any of the 55 samples. Boxplots highlight that all treatments show higher location and dispersion towards control group (Figure 6), so significant differences are expected. The null that location parameters of "ARI scores" are the same in each group is rejected, as the Kruskal-Wallis rank sum test is 13.8863 and its P value is 0.007667. The Kruskal-Wallis multiple comparison test show that the null is rejected at 5% significance level when comparing Group 2 and Group 4 to control and at 1% significance level when comparing Group 2 and control (Table 2).

4. Discussion

ARI score results showed that the best composite resin retention to the enamel surface occurred in the control group

Table 2: Results of Kruskal-Wallis multiple comparison test, treatment groups versus control (two-tailed).

Comparisons	Observed diff.	*P* value					
		10%		5%		1%	
		Critical diff.	Difference	Critical diff.	Difference	Critical diff.	Difference
Control group—Group 2	23,636	15,312	TRUE	17,063	TRUE	20,653	TRUE
Control group—Group 3	14,909	15,312	FALLS	17,063	FALLS	20,653	FALLS
Control group—Group 4	17,273	15,312	TRUE	17,063	TRUE	20,653	FALLS
Control group—Group 5	12,364	15,312	FALLS	17,063	FALLS	20,653	FALLS

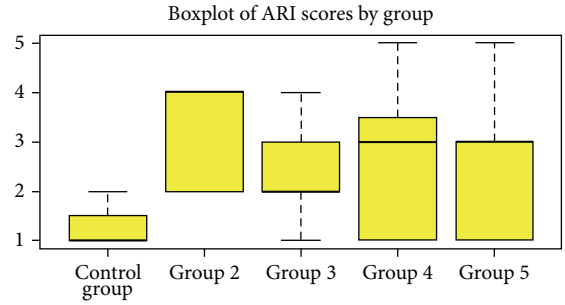

Figure 6

(Group 1), in which the enamel surfaces were prepared with acid etching alone. Groups 3 and 5, in which the enamel surfaces were treated with laser before acid etching, had better retention of the composite material to the tooth surface compared with Groups 2 and 4. This finding can be explained by the fact that laser irradiation destroys enamel prisms in an indifferent way: the core of the prism as well as the walls is destroyed (Class 3 in the Silverstone classification), resulting in the typical lava flow appearance of the enamel surface under SEM [20, 21]. This surface is poorly wettable by the bonding material; when phosphoric acid is applied to this surface, the acid attacks and regularizes the enamel's surface, increasing the microinfiltration capacity of the bonding material.

No cracks were observed at the periphery of the bracket attachment in any of the 55 samples, confirming that debonding forces did not damage the enamel surface. This lack of damage probably resulted from the use of an appropriate adhesive system and a specific instrument designed for bracket debonding. Moreover, for samples in which part of the composite remained on the tooth surface and part on the bracket, the group treated with laser alone did not have as homogeneous an adhesive area after debonding as that of the samples treated with acid. This finding confirmed the Silverstone Class 3 classification of enamel surfaces treated with Er:YAG laser.

Bishara and Trulove believed that bond failure at the enamel-adhesive interface was preferable to failure at other locations, because it leaves less residual adhesive and consequently requires less chair time for removal [25]. Several years later, the same author demonstrated that bond failure at the bracket-adhesive surface was better than at the enamel-adhesive interface, because it reduced the risk of enamel fracture and crazing during debonding [26]. We found that acid etching produced the most instances of debonding at the bracket-adhesive surface. We believe that reducing the risk of damage to the pulp is crucial when the debonding procedure is applied in orthodontics.

Authors have reported that laser etching is a valuable method comparable to the classical acid etching procedure [29–32]. These studies were based on shear bond strength measurements; the different laser irradiation protocol makes comparisons with the present study difficult. Moreover, as stated by Contreras-Bulnes et al., this method can result in substantial enamel loss [24]. Our results support those of Martínez-Insua et al., who reported that adhesion to dental hard tissues after Er:YAG laser etching is inferior to that obtained after conventional acid etching [33].

5. Conclusion

The use of Er:YAG laser *alone* showed no significant advantages over phosphoric acid etching in the bonding procedure for orthodontic brackets. Taking into account the cost and the additional time required to use the laser, this technology does not currently represent an added value for orthodontists in improving resin adhesion.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

SOPROLIFE System: An Accurate Diagnostic Enhancer

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Objectives. The aim of this study was to evaluate a light-emitting diode fluorescence tool, the SOPROLIFE light-induced fluorescence evaluator, and compare it to the international caries detection and assessment system-II (ICDAS-II) in the detection of occlusal caries. Methods. A total of 219 permanent posterior teeth in 21 subjects, with age ranging from 15 to 65 years, were examined. An intraclass correlation coefficient (ICC) was computed to assess the reliability between the two diagnostic methods. *Results.* The results showed a high reliability between the two methods (ICC = 0.92; IC = 0.901–0.940; $P < 0.001$). The SOPROLIFE blue fluorescence mode had a high sensitivity (87%) and a high specificity (99%) when compared to ICDAS-II. *Conclusion.* Compared to the most used visual method in the diagnosis of occlusal caries lesions, the finding from this study suggests that SOPROLIFE can be used as a reproducible and reliable assessment tool. At a cut-off point, categorizing noncarious lesions and visual change in enamel, SOPROLIFE shows a high sensitivity and specificity. We can conclude that financially ICDAS is better than SOPROLIFE. However SOPROLIFE is easier for clinicians since it is a simple evaluation of images. Finally in terms of efficiency SOPROLIFE is not superior to ICDAS but tends to be equivalent with the same advantages.

1. Introduction

Dental caries is a preventable and reversible infectious disease process [1, 2] to which people are susceptible throughout their lifetime [2]. Despite the benefits of its prevention through fluorides, toothpastes, sealants, improvements in diet, oral health education, and dental care [3], dental caries still remains a major problem worldwide [4] affecting 60–90% of schoolchildren and the vast majority of adults [5, 6]. Its prevalence is around 80% worldwide [7]. Molars and premolars are the most vulnerable teeth of caries attack related to the morphology of their occlusal surfaces [8] and the difficulty of plaque removal [9]. Many dentists continue to intervene when caries are still at enamel level [10]. For that reason, accurate preoperative diagnosis of caries depths and early occlusal caries detection are important to establish adequate preventive measures and avoid premature tooth treatment by restoration [9].

To date, there are two major techniques aimed at helping clinicians in detecting caries on occlusal surfaces [11] represented by visual examination and by light-based caries diagnostic tools as fiber optic transillumination (FOTI), DIAGNODent tool (KaVo), and SOPROLIFE. Visual examination of caries has progressed by establishing the international caries detection and assessment system (ICDAS) [12]; indeed, ICDAS-II, the second version, was improved and developed to provide a standardized system [13] to enable clinicians to diagnose and detect the first visual change in enamel leading to better information for clinical management [14, 15].

All diagnostic tools for detection and quantification of dental caries have to obey safety regulations, detect and differentiate shallow and deep lesions, and make monitoring possible by taking precise and quantitative measurement; in addition they have to be cost-effective and user-friendly [13].

The principle of FOTI is used since the seventies [16]. This technique uses a narrow beam of bright white light

Table 1: International caries detection and assessment system criteria used in visual examination [11].

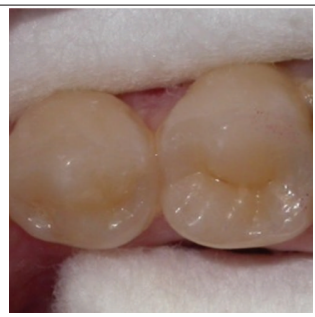
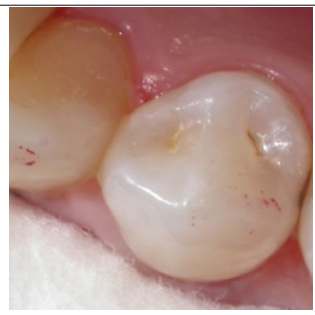
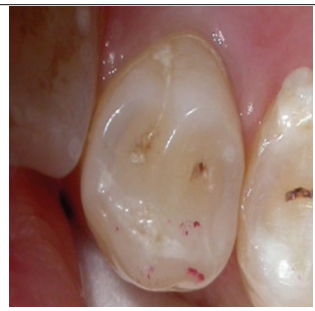
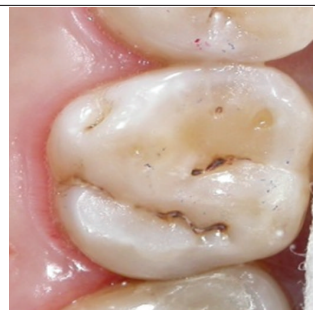
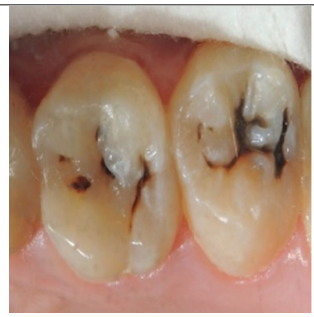
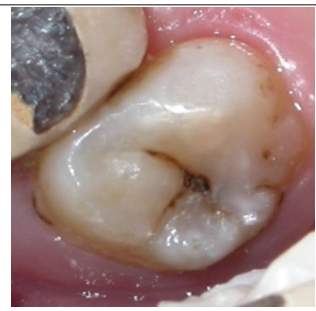
Six-point scale categories	Criteria	Clinical lesions
0	Sound tooth surface.	
1	First visual change in enamel.	
2	Distinct visual change in enamel.	
3	Microcavitation in enamel.	
4	Underlying dark shadow from dentine with or without cavitation.	

Table 1: Continued.

Six-point scale categories	Criteria	Clinical lesions
5	Distinct cavity with visible dentine.	
6	Extensive distinct cavity with visible dentine.	

that is directed across areas of contact between the proximal surfaces and the disruption of crystal structure that deflects the light beam and thus produces shadows [1]. The DIAGNODent tool is based on laser fluorescence and detects porphyrins involvement areas; it appears to measure caries lesion rather than crystalline demineralization [17]. SOPROLIFE is a more recently released device using a light-induced fluorescence evaluator for diagnostic and treatment (LIFE D.T); it was developed and based on the imaging and autofluorescence of dental tissues [18, 19].

Till now, no study has looked at the reproducibility of the SOPROLIFE in the detection and assessment of occlusal caries. Therefore we designed a clinical study with the aim of evaluating the clinical sensitivity and specificity rates of SOPROLIFE as opposed to ICDAS for the detection of initial occlusal caries in noncavitated enamel in permanent premolars and molars.

2. Materials and Methods

2.1. Sample Patients' Selection. This study was conducted over 2 months from March 7 to May 10, 2013. Twenty-one patients were randomly selected (based on their arrival order) from all patients attending the Aesthetic and Restorative Dentistry Department of the Dental School of Lebanese University. Inclusion criteria were age between 15 and 65 years, with no gender restriction, and patients with fully unrestored dental arches.

Exclusion criteria were patients with posterior restorations on molars or premolars or poor oral health with chronic or acute dental infection. In addition patients with a significant past or current medical problem history were not considered for the study, that is, patients with conditions that may affect oral health or oral flora (i.e., diabetes, HIV, and heart conditions which require antibiotic prophylaxis) or taking medication that may affect the oral flora or salivary flow; pregnant or breastfeeding women were also excluded. The subjects who met the criteria were informed of the purpose of the study and verbal consent from the patient was obtained before the examination session.

Examiners start evaluating patients using the ICDAS. After finishing all the samples, they did the work using SOPRO. One should note that patients ID was hidden when working with SOPRO.

2.2. Observers. Two independent dentists (Mona Zeitouny and Mireille Feghaly) specialized in restorative and aesthetic dentistry randomly examined each tooth by two different methods of caries assessments consecutively and without knowing the results of each method: the visual examination and assessment using the ICDAS-II criteria (Table 1) [20] and the use of the light fluorescence device SOPROLIFE (SOPRO, ACTEON Group, La Ciotat, France). This method involves an intraoral LED light-emitting diode camera offering the ability to detect and locate differences in density, structures, and/or chemical composition of a biological tissue.

Twenty days prior to the initiation of the study, calibration sessions were arranged for the 2 operators and the two methods separately in the examination site. Observers were trained using 100 premolars and molars cleaned without sealants or restorations. Each observer examined each tooth and noted the results. Then, the observers compared the results between them and reviewed the discrepancy cases for calibration until the two observers reach a full concordance rate.

2.3. Tooth Cleaning. Before examination, the occlusal surfaces of each tooth were cleaned for 10 seconds with a water powder jet cleaner and sodium bicarbonate powder (EMS) (ProphyFlex II, KaVo and Biberach; Germany) and then rinsed by an air water spray for another 10-second period in order to remove any powder remnants from the fissure. Following this preparation step of the tooth surfaces, all examinations were conducted under standard conditions in a professional dental light with a front-surface dental mirror and an oil-free air syringe for drying teeth during 5 seconds. The drying procedure is a requisite both for the ICDAS-II evaluation and for the use of the SOPRO device.

2.4. Visual Examination. The visual examination was performed using the ICDAS-II criteria, which provides a standardized method of lesion detection. The ICDAS-II detection codes for coronal caries range from 0 to 6 depending on the

Table 2: Scores of SOPROLIFE in blue fluorescence mode [13].

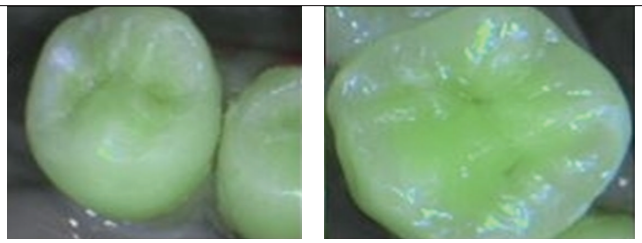
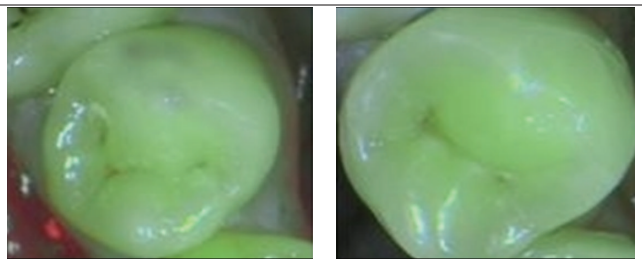
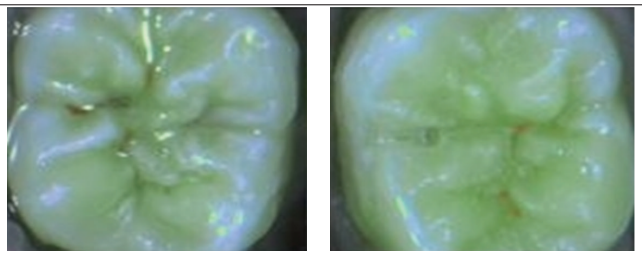
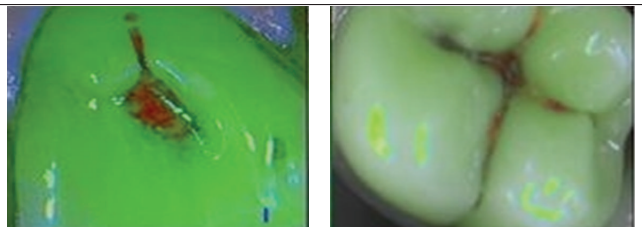
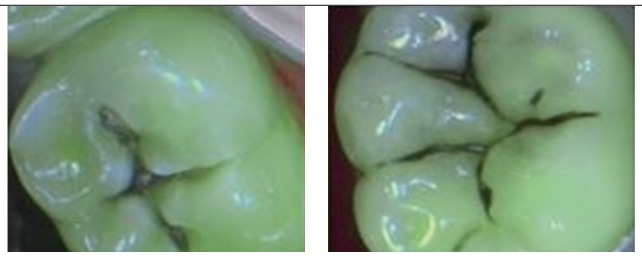
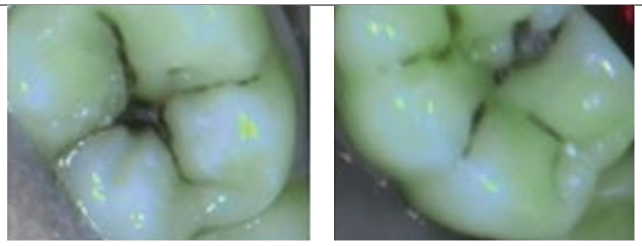
Five-point scale categories	Criteria	Clinical lesions
0	Fissure appears as shiny green; enamel appears sound. A graphite-pencil-colored thin shine/line is rarely observed.	
1	Tiny, thin red shimmer in the pit and fissure system is viewed. No red dots appeared.	
2	In addition to tiny, thin red shimmer in pits and fissures possibly coming up the slopes darker red spots confined to the fissure are visible. There was no surface roughness.	
3	Dark red extended areas are confined to the fissures. Slight roughness is possible.	
4	Dark red areas are wider than fissures. Surface roughness occurs. Possibly grey or rough grey zone may be visible.	
5	Obvious enamel breakdown with visible dentine was observed.	

Table 3: Characteristics of the study population.

Characteristics	N (%) or mean (SD)
Patients age	30.61
Male patients	11 (52.4%)
Female patients	10 (47.6%)
Permanent molars	98 (44.7%)
Permanent premolars	121 (55.3%)

severity of the lesion with the corresponding clinical views (Table 1).

In this study, we used the SOPROLIFE light-induced fluorescence evaluator system (SOPRO, ACTEON Group, La Ciotat, France) operating in the blue fluorescence mode, in which the system uses four white LEDs, and the magnification mode I with the disposable intraoral protection sheets and the intraoral tip. The blue LED, selected by the device, emits at a 450 nm-wavelength which excites a light fluorescence signal re-transmitted by dentine. The spectrum of the fluorescence signal is green when the dentine is healthy and dark red when the dentine is infected (according to the SOPROLIFE manufacturer's instructions). The images were recorded with the SOPRO IMAGING software. An HP 620 Notebook was used to collect the data.

When evaluating occlusal fissure areas in the SOPROLIFE blue fluorescence mode, we used the SOPROLIFE blue fluorescence mode score description as presented in Table 2.

Code 0 was given when the fissure appears shiny green, the enamel appears sound, and there are no visible changes. Code 1 was selected if a tiny, thin red shimmer in the pits and fissure system is observed, which can slightly come up the slopes (walls) of the fissure system. No red dots appeared. At code 2, darker red spots confined to the fissure are visible. For code 3 dark red spots have extended as lines into the fissure areas but remain confined to the fissures. A slight beginning roughness of the more lined red areas can be visible. If the dark red (or red-orange) extends wider than the confines of the fissures, code 4 was given. Code 5 was selected if obvious openings of enamel were seen with visible dentin [13].

2.5. Data Collection. Each tooth was evaluated and scored for lesions severity using a seven-category scale (0–6) according to ICDAS-II and a six-category scale (0–5) according to SOPROLIFE.

2.6. Statistical Analysis. Data were analyzed using the SPSS program (version 17, SPSS Inc., Chicago, IL, USA). In all analyses, a P value < 0.05 was considered significant. Demographic data are presented as mean ± one standard deviation (SD). Interobserver reproducibility with each examination method and between methods was assessed using intraclass correlation coefficients (ICCs). ICC values equal to 0 represent agreement equivalent to that expected by chance, while 1 represents full agreement. ICC values between 0 and 0.2 indicate poor agreement, values between 0.3 and 0.4 indicate fair agreement, values between 0.41 and 0.6 indicate moderate agreement, values between 0.61 and 0.8 indicate strong agreement, and values greater than 0.8 indicate almost perfect agreement. In addition, a Bland and Altman analysis was done to show graphically the difference between the two methods. Sensitivity and specificity of the new diagnostic system for detecting caries in noncavitated lesions were calculated by reference to the ICDAS-II values. Since we are interested in noncavitated lesions, the calculation was made by dividing scores of the two diagnostic methods into two groups: group 1 which included scores 0 representing healthy teeth without caries and group 2 which included scores 1 and 2 both representing visual change in enamel.

3. Results

This study compared the SOPROLIFE device (in blue fluorescence mode) to the ICDAS-II in the detection of caries lesions. Twenty-one patients were evaluated in this study and a total of 219 teeth (98 permanent molars and 121 permanent premolars), without sealants or restoration, were examined. The patient sample consisted of 10 women and 11 men with age ranging from 15 to 65 and involved mostly young adult patients in their thirties (Table 3).

3.1. ICDAS-II and SOPROLIFE Scores Distribution. The recorded data by each observer are presented in Table 4. Most lesions were noted in the 0 to 2 range of ICDAS-II criteria or in the 0 to 5 range of SOPRO blue fluorescence codes.

3.2. Interobserver Reproducibility. The reproducibility of measurements by each observer was first calculated by the means of ICC for each observer and for each diagnostic method (ICDAS-II and SOPROLIFE) as shown in Table 5. The level of interobserver agreement was found to be high both for visual ICDAS-II scored examination (ICC = 0.972; $P < 0.001$) and for SOPROLIFE (ICC = 0.979; $P < 0.001$).

3.3. Agreement between ICDAS-II and SOPROLIFE Methods. Since each observer examined and scored the same teeth by both ICDAS-II and SOPROLIFE, the means of the two measurements done by each observer were calculated for each tooth and diagnostic method and used to determine agreement and reliability between the two methods. The reliability between methods was computed using the intracoefficient correlation scale; here we considered the ICDAS-II and SOPROLIFE scales as quantitative variables. Means values for each method (ICDAS II mean = 1.69 ± 1.48 and SOPROLIFE mean = 1.56 ± 1.52) did not differ significantly and a high intraclass correlation coefficient was found (ICC = 0.92; CI = 0.901–0.940; $P < 0.001$). Thus, our results showed a high agreement between the two methods of caries detection.

Figure 1 shows the Bland-Altman analysis. The x-axis shows the mean of the results of the two methods ([SOPRO + ICDAS-II]/2), whereas the y-axis represents the absolute

Table 4: Distribution of the ICDAS-II and SOPROLIFE blue fluorescence mode scores by both observers.

Method	Observer	0 (*n*)	1 (*n*)	2 (*n*)	3 (*n*)	4 (*n*)	5 (*n*)	6 (*n*)
ICDAS-II score ($n = 219$)	1	56	56	61	16	10	20	—
	2	45	59	60	20	10	21	—
SOPROLIFE blue fluorescence score ($n = 219$)	1	51	57	64	16	10	21	—
	2	68	52	60	11	7	21	—

Table 5: Interobserver repeatability among the two observers.

Type of examination	ICC* (CI† 95%)
ICDAS-II	0.972‡ (0.964–0.979)
SOPROLIFE	0.979‡ (0.972–0.984)

*ICC = intraclass coefficients.
†CI = confidence interval.
‡P value < 0.001.

difference between the two methods ([SOPRO − ICDAS-II]). Our results showed an acceptable discrepancy between methods (Figure 1).

3.4. Sensitivity and Specificity for the SOPROLIFE. In this study we further attempted to estimate both sensitivity and specificity of the SOPROLIFE blue light irradiation in regard to the visual examination score ICDAS-II used as a reference. Sensitivity was measured as the proportion of actual caries lesions which are correctly diagnosed by SOPROLIFE in regard to ICADS-II, whereas specificity was measured as the proportion of noncarious lesions which were correctly diagnosed by SOPROLIFE in regard to that of ICADS-II. For this purpose, we considered the following two groups: the noncarious (sound tooth surface) lesion group that comprised the 0 scores for each method and the visual change in enamel group that included both score 1 and score 2 groups for each method. These results showed that SOPROLIFE detects noncarious lesions in 88% (specificity measurement) of the cases diagnosed by ICDAS-II. Visual change in enamel was detected by SOPROLIFE in 93% (sensitivity measurement) of the cases detected by ICDAS-II (Table 6).

4. Discussion

The present study assessed and compared the newly marketed caries lesion detection tool SOPROLIFE diagnostic mode to the ICDAS-II system.

The results of this study found an almost perfect agreement among the two methods of caries detection with no statistical significant differences between scoring with visual examination and LED fluorescence. This indicates that the diagnosis made with visual examination is roughly the same as the diagnosis made by SOPROLIFE. In addition, according to our results, the number of teeth with 0 score was greater when using fluorescence LED with no statistical difference.

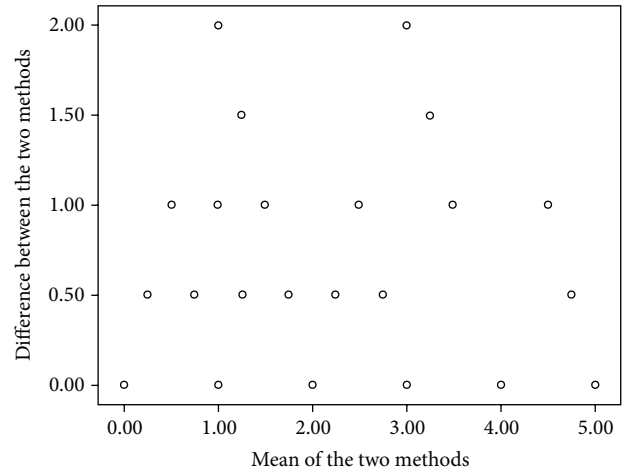


Figure 1: Bland-Altman analysis.

The perfect agreement between the two techniques found in our study has been demonstrated in previous study [13]. The visual examination is routinely used for detecting caries in dental clinics and was also used in recent studies comparing the efficacy of various visual aids. It has the benefit that it is quick and easy to perform, does not need expensive equipment, and can be completed without unnecessary radiation or fluorescence [14]. On the other hand, in the results from *in vitro* study conducted to determinate which nondestructive diagnostic method is clinically applicable and reliable at resolving early enamel changes in occlusal fissure caries created in the laboratory, SOPROLIFE demonstrated only additional light scattering due to the demineralization process [21]. *In vitro* and *in vivo* studies showed that different fluorescence signals emitted by SOPROLIFE were a helpful guide for caries detection and excavation [19].

Despite the clinical comparable results between the two diagnostic methods found in our study and in literature, the visual examination presents many limitations in its use. Indeed, one of its limitations is that it requires subjective evaluations to be made by the practitioner; lesions can go undetected because teeth are typically examined by the naked eye. In addition, studies showed that training dental examiners is an essential component of good quality control in dental research [22]. The examiners should be experienced dentists with an interest in cariology and the teeth should be well cleaned for a better visual examination [23]. Stained sites, areas of fluorosis, or developmental defects could be incorrectly scored as caries [9]. By meticulously examining clean

Table 6: Validity of the SOPROLIFE regarding ICDAS-II.

Tools	Group 1* % (*n*) of teeth scored as noncarious and non cavitated	Group 2† % (*n*) of teeth scored as carious and non cavitated	Sensitivity‡§b	Specificity‡‖b
ICDAS-II	25.5 (56)	53.3 (117)		
SOPROLIFE	29 (64)	52 (114)	93%	88%

*Group 1: including score 0.

†Group 2: including scores 1 and 2.

‡Sensitivity and specificity calculated by taking the ICDAS-II as a gold standard.

§Sensitivity measured the proportion of actual caries lesions which are correctly diagnosed by SOPROLIFE regarding ICADS-II.

‖Specificity measured the proportion of noncarious lesions which are correctly diagnosed by SOPROLIFE regarding ICADS-II.

bTrue negative results = 46; true positive results = 104; false negative results = 8; false positive results = 6.

dry teeth, sensitivity of a visual examination can be improved after a short training period [9]. Furthermore, meticulous visual inspection with a good operation light, a dry tooth, and a probe can render good sensitivity and specificity values [14]. The readings may also be influenced by several factors such as calculus, plaque and prophylactic pastes, and nonconsistent cleaning procedures [12]. Therefore, caries detection by eyesight is better at an advanced stage than early and presents many limitations related to the experience of the examiner and to the preparation procedure of the teeth examined. Consequently, diagnosis of the caries process by visual inspection is partial and auxiliary methods are needed as adjunct to conventional examination for identifying and quantifying such lesions [12, 24]. In addition, one other disadvantage of ICADS-II is that no images can be taken in order to save the findings for longitudinal monitoring.

In contrast, with SOPROLIFE system, the lesion and its real topography can be seen in a magnified enlarged view [13]. Several studies have shown that the additional observation with the SOPROLIFE camera might also prevent unnecessary operative interventions based on high fluorescence scores due to the better visibility [23, 25]. Due to that "visibility" of the lesion, the interpretation of higher fluorescence answers is easier [26]; the observation capacity of the SOPROLIFE system should guide the clinician toward a more preventive and minimally invasive treatment strategy with monitoring lesion progression or remineralisation over time and not tempt him/her to overtreat a lesion [27].

When comparing the measurements between the two examiners for both methods, our results demonstrated a high reproducibility among the two methods of diagnosis. These results indicated similarity in diagnosis among the 2 observers with both techniques. Despite the different degrees of experience in detecting caries between the two observers, this high interobserver agreement could result from the fact that the observers were from the same department and had a suitable training and calibration session before starting teeth's examination.

In the current study, ICDAS-II was set as "gold standard" [14] due to validated relationship between its codes and the histological depth of a carious lesion as in many other studies [13, 28]. In addition, several studies have shown good reproducibility and accuracy of ICDAS-II for occlusal caries detection in permanent teeth [29] especially caries lesions in the outer half of the enamel [29].

Our results show a high sensitivity and specificity of SOPROLIFE blue fluorescence mode consistently with other studies [13], probably due to its better visibility.

Finally sensitive caries diagnostic tools can serve not only for early detection but also for monitoring of caries lesions to confirm the success of prevention and remineralisation efforts. In order to limit diagnostic errors resulting not only from failure to detect caries, but also from unnecessary preparing of healthy fissures, it is vital to enhance the visual examination (ICDAS-II method) with other sensitive and specific methods as the SOPROLIFE system.

5. Conclusion

Compared to the most used visual method in the diagnosis of occlusal caries lesions, the finding from this study suggests that SOPROLIFE can be used as a reproducible and reliable assessment tool. At a cut-off point, categorizing noncarious lesions and visual change in enamel, SOPROLIFE shows a high sensitivity and specificity. We can conclude that financially ICDAS is better than SOPROLIFE. However SOPROLIFE is easier for clinicians since it is a simple evaluation of images. Finally in terms of efficiency SOPROLIFE is not superior to ICDAS but tends to be equivalent with the below advantages.

(i) High-resolution fluorescence images are likely to provide reliable scores. The better visibility of such images could prevent unnecessary operative intervention.

(ii) We can compare images (before and after).

SOPROLIFE may suffer from interference since it is light based. It might also give false positive results if images are magnified above a certain threshold. Both effects are not elaborated within this study.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

The Effects of CO_2 Laser with or without Nanohydroxyapatite Paste in the Occlusion of Dentinal Tubules

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The aim of this study was to evaluate a new treatment modality for the occlusion of dentinal tubules (DTs) via the combination of 10.6 μm carbon dioxide (CO_2) laser and nanoparticle hydroxyapatite paste (n-HAp). Forty-six sound human molars were used in the current experiment. Ten of the molars were used to assess the temperature elevation during lasing. Thirty were evaluated for dentinal permeability test, subdivided into 3 groups: the control group (C), laser only (L−), and laser plus n-HAp (L+). Six samples, two per group, were used for surface and cross section morphology, evaluated through scanning electron microscope (SEM). The temperature measurement results showed that the maximum temperature increase was 3.2°C. Morphologically groups (L−) and (L+) presented narrower DTs, and almost a complete occlusion of the dentinal tubules for group (L+) was found. The Kruskal-Wallis nonparametric test for permeability test data showed statistical differences between the groups ($P < 0.05$). For intergroup comparison all groups were statistically different from each other, with group (L+) showing significant less dye penetration than the control group. We concluded that CO_2 laser in moderate power density combined with n-HAp seems to be a good treatment modality for reducing the permeability of dentin.

1. Introduction

Dentin hypersensitivity (DH) arises from exposed dentin in response to tactile, thermal, osmotic, evaporative, and chemical stimuli, which cannot be attributed to any other form of dental defect or pathology. DH is characterized by short, sharp pain and is often faced as a bothering symptom in dental clinics [1]. Basically, exposure of the dentin results from one of two processes, either removal of the enamel covering the crown of the tooth or denudation of the root surface by loss of cement and overlying periodontal tissues [2, 3].

Hypersensitive teeth have a larger number and wider diameter of exposed DTs than normal teeth. That is the reason why treatment modalities often focus on decreasing the radius of the open dentinal tubules (DTs) [4]. Scanning electron microscopic (SEM) examinations of human DTs showed a number of approximately 20,000/mm^2 at the surface of peripheral dentin [5]. Isik et al. [6] stated that, on untreated dentin, the diameter of DTs ranges from 1.76 to 2.12 μm.

In 2006, Bartold [7] postulated that 14.3% of all patients have some degree of sensitivity. Incidence peaks around the third decade of life with no gender preference [8, 9]. Maxillary

premolars are the most commonly affected teeth and cold drinks are most often the triggering factor [10, 11]. DH prevalence seems to range from 60% to 98% in patient with periodontitis [12]. So dentin hypersensitivity is a widespread and common problem.

In spite of extensive research, DH mechanism and management are still only partially understood. One mechanism in the treatment of DH is hydrodynamic one; here the treatment focus is on desensitizing agents and dentifrices mainly containing fluorides that have the ability to seal or occlude the DTs through calcium fluoride crystal precipitation. Another one is neural, by decreasing the activity of the dentinal sensory nerve. Potassium nitrate is mainly used for this mechanism. Until now, no treatment certainly eliminates DH [10, 13].

In 1935, Grossman [14] listed the basic requirements that an ideal dentifrice or desensitizer should have, which are still valid now: nontoxic material, not irritating the pulp, being easy to apply and spread, being consistently effective, being permanently active, and having rapid performance, and it should not cause tooth discoloration. Considering possible materials to use for this purpose, nanohydroxyapatite (n-HAp) could be a good option because of its similar composition to tooth and bone. It is a widely accepted material in dentistry and in medicine, because it has a very high level of biocompatibility and bioactivity [15, 16]. n-HAp particle diameter is in the nanometer scale, which is much smaller than that of the DTs. In general, surface area and chemical reactivity of the material increase with decreasing particle size. Assuming equal masses of nano- and micrometer particle diameter of the same material, the surface area and chemical reactivity are approximately 1000-fold greater [17].

Since the invention of the laser by Maiman [18] in 1960, researchers have investigated laser applications in dentistry, and since that time, lasers have added additional revolutionary treatment options for both hard and soft tissue applications in dentistry.

The mechanisms by which lasers act on tissue depend on factors of the tissue itself and the laser parameters. Lasers have also been used in the treatment of DH. High output power laser systems such as neodymium: yttrium-aluminum-garnet (Nd:YAG), erbium: yttrium-aluminum-garnet (Er:YAG), and carbon dioxide (CO_2) can decrease or even eliminate dentinal pain due to their ability to occlude DTs [19, 20]. Diode laser achieved good results in DH treatment through causing stenosis of the DTs and reduced dye penetration across the dentin [21]. Pashley et al. [22] reported that CO_2 laser irradiation is able to occlude DTs and decrease dentinal permeability by reducing hydraulic conductance.

The selective absorption of 10.6 μm CO_2 laser by hydroxyapatite paste makes this wavelength the appropriate candidate for treatment of DH. To our knowledge, there are no studies addressing the combination of a CO_2 laser and a nanoparticle hydroxyapatite paste with this extremely small particle size. This small particle size possesses the ability to enter inside the DTs to increase [23] its surface area and to promote its absorption onto tooth surfaces.

This study investigates in vitro the effects of CO_2 laser with or without n-HAp on dentin permeability, temperature elevation, and morphology.

2. Materials and Methods

2.1. Sample Preparation. In this study 46 sound extracted human molars were selected (due to periodontal indications) after approval of the Ethics Review Committee of the Medical University of Vienna on research (EK Nr: 980/2009). Thirty molars were used for the permeability test, six samples for the SEM examination, and the last ten teeth for temperature measurement. After teeth apices were mounted in acrylic resin, two horizontal sections were made using a diamond saw blade (915 DC, Meisinger, Germany), which is mounted on a low speed hand piece (W&H A 25 RM, Dabi Atlante, Austria) under running distilled water. One section was done at the cement-enamel junction and the second 3 mm apical to the first one. The cementum was removed by a periodontal curette (4L-4R, GC-AMERICAN, USA) before the second sectioning was done. Samples of 3 × 4 mm area were obtained from each molar. The teeth were immersed in 1% citric acid solution for 5 minutes [24] for smear layer removal, then washed with distilled water in an ultrasonic bath for 15 minutes, and dried with gauze (Figure 1).

The surface and cross section morphology of all samples were evaluated using SEM (Tabletop Microscope TM-1000, Hitachi high technologies corp., Tokyo, Japan). Six samples were used; three of them were utilized for assessment of surface morphology and the other three samples were used to examine the cross section morphology after different treatment modalities. For the cross section examination, the three samples were fractured with a dental chisel after making a groove at the pulpal side of the sample opposite to the lasing side, with the aid of a diamond bur mounted on a high speed air motor with water spray.

A whole tooth was used for the temperature measurement experiment. The lasing was done after cementum removal and marking an area of 3 × 4 mm for lasing. A hole was made with a diamond fissure bur in the root surface opposite to the lasing area, until reaching the pulp cavity. The teeth were mounted in acrylic resin at the crown portion (Figure 2).

2.2. Samples Grouping and Treatment. Thirty-six exposed DTs samples were divided into three groups ($n = 12$) (Table 1), the control (C), laser (L−), and laser plus n-HAp (L+), two per group for the SEM and ten for each group for the permeability test. The (C) group samples received no treatment after surface conditioning with 1% citric acid. For the group (L−) the samples were irradiated with a CO_2 laser only, and for the (L+) group the CO_2 laser irradiation was done after applying the n-HAp paste (M K Impex Corp., Ontario, Canada). With an average particle size of 60 nm, the paste was prepared by mixing the n-HAp powder with distilled water, and then it was added to the dentin surface by a microbrush 10 times over a period of 10 minutes with hand pressure. After that, excess paste was brushed away. The samples were irradiated with a CO_2 laser (OpusDuo EC,

Table 1: Sample grouping and treatment.

Groups	Laser	n-HA paste	Time (s)	Interval (s)	Power (W)	Spot size (mm)	Pd[a] (W/cm^2)
C[b]	—	—	—	—	—	—	—
L–[c]	CO_2	–	6X (5 s)	5X (20 s)	0.65	0.4	129.33
L+[d]	CO_2	+	6X (5 s)	5X (20 s)	0.65	0.4	129.33

[a]Power density.
[b]Control group.
[c]Laser only group.
[d]Laser plus nanoparticle hydroxyapatite paste group.

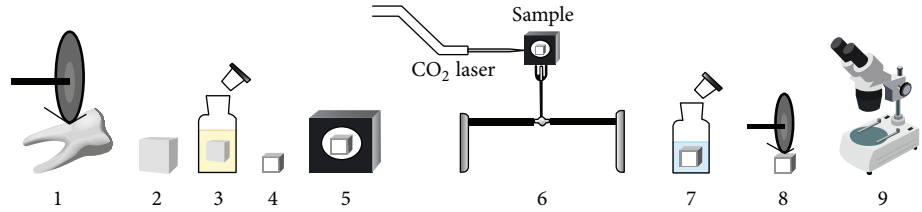


Figure 1: Permeability test setup and method. 1: tooth sectioning. 2: samples collection and cementum removal. 3: immersion in 1% citric acid for 5 min. 4: marking of the lasing area and nail varnish coating. 5: mounting in acrylic resin mold. 6: lasing, 7: immersion in 2% methylene blue for 1 hr. 8: longitudinal sectioning. 9: stereomicroscopic examination.

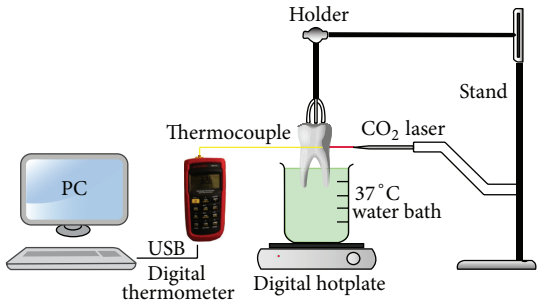


Figure 2: Experimental setup for temperature measurement.

Lumenis Germany GmbH), 10.6 μm wavelength, 0.65 W, in a continuous mode perpendicular to dentin surface, with 5 mm defocus distance, and a power density of (129.33 W/cm^2). The lasing was done according to Moritz et al. [20], 6 times for 5 s with 20 s interval for cooling in between. After treatment, the samples were rinsed with a distilled water spray for 15 seconds.

2.3. Dye Penetration Test. The specimens were coated with three layers of nail varnish except in the marked area and then were immersed in an aqueous solution of 2% methylene blue dye for 1 hour at room temperature. The samples were then washed under tap water, dried, and cut longitudinally and the cross section of each sample was examined with a stereomicroscope (Hamilton, Altay Scientific, Rome, Italy).

The permeability test was evaluated by using the measure pictures V 1.0 software (CAD-KAS Kassler Computer software GbR, Germany). A stereomicroscope under the magnification of ×50 was used to measure the length of dye penetration in the DTs from the outer surface of the root toward the pulp chamber. For the standardization of the samples measurements, the length of dye penetration inside the dentin was divided by the whole thickness of the sample. Then the results multiply by 100%.

2.4. Temperature Measurement. The measurements were done opposite to the lasing area under the following conditions. Part of the root was immersed in water, which was heated by an accurate digital hotplate (Cemaric*, Thermo Scientific Inc., MA, USA) for teeth temperature stabilization at 37 ± 0.5°C while being immersed in water. A K type thermocouple was used, which was connected to a digital multilogger thermometer (Amprobe TMD-56, Everett, WA, USA), with basic accuracy of ±0.05%, through a universal serial bus controller connected to a computer software (Amprobe multiline V3.0). The temperature recording was done every second. A thermal compound of 5.6 W/MK thermal conductivity (Arctic MX-2, China) was injected inside the pulp chamber, to confirm a contact between the thermocouple and the dentin surface during temperature measurements. Then a horizontal tooth sectioning through the premarked lasing area was done to measure the thickness of all samples by a vernier caliper (TOPEX Sp. z o.o. S.K., Warsaw, Poland), from the peripheral dentin surface to the pulpal one.

3. Results

3.1. Dye Penetration Test. The data obtained from dye penetration from all the experimental groups were statistically analyzed using SPSS Statistics 20 (IBM Corp., NY, USA). Descriptive statistics were done to obtain the means and standard deviations (Table 2).

Group (C) showed an obvious dye penetration approaching the pulpal side of the dentin with a mean of 86.52%, while (L+) exhibited only a slight penetration of the dye beyond

Table 2: Mean of percentage of dye penetration and standard deviation in each group.

Groups	Mean	Std. deviation
C[a]	86.52%	20.10
L−[b]	49.39%	43.29
L+[c]	16.22%	23.47

[a]Control group.
[b]Laser only group.
[c]Laser plus nanoparticle hydroxyapatite paste group.

the dentin surface with a mean of 16.22%. In group (L−), the mean was 49.39% which seemed to have dye penetration in the DTs.

To check if the obtained data distribution is normal, the Shapiro-Wilk test was implemented, and the test statistics showed that data were not normally distributed ($P < 0.05$). To examine if the groups were statistically different, the Kruskal-Wallis nonparametric test was used and the obtained descriptive level was 0.002, which revealed that the groups were significantly different. For intergroups comparisons the post hoc Dunnett multiple comparison test was performed. The group (L+) possessed highly significant less dye penetration compared to the control group (C) ($P < 0.001$) (Figure 3); (L+) and (L−) groups were also statistically different ($P < 0.05$). Finally the laser group was significantly different compared to the control group (C) ($P < 0.05$).

3.2. SEM Evaluation. The SEM showed the morphological characteristics of all different groups of the experiment. In group (C), the DTs were widely open and the whole area was free from smear layer (Figures 4(a) and 5(a)). The specimens of group (L−) (Figure 4(b)) showed a smaller diameter of DTs which is also noted in the cross section micrograph (Figure 5(b)). A large number of DTs were occluded due to the melting effect of the CO_2 laser. In the combined group (L+) (Figure 4(c)), most tubules were occluded by n-HAp, and there were signs of melting of the n-HAp. The cross section micrograph of the combined group (L+) (Figure 5(c)) showed melting of the n-HAp, penetration into the tubules, and formation of plug over it.

3.3. Temperature Measurements. Laser irradiation showed a mean temperature rise of 2.33°C ± 0.56°C, and the maximum temperature rise was 3.2°C. The corresponding average dentin thickness was 2.19 mm ± 0.24 mm. The curve of the temperature elevation course (Figure 6) showed a temperature increase of 2.5°C, revealing a favorable temperature drop in the 20-second interval between each 5-second lasing cycle.

4. Discussion

Various studies have been conducted to evaluate the effect of n-HAp on enamel and dentin remineralization [25, 26]. In this study we are trying to assess the effectiveness of this material in the treatment of DH. In this work, the ability of a CO_2 laser to melt the nanoparticle hydroxyapatite paste and fuse the open DTs was examined regarding the morphology and permeability of dentin. Additionally, temperature measurements were performed.

The results of the three groups in this study showed that (L+) group has the greater decrease in dentin permeability. This outcome was supported by SEM as most of DTs were sealed by n-HAp plugs.

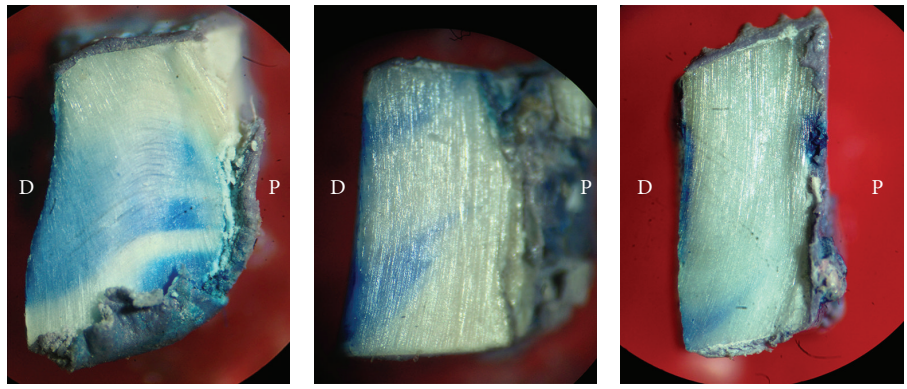
The (L+) group reduced the permeability more than (L−). This may indicate that the addition of n-HAp was effective in reducing dentinal permeability. In the (L−) group dye penetration inside the dentin was 49%. These results can be compared to Matsui et al. [21] who recorded a 41% dye extension inside the tooth dentin thickness. Bonin et al. [27] reported a reduction in the dentinal permeability with 1 W using CO_2 laser alone. In the present study the n-HAp paste and the CO_2 laser with 0.65 W proved to reduce the dentinal permeability to an acceptable level at a lower power setting.

In this study, 1% citric acid was used to remove the smear layer leaving a clean surface free of tubules plugs and without surface damage (Figure 4(a)). Citric acid is one of the agents used to remove the smear layer from the dentin surface [28, 29] which may simulate DH in vivo conditions due to its presence in juices, vegetables, and fruits [30]. Samples were stored without adding any antiseptics, as they may affect the dentin permeability due to mineral trapping inside the DTs [31].

On SEM micrographs, for group (L+) most of the DTs were occluded. The n-HAp particles appeared to be melted, recrystallized, and trapped inside the tubules forming a plug inside their orifices (Figure 5(c)). These plugs resisted the 15 s air-water spray that was done after the lasing, which remained bonded to the DTs (Figure 4(c)). This may prove the ability of the CO_2 laser in melting and bonding the n-HAp particles to the DTs. In pilot experiments of this study SEM shows that adding n-HAp paste only did not resist the 15 s air-water spray. Leave open DTs like the SEM of the control group (C) with some remnant of the n-HAp paste remaining on the dentin surface (Figure 7). The control group was chosen to be representative of open DTs. We may say that the combined treatment or the indirect method as first stated by Moritz et al. [20, 32] is a step forward to reduce the shortcoming of either treatment alone [23, 24, 33].

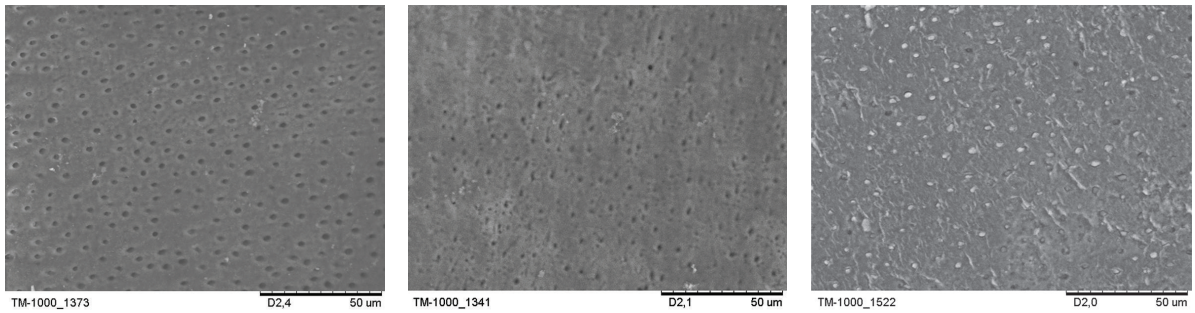
As shown in the (L−) micrograph (Figure 4(b)), dentin surface melting and narrowing of DTs diameter had been also noted. Similar results were presented by Cakar et al. [24] demonstrating a reduction of DTs diameter after exposure to CO_2 laser. In the (L+) group micrograph, the melting of n-HAp may be due to the nanometer particles size of the n-HAp which reveals a large surface area promoting absorption on the dentin surface [23].

We measured the temperature to ensure that our parameter is within the pulp safety limit of a 5.5°C temperature increase as reported by Zach and Cohen [34]. The maximum recorded value was a temperature increase of 3.2°C inside the pulp chamber, with an average dentin thickness of 2.19 mm. This result is in agreement with Moritz et al.'s [20] who recorded a 2.5°C as maximum temperate increase. As the heat dissipated to the surrounding medium, the air or the water [35], we made a hole opposite the lasing area, so that the



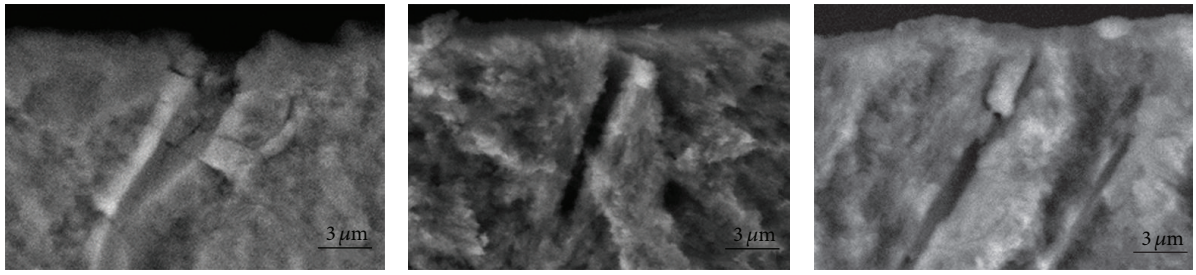
(a) (b) (c)

Figure 3: Dye penetration test. D: dentin surface side; P: pulpal side. (a) Control group (C), (b) laser only (L−), and (c) the laser plus nanohydroxyapatite paste (L+). The (C) group shows a full length dye penetration while for the (L+) group the dye is confined mainly to the surface with very slight dye penetration.



(a) (b) (c)

Figure 4: Dentine surface scanning electron microscope (SEM) micrographs. (a) Control group (C), (b) laser only (L−), and (c) the laser plus nanohydroxyapatite paste (L+). The original magnification was ×1200.



(a) (b) (c)

Figure 5: Cross-sectional scanning electron microscope (SEM) micrographs. (a) Control group (C), (b) laser only (L−), and (c) the laser plus nanohydroxyapatite paste (L+). The original magnification was ×5000.

thermocouple inserted inside the pulp chamber was isolated by the tooth structure and may not detect the heat dissipated to that medium. We used a whole tooth instead of sectioned dentin samples for temperature measurement, such as what we had done in the permeability test. According to the basic law of thermodynamics, $dQ = mcdT$, where dQ is the heat content, m represents tooth mass, c is the heat capacity, and dT is the linear change in the temperature. The sample with a smaller mass may exhibit a higher temperature after the lasing procedure. A thermal compound was used inside the pulp chamber to prevent a gap formation between the thermocouple and the dentin.

According to the manufacturer specifications, the n-HAp used in this study is water insoluble. This may give favorable expectations, and while Romano et al. [35] used the calcium hydroxide paste in combination with CO_2 laser, they stated that the stability of this paste is still a point for open discussion due to the solubility of this material.

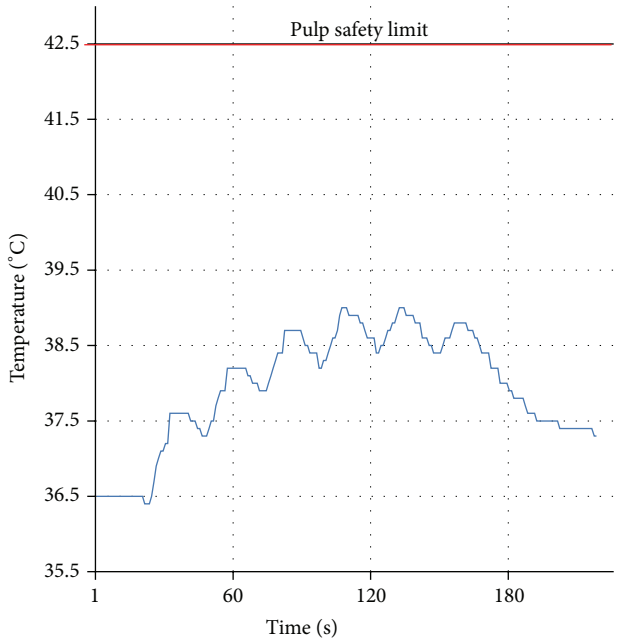


Figure 6: Temperature curve. A temperature increase of 2.5°C for the most representative sample. The six cycles of lasing are clearly visible.

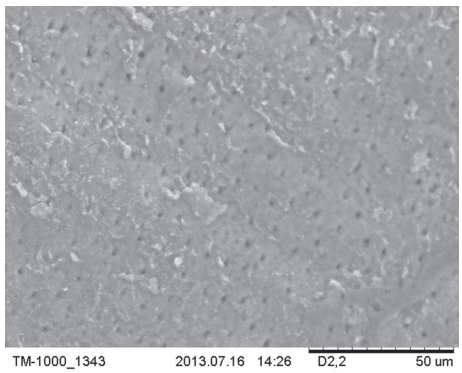


Figure 7: SEM micrograph of a pilot sample in which n-HAp only is applied on DTs. Showing open DTs like the SEM of the control group (C) with some remnant of the n-HAp paste remaining on the dentin surface. The original magnification was ×1200.

Due to its high absorption in both hydroxyapatite and water, the CO_2 laser with a wavelength of 10.6 μm has high absorption coefficient in dentin (800 cm^{-1}), presenting satisfactory superficial interaction. This kind of interaction is required for sealing DTs to reduce fluid passage across the dentin and consequently DH pain relief [35, 36].

Due to the presence of the dental pulp in vivo, this work needs to be confirmed clinically. The presence of pulp circulation in vivo may be of advantage in reducing the temperature elevation due to lasing as it acts as a heat sink that dissipates the generated heat [37]. Also, odontoblast cells in the dental pulp of primates and dogs could be indirectly activated by low power CO_2 laser leading to reactional dentinogenesis [38, 39].

DH treatment by laser seems to be simple, quick, and effective [21]. He et al. [40] concluded through his systematic review that laser treatment with correct and controlled parameters will not lead to adverse effects. Our experiment with 0.65 W of moderate power density showed that there was no damage, carbonization, or cracks on the dentin surface, which is in accordance with previous studies employed for DH treatment [32, 35, 41].

This experiment showed that n-Hap, melted and plugged over most of the DTs, reduced dentinal permeability with an acceptable temperature increase using a CO_2 laser of 0.65 W with a moderate power density.

5. Conclusions

Based on the results of the present study, the combination of nanohydroxyapatite paste and a CO_2 laser of moderate power density occluded the dentinal tubules and reduced the permeability of exposed dentin. This preliminary experiment gives primary evidence of a new treatment modality for dentin hypersensitivity. Further comprehensive clinical studies are needed to assess the clinical potential of this combined treatment.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Today Prospects for Tissue Engineering Therapeutic Approach in Dentistry

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In dental practice there is an increasing need for predictable therapeutic protocols able to regenerate tissues that, due to inflammatory or traumatic events, may suffer from loss of their function. One of the topics arising major interest in the research applied to regenerative medicine is represented by tissue engineering and, in particular, by stem cells. The study of stem cells in dentistry over the years has shown an exponential increase in literature. Adult mesenchymal stem cells have recently been isolated and characterized from tooth-related tissues and they might represent, in the near future, a new gold standard in the regeneration of all oral tissues. The aim of our review is to provide an overview on the topic reporting the current knowledge for each class of dental stem cells and to identify their potential clinical applications as therapeutic tool in various branches of dentistry.

1. Introduction

Anatomical structures of the mouth undergo several physiologic and pathologic modifications which can determine damages towards both hard and soft tissues [1]. One of the purposes of the scientific research in medical field is to provide techniques and materials to repair the loss of damaged tissues. In recent years a new approach based on tissue engineering is now adding the current treatment protocols. Tissue engineering was introduced in the 1990s and consists of an ensemble of techniques and procedures aimed at the regeneration of biological tissues [2] based on a triad derived from the three major components of tissues: cells, their ECM, and a signalling system [2].

Stem cells are generally defined as clonogenic cells capable of both self-renewal and multilineage differentiation [3] and have been identified from three main sources: embryonic stem cells, adult stem cells, and induced pluripotent stem cells [1]. Embryonic stem (ES) cells are pluripotent cells derived from blastocyst-stage embryos; pluripotent stem cells have not undergone complete differentiation and retain the capacity to divide into any of the three germ layers (endoderm, ectoderm, and mesoderm) but not into extraembryonic tissue [4]. Adult, somatic or postnatal stem cells reside amongst differentiated cells within a number of organs in the body where they play a role in tissue maintenance, renewal, and repair. They are multipotent stem cells and are more restricted in their differentiation capacity when compared with embryonic stem cells [1]. Induced pluripotent stem (iPS) cells are the product of somatic cell reprogramming to an embryonic-like state through genetic manipulation [5]. They have been first developed from adult mouse cells and then from adult human cells [5, 6]. Several types of adult stem cells have been isolated from teeth (Figure 1), including dental stem cells (DPSCs) [7], periodontal ligament stem cells (PDLSCs) [8], stem cells from human exfoliated deciduous teeth (SHEDs) [9], dental follicle progenitor stem cells (DFPCs) [10], and stem cells from apical papilla (SCAPs) [11].

In order to use stem cells in tissue engineering procedures, the presence of a scaffold and growth factors is necessary [2]. An ideal scaffold should support the attachment, migration, proliferation, and spatial organization of cells required for structural and functional replacement of the target tissue [12]. Growth factors (GFs) are peptide molecules which transmit signals to control cell behavior and activity interacting with specific receptors located on the surfaces of cells [13].

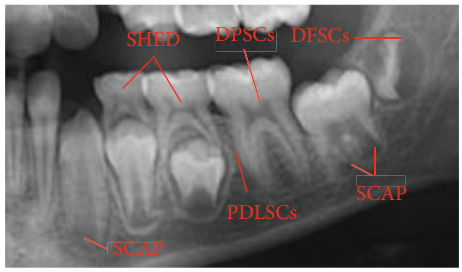

Figure 1: Dental stem cells' sources.

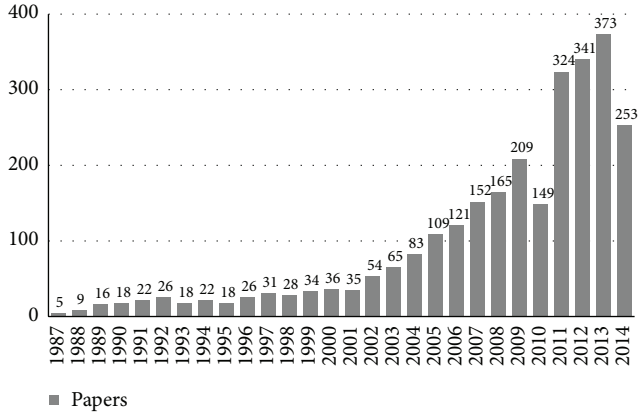

Figure 2: Number of papers dealing with stem cells in dentistry published during the last 27 years.

In recent years there has been an exponential increase in the number of publications dealing with stem cells (Figure 2). The focus of stem cells research in dentistry is the regeneration of missing oral tissues like, for example, dentine-pulp complex, maxillary bone, and periodontal ligament [14]. A further interest of dentistry towards stem cells is due to the fact that it is possible to isolate and harvest them from dental tissues; the oral cavity must be considered as a source of stem cells as well as a site of application.

The aim of our review of literature was to define various dental stem cells types and outline their possible modalities of clinical application for tissue regeneration.

2. Material and Methods

In order to perform our revision we consulted PUBMED database initially performing several test searches. Afterwards we decided to carry out the ultimate search by entering "STEM CELL" as main inquiry term, "AND" as default Boolean operator, and "IN DENTISTRY" as secondary inquiry term and we added four search filters offered by the same database. Therefore, review articles were excluded by the results even if these have been studied for the purpose of completeness of the research. Then we performed a second exclusion step by reading articles' title and abstract and a third exclusion step by reading original manuscripts. In our work mainly papers focused on in vivo studies on stem cells in tissue engineering applications were considered in order to investigate on the current knowledge about feasible usage of stem cells as regenerative tool for therapeutic purposes.

Our primary search resulted in a total number of 586 articles including 129 revisions on the subject. We considered 116 papers by reading title and abstract and we finally included 40 papers by analyzing the complete manuscript content.

3. Results and Discussion

Data extracted from the analysis of the selected articles are summarized in Table 1.

3.1. DPSCs. Adult human dental stem cells were first identified and isolated in 2000 by Gronthos et al. [7] from normal impacted third molars' pulp and were characterized as clonogenic and highly proliferative, being able to form in vitro calcified sporadic nodules [7].

DPSCs are shown to anatomically locate in a perivascular niche within the pulp tissue [15] and to possess self-renewal capabilities and multipotent differentiating ability: they can differentiate in vitro into odontoblasts, adipocytes, neural cells, osteoblasts, chondrocytes and myoblast-like cells [16–20]. Interestingly DPSCs have also been reported to show immunomodulatory properties in vitro and in vivo on mouse [21]. Although they share several characteristics with bone marrow mesenchymal stem cells (BMMSCs), DPSCs show reduced osteogenic and adipogenic potentials in vitro when compared to BMMSCs [7].

Human DPSCs can also be successfully isolated and characterized from inflamed pulp tissue [22], from supernumerary teeth [23] and from natal teeth [24]. Moreover several studies have isolated and characterized stem cells and subpopulations of progenitor cells in the dental pulp of different animal species [25–27].

The banking of DPSCs by cryopreservation in liquid N2 is clinically possible for future usage providing a good prospective in future regenerative dental and medical treatment [28, 29]. DPSCs have been successfully isolated from cryopreserved healthy molar and premolar teeth, as well as from their undigested dental pulp tissue [30–33] and also from diseased but vital teeth [34].

DPSCs' possible employment as therapeutic tool in regenerative endodontics is supported by several in vivo studies which showed that human DPSCs transplanted under the skin of immunocompromised mice formed pulp/dentin-like tissue complexes after odontoblastic differentiation [7, 35–37]. Different scaffolds were used in these studies. Gronthos et al. used a hydroxyapatite/tricalcium phosphate (HA/TCP) ceramic powder scaffold [7]. Demarco et al. used Poly-L-lactic acid (PLLA) scaffolds prepared in pulp chambers of extracted human third molars using salt crystals or gelatin spheres as porogen (PLLA/tooth slice scaffold). This study showed that dentin-related morphogen factors influence the differentiation of stem cells toward an odontoblast-like cell phenotype [35]. Prescott et al. and Johnson et al. used a collagen scaffold and Dentin Matrix Protein-1 (DMP1) [36, 37] which is a growth factor that is primarily found in dentin and bone and has been implicated in the regulation

Table 1: In vivo studies dealing with dental stem cells applications in tissue engineering procedures.

Type of cell	Origin	Year	Reference	Scaffold	GFs	Animal model	Type of differentiation
DFSC	Bovine	2002	Handa et al. [52]	HA ceramic powder		Mice	Cementoblastic
	Human	2011	Honda et al. [53]	Pellet culture system		Rat	Osteoblastic
	Pig	2008	Tsuchiya et al. [54]	β-TCP		Mice	Osteoblastic
	Human	2005	Morsczeck et al. [10]	HA ceramic powder		Mice	Osteoblastic
	Human	2010	Yagyuu et al. [55]	Porous ceramic discs		Rat	Cementoblastic
DPSC	Dog, pig	2004	Iohara et al. [41]	Pellet culture system	BMP-2	Dog	Odontoblastic
	Human	2011	Alsanea et al. [37]	Collagen/dentin wafer	DMP-1	Mice	Odontoblastic
	Pig	2008	Prescott et al. [36]	Collagen/tooth slice	DMP-1	Mice	Odontoblastic
	Pig	2012	Kodonas et al. [44]	Collagen/PLGA		Pig	Odontoblastic
	Human	2011	Chan et al. [50]	Peptide hydrogel		Mice	Osteoblastic
	Human	2010	Demarco et al. [35]	PLLA/tooth slice and porogens		Mice	Odontoblastic
	Human	2000	Gronthos et al. [7]	HA/TCP ceramic powder		Mice	Odontoblastic
	Human	2010	Kraft et al. [51]	HA/TCP granules		Mice	Osteoblastic and odontoblastic
	Dog	2013	Wang et al. [45]	HA ceramic powder		Dog	Odontoblastic
	Dog	2013	Khorsand et al. [56]	Bovine bone granules		Dog	Osteoblastic, cementoblastic, and fibroblastic
	Rabbit	2008	El-Backly et al. [43]	DL-lactide-CO-glycolide		Rabbit	Odontoblastic
	Pig	2012	Zheng et al. [42]	β-TCP		Swine	Odontoblastic
	Rat	2013	Tsujigiwa et al. [46]	β-TCP particles		Mice	Odontoblastic
	Human	2014	Syed-Picard et al. [40]	3D scaffoldless DPSCs/root canal		Mice	Odontoblastic
	Human	2005	Laino et al. [48]	Fibrous bone obtained in vitro		Mice	Osteoblastic
	Human	2007	d'Aquino et al. [49]	Bone chips obtained in vitro		Mice	Osteoblastic
PDLSC	Dog	2012	Suaid et al. [57]	Collagen sponge		Dog	Cementoblastic and fibroblastic
	Dog	2012	Wang et al. [58]	HA ceramic particles		Mice	Fibroblastic
	Human	2004	Seo et al. [8]	HA/TCP ceramic particles		Rat	Cementoblastic and fibroblastic
	Dog	2009	Kim et al. [59]	HA/TCP granules		Dog	Osteoblastic
	Human	2011	Park et al. [60]	Macroporous biphasic calcium phosphate		Mice	Cementoblastic
	Human	2013	Ji et al. [61]	Human dentine bocks		Mice	Cementoblastic and fibroblastic
	Dog	2014	Yu et al. [62]	Bovine bone granules		Dog	Osteoblastic
	Rat	2014	Han et al. [63]	Adsorbable gelatin sponge		Rat	Osteoblastic, cementoblastic, and fibroblastic
	Human	2006	Sonoyama et al. [25]	HA/TCP blocks		Swine	Cementoblastic and fibroblastic
SHED	Human	2012	Wang et al. [64]	Ceramic bovine bone (CBB); fibrin gel		Mice	Osteoblastic
	Human	2010	Sakai et al. [65]	PLLA/tooth slice		Mice	Odontoblastic
	Human	2013	Rosa et al. [66]	Peptide hydrogel or rH collagen in root canal		Mice	Odontoblastic
	Human	2013	Alkaisi et al. [67]	Cell pellets		Rat	Osteoblastic
	Human	2014	Kim et al. [68]	Macroporous biphasic calcium phosphate	FGF-2	Mice	Odontoblastic
	Human	2010	Casagrande et al. [69]	PLLA/tooth slice		Mice	Odontoblastic
	Human	2008	Cordeiro et al. [70]	PLLA/tooth slice		Mice	Odontoblastic
	Human	2003	Miura et al. [9]	HA/TCP ceramic powder		Mice	Osteoblastic and odontoblastic
	Human	2008	Seo et al. [71]	HA/TCP particles		Mice	Osteoblastic
SCAP	Human	2006	Sonoyama et al. [25]	HA/TCP blocks		Swine	Odontoblastic
	Human	2012	Abe et al. [72]	HA/TCP particles		Mice	Osteoblastic
	Human	2010	Yagyuu et al. [55]	Porous ceramic discs		Rat	Odontoblastic

of mineralization processes [38]. A very interesting suitable scaffold for regenerative endodontics is a self-assembling peptide hydrogel that can be poured into a pulp chamber and which self-polymerizes under physiological conditions to form a solid gel capable of supporting cell growth and differentiation [39]. Its application is highly attractive from an endodontic stand-point as a liquid may be expected to conform more easily to the variable shape of a pulp chamber than would a solid or even moldable scaffold. Human DPSCs have been reported to generate pulp-dentin-like complex also arranged in 3D scaffoldless structures in human root canals implanted subcutaneously into mice [40].

Animal DPSCs have also been tested in several in vivo experiments for regenerative endodontics [41–46]. DPSCs might have possible application in bone regenerative procedures. Nakamura et al. reported that rat dental pulp cells have the potential to generate mineralized tissue via the osteoblastic phenotype, on titanium, in vitro [47]. Some investigators reported the successful formation of lamellar bone in vivo by inducing human DPSCs to synthesize bone tissue in vitro and then transplanting it subcutaneously into mice, without needing of a scaffold support as the transplanted fibrous bone was an already formed hard tissue [48, 49]. Chan et al. used a self-assembling peptide hydrogel scaffold seeded with DPSC to create mineralised bone-like tissue pieces containing blood capillaries [50]. DPSCs with a mature osteogenic phenotype have been reported to be more responsive to pulsating fluid shear stress than osteogenically immature DPSCs and produce more bone in vivo suggesting that DPSCs with a mature osteogenic phenotype might be preferable for bone tissue engineering, because they might be able to perform mature bone cell-specific functions during bone adaptation to mechanical loading in vivo [51].

Nishino et al. reported a possible application in soft tissue regenerative medicine for human DPSCs associated with Basic Fibroblast Growth Factor (b-FGF), which where shown to accelerate the wound healing of a skin defect of a mice [73]. A study performed by Khorsand et al. showed that Dog DPSCs seeded on bovine bone granules possess periodontium and bone forming ability in periodontal canine defects [56].

3.2. PDLSCs. Adult stem cells from human periodontal ligament (PDL) of healthy permanent teeth were first isolated and characterized in 2004 by Seo et al. [8]. PDLSCs possess classic characteristics of stem cells (i.e., small size, slow cellular cycle, and several stem cells markers' expression) [8, 74] and show a faster cell growth and superior clonogenic capabilities compared with BMMSCs [75].

PDLSCs were shown to possess multilineage differentiation ability in vitro into osteoblast-like cells, cementoblast-like cells, adipocytes, and collagen-forming cells [8, 75, 76] although their osteogenic potential was found to be lower than their bone marrow and pulp tissue counterparts in vitro [8, 75]. PDLSCs have been reported to differentiate into chondrocyte-like cells in chondrogenesis-inducing media with the addition of Transforming Growth Factor β3 (TGF-β3) [75] and by adding TGF-β3 and BMP-6 to the culture [77]. They have also been reported to possess immunomodulatory functions which might lead to new possible application fields [78].

PDLSCs have been isolated from inflamed regenerating periodontal tissue obtained from intrabony defects during flap surgery and showed similar proliferating and differentiation properties, an increased migratory capacity, and a lower osteoblastic differentiation ability when compared to healthy PDLSCs [60, 79]. Stem cells isolated from periodontitis-affected periodontal tissue were even shown to differentiate into highly proliferative neural precursors in vitro [80].

Several authors investigated the differences between PDLSCs isolated in permanent or in deciduous teeth; deciduous PDLSCs were found to have a higher proliferative rate [61, 81] and both cell types display multipotentiality toward adipocytes, osteoblasts, and chondrocytes with some differentiation potential differences among them [82]. Silvério et al. reported that deciduous PDLSCs have higher ability to differentiate into adipocyte-like cells, rather than osteoblast-like cells, compared to permanent PDLSCs [81]. Conversely Ji et al. reported that deciduous PDLSCs are more apt than permanent PDLSCs to differentiate into both osteoblasts and adipocytes under appropriate differentiating in vitro conditions [61].

Several studies have isolated and characterized stem cells and subpopulations of progenitor cells in the PDL of different animal species [58, 83, 84].

FGF-2 was found to increase proliferation of the human PDLSCs cultures [85]. Moreover it has been shown that TGF-β1 combined with PDGF-BB and IGF-1 stimulated mitogenesis and enhanced the adhesion of human PDL cells to human periodontally diseased root fragments treated by scaling and root conditioning with a citric acid and tetracycline solution [86]. Swine PDLSCs have been reported to be induced by BMP-2 to form mineralized nodules and by FGF-2 to form tube-like vascular structures [84].

Several in vivo studies have been performed on PDLSCs. Human PDLSCs mixed with HA/TCP ceramic particles have been shown to be capable of generating a cementum/PDL-like complex, characterised by a layer of aligned cementum-like tissues and clearly associated PDL-like tissues, when transplanted into periodontal defects surgically created in rats [8]. PDLSCs from both healthy and inflamed human PDL mixed with macroporous biphasic calcium phosphate have been reported to create a typical cementum-like/PDL structure after transplantation into immunocompromised mice. However, the degree of cementum regeneration induced by the inflamed DPSCs was significantly lower than that induced by healthy PDLSCs [60]. Stem cells from human deciduous and permanent PDL have been compared in vivo; deciduous PDLSCs cell sheets combined with dentin blocks transplanted into the peritoneal cavity of nude mice were able to generate regularly arranged PDL-like fibrous tissue that interfaced with new cementum-like tissue formed on the surface of the dentin block. In contrast, there was only PDL-like tissue regeneration, without cementum formation, in transplanted human permanent PDLSC cell sheets [61].

Canine PDLSCs have been reported to generate a cementum/PDL-like complex if seeded on a HA scaffold and

transplanted into immunocompromised mice [58] and also if applied into furcation defects on dog using a collagen scaffold [57]. Furthermore PDLSCs from dog have been found to promote bone regeneration mixed with HA/TCP carriers in surgically created peri-implant saddle-like defects [59] and supported by bovine bone granules in sinus floor augmentation on dog [62].

Rat PDLSCs seeded on a gelatin sponge have been referred to promote bone, PDL, and cementum formation in vivo on rat [63].

3.3. SHED. SHED represent a distinctive population of multipotent stem cells from the remnant pulp of exfoliated deciduous teeth [9] which derive from a readily accessible tissue source as human deciduous teeth that are expendable and routinely exfoliated in childhood with little or no morbidity to the patient [9, 70, 71].

Although both are extracted from pulp tissue, SHED and DPSCs exhibit significant differences regarding proliferative capacity and gene expression, which can potentially affect their mechanisms of differentiation [87]. SHEDs express mesenchymal stem cell markers such as DPSCs, but exhibit a significantly higher positivity for CD146, a multipotency related marker for mesenchymal stem cells whose expression denotes less differentiated lineages which may have a higher differentiating capacity [64]. SHEDs exhibit a higher proliferation rate than DPSCs in vitro [9, 64] and the capacity to differentiate into several mesenchymal lineages, such as osteoblasts, odontoblasts, adipocytes, chondrocytes, and myocytes and expressed neuroprogenitor markers [9, 64, 88, 89]. Stem cells from deciduous teeth pulp are obtained easier in teeth with advanced resorption process probably because of the modifications in the ECM performed by the high quantities of cytokines produced by circulating mononuclear cells involved in the resorptive phenomenon [90].

In vitro tests showed that SHEDs have a higher capacity than DPSCs for osteogenic and adipogenic differentiation [9, 64].

In vivo studies carried out by implanting tooth slice/PLLA scaffolds containing SHED into the subcutaneous tissue of immunodeficient mice, showed that SHEDs possess the ability to develop a dental pulp-like tissue and vascular structures anastomosed with the mouse vasculature. In this particular study model dentin-derived morphogenic signals are necessary and sufficient to induce the differentiation of stem cells into odontoblasts [69, 70]. Another scaffold model supporting the odontoblastic differentiating ability of SHED consisted of peptide hydrogel or human recombinant collagen (rH collagen) injected into human tooth root and transplanted into mice [66]. The ability of forming pulp-like tissue in vivo was also reported by using a macroporous biphasic calcium phosphate scaffold and fibroblast growth factor-2 (FGF-2) with SHED from inflamed deciduous teeth [68].

The capacity of osteogenesis of SHED was supported by in vivo experiments in which SHED, arranged in cell pellets or mixed with ceramic bovine bone or HA/TCP scaffolds and transplanted into animal models, underwent osteoblastic differentiation and determined bone tissue formation [9, 64, 67, 71]. SHED showed a higher bone forming ability in vivo than DPSCs when transplanted in the same experimental conditions [64]. SHEDs are reported to have significant immunomodulatory properties in vitro and in vivo when transplanted in mice [91].

3.4. DFSCs. DFSCs have been isolated and characterized by Morsczeck et al. from normal human impacted third molars [10]. They show a typical fibroblast-like morphology and express mesenchymal stem cell markers [10]. DFSCs were able to differentiate in vitro in PDL-like structures or calcified nodules with bone- or cementum-like attributes [10]. Honda et al. found that DFSCs demonstrate osteogenic-, adipogenic-, and periodontium-like tissues differentiation capacity in vitro after induction but they are not able to differentiate in chondrocytes [53] while Kémoun et al. reported that they can differentiate into osteoblasts, chondrocytes, and adipocytes [92]. DFSCs have also been successfully cultured into a serum-free medium [93].

DFSCs have been tested in vivo in several studies [10, 52–55]. Human DFSCs have been transplanted with HA powder into immunocompromised mice and generated a structure lining the surfaces of the HA particles, which are comprised of fibrous or rigid tissue. In this study no cementum or bone formation was found in histological sections [10]. Pellets of human dental follicle cells have been reported to be able to regenerate critical size bone defects in rats' calvaria [53]. Human DFSCs cells mixed with porous ceramic discs showed hard tissue-forming potential in immunocompromised rats [55]. Porcine DFSCs mixed with β-TCP formed mineralized bone-like tissue subcutaneously in immunodeficient mice [54]. Bovine DFSCs mixed with HA powder and transplanted into immunocompromised mice generated cementum-like mineralized tissue on the border of HA beads and a ligament-like fibrous tissue interfaced these areas [52].

3.5. SCAPs. SCAPs were isolated and characterized by Sonoyama et al. from immature roots of normal human impacted third molars [11].

They express mesenchymal stem cell markers, embryonic stem cell markers, and also neurogenic markers [11]. Unlike DPSCs and other MSCs, SCAPs are telomerase-positive, a characteristic of embryonic stem cells, which suggests a notably immature state of differentiation [25, 94]. SCAPs are able to differentiate into multiple mesenchymal lineages (osteoblasts, odontoblasts, adipocytes, chondrocytes, and smooth muscle cells) and neural lineage in vitro [25, 72, 95] and have higher proliferation ratio and mineralization ability than DPSCs whereas the adipogenic potential of SCAPs is weaker than BMMSCs [11, 95]. Similarly to other dental stem cells, SCAPs have been reported to have immunomodulatory characteristics [96].

Human SCAPs were transplanted into immunocompromised mice using particles of HA/TCP as a carrier and generated a typical dentin structure. In the same study both human SCAPs and PDLSCs have been transplanted in a minipig model to generate a root/periodontal complex

Table 2: The possible clinical application fields of dental stem cells and tissue engineering.

Cell type	Possible clinical application
DPSCs	(i) Regenerative endodontics (ii) Bone regeneration
SHED	(i) Regenerative endodontics (ii) Bone regeneration
SCAP	(i) Regenerative endodontics (ii) Bone regeneration
PDLSCs	(i) Periodontal regeneration (ii) Bone regeneration
DFSCs	(i) Periodontal regeneration (ii) Bone regeneration

capable of mimicking a biophysiological root/periodontal setup in vivo [25]. Another in vivo study reported that human SCAPs mixed with porous ceramic discs show hard tissue-forming potential transplanted into immunocompromised rats [55]. The human SCAPs' ability of generating bone tissues has been reported in a study in which they were transplanted into immunodeficient mice with a HA scaffold [72].

4. Conclusion

Dental stem cells are an easily obtainable source of multipotent cells and in vivo studies on animal models confirmed the significative outcomes of in vitro studies. Our review reports encouraging results concerning the scientific research on dental stem cells, particularly regarding their possible employment, together with scaffolds and GFs, as therapeutic tool in various branches of dentistry.

According to their differentiation capacity, every oral stem cell type represents a determined source for a specific application field (Table 2). The highest number of articles on this topic focuses on DPSCs, which are good candidates in regenerative endodontics for pulp organ regeneration into necrotic or vital but diseased teeth as well as for the induction of dentin tissue repair among exposed pulp. Even more recently discovered cells as SCAP and SHED can be suggested for use in regenerative endodontics. DPSCs, SHED, SCAPs, PDLSCs, and DFSCs are good candidates for improving the existing regenerative procedures of craniofacial bone defects together with already reliable scaffolds and/or GFs. PDLSCs and DFSCs can be proposed as adjuvants tools for periodontal regeneration procedures as GTR technique. Furthermore dental stem cells may provide innovative solutions also in other medical branches thanks to their multipotent differentiation ability and immunomodulatory properties.

Despite all, at present, there are no in vivo studies on humans supporting the reliability for therapeutic use and further evidence is required to demonstrate the possibility of using dental stem cells as a therapeutic tool for daily clinical practice.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Maurizio Bossù and Andrea Pacifici equally contributed to this work.

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Clinical Study

Oral Crest Lengthening for Increasing Removable Denture Retention by Means of CO_2 Laser

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The loss of teeth and their replacement by artificial denture is associated with many problems. The denture needs a certain amount of ridge height to give it retention and a long-term function. Crest lengthening procedures are performed to provide a better anatomic environment and to create proper supporting structures for more stability and retention of the denture. The purpose of our study is to describe and evaluate the effectiveness of CO_2 laser-assisted surgery in patients treated for crest lengthening (vestibular deepening). There have been various surgical techniques described in order to restore alveolar ridge height by pushing muscles attaching of the jaws. Most of these techniques cause postoperative complications such as edemas, hemorrhage, pain, infection, slow healing, and rebound to initial position. Our clinical study describes the treatment planning and clinical steps for the crest lengthening with the use of CO_2 laser beam (6–15 Watts in noncontact, energy density range: 84.92–212.31 J/cm^2, focus, and continuous mode with a focal point diameter of 0.3 mm). At the end of each surgery, dentures were temporarily relined with a soft material. Patients were asked to mandatorily wear their relined denture for a minimum of 4–6 weeks and to remove it for hygienic purposes. At the end of each surgery, the deepest length of the vestibule was measured by the operator. No sutures were made and bloodless wounds healed in second intention without grafts. Results pointed out the efficiency of the procedure using CO_2 laser. At 8 weeks of post-op, the mean of crest lengthening was stable without rebound. Only a loss of 15% was noticed. To conclude, the use of CO_2 laser is an effective option for crest lengthening.

1. Introduction

The oral rehabilitation of patients after loss of teeth has made significant progress recently. Lack of an adequate residual alveolar ridge and basal seat severely compromises the success of prosthodontic treatment [1]. Vestibuloplasty, ridge augmentation, and different types of implants were used to overcome the problems of flat alveolar ridge [2]. A way of increasing the stability of the prosthesis in this circumstance is to deepen the vestibule [3]; it generally involves increasing by deepening of vestibule without any addition of bone [1]. In the preimplant era, vestibuloplasty was applied to deepen the vestibule with the aim of lining the vestibular trough with functional mucosa in order to form a valve-type margin [4].

Various surgical techniques for vestibular lengthening and vestibuloplasty have been described and advocated. Commonly used in practice, they have the drawback of being associated with a loss of the gained alveolar ridge height of 50% as a result of scar contraction. The patient has to endure pain due to the open wound surfaces and is limited in terms of food intake. In addition, patients often have to reattend the dental practice because they develop pressure sores owing to scar contraction. In the worst-case scenario, the relined denture was not worn by the patient, resulting in conditions similar to the pretreatment situation [5]. The laser technology enables practitioners to achieve a more sustainable result, causing minimal pain to the patient, without the disadvantages of the conventional surgery. The aim of our study is to describe a new method for oral crest lengthening and to evaluate its efficiency and stability.

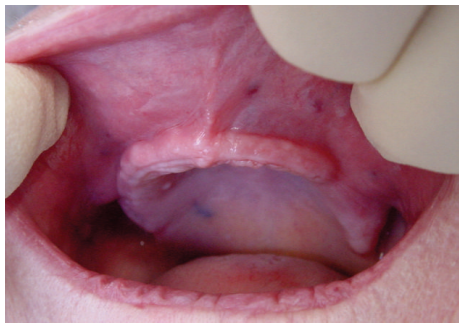

Figure 1: View of the maxilla crest showing a reduced length.

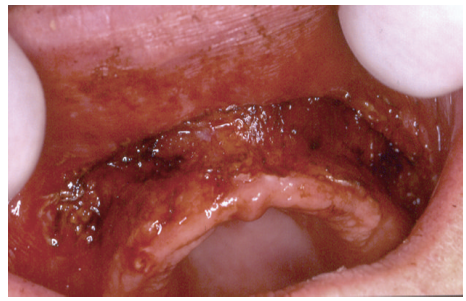

Figure 2: View of the operated site during surgery. The site is bloodless. Neither suture nor graft was necessary.

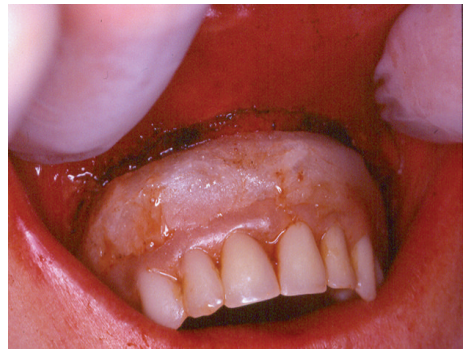

Figure 3: The removable denture was temporarily relined with a soft acrylic material until the bottom of the vestibular deepening. Patients were advised to keep the denture in mouth during the 6 weeks of post-op.

2. Patients and Methods

2.1. Patients. Sixty-nine healthy edentulous patients (41 females and 28 males) who needed a crest lengthening in order to improve the stability and retention of their removable dentures were included in this study. The age range of patients was 61–82 years with an average age of 68 years.

Exclusion and inclusion criteria: only patients who had enough distance between the bottom of the vestibule before surgery and nasal spine and with the zygomatic process were reported in this study.

All selected patients were healthy. High-risk cases were excluded because of the absence of suturing of surgical sites at the end of surgeries. According to the declaration of Helsinki and ethic committee recommendations, the decisions for surgery were made after informing patients about the different steps of each surgery, risks, and expected postoperative discomforts. Each surgery was performed only after receiving signed consent of the patient. Only maxilla was included for surgeries (Figure 1) to avoid the risk of trauma of the chin nerves at the mandible. Clinical examination by palpation and the results of X-ray CT scan generating a three-dimensional image of maxilla bone allowed us to make a decision for the surgical option for patients. Only cases for which we were able to perform a vestibular lengthening greater than 10 mm were admitted for this study. No medications were prescribed before surgeries. Due to the financial situation of the patients and the difficulty to afford the cost of prosthetic implant treatments, a surgery by using carbon dioxide laser (CO_2) was performed.

2.2. Laser. CO_2 laser beam (Smart US20 D Laser 10 600 nm, High Tech Laser, Herzele, Belgium) was used at the following setting: 6–15 Watts, energy density range: 85–212 J/cm^2, continuous, noncontact, and focus mode, and with a focal point diameter of 0.3 mm.

2.3. Surgical Procedure. We used a CO_2 laser machine (10.6 μm) and all treatments were performed in one session. All reported patients were healthy without high-risk cases. Before each surgery, a local anesthesia (articaine with vasoconstrictor) was made. All medical staff wore specified glasses to protect their eyes against CO_2 laser beam. Patient wore protective eye glasses, or wet and sterile compressive gauzes were applied over the patient's eyes. Surgical procedures were as follows. The incision by CO_2 laser was always started from the border of attached mucosa of the crest. The incision progressed by leaving 1 mm of soft tissues covering bone surfaces of the maxilla (Figure 2). The incision was carried out with a strict respect of the anatomy of the surface of the maxilla by keeping a parallel trajectory of incisions to the bone surfaces. The incisions for crest lengthening were always stopped 2 mm before the bone surface of the bottom of the vestibule (zygomatic process or nasal spine) (Figure 2) in order to avoid any future discomfort caused by an intensive contact between the edge of the prosthesis and the bone of the vestibule. Sometimes, the incised zone was made up of a very large area from one tuberosity to the other. At the end of surgeries, all old dentures were relined in mouth by a soft acrylic material (Silagum, automix comfort soft relining, DMG inc., Hamburg, Germany) (Figure 3). Patients were asked to mandatorily wear their temporary relined denture for a minimum of 6 weeks after surgery. We allowed patients to remove dentures only for the time of cleaning or for hygienic purposes. At the end of each surgery, the deepest length of the vestibule was measured by means of digital calipers with a precision of 0.01 mm (Caliper digital IP 67 300 mm, Helios-Preisser company, Gammertingen, Germany). No sutures were made and the bloodless wounds healed in second intention without grafts. The aim of the surgery is to remove mussels' attachments in order

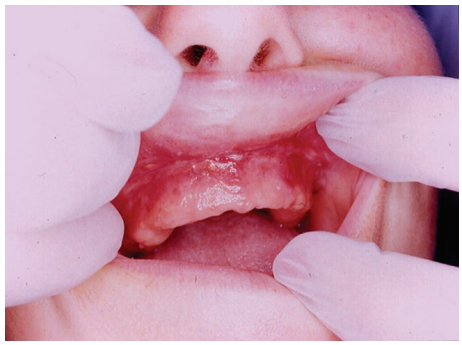

Figure 4: View of the healed site after 4 weeks post-op. The vestibular lengthening is stable. The healing of the crest was satisfactory.

to increase crest length. The defocus irradiation mode was only used to provoke the coagulation of bleeding areas. The tissue carbonization was gently removed from the operated tissue surfaces. The wound was left without any make-up and without wound dressing.

Postsurgical medication was prescribed: adapted antibiotics with respect to patient allergy risks, nonsteroid anti-inflammatory, analgesics, and a mouthwash solution (chlorhexidine 0.2%).

2.4. Follow-Up. Patients were recalled after 2, 4, 6, 8, and 10 weeks. The control concerned the evaluation of healed tissue and the measurement of the deepest vestibular length.

The percentage of success for each surgery has been calculated as follows.

Vestibule length after 2, 6, and 10 weeks divided by the initial vestibule length at the end of surgery = percentage of maintained vestibular lengthening.

ANOVA paired statistical test with Bartlett's test for equal variances (Bartlett's statistic corrected and a posttest: Newman-Keuls multiple comparison test) were used to compare results using the software Graph pad Prism (GraphPad Software, Inc., San Diego, California, USA).

3. Results

Pain, postoperative edema, and swelling were always present during the postoperative period. In some cases, the complete healing of wounds with a normal tissular aspect was reached after 4 weeks of post-op (Figure 4). During the healing period, the wounds appeared recovered by a layer of fibrin with a white or grey aspect. Despite the healed aspect of the operated area (between 2 and 4 weeks), patients were recommended to wait a minimum of 10 weeks before starting performing a new removable denture or a permanent relining. All operated areas showed a good quality of healing without scar formation.

The means and standard deviations of vestibular lengths at the end of surgeries, after 2, 6, and 10 weeks of post-op, were 15.56 ± 2.72 mm (initial lengths), 14.20 ± 1.9 mm (after 2 weeks of post-op), 11.53 ± 3.1 mm (after 6 weeks), and 11.39 ± 2.6 mm (at 10 weeks of post-op) (Figure 5).

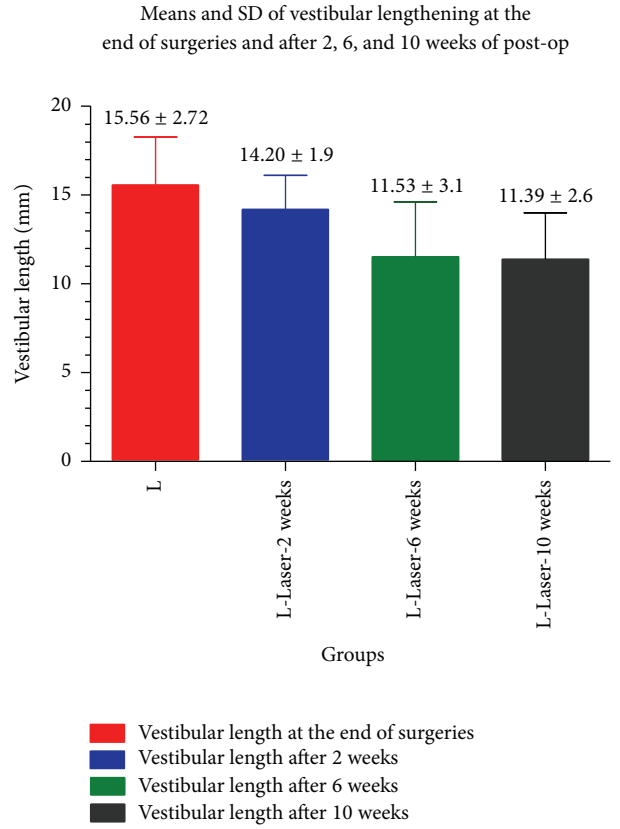

Figure 5: The means and standard deviations of vestibular lengths at the end of surgeries, after 2, 6, and 10 weeks post-op were 15.56 ± 2.72 mm (initial lengths), 14.20 ± 1.9 mm (after 2 weeks of post-op), 11.53 ± 3.1 mm (after 6 weeks), and 11.39 ± 2.6 mm at 10 weeks of post-op. The difference between means of vestibular lengthening in all groups is significantly different except between means of vestibular lengthening after 6 and 10 weeks of post-op. The loss in the initial vestibular deepening continues to increase significantly until 6 weeks post-op and it becomes statically stable.

The difference between means of vestibular lengthening in all groups is significantly different except between means of vestibular lengthening after 6 and 10 weeks. The loss of the initial vestibular lengthening increased continuously and significantly during the first 6 weeks of post-op and then became statically stable (P value <0.0001, P value: 0.0012, R square: 0.3189, ANOVA test with Bartlett's test for equal variances, Bartlett's statistic corrected with a posttest, and Newman-Keuls multiple comparison test) (Table 1).

The rebound between initial vestibular lengths at the end of surgeries and those at 2, 6, and 8 weeks of post-op may be explained by the tissue remodeling during mussels reattachment.

Failures were reported for three male patients because they could not keep their relined denture in mouth during the postsurgical period (Table 2).

4. Discussion

When alveolar ridge resorption occurs in the edentulous mandible, the surface of the attached mucosa on the ridge

Table 1: Results of statistical analysis for all groups are shown. Means are statistically different except for means of vestibular lengthening results at 6 and 10 weeks.

ANOVA test				
P value	<0.0001			
P value summary	* * *			
Are means significantly different? ($P < 0.05$)	Yes			
Number of groups	4			
F	42,44			
R square	0,3189			
Newman-Keuls multiple comparison test	Mean difference	q	Significant? $P < 0.05$?	Summary
L-Laser-10 weeks vs L	−4.170	13.24	Yes	* * *
L-Laser-10 weeks vs L-Laser-2 weeks	−2.810	8.922	Yes	* * *
L-Laser-10 weeks vs L-Laser-6 weeks	−0.1400	0.4445	No	ns
L-Laser-6 weeks vs L	−4.030	12.80	Yes	* * *
L-Laser-6 weeks vs L-Laser-2 weeks	−2.670	8.477	Yes	* * *
L-Laser-2 weeks vs L	−1.360	4.318	Yes	**

Table 2: Sexes and number of succeeded and failed surgeries are shown.

	Number of patients	Number of succeeded surgeries	Number of failed surgeries
Females	41	41	0
Males	28	25	3

decreases [6]. In this situation, the connection of the mucosa and muscles near the seat of the complete denture plays an important role in prosthesis retention and stability. It has been suggested that expansion of the denture-bearing area by means of a vestibuloplasty would reduce denture load per square unit of supporting bone and thus reduce the bone resorption caused by transfer of occlusal forces [7].

Many procedures for vestibuloplasty were proposed to overcome the problems of flat alveolar ridge [2]. Vestibular lengthening as a way of increasing the stability of the prosthesis was proposed to deepen the vestibule [3]. This generally involves increasing by deepening of vestibule without any addition of bone [1].

Various surgical techniques for vestibular lengthening and vestibuloplasty have been described and advocated. They have the drawback of being associated with a loss of the gained alveolar ridge height of 50% as result of scar contraction [5]. Previous procedures for vestibuloplasty and crest lengthening proposed the use of grafts (mainly skin graft) [3, 6, 7] or other materials to cover and protect the wound during the healing period. Laser technology enables practitioners to achieve oral surgeries in a bloodless field and without any need for suturing or graft in healthy patients [8–11]. Many authors proposed the use of CO_2 laser beam for the surgery of vestibular lengthening. The proposed procedures were able to manage a partial and limited vestibular lengthening [12–15]. In our study, we developed a new procedure able to treat a large area of the crest or to manage a global and total vestibular lengthening of the maxilla. Moreover, our surgical protocol pointed out the advantages of using laser beam that can be resumed as follows: an easy and quick procedure, a bloodless surgery, no need for suturing or for graft techniques, and a good quality of healed tissue.

Additionally, the use of CO_2 laser beam reduces and minimizes dramatically scar formation due to the sparse presence of myofibroblasts in the lased wound. It has been shown that the number of myofibroblasts in CO_2 laser wounds is three times less than that found in scalpel wounds [16–18]. The result is an appreciated quality of healing in lased tissues without scar formation. This added value of our use of CO_2 laser for the management of vestibuloplasty resolves the reported problems in conventional procedures (scalpel use): scars in healed tissue and the 50% of rebound.

Moreover, it has been reported that healed mucosa is enriched in collagen induced by laser beam in mast cells and myofibroblasts. The enrichment of lased mucosa by collagen is helpful and allows healed gingival crest to have more resistance to stress caused by dentures [19].

5. Conclusion

CO_2 laser with $\lambda = 10.6\ \mu$m was effective in crest lengthening procedures and presented some advantages: quick and simple method of applying without any need for suturing or graft techniques. In this way, CO_2 laser can be reported to be an effective option in improving vestibuloplasty.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Antimicrobial Photodynamic Therapy and Dental Plaque: A Systematic Review of the Literature

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Background. The aim of this study was to perform a systematic review of the literature on the efficacy of antimicrobial photodynamic therapy (PDTa) on cariogenic dental biofilm. *Types of Studies Reviewed*. Studies *in vivo*, *in vitro*, and *in situ* were included. Articles that did not address PDTa, those that did not involve cariogenic biofilm, those that used microorganisms in the plankton phase, and reviews were excluded. Data extraction and quality assessments were performed independently by two raters using a scale. *Results*. Two hundred forty articles were retrieved; only seventeen of them met the eligibility criteria and were analyzed in the present review. Considerable variability was found regarding the methodologies and application protocols for antimicrobial PDTa. Two articles reported unfavorable results. *Practical Implications*. The present systematic review does not allow drawing any concrete conclusions regarding the efficacy of antimicrobial PDTa, although this method seems to be a promising option.

1. Background

Dental caries has a multifactor etiology, including cariogenic microorganisms in the oral cavity. These microorganisms use a glycolytic pathway to produce acids that are capable of demineralizing tooth enamel and dentin. Some microorganisms use sucrose as substrate for the production of intracellular and extracellular polysaccharides, which are highly cariogenic [1, 2]. Moreover, a large portion of periodontopathogenic bacteria is found in dental biofilm (plaque), which underscores the considerable contribution of this substance in the development of adverse health conditions of the oral cavity.

Dental biofilm is a three-dimensional structure of bacterial communities adhered to the tooth surface [3]. Microcolonies of bacterial cells account for 15 to 20% of dental biofilm and the rest is composed of exopolysaccharides, water, proteins, salts, and the cell fragments [4, 5]. Pores or channels of water among the bacterial microcolonies serve as a primitive circulation system, allowing the passage of nutrients and other agents, which affect the distribution and movement of molecules in biofilm [3]. The constitution of biofilm protects colonizing species from adverse factors in the environment, such as defense mechanisms of the host and potentially toxic substances (lethal chemical agents and antibiotics) [4]. Moreover, slow-growing cells, which are one of the characteristics of bacteria found in deeper portions of biofilm, are less sensitive to antimicrobial agents and the ability of bacteria in the biofilm to produce neutralizing agents that protects neighboring organisms [3]. Thus, studies have described an increase in resistance to antibiotics, due to their inadequate or excessive use [6, 7], as well as difficulties concerning the access of topical agents with effectiveness against the biofilm [8].

Chlorhexidine is a cationic broad-spectrum antimicrobial agent that has been widely studied and proven effective at controlling dental biofilm [9]. This effectiveness is directly related to a property denominated substantivity, by which

the molecule remains adhered to tissues and has antibacterial action for up to 12 hours [10]. However, side effects lasting for more than 14 days are associated with chlorhexidine, such as pigmentation of the teeth and mucosa, an increase in the formation of supragingival calculus, a temporary loss of the sense of taste, a burning sensation, and dry mouth [9].

Antimicrobial photodynamic therapy (PDT) has emerged as an alternative to antibiotics for the treatment of microbial infections [6]. With this method, a photosensitizing agent is activated by light at a specific wavelength that corresponds to maximum absorbance by the substance, resulting in the production of free radicals, singlet oxygen, and other reactive oxygen species, which have a toxic effect on bacterial cells, leading to cell death without causing harm to the host [6, 7, 11–13]. This minimally invasive method is effective against resistant bacteria [14], has a rapid effect on the target organisms [15, 16], and does not lead to the development of resistance mechanisms [6, 17]. Moreover, antimicrobial PDT is selective and painless and does not affect the patient's sense of taste [18].

Most oral bacteria do not absorb visible light from some type of low-power laser light. Therefore, nontoxic optical agent absorption used to be fixed at the bacterial walls, attracting to itself the laser at the moment of irradiation. It is essential to have antimicrobial action. Reactive oxygen species released by the association between the dye and the light source causes damage to various cellular structures, but primarily to DNA and cytoplasmic membrane [12], which affects differently gram-positive and gram-negative bacterias [34]. The cellular destruction depends on the association between the dye and the light source [7, 17]. The effect of the dye is influenced of the kind, dose and site application. The efficacy of light can be influenced by wavelength, power density, energy fluence and the amount of oxygen available for the combination of both (dye and light) [11].

Different types of photosensitizers and light sources under various conditions have been used for the realization of photodynamic antimicrobial therapy [13]. For its use, a photosensitizer should have photophysical, chemical, and biological characteristics appropriate [11] among which is the ability to become an active drug and provide singlet oxygen, a broad spectrum of action, and affinity for microorganisms; low affinity for host cells promotes low mutagenicity and cytotoxicity associated with low possibility of developing resistant strains of microorganisms [11]. The light source, to be adequate, must present low power situated in the visible portion of the electromagnetic spectrum and specific wavelength resonant to dye. The wavelength depends on the dose and the depth of action of the photosensitizer used [11].

However, bacteria in biofilm have been demonstrated to be less affected by PDT than those in the plankton phase [35]. While a number of authors working with different light sources and photosensitizing agents report the efficacy of PDT in controlling dental biofilm by reducing the bacteria viability [7, 13, 14, 16, 22–24, 36–38], there is a lack of scientific evidence regarding the actual effectiveness of this method. Thus, the aim of the present study was to perform a systematic review of the literature on the efficacy of antimicrobial PDT on dental biofilm.

2. Methods

Articles addressing the effect of antimicrobial PDT with the use of a photosensitizing agent on known cariogenic biofilm formed mainly by streptococci of the *mutans* group and/or lactobacilli were included, with no restrictions placed on the method employed or year of publication. Reviews of the literature, studies involving only bacteria in the plankton phase, and studies involving an animal model were excluded.

2.1. Search Strategy. Searches were made of the Pubmed, Web of Science, Scopus, Lilacs, and Cochrane Library databases in October and November 2013 as well as March 2014. A manual search of the references of each article retrieved was also performed in an attempt to find further articles that were not in the electronic databases. Each database was searched from its inception to March 2014. The search was performed by two researchers and limited to studies involving human subjects published in the English language, using the following keywords: ("Biofilms"[Mesh] OR "Dental Plaque"[Mesh]) AND (photodynamic therapy OR antimicrobial photodynamic therapy OR light therapy).

2.2. Data Extraction and Evaluation of Methodological Quality. A total of 23 articles were retrieved from the databases and four additional articles were retrieved from the manual search of the reference lists. Following the reading of the title and abstract of each article, two independent raters (GCS and DSBO) selected studies for the full-text analysis. Interexaminer agreement was 96%. Thirty-two articles were selected for the full-text analysis due to insufficient information in the abstract to support the decision regarding eligibility. Articles that did not address antimicrobial PDT, those that did not involve potentially cariogenic biofilm, those that used microorganisms in the plankton phase, and reviews of the literature were excluded. After the full-text analyses, seventeen articles were included in the present systematic review (Figure 1).

Data extraction and the evaluation of methodological quality were performed by two independent raters (GCS and DSBO). The evaluation involved the use of a chart considering the sample (sample size calculation = 1; randomization = 1), study design (*in vivo* = 3; *in situ* = 2; *in vitro* = 1), control group (present = 1; absent = 0), blinding (double-blind = 2; single-blind; absent = 0), and repetition of the experiment (yes = 1; no = 0). The maximum score was 9 points. Disagreements between the raters were discussed and resolved by consensus. The determination and critical analysis of the quality of the articles allowed suggestions for improvements in future studies.

3. Results

Among the total of 240 articles retrieved during the original search of the databases and references lists, seventeen were selected for the present systematic review for addressing the efficacy of antimicrobial PDT on biofilm with cariogenic potential. All seventeen articles described either *in vitro* or *in situ* studies. Table 2 offers a summary of the findings.

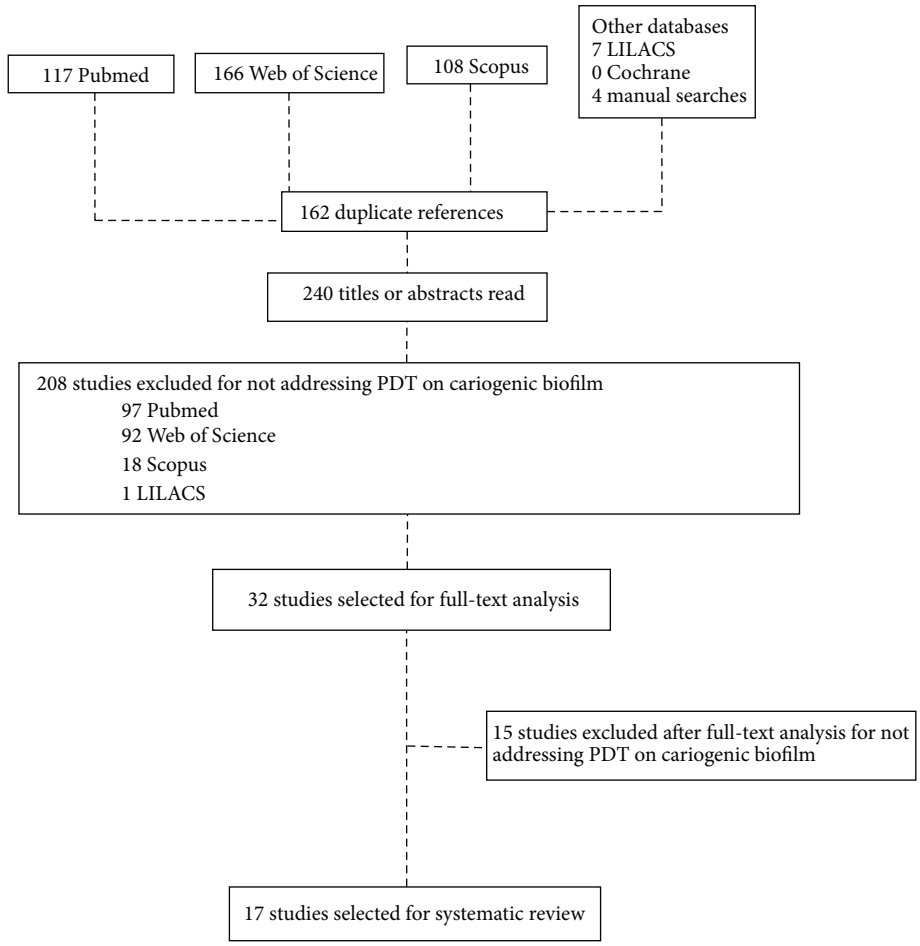

Figure 1: Flowchart demonstrating selection process of studies on PDT and dental biofilm.

Considerable variability among the articles was found regarding the photosensitizing agent. Toluidine blue was the most commonly employed. Each study used a specific light source (LED, laser, and light bulb), power, and application protocol.

Biofilm was cultivated in human saliva in three studies [14, 22, 32], natural human biofilm was used in four studies [19, 21, 27, 30], and synthesized biofilm was used in ten studies [16, 20, 23–26, 28, 29, 31, 33].

Two articles reported unfavorable results regarding the reduction of microorganisms in dental biofilm with the use of antimicrobial PDT [25, 30].

In the analysis of methodological quality, scores ranged from 1 to 5 points. The main drawbacks were related to the sample size calculation, randomization of the sample, blinding, and repetition of the experiment (Table 1).

4. Discussion

The analysis of the articles revealed the frequent lack of randomization of the specimens studied and failure to calculate the appropriate sample size. These data indicate possible selection bias. Moreover, divergent effects may have derived from systemic alterations in the different specimens and there is no possibility of reproducing the studies.

The majority of articles evaluated antimicrobial PDT on dental biofilm using an *in vitro* study, which is not the best design for arriving at adequate scientific evidence, although this model has led to significant advances in the study of dental biofilm [39]. *In vitro* models tend to involve a small number of species of microorganisms and laboratory conditions that may not adequately reflect the physiological situation in the oral cavity [40]. Factors such as salivary flow, the capacity of antimicrobial substances to adhere to the film on the teeth or the surface of soft tissues, and the interaction of noncultivatable bacteria cannot be modeled in an *in vitro* experiment [41].

The presence of polymeric extracellular substances, composition of the cell wall, growth rate, metabolic activity, and gene expression offer natural biofilm protection from the action of antimicrobial agents [42]. Moreover, nutritional status, temperature, pH, and undereffective exposure to antimicrobial agents can enhance bacterial resistance to this type of treatment [4, 43]. As biofilm is dependent on a number of factors, the use of a synthesized biofilm may not demonstrate the same scientific evidence as natural biofilm.

Table 1: Quality scores of articles selected based on proposed evaluation scale.

Authors and year	Sample	Study design	Control group	Blinding	Repetition of experiment	Total
Wilson et al., 1995 [19]	0	1	1	0	0	2
Wilson et al., 1996 [20]	0	1	0	0	0	1
Wood et al., 1999 [21]	0	2	1	0	0	3
O'Neill et al., 2002 [22]	0	1	1	0	0	2
Zanin et al., 2005 [16]	0	1	1	0	0	2
Wood et al., 2006 [23]	0	1	1	0	1	3
Zanin et al., 2006 [24]	1	1	1	0	0	3
Müller et al., 2007 [25]	0	1	1	0	1	3
Steinberg et al., 2008 [26]	0	1	1	0	1	3
Lima et al., 2009 [27]	1	1	1	1	0	4
Schneider et al., 2012 [14]	0	1	1	0	0	2
Chen et al., 2012 [28]	0	1	1	0	0	2
Silva et al., 2012 [29]	0	1	1	0	1	3
Teixeira et al., 2012 [30]	1	2	1	1	0	5
Pereira et al., 2013 [31]	0	1	1	0	0	2
Al-Ahmad et al., 2013 [32]	0	2	1	0	1	4
Araújo et al., 2014 [33]	0	1	1	0	1	3

Dental biofilm has an organized structure formed by different types of microorganisms, which give the substance a complex, protective trait. Thus, studies employing biofilm composed of a single genus of microorganisms [14, 16, 19, 20, 22–24, 26, 28, 29, 31] may not demonstrate the actual effect of antimicrobial PDT on dental biofilm in the oral cavity.

Although the majority of studies report favorable results with the use of antimicrobial PDT to reduce the volume of cariogenic microorganisms in the oral cavity, the articles offered a considerable variety of photosensitizing agents, light sources, application protocols, and methods for evaluating the effectiveness of the technique. This hinders the comparison of the findings, the reproducibility of events, and the determination of possible causality between the reduction in microorganisms and antimicrobial PDT. Moreover, the variations among the methods employed hamper the establishment of a possible protocol for the application of antimicrobial PDT on cariogenic biofilm.

Most of the articles included in this review used as photosensitizing dyes phenothiazine (methylene blue and toluidine blue). The physicochemical properties of the photosensitizers are important to the efficacy of photodynamic therapy. The ability of a component to absorb incident light does not mean it can act as a photosensitizer. Other requirements are important, such as having no toxic characteristics to the host cell, presenting toxicity only after activation by light, staying excited long enough to allow its interaction with neighboring molecules, producing cytotoxic species capable of causing bacterial killing time, and having high solubility in water [8, 44, 45].

In oral antimicrobial photodynamic therapy, toluidine blue and methylene blue photosensitizing agents are the most commonly used [11, 12], since they have a high degree of selectivity for damage for gram-positive and gram-negative bacterias [46–48]. What determines the selectivity of this type of dye microbial cells is the interaction between the positive charges of the dye and the negative charges of the outer surface of the microbial cell [49]. The dye methylene blue is a prototype of phenothiazine derivatives and their use is attested almost a century and its relatively low toxicity to humans [50]. However, Wood et al. 2006 [23] noted the erythrosine better efficiency when compared to methylene blue and Photofrin on *Streptococcus mutans* biofilm.

The concentration of photosensitizers is still controversial. Al-Ahmad et al. 2013 [32] using different concentrations of toluidine blue (5, 10, 25, and 50 mg/mL^{-1}) found that the antimicrobial effect can be observed at lower concentrations.

The first light sources used in photodynamic therapy were conventional lamps with noncoherent, polychromatic light and a strong thermal component. With the development of lasers, which have particular characteristics, such monochromaticity, coherence, and collimation, the light source proved to be more efficient to photodynamic therapy. Diode lasers have resonant wavelength absorption band of most currently used dyes, act continuously, and are less portable and low in cost [51]. Currently, the light of a specific wavelength, sources which are most commonly applied in PDT are helium-neon (HeNe) lasers, (633 nm) gallium-aluminum-arsenide (GaAlAs) diode lasers (630–690, 830, or 906 nm), and argon lasers (488–514 nm).

In this review, the majority of included studies used HeNe laser and LED (light emitting diode). LEDs are another alternative source of laser light and differ by presenting divergent beam, low thermal component and monochromatic light [51], and low cost [16]. Additionally, LED sources are present in the dental routine and can be used in PDT without requiring the acquisition of new equipment. However, no difference regarding the efficacy of these two types of light source for photodynamic therapy was observed [16].

Table 2: Summary of data reported in selected articles addressing effectiveness of antimicrobial PDT on potentially cariogenic biofilm.

Authors and year of publication	Sample	Photosensitizing agent	Light source	Microorganisms/biofilm	Application protocol	Outcome
Wilson et al., 1995 [19]	Natural dental biofilm; 10 volunteers; prereduced Calgon Ringer's solution	$AlPcS_2$ TB	GaAlAs laser 660 nm (11 mW) HeNe laser 632.8 nm (7.3 mW)	*Streptococci*	60 s and 180 s	HeNe/TB combination was more effective at reducing viable microorganisms
Wilson et al., 1996 [20]	Synthesized biofilm in 4 days; single species; HA discs	$AlPcS_2$	GaAlAs laser 660 nm	*S. sanguinis*	0.8 J (4.1 J/cm^2)	Reduction in viable microorganisms
Wood et al., 1999 [21]	Natural dental biofilm in 7 days; 8 volunteers; devices were attached to surface of maxillary first or second molars	PPC	Tungsten white bulb 600–700 nm (400 w; 22.5 mW/cm^2)	Natural human dental biofilm (species not listed)	30 minutes	Reduction in viable microorganisms
O'Neill et al., 2002 [22]	Biofilm formed from human saliva from 10 volunteers; multispecies biofilms grown on discs prepared from cellulose nitrate membrane filters for 24 h	TB	HeNe laser 632 nm (35 mW)	*Streptococci*	15 minutes (31.5 J; 81.9 J/cm^2)	Reduction in viable microorganisms
Zanin et al., 2005 [16]	Synthesized biofilm in 3, 7, and 10 days; HA discs; single species	TB	HeNe laser 632.8 nm (32 mW) LED 620–660 nm	*S. mutans*	5 (49 J/cm^2), 15 (147 J/cm^2) and 30 (294 J/cm^2) minutes	LED/TB on biofilm for 3 (147 J/or 294 J/cm^2) and 7 days (49 J/cm^2 or 294 J/cm^2) For 10 days, no difference between HeNe and LED
Wood et al., 2006 [23]	Synthesized biofilms in 48, 120, 168, 216, and 288 hours; steel discs; single species	ER MB Photofrin	Tungsten white light 40 W (ER—22.7 mW/cm^2, 500–550 nm; MB and Photofrin— 22.5 mW/cm^2, 600–650 nm)	*S. mutans*	15 minutes of irradiation	Reduction in viable microorganisms Greater effectiveness with ER
Zanin et al., 2006 [24]	Synthesized biofilm in 3, 5, and 7 days; single species; bovine enamel discs	TBO	Red LED 620–660 nm (32 mW)	*S. mutans, S. sobrinus, S. sanguinis*	7 minutes of irradiation 85.7 J/cm^{-2}	Reduction in viable microorganisms
Müller et al., 2007 [25]	Synthesized biofilm in 64.5 h; multispecies; bovine enamel discs	MB	Gasiform ozone and laser 665 nm (75 mW)	*S. sobrinus, S. oralis*	60 s of irradiation	No reduction in viable microorganisms

Table 2: Continued.

Authors and year of publication	Sample	Photosensitizing agent	Light source	Microorganisms/biofilm	Application protocol	Outcome
Steinberg et al., 2008 [26]	Synthesized biofilm in 24 h; single species; microplates	H_2O_2	Blue xenon lamp 400–500 nm	*S. mutans*	30 s of irradiation (34 J/cm^2) 60 s (68 J/cm^2)	Reduction in viable microorganisms
Lima et al., 2009 [27]	Natural dental biofilm for 7 days; 20 volunteers; palatal devices containing slabs of human dentin	TB	LED 620–660 nm	*Streptococci, Lactobacilli*	5 minutes of irradiation (47 J/cm^{-2}) and 10 minutes (94 J/cm^{-2})	Reduction in viable microorganisms with LED/TB and LED (94 J/cm^{-2}) alone
Schneider et al., 2012 [14]	Biofilm from saliva from 4 volunteers and growth in 4 hours Glass surface Single species	Phenothiazine chloride	Diode laser 660 nm (100 mW)	*S. mutans*	2 minutes of irradiation	Reduction in viable microorganisms
Chen et al., 2012 [28]	Synthesized biofilm in 24 hours Single species Stainless steel discs	Nanoparticles of ER + CS	Green LED 540 ± 5 nm (22m W/cm^2)	*S. mutans*	ER/CS nanoparticles for 12 h followed by dose of 50 J cm^2	Reduction in viable microorganisms
Silva et al., 2012 [29]	Synthesized biofilm in 24 hours Single species Bovine dentin discs	Photogem hematoporphyrin derivative	Biotable LED 610–650 nm	*S. mutans*	25 minutes of irradiation (75 Jcm^{-2}) and 50 minutes of irradiation (150 Jcm^{-2})	Reduction in viable microorganisms
Teixeira et al., 2012 [30]	Natural dental biofilm in 7 days Acrylic palatal device with human enamel slabs 21 volunteers	TB	Red LED 620–660 nm	*S. mutans*	15 minutes of irradiation (55 J cm^{-2})	No reduction in viable microorganisms
Pereira et al., 2013 [31]	Synthesized biofilm in 48 hours Single species Acrylic resin discs	ER and RB	Blue LED 455 ± 20 nm (200 mW)	*S. mutans, S. sanguinis*	95 Jcm^{-2} (36 J and for 180 s)	Reduction in viable microorganisms
Al-Ahmad et al., 2013 [32]	Culture of microorganisms from the saliva of a volunteer of 45 years old	TB	Broadband VIS + wIRA radiator with a water-filtered (580–1400 nm)	Salivary bacteria (species not listed)	220 mW cm^{-2} For 1 min	Reduction in viable microorganisms
Araújo et al., 2014 [33]	Synthesized biofilm in 7 days Single species Polystyrene 96-well plates	Curcumin	Blue LED 450 nm	*S. mutans, L. acidophilus*	5 minutes 5, 7 J/cm^2 (19 mW/cm^2)	Reduction in viable microorganisms

$AlPcS_2$: aluminum disulfonate phthalocyanine; GaAlAs: gallium-aluminum-arsenide; HA: hydroxyapatite; HeNe: helium/neon; LED: light emitting diode; PPC: pyridinium Zn(II) phthalocyanine; TB: toluidine blue; ER: erythrosine; TB: toluidine blue; MB: methylene blue; H_2O_2: hydrogen peroxide; CS: chitosan; ER: erythrosine; TBO: toluidine blue; RB: rose bengal; VIS + wIRA: visible light together with water-filtered infrared-A.

Considering the wavelength, blue light has been shown to be more efficient to be used in conjunction with a dye than red light [52, 53]. However, the use of different types of light sources by authors evaluated in this review shows that there is no consensus regarding the type of light and the parameters to be used permanently in photodynamic therapy to control biofilm.

Two studies reported unfavorable results regarding the effect of antimicrobial PDT on dental biofilm [25, 30]. The authors attribute these findings to an increase in resistance to this technique among bacteria in biofilm and/or multispecies biofilm [25, 30] as well as the thickness [30] and age of the biofilm [25]. Similarly, resistance to antiseptics, such as chlorhexidine, has been described [54]. Moreover, antimicrobial efficacy is believed to be dose dependent [55], which would explain the lack of a positive effect, as antimicrobial PDT was only applied once in both studies [30]. The difficulty for antimicrobial agents, photosensitizing agents, and light to penetrate the deeper layers of biofilm limits their effectiveness [25, 30]. However, studies have demonstrated that although antimicrobial PDT is threefold to fourfold less effective on thick, multispecies biofilm, antibiotics are as much as 250-fold less effective [56]. There are alternatives that can enhance the effectiveness of antimicrobial PDT, such as the selection of photosensitizing agents capable of penetrating the matrix of the biofilm, the use of photomechanical waves to optimize the penetration of the photosensitizing agent [57], and internal irradiation of the biofilm using an optic fiber [22].

Some of the included studies investigated alternatives to optimize antimicrobial photodynamic therapy when applied in biofilm. The use of the visible light in combination with water-filtered infrared-A (VIS + wIRA) [32] showed satisfactory results, while the use of ozone gasiform [25] was not effective. The use of curcumin in biofilm decreased from 95 to 99.9% of viable microorganisms, depending on the concentration of the photosensitizing agent; however, when applied to carious dentin, their effectiveness was probably reduced by the difficulty of penetration of the photosensitizing agent [33].

Suggestions for Future Research. Despite the number of studies on antimicrobial PDT, greater knowledge is needed regarding the effectiveness of this form of treatment. Studies with methodological standardization, randomization, an adequate sample size, reproducibility, and adequate data analysis are needed. Moreover, the effectiveness of antimicrobial PDT on multispecies biofilm under real conditions, such as in an *in situ* and *in vivo* design, is needed to gain a better understanding of the action mechanism of this treatment modality and the determination of a possible application protocol.

5. Conclusion

The present systematic review of the literature does not allow drawing any concrete conclusions regarding the efficacy of antimicrobial PDT due to the contradictory findings and methodological differences. Although this method seems to be a promising option for reducing the quantity of cariogenic microorganisms in dental biofilm, there is no sufficiently strong scientific evidence to support this association.

Further experimental studies with methodological standardization, the use of natural human biofilm, and an *in vivo* design are needed to gain a better understanding of the mechanisms, indications, and possible side effects of antimicrobial photodynamic therapy.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Ms. G. C. Santin contributed in the systematic review, analysis of studies, and paper writing. Ms. D. S. B. Oliveira contributed in the systematic review, analysis of studies, and paper writing. R. Galo contributed in the analysis of studies and paper writing. M. C. Borsatto contributed in paper writing. S. A. M. Corona contributed in paper writing.

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Research Article

Treatment of Dentinal Hypersensitivity by means of Nd:YAP Laser: A Preliminary *In Vitro* Study

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Objective. The aim of this study is to evaluate the effectiveness of Nd:YAP laser to seal dentinal tubules at different parameters. *Material and Methods*. 24 caries-free human wisdom impacted molars were used. The crowns were sectioned transversally in order to totally expose the dentin. The smear layer was removed by a 1 min application of EDTA. Each surface was divided into four quadrants, but only three quadrants were irradiated at a different output power setting (irradiation speed: 1 mm/sec; optical fiber diameter: 320 μm; tangential incidence of beam and in noncontact mode). Samples were smeared with a graphite paste prior to laser irradiation. All specimens were sent for SEM analysis. Pulp temperature increases in additional twenty teeth were measured by a thermocouple. *Results*. Morphological changes in dentin surfaces depend on the value of used energy density. Higher energy densities (2 W–4 W; 200–400 mJ; pulse duration: 100 m sec.; and 10 Hz) induce higher dentin modifications. Our results confirmed that Nd:YAP laser irradiations can lead to total or partial occlusion of dentin tubules without provoking fissures or cracks. Measurements of pulp temperature increases showed that Nd:YAP laser beam can be considered as harmless for pulp vitality for following irradiation conditions: 2 W (200 mJ) to 4 W (400 mJ) with an irradiation speed of 1 mm/sec; fiber diameter: 320 micrometers; 10 Hz; pulse duration: 100 m sec; noncontact mode and in tangential incidence to exposed dentin. The perpendicular incidence of the laser beam on exposed dentin may injure pulp vitality even at low output power of 3 W. *Conclusions*. Nd:YAP laser beam was able to seal the dentin tubules without damaging dentinal surfaces and without harming pulp vitality. Nd:YAP laser is effective and may be safely used for future *in vivo* treatments of dentinal hypersensitivity under certain conditions.

1. Introduction

Dentinal hypersensitivity (DH) is described in the literature as a "pain derived from exposed dentin in response to chemical, thermal tactile, or osmotic stimuli which cannot be explained as arising from any other dental defect or disease" [1].

Dentinal hypersensitivity is a quite common problem. Que et al. [2] pointed out a prevalence of dentinal hypersensitivity varying between 2–8% and 74%. Many solutions have been proposed and tested to treat DH but few of them are really successful [3]. DH is a very annoying disease which can have a negative influence on the quality of life, oral hygiene, and treatments like cleanings with ultrasonic instruments.

The etiology of this disease remains unknown, but the most common accepted theory is the fluid movements/hydrodynamic theory proposed by Braennstrom and Astroem, which involves the fluids movements of the tubules. These movements of the fluids are direct reactions of thermal, chemical, osmotic, and mechanical stimuli [4]. The odontoblastic processes are indeed rounded by dentinal fluid coming from the pulp complex, which forms 22% of the dentinal volume [5], and some studies reported that sensitive

dentine contain 8 times more tubules, but also wider tubules, than not sensitive teeth [5–7].

It is considered that an ideal desensitizing agent for dentin hypersensitivity should not irritate or endanger the pulp; it should be relatively painless, easily applied, rapid, and permanently effective, and it should not discolor the teeth [8, 9].

The desensitizing methods in use inhibit the pain by trying to avoid any fluid movement or by having an influence on the nerve [10]: sealing dentinal tubules with a coating mechanism that can alter the tubule contents by coagulation, by protein precipitation, or by the creation of insoluble calcium complexes.

Only potassium salts (potassium nitrate) and possibly lasers can have a direct influence on the nerve excitability by disturbing the nerve transmission [11, 12].

Concerning the use of laser for the treatment of laser hypersensitivity, Sgolastra et al. [13] reported that the mechanisms of action of the laser allowing efficient treatment of DH are

(1) coagulation of proteins of the fluid inside the dentinal tubules; this will diminish the fluids movements;

(2) occlusion of tubules through partial submelting of the denuded dentine;

(3) discharging of internal tubular nerve.

Those interesting effects may be acceptable for the clinical use of laser for DH treatment if it is used safely without pulp damage [13, 14].

The aim of our study is to evaluate the ability of the Nd:YAP laser (1340 nm) to induce dentinal melting, to provoke the occlusion of the dentinal tubules, and to determine the safe irradiation conditions.

2. Material and Methods

2.1. SEM Study. Teeth used in this *in vitro* study were extracted caries-free adult human impacted molars wisdom teeth. Patient age range is 18 to 25 years. Reasons for the extractions were not related to the purpose of this study. 44 caries-free adult human molars were kept in a balanced salt solution at 4°C. 24 teeth were used for the SEM study and 20 were used for the temperature increase study. The external surfaces were cleaned using a scaler, and then crowns were immediately sectioned transversally at low speed (300 rpm) using a precision sectioning 20 LC diamond blade (Isomet Low Speed Saw, Buehler Ltd., Lake Bluff, IL, USA) in order to totally expose the dentin. The anatomical crown and apical part of each root were separated. 3 mm thick dentin discs will be obtained through this procedure. The exposed dentinal surfaces of these discs were polished with Soft-Lex discs 3 M Espe (coarse-grit disc and medium-grit) using a handpiece speed of 12000 rpm for 20 seconds. Then, samples were rinsed with cool water and dried with a five-second air blast.

Each surface was divided into four quadrants with a standard grit diamond bur (C 4, 10 mm long, standard grit, Crosstech Diamond Instruments Ltd., Thailand) under cooling water.

The smear layer was removed by a one-minute application of 18% ethylene diamine tetra-acetic acid (EDTA) (Ultradent Products Inc., USA). Teeth were rinsed with distilled water and immediately irradiated at different energy densities.

The exposed dentins were irradiated with Nd:YAP laser (LOKKI, Lobel Medical, Les Roches de Condrieu, France) as follows: pulsed mode, fiber diameter: 320 μm, tangential incidence of the beam, and in noncontact mode (the distance between the optical fiber and the irradiated surface was 1 to 2 mm). Delivered output power was ranged from 0.9 W to 10 W. The output powers available are predetermined by the manufacturer, so there are only 9 different output powers available on the apparatus (LOKKI laser model): 0.9 W–5 Hz and 0.2 m sec per pulse; 1.4 W–5 Hz and 0.2 m sec; 1.8 W–5 Hz and 0.2 m sec; 2 W–10 Hz and 0.1 m sec; 3 W–10 Hz and 0.1 m sec; 4 W–10 Hz and 0.1 m sec; 5 W–30 Hz and 0.33 m sec; 7.5 W–30 Hz and 0.33 m sec; and 10 W–30 Hz and 0.33 m sec per pulse. Eight teeth were used for each power density. We used a large choice of irradiation parameters for the Nd:YAP laser because of the absence of information in the literature about this kind of laser wavelength. The specimens were placed on a flat surface, the optical fiber was moved by the operator tangentially at approximately 1 mm/sec speed, and the speed was controlled and appreciated by the operator with possible human error. On each tooth, we only irradiated 3 different quadrants. The fourth quadrant was kept as a control without any laser irradiation; it was only treated with EDTA 18% for one minute.

Before laser irradiation, exposed dentine of three quadrants was smeared with a graphite paste prepared by mixing distilled water and fine grain (particle size: 5–25 μm) graphite powder (Pressol, Nuremberg, Germany) as an enhancer. The particle size is larger than the diameter average of dentinal tubules. At the end of the irradiations, samples were carefully rinsed with distilled water in order to eliminate the residual graphite.

An SEM (JSM 7500F, JEOL, Tokyo Japan) study was done in order to find the optimal irradiation parameters of the Nd:YAP laser. The selection criteria were its ability to induce dentinal melting and/or the sealing of tubules without inducing cracks or morphological dentinal destruction. After the metallization of all samples, we used a constant magnification of ×3000 for all SEM examinations.

2.2. Temperature Increase Study. We used 20 teeth for the pulp temperature increase measurements. For this part of the study, we decided to only test the optimal irradiation parameters resulting from the SEM analyses that were able to occlude the majority of dentinal tubules.

The cement surfaces of teeth were cleaned using a scaler, and then the cement layer was removed gently with a diamond bur (approximately 100 μm) (C 4, 10 mm long, standard grit, Crosstech Diamond Instruments Ltd., Thailand) in order to totally expose the dentinal tubules. Crowns were sectioned transversally at low speed (300 rpm) using a precision sectioning 20 LC diamond blade (Isomet Low Speed Saw, Buehler Ltd., Lake Bluff, IL, USA) in order to

totally expose and open the cameral pulp chamber below the level of the enamel cement margin. The pulp tissue was removed, and the access cavity was cleaned and filled by a special thermoconductor paste having the same thermal conductivity as human tissue: $0.4\,\mathrm{cal\,s^{-1}\,m^{-1}\,K^{-1}}$. This is comparable to the thermal conductivity of soft tissues (0.2–$0.5\,\mathrm{cal\,s^{-1}\,m^{-1}\,K^{-1}}$), depending on hydration [15]. Each tooth was placed into a warm bath at constant temperature of 37°C. A sensor of the K-type thermocouple (K-type thermocouples HH806AWE Omega, Manchester, UK) was placed into the pulp chamber against the dentinal wall in regard to the future irradiated zone by occlusal access. The second sensor of the thermocouple was placed into the warm bath in order to control the constancy of water temperature at 37°C. We started each measurement after verification that intrapulpal temperature was stable at 37°C.

The graphite paste was applied on external dentinal surfaces below the cervical border (enamel/dentine junction) on a surface of h: 2 mm ×L: 5 mm.

The treatment of each area covered by the graphite was performed with the Nd:YAP laser beam in tangential incidence with an approximate speed of 1 mm/sec. Between two successive temperature measurements, we also took care to wait enough time in order to allow irradiated dentin to have a thermal relaxation and to allow the pulp chamber to once again stabilize its temperature at 37°C.

We performed 6 measurements per irradiation parameter.

According to the study of Zach and Cohen [16], we considered the temperature increase as safe when it was below the trigger temperature of 3°C.

3. Results

3.1. SEM Analysis. The unlased dentin of the control groups which were only treated with EDTA showed a dentinal surface without the smear layer and wide open tubules (Figure 1).

Dentinal surfaces irradiated by means of Nd:YAP laser beam showed different structural changes depending on the delivered power. We observed a direct correlation between the power settings and the tubules occlusion of the exposed dentin.

The output power that ranged from 0.9 W to 1.4 W did not allow occlusion of tubules (Figure 2).

The output power that ranged from 1.8 W to 2 W induced a tubules narrowing and some total tubules occlusion (Figure 3).

Only output power ranging from 3 W to 4 W can induce a total occlusion of tubules (Figure 4).

Higher power settings ranging between 5 W and 10 W with reduced pulse duration induced limited total occlusion or tubules narrowing (Figure 5).

3.2. Temperature Increase. In this part of the pulp temperature increase, we decided to only test the optimal irradiation parameters resulting from the SEM study that were able to occlude the majority of dentinal tubules: 2 W, 3 W, 4 W, and 5 W in pulsed mode.

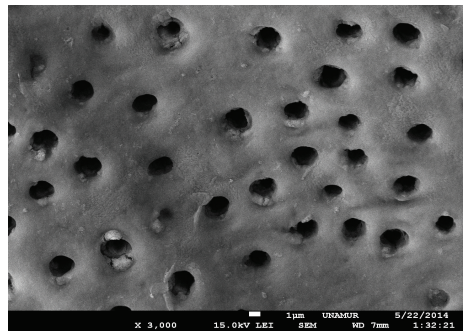


Figure 1: SEM view of unlased dentin (control) treated only with EDTA (18%) during one minute. The dentin is not covered by the smear layer. The tubules are open. Magnification: 3000x.

The output powers ranging between 2 W and 4 W used with a tangential incidence of the laser beam induced a pulpal temperature increase lower than the trigger point of 3°C, while higher power settings induced temperature increases above 3°C (Figure 6). It is interesting to note that output parameters considered as harmless (3 W and 4 W) generated pulpal temperature increase higher than 3°C when the incidence of the laser beam is used perpendicularly to the dentinal surfaces (Figure 6).

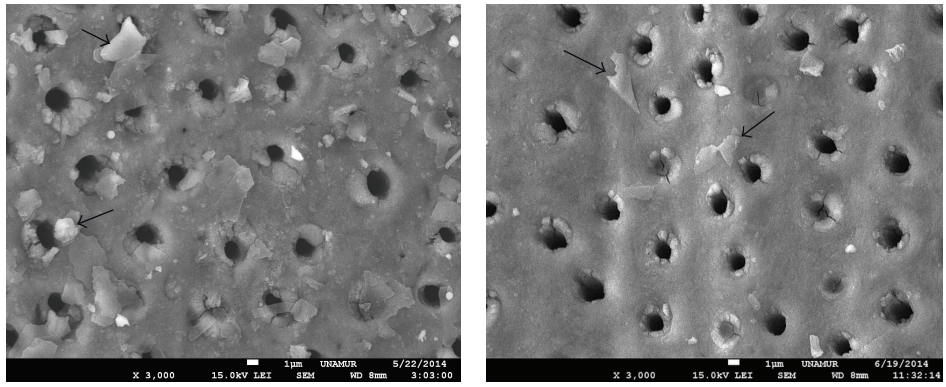
All values passed the normality test (Kolmogorov-Smirnov test with Dallal-Wilkinson-Lillie for P value). Table 1 shows the means and standard deviations of pulpal temperature rise for each irradiation condition.

4. Discussion

We selected young wisdom teeth in our study with the aim of obtaining samples that were as homogenous as possible with similar degrees of dentinal calcification in order to evaluate the effectiveness of Nd:YAP laser to melt dentine and to close wide open tubules.

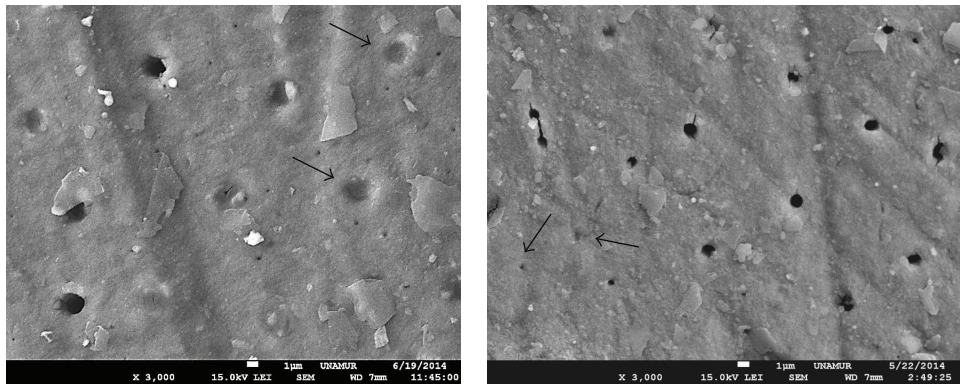
Ethylene diamine tetra acetic acid (EDTA) is a chelating agent of calcium ions that induces the demineralization of the dentine and the smear layer removal [17]. We decided to treat all exposed dentinal surfaces of samples with EDTA in order to have tubules totally open [18]. In this way, we wanted to simulate the same clinical situation of open tubules causing the dentinal hypersensitivity.

The physical properties of each laser wavelength influence the level of absorption and interaction with each tissue. The Nd:YAP laser wavelength is not well absorbed by hard dental tissue to be able to heat sufficiently the surface of the dentine without inducing a pulp overheating. For this reason, we decided to use the graphite paste applied on the surface of the dentine in order to use a lower output power of the laser than would be necessary without the graphite paste [19]. The absorption of Nd:YAP laser beam by the graphite generates a sudden increase in temperature that would be able to provoke an immediate superficial dentinal melting leading to partial occlusion or narrowing of dentinal tubules. We also used



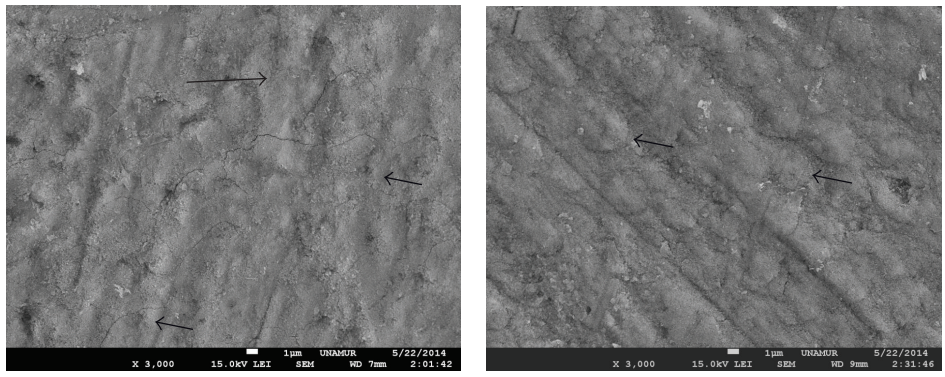
(a) (b)

Figure 2: SEM view of Nd:YAP lased dentin treated previously with EDTA (18%). SEM view of exposed dentine with Nd:YAP laser beam at 0.9 W (a) and 1.4 W (b). We can only notice a slight tubules narrowing. Arrows show the graphite particles still existing on the dentinal surface (not disintegrated by the laser beam). Magnification: 3000x.



(a) (b)

Figure 3: SEM view of Nd:YAP lased dentin treated previously with EDTA (18%). SEM view of exposed dentine with Nd:YAP laser beam at 1.8 W (a) and 2 W (b). We can notice a tubules narrowing. Arrows show some occluded tubules. Magnification: 3000x.

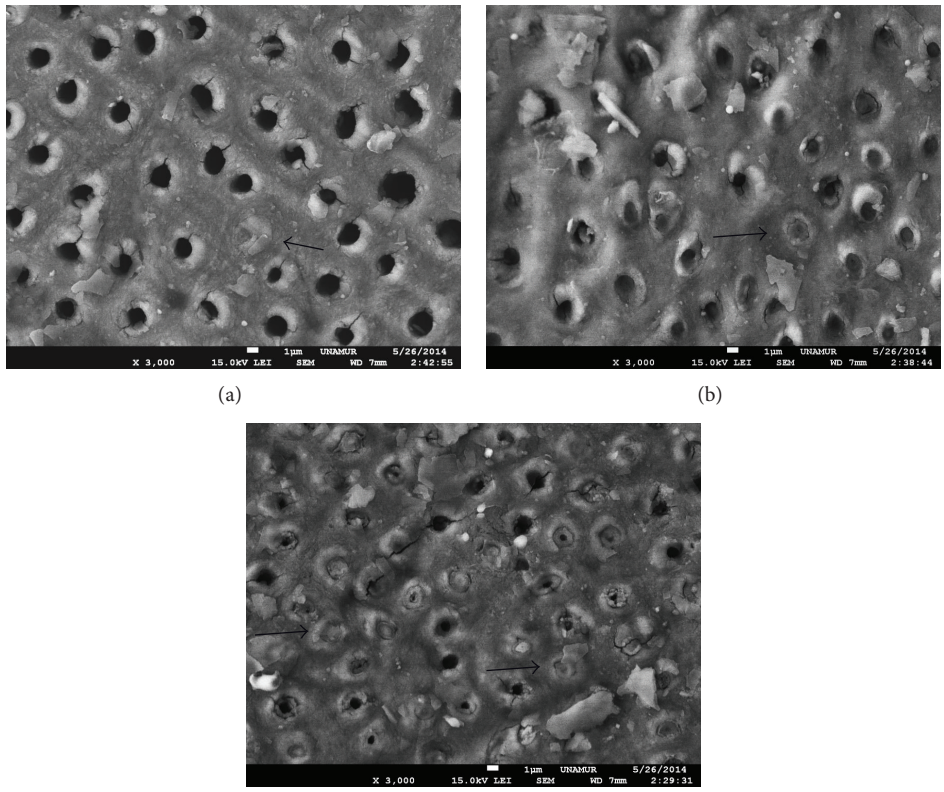


(a) (b)

Figure 4: SEM view of Nd:YAP lased dentin treated previously with EDTA (18%). SEM view of exposed dentine with Nd:YAP laser beam at 2 W (a) and 3 W (b). We notice a total occlusion of tubules. Arrows show some total occluded tubules. Magnification: 3000x.

pulsed modes in order to allow dentin to have a thermal relaxation. We selected the noncontact mode to avoid the damage of optical fiber from the heating of the graphite paste.

Our laser beam struck the dentinal surface at a tangential angle, with the aim of avoiding a direct pulp exposure by the nonabsorbed part of the beam by dentin. We selected the optimal irradiation conditions generating a pulpal temperature increase below 3°C [16]. The tangential mode is indicated because the reduction of the incident angle towards the refractive angle of the tissue surface increases the potential



(a) (b)

(c)

Figure 5: SEM view of Nd:YAP lased dentin treated previously with EDTA (18%). SEM view of exposed dentine with Nd:YAP laser beam at 5 W (a), 7.5 W (b), and 10 W (c). We can notice a tubules narrowing specifically in (b) and (c). Arrows show some occluded tubules. Magnification: 3000x.

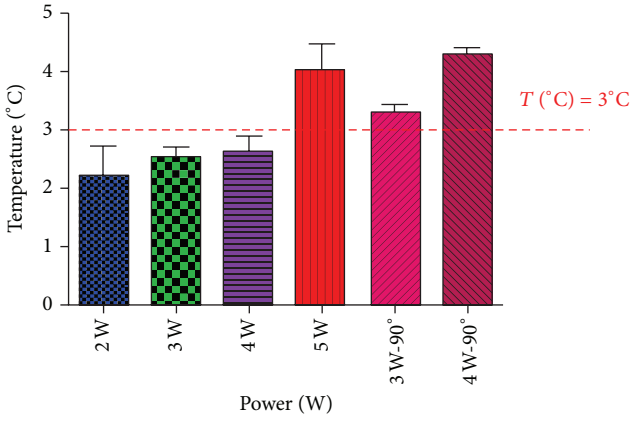


Figure 6: Pulp temperature rise during Nd:YAP laser irradiation of exposed dentine for tubular occlusion. The output powers ranging between 2 W and 4 W used with a tangential incidence may be considered as harmless for the pulpal vitality.

for true light reflection with an important reduction of pulp absorption of incident beam. Our results showed that this precaution was justified. In fact, some harmless parameters (3 W and 4 W used with a tangential incidence) showed a dramatic increase in pulp temperature (higher than 3°C) when they were applied in perpendicular incidences. However, it remains a potential bias for recording the elevation of the pulp temperature. Furthermore, in case of using a perpendicular incidence, we may provoke an artificial pulp temperature increase because the 1430 nm wavelength is well absorb by the metal and can induce possible electromagnetic interferences with the metallic sensor of the thermocouple.

In previous studies, authors demonstrated the possibility to occlude dentinal tubules by means of different wavelengths. Kim et al. [20] demonstrated the feasibility of tubules occlusion by using a CO_2 laser and nanocarbonate apatite, while Han et al. [21] also succeeded in occluding dentinal tubules by replacing the CO_2 laser by an Er:YAG laser.

Umana et al. [19] succeeded in occluding dentinal tubules using diode lasers (810 nm and 980 nm) in combination with graphite paste. Farmakis et al. [22] showed the possibility to occlude tubules using the combination of bioglass and Nd:YAG laser.

Further studies should be conducted in order to evaluate the clinical efficiency of Nd:YAP laser for dentinal hypersensitivity treatment. It is also necessary to evaluate the clinical persistence of this treatment using Nd:YAP and graphite paste.

Table 1: Means and standard deviations for each irradiation condition. All groups passed the normality test of Kolmogorov-Smirnov test (with Dallal-Wilkinson-Lillie for *P* value).

	2 W	3 W	4 W	5 W	3 W—90°	4 W—90°
Number of values	6	6	6	8	8	8
Mean	2,180	2,525	2,620	4,038	3,300	4,300
Std. deviation	0,5450	0,3500	0,2864	1,269	0,1414	0,1000
Std. error	0,2437	0,1750	0,1281	0,4488	0,1000	0,05774
KS normality test						
KS distance	0,1871	0,1723	0,2722	0,1951	0,1788	0,1812
P value	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10
Passed normality test (alpha = 0.05)?	Yes	Yes	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns	ns	ns

5. Conclusions

Under the limitations of this study, the combination of an Nd:YAP laser and a graphite paste is able to induce tubule occlusion, and it may be recommended for a future safe clinical application. Our results pointed out that the following parameters can be considered as efficient for tubules occlusion and harmless for dental pulp: 2 W (200 mJ) to 4 W (400 mJ) with an irradiation speed of 1 mm/sec; fiber diameter: 320 micrometers; 10 Hz; pulse duration: 100 m sec; non-fiber contact mode and in tangential incidence to exposed dentin. The perpendicular incidence of the laser beam on exposed dentin may injure pulp vitality even at low output power of 3 W.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Pulp Revascularization of Immature Permanent Teeth: A Review of the Literature and a Proposal of a New Clinical Protocol

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Tissue engineering is a growing field. In the near future, it will probably be possible to generate a complete vital tooth from a single stem cell. Pulp revascularization is dependent on the ability of residual pulp and apical and periodontal stem cells to differentiate. These cells have the ability to generate a highly vascularized and a conjunctive rich living tissue. This one is able to colonize the available pulp space. Revascularization is a new treatment method for immature necrotic permanent teeth. Up to now, apexification procedures were applied for these teeth, using calcium dihydroxide or MTA to produce an artificial apical barrier. However, the pulp revascularization allows the stimulation of the apical development and the root maturation of immature teeth. Two pulp revascularization techniques are used in the literature, one using calcium dihydroxide and the second using a triple antibiotic paste. Based on these two different pulp revascularization protocols, which obtain the desired therapeutic success, the literature will be reviewed and analyzed according to the relevance of their choice of materials. Based on the literature, we propose a new relevant protocol and a new mixture of antibiotics.

1. Introduction

Tissue engineering is a growing field. In the near future, it will probably be possible to generate a complete vital tooth from a single stem cell. Stem cells are in fact totipotent cells, which have the capacity to proliferate and to produce cells, which are capable of differentiating into specialized cells.

Two types of stem cells exist: embryonic stem cells and adult stem cells (or postnatal cells) [1]. Concerning pulp revascularization, mature stem cells are rather of interest. These cells are found in many sites of the dental element: in the pulp, in the apical papilla, and in the periodontal ligament [1, 2]. These clonogenic cells, rapidly differentiating, have the capacity of inducing dentin-pulp regeneration if differentiating into appropriate cells. In addition, the pulp, which is a product from migration of the neural crest, would probably be a very good candidate to allow nerve regeneration [1]. Regarding the daily practice, it is imperative to find ways to save as much as possible the vitality of stem cells from the dental element and induce their differentiation.

Pulp revascularization is dependent on the ability of residual pulp and apical and periodontal stem cells to differentiate [3–5]. These cells have the ability to generate a highly vascularized and a conjunctive rich living tissue. This one is able to colonize the available pulp space. Subsequently, these stem cells will differentiate into newly formed odontoblasts that will induce an apposition of hard tissue. The nature of this latter is unknown yet [1].

Revascularization is a new treatment method for immature necrotic permanent teeth. Indeed, it would provide, after treatment, a vital tooth that would be able to complete its root maturation. Up to now, apexification procedures were applied for these teeth:

(i) using calcium dihydroxide to induce the formation of an apical calcified barrier;

(ii) using mineral trioxide aggregate (MTA) to produce an artificial apical barrier.

Both methods have shown to be effective regarding the narrowing of the apical foramen of an immature tooth. However,

the pulp revascularization allows also the stimulation of the apical development and the root maturation of immature teeth (root growth and thickening of dentinal walls and natural apexification).

Indications for treatment of pulp revascularization are the presence of deep caries or trauma inducing a stop in the development of root canal of an immature tooth. It is important to keep in mind that an endodontic treatment on an immature tooth, often necessary up to now, involves a root canal treatment on an open apex tooth with thin and fragile walls. This will involve the persistence of a weakened tooth with often a reserved long-term prognosis due to the remaining of an intrinsic fragility and to the difficulty to obtain a good sealing of an open apex. Revascularization technique would allow the growth of root and thus avoiding the remaining of thin and fragile walls. It will reduce the risk of root fracture [6]. This is not the case with apexification treatment.

Immature teeth with a large open apex and short roots seem to be more conducive to the successful treatment of pulp revascularization.

A great importance is given to maintaining the vitality of a tooth in order to keep a possibility of "alert" signal in case of pathogenic stimuli. Losing its innervation and vascularization, a tooth is more vulnerable to any lesion. The maintaining of dental vitality allows better defenses in case of future possible infections.

This pulp revascularization is used for necrotic immature permanent teeth. Even if pulp has lost its vitality, residual pulp stem cells are able to survive. Apical papilla stem cells can also survive to an apical lesion thanks to an abundant blood supply [1, 6–8].

2. Operative Protocol

Two pulp revascularization techniques are found in the literature: one using calcium dihydroxide (Table 1) and another using a triple antibiotic paste (Table 2) for disinfection of pulp necrosis. Both are two-step procedure.

Second step takes place two or three weeks after the first one, only if the tooth is asymptomatic and if there is a visual reducing of the apical lesion.

In pulp revascularization, at three months postoperative, the tooth is normally asymptomatic and about nine months later X-ray radiography shows an increasing thickness of dentinal walls and an apical closure. Root development and apical closure may be visible after three months.

Based on these two different pulp revascularization protocols, which obtain the desired therapeutic success according to their authors, the literature is reviewed and analyzed according to the relevance of their choice of materials. The objective is to define a protocol that would seem to be the most adapted.

3. Discussion

The success of pulp revascularization treatment depends on three elements: root canal disinfection, the presence of a scaffold (blood clot), and hermetic coronary filling [2].

The generation of a functional tissue requires three key elements: stem cells, growth factors, and a scaffold [9].

It is known that the quality of root canal restoration is questionable when residual bacteria are present in the canal. This one could proliferate and eventually induce a reinfection. Therefore, it is essential to have an immune system of quality, major canal disinfection, and a coronary and apical filling allowing no recontamination.

The first part of discussion will be concerned with instrumentation in root canal. Then, the discussion will mention points of divergence between both protocols: irrigation, disinfection, and pulp-capping material. Finally, tissue obtained after pulp revascularization treatment will be mentioned.

3.1. Instrumentation. Most of authors agree to advocate no instrumentation procedure. Using root canal instrument could not only increase fragility of dentin walls but also injure stem cells present in the apical area of these dentin walls. These also contain growth factor imprisoned during dentinogenesis. Growth factor and other cells essential for the regeneration process could also be eliminated by instrumentation. Two types of cells are required to achieve a normal root development: odontoblasts and epithelial cells of Hertwig's sheath. These two cell types are present in abundance in the apical area of immature teeth and are able to resist inflammation phenomena [1, 6–8]. These cells will be able to differentiate into secondary odontoblasts that will generate dentin on root canal walls [1] and thus allow root maturation. No instrumentation procedure remains consistent with vital stem cells preservation and avoids weakening of already thin root canal walls [4, 10, 11].

According to the study of Cehreli et al. [12], even if the number of cases is not sufficient to be statistically significant, it can be noticed that some patients have regained tooth sensitivity (vitality) after treatment. That was observed only in no instrumentation treatment cases.

Thus, elements mentioned so far in favor of no instrumentation protocol seem to be more advised.

3.2. Irrigation. Irrigators play a role of primary disinfection. They should have a maximal bactericidal and bacteriostatic effect while having a minimal cytotoxic effect on stem cells and fibroblasts to allow their survival and ability to proliferate.

Pulp infection can usually spread to the apical region and create a canal acidic environment. This one is not conducive to the creation of tissue regeneration. Bacterial invasion of root canal system causes the formation of bacterial biofilms. Those hang on root canal walls, entrance of dentinal tubules, and in the apical area containing more complex anatomical crevices. At these locations, bacterial biofilms are more resistant to disinfection procedures. Bacteria existing in depth and within the biofilm are in lag phase and therefore refractory to action of antibiotics and irrigators. To ensure optimal root canal disinfection for tissue regeneration, it is necessary to disrupt or eliminate biofilms. Using a tool such as fine "interdental brush" could probably be useful to disrupt biofilms without injuring hard dental tissue. However, the

Table 1: Pulp revascularization using calcium hydroxide.

(a)

First step
Local anesthesia
Isolation of the tooth with a rubber dam
Opening of the pulp chamber to canal entrance (pulpotomy)
Irrigation of root canal (often with 10 mL sodium hypochlorite at 2.5%)[a]
No instrumentation in root canal
Preparation of calcium hydroxide paste[b]
Insertion of the paste in the pulp chamber and in the coronary part (third or half) of root canal (with a cotton ball)
Sealing of the access cavity with a temporary filling

[a]According to authors, nature and concentration of the irrigator can vary.
[b]$Ca(OH)_2$-sterile water in a 3 : 1 ratio.

(b)

Second step (two or three weeks later if asymptomatic tooth and/or absence of fistula)
Local anesthesia without vasoconstrictor[a]
Isolation of the tooth with a rubber dam
Opening the tooth to have a access to root canal
Removal of the calcium hydroxide paste
Copious irrigation of root canal with sodium hypochlorite
Rinsing root canal with sterile water
Drying root canal with paper cones
An apical bleeding is caused by irritation of the apical region with a 15 K-file lime[b]
Preparation of mineral trioxide aggregate (MTA) and its placement on the clot in order to form a hermetic sealing
Place a wet a cotton ball on MTA filling
Sealing of the cavity with a temporary filling

[a]In order to not inhibit the future apical bleeding.
[b]It takes 15 minutes to obtain a blood clot. If a root canal is not bleeding, it is possible to transfer blood from one root canal to another. Blood level must be at least 2-3 mm below the cement-enamel junction.

disadvantage of this kind of tool is the potential risk of leaving nonbiocompatible residues (hairbrush) into root canal.

Activating the irrigation solution within the root canal system is the only possibility to realize disintegration of the bacterial biofilm in noninstrumented areas. It justifies the use of endosonics means. They generate a process of cavitation that induces a temperature increase of the irrigator and currents propelling the irrigator in all crevices. The whole have the effect of potentiating the efficacy of irrigator in order to disintegrate bacterial biofilm [13]. However, during this activation, it is essential to avoid touching the canal walls with endosonic tool in order to respect the decision to avoid any contact between dentinal walls and instruments.

(A) Hydrogen Peroxide. Solvent properties of hydrogen peroxide are almost nonexistent, but it has an interesting hemostatic action. Hydrogen peroxide is antiseptic by release of oxygen radical. Unfortunately, his action is too short and quickly neutralized by organic debris. Moreover, it requires a rinse to reduce pain and possible postoperative gaseous emphysema.

(B) Chlorhexidine. Chlorhexidine 2% gel was proposed as a temporary medication. It has good action on candida and gram$^+$ bacteria by the carryover effect. Indeed, its positively charged molecules confer the property of being adsorbed by the dentin walls and thus allow release of chlorhexidine for at least two to twelve weeks, preventing reinfection of the root canal during this period [14]. Despite this advantage, chlorhexidine does not have an effective dissolving action.

(C) Sodium Hypochlorite. So far, sodium hypochlorite remains irrigator reference in endodontic. It has a solvent action on necrotic tissue and an antiseptic effect widely demonstrated [15]. However, it must be supplemented by a desalting. Recommended concentrations vary between 0.5% and 5.25% [16–19]. Cytotoxicity of sodium hypochlorite is proportional to its concentration. The concentration of 2.5% seems to be the best compromise between efficiency and lack of toxicity [20]. Furthermore, Cunningham showed that elevation of the temperature at 37°C of the 2.5% sodium hypochlorite solution potentiates its solvent power and its

Table 2: Pulp revascularization using a triple antibiotic paste (TAP).

(a)

First step
Local anesthesia
Isolation of the tooth with a rubber dam
Disinfection of the tooth with 10% povidone-iodine (iso-Betadine) before opening it[a]
Opening of the pulp chamber to canal entrance (pulpotomy)
Irrigation of root canal[b] with 20 mL sodium hypochlorite (1.25%–5.25%) then with physiological serum and finally with 2% chlorhexidine
No instrumentation in root canal
Drying root canal with paper cones
Insertion of the triple antibiotic paste[c] into root canal
Place a cotton ball at the root canal entrance
Sealing of the access cavity with a temporary filling

[a]According to the authors, disinfection is done or not.
[b]According to the authors, irrigation may vary.
[c]Mixture of equal proportion of three antibiotics: metronidazole, ciprofloxacin, and minocycline bonded with propylene glycol. Minocycline may be replaced by cefaclor to avoid inducing coloration.

(b)

Second step (two or three weeks later if asymptomatic tooth and/or absence of fistula)
Local anesthesia without vasoconstrictor[a]
Isolation of the tooth with a rubber dam
Disinfection of the tooth with 10% povidone-iodine (iso-Betadine) before opening it[b]
Opening the tooth to have a access to root canal
Removal of the triple antibiotic paste using irrigation with sodium hypochlorite (1.25%–5.25%) then with physiological serum and finally with 2% chlorhexidine[c]
An apical bleeding is caused. Blood level must be at the cement-enamel junction.
Preparation of mineral trioxide aggregate (MTA) and its placement on the clot[d] in order to form a hermetic sealing
Place a wet a cotton ball on MTA filling
Sealing of the cavity with a temporary filling

[a]In order to not inhibit the future apical bleeding.
[b]According to the authors, disinfection is done or not.
[c]Irrigation is done in order to make space for the future blood clot.
[d]It takes 15 minutes to obtain a blood clot.

efficiency becomes comparable to that of the solution to 5, 25% [21].

(D) Iodine. Iodine is bactericide, antifungal, antiviral, sporicidal, and sedative. Purulent secretions and blood do not inactivate it [22]. Its disadvantage is that it colors dental tissues in brown [23].

(E) Ethylene Diamine Tetraacetic Acid (EDTA) + Irrigators. Chelators are weak acids, which react with the mineral portion of dentinal walls. They replace calcium ions with sodium ions, which combine with the dentin to give soluble salts. EDTA-type chelating allows better wettability of the irrigator and a removal of the smear layer [24, 25].

According to Trevino who studies effects of irrigants on the survival of human stem cells of the apical papilla, the use of EDTA before irrigators would allow maximum survival of these cells [26]. 17% of EDTA is often used in cases of bacterial infection to remove the smear layer and allow access to the entrance of dentin tubules (allowing a better chance of joining tissue of regeneration) and induce a better penetration of the irrigator (increases wettability of the irrigator) and of root canal medications [24, 25]. EDTA is also a "sealer" that maximizes bacteriostatic and bactericidal effects of different agents. Its chelating effect would allow the release of growth factors imprisoned in the dentin during dentinogenesis. That would stimulate the proliferation of stem cells [27, 28]. Since EDTA appear to have many advantages, it is important to know how to combine the irrigators. Ring et al. have compared effects of chlorhexidine and hypochlorite after treatment with EDTA [29]. They show that there is no survival stem cell after using a combination of EDTA and 2% chlorhexidine. Moreover, precipitates chlorhexidine salts are formed and maintained in root canal. These precipitates can be toxic and prevent cell adhesion to the canal wall. The combination of EDTA

and 6% of hypochlorite seems to moderately reduce vitality of stem cells. It is also recommended to rinse with saline after irrigating in order to minimize the risk of possible precipitates and to remove residual debris and remain of irrigant [26].

3.3. Disinfection

(A) Calcium Dihydroxide. Calcium dihydroxide, $Ca(OH)_2$, is a strong base (pH = 12.5–12.8); its ionic dissociation in Ca^{2+} and OH^- induced genesis of hard tissue (apexification, tertiary dentin) and has an antibacterial effect by the release of ion OH^- [30]. These ions OH^- damage the cytoplasmic membrane, suppress the bacterial enzyme activity, denature proteins, damage DNA and thus inhibit any replication, and inactivate endotoxins. However, it seems that they have no power over biofilms [31].

Calcium dihydroxide has a low coefficient of dissociation (0.17), which is a good clinical feature since it allows a long-term release of Ca^{2+} and OH^-. Seven days seem sufficient to reduce the bacterial load in root canal at a level of negative culture [32].

According to Nosrat et al. [6], it appears that the basic pH of calcium dihydroxide denatures proteins and could induce necrosis of apical tissue. In any way, it allows thickness increasing of dentinal walls [6, 33].

However, it seems that the dentine (consisting of hydroxyapatite), residues of pulp necrosis, and inflammatory exudates decrease its antibacterial power. For this reason, its effectiveness of disinfection is discussed for in vivo application [34]. Calcium dihydroxide would not be effective on *Enterococcus faecalis*. Acids bacterial products and phosphates from hydroxyapatite of the dentin that limit the diffusion of ions H^+ and OH^- rapidly neutralize its pH [34].

According to some research, $Ca(OH)_2$ would increase the expression of some kind of kinases (extracellular signals by phosphorylation), which are indicators of proliferation of stem cells from pulp and ligament [35]. Therefore, used in usual concentrations, it would not be cytotoxic for stem cells and would support their proliferation [36]. However, tricalcium silicates cements, such as MTA, $Ca(OH)_2$, or Biodentine, have a weakening effect on dentin because of their pH [4, 5]. These damages would be repairable over time but only for MTA and Biodentine [33].

A study realized on cells' cultures showed the direct effect of intracanal medications on stem cells from apical papilla. Calcium dihydroxide used at a concentration of 0.01 mg/mL for canal disinfection allows survival of 100% of the apical stem cells. Even at higher concentration, 1 mg/mL, $Ca(OH)_2$ would also give a maximal survival of stem cells. At the same concentration, antibiotics paste only allows between 33% and 56% cells survival. Used in normal concentrations, antibiotics paste is more toxic than $Ca(OH)_2$, unless if they are used in appropriate concentrations (lower concentrations) [36].

(B) Triple Antibiotic Paste (TAP). According to Chuensombat et al. [37] who studies in vitro antibacterial efficacy and cytotoxic effects of a triple antibiotic paste, it appears that an antibiotic used alone is less cytotoxic than the use of a mixture of antibiotics. To eliminate bacteria belonging to the spectrum of an antibiotic, this one should be used at a minimum concentration of 25 μg/mL. No antibiotics have a spectrum large enough to be active against all types of bacteria present in root canals and apical regions; a combination of antibiotics is essential to cover a maximum range of action. Antibiotics pastes must be used in proper concentration for a balance between a lower cytotoxicity against stem cells (cytotoxicity increases with dose) and a maximum bacterial disinfection. An in vitro study has shown that a TAP concentration of 39 μg/mL would be best for application in disinfection root canal [37].

Hoshino and Takushige showed [38] that mixture paste of three antibiotics with propylene glycol put into root canal with a Lentulo and at a concentration of 20 μg/mL decreases by more than 99%; the average number of bacterial colonies is present [38]. Another in vitro study conducted by Hoshino et al. shows that each antibiotic used alone is ineffective against bacteria present in pulp, dentine, and apical lesions, while the trio of antibiotics allows complete sterilization of germs [38, 39]. Sato et al. developed triple antibiotic paste [40]. Expected to cover at best different root canal bacteria, the three antibiotics consisting of the paste are minocycline (spectrum of $gram^+$ and $gram^-$), ciprofloxacin (spectrum of $gram^+$ and $gram^-$), and metronidazole (spectrum of anaerobic bacteria and protozoa) [37].

Acid pH of minocycline is not favorable to cultivation of stem cells; it would probably facilitate cell permeability of the antibiotic, which would keep long-term cytotoxicity. Ciprofloxacin has also an acid ph. Metronidazole is the only antibiotic of the mixture to have a neutral pH and thus it has no cytotoxicity for needed stem cells [37].

The triple antibiotic paste seems to be biocompatible but its current problem is the possible bacterial resistance.

Minocycline is a semisynthetic tetracycline derivative with a similar action spectrum. It may be replaced by cefaclor in order to avoid any risk of unaesthetic coronary coloring [41] because minocycline binds to ions Ca^{++} by chelation and form insoluble complexes [42]. However, cefaclor appears to be less effective against enterococci. An alternative could be to previously seal the dentinal tubules of the pulp chamber (etching and bonding) [4].

Tetracycline would have ability to inhibit collagenase and metalloproteinases; it is not cytotoxic and is capable of increasing the level of interleukin-10 (anti-inflammatory cytokine). Replacing minocycline by cefaclor due to coronary coloring, we will not be deprived of the benefits of this tétracycline dérivative? Should we not rather choose directly the option of sealing dentin tubules to avoid coronary discoloration? [4].

Metronidazole and ciprofloxacin could induce the formation of fibroblasts [4].

According to Bose et al., the use of triple antibiotic paste shows the highest percentage increase in thickness of the dentinal canal walls compared to the two other intracanal medications (calcium dihydroxide and formocresol) [43].

Enterococcus faecalis is a bacterium of the most importance because it is present in infection resistant to apical treatments [44]. Current enteric bacterium, gram-positive,

can survive and grow in dental root canal without requiring the presence of other bacteria. This bacterium has the ability to invade and survive easily in the dentinal tubules. According to Adl et al., antibiotics have a better action against *Enterococcus faecalis* than calcium dihydroxide. Indeed, the triple antibiotic powder (metronidazole, ciprofloxacin, and minocycline) combined with a saline solution shows the lowest minimum inhibitory concentration (MIC) against *Enterococcus faecalis* (MIC = 77.5 μg/mL). The second place is for a combination of triple antibiotic paste and 2% chlorhexidine with similar results than a combination of minocycline and saline (MIC = 325 mg/mL). The least effective group is combination of calcium dihydroxide and chlorhexidine (MIC = 195 000 μg/mL). Calcium dihydroxide combined with saline is absolutely not effective against *Enterococcus faecalis*. Triple antibiotic paste is very effective against bacteria often present in apical lesion and minocycline seems to be its most active component. However, *Enterococcus faecalis* is not a prevalent bacterium in primary infection of permanent immature teeth [44]. *E. faecalis* is also present in the endocarditis, based on the antibiotic treatment for this disease; we can imagine using similar components for pulp regeneration. Furthermore, their bactericidal spectrum is similar to that commonly used for root disinfection [45, 46].

Pinheiro et al. [47] reported twenty isolated kinds of bacteria in filled root canal with persistent apical lesion. It appears that these highly resistant bacteria are apparently sensitive to tetracycline and doxycycline [47].

Since the choice would seem to lead to pulp revascularization using antibiotic paste, it is important to find antibiotics with neutral ph. Indeed, this would be a favorable environment for stem cells differentiation. Moreover, physical properties of dentin walls could be affected by leaving an acid component for long term in root canal. Therefore, the best will be antibiotics with neutral pH and covering spectrum of minocycline and ciprofloxacin.

An alternative could be the use of a chloramphenicol solution stabilized at neutral ph. Chloramphenicol is used in the absence of alternatives for the local treatment of conjunctivitis, keratitis, and corneal ulcers. Unfortunately, it seems little used due to the risk of adverse effects that must be taken into account.

Other combinations of antibiotics used for infective endocarditis [46] may be considered for their cellular tolerance and their neutral pH. The use of ampicillin (active on bacteria gram$^+$ and gram$^-$) combined with gentamycin (active on bacteria gram$^-$) was proposed. On the other hand, a recent study [46] proposed the following combination for its safety and efficiency ampicillin with ceftriaxone (cephalosporin, third generation). Therefore, we can propose the same association combined with metronidazole for anaerobic germs.

A second proposition can be done. The combination of metronidazole, penicillin G [45], and streptomycin [48] (efficient against gram$^-$ as *E. Coli* and gram$^+$ as *Staphylococcus aureus*). A third proposition may be metronidazole, ceftriaxone, and amikacin [49] (gram$^-$).

3.4. Pulp-Capping Materials (MTA and Biodentine). After disinfection step, a suitable scaffold to encourage generation of new tissue must fill the root canal. At the same time, coronary access must be sealed to prevent further reinfection [50].

Before discussing the possibilities of capping root canal, an issue arises. Induction of the root canal bleeding is done to bring in situ fibrin, platelets, and growth factor. All these elements are indispensable to formation of tissue regeneration. It would also create a matrix from which the growth of new vital tissue is possible into root canal space. The question is could previously prepared platelet rich fibrin (PRF) be included in root canal during bleeding [26]. This would contribute to bring more growth factors and to create a biological tissue scaffold, which promotes tissue growth (reduction of waiting time in comparison with time required for the formation of coagulum).

In vitro studies have demonstrated that calcium dihydroxide and MTA, with their high pH, exert a severe weakening effect on dentin walls during a period of two weeks to two months [51]. However, samples sealed with MTA seem to recover their mechanical properties as fracture toughness after one year. It is not the case with calcium dihydroxide [33].

Biodentine has the same mechanical characteristics as human dentin. Moreover, upon application of this material in a cavity, it seems to fully expand and fill the space by its plasticity [52]. Another advantage is absence of coloring the cervical area unlike MTA, excepted using white MTA.

3.5. The Tissue Regeneration. Claus et al. [53] and Ritter et al. [15] described histological tissue regeneration in animals. They described the existence of a significant neovascularization and the presence of connective cells [15, 53]. Through studies on animal cuts, the apposition material-inducing thickening of root walls may be of different nature dentin, cementum, or even bone [54]. Therefore, this procedure is not a process of pulp revascularization but a process of tissue regeneration. The inability to obtain sections of human teeth after revascularization is a handicap for understanding and validating this process. Only radiographic assessments of in vivo clinical studies and the use of a laser quantifying blood flow (laser Doppler flowmetry) can give us an idea of treatment success [15, 55]. Testing vitality with cold also seems to be a good indicator of success.

Through the analysis of articles in the literature, a new protocol could be proposed (Table 3).

4. Conclusion

Following the analysis of pulp revascularization approaches discussed so far, before opening the tooth, it seems effective to isolate the tooth with a rubber dam and disinfect it with 10% povidone iodine (iso-Betadine) to maximal reduce of oral bacterial concentration.

After opening of the pulp chamber, no root canal instrumentation is still recommended to avoid altering dentinal walls and stem cells present on their surfaces. However, the use of a breast nerve may be useful to remove majority of the infected and necrotic pulp without damaging the root walls of immature teeth.

Table 3: New protocol.

(a)

First step
Local anesthesia
Isolation of the tooth with a rubber dam
Disinfection of the tooth with 10% povidone-iodine (iso-Betadine) before opening it
Opening of the pulp chamber to canal entrance (pulpotomy)
Application of Biodentine on dentinal tubules of the pulp chamber[a]
Root canal disinfection with 17% EDTA following by 2.5% sodium hypochlorite warming at 37°C
Drying root canal with paper cones
Insertion of the triple antibiotic paste[b] into root canal with a Lentulo[c]
Place a cotton ball at the root canal entrance
Sealing of the access cavity with a temporary filling

[a]It is important to keep root canal entrance accessible. This action is intended to seal dentin tubules in order to avoid any subsequent medicine staining.
[b]Mixture of equal proportion of three antibiotics: metronidazole, ciprofloxacin, and minocycline bonded with propylene glycol (concentration of 0.39 μg/mL).
[c]Without overflow at the pulp chamber to avoid any future staining.

(b)

Second step (two weeks later if asymptomatic tooth and/or absence of fistula)
Local anesthesia without vasoconstrictor[a]
Isolation of the tooth with a rubber dam
Disinfection of the tooth with 10% povidone-iodine (iso-Betadine) before opening it[b]
Opening the tooth to have a access to root canal
Removal of the triple antibiotic paste using irrigation with 2.5% sodium hypochlorite then with physiological serum
An apical bleeding is caused. Blood level must be at the cement-enamel junction After filling root canal with blood, previously prepared PRF can be add
Twelve minutes later, application of Biodentine on the clot formed around PRF in order to close access to root canal
Final hermetic filling after hardening of Biodentine

[a]In order to not inhibit the future apical bleeding.

Primary irrigation with EDTA combined with 6% sodium hypochlorite seems to be the best solution as EDTA (little cytotoxic and opening dentin tubules) allows better penetration of the irrigants (and medications) in root canal crevices and tubules. A release of growth factors imprisoned during dentinogenesis could be expected. Sodium hypochlorite remains irrigator base for root canal disinfection. If using a 2.5% sodium hypochlorite concentration, its effectiveness and its solvent power may be potentiated by warming at 37°C. It also seems that rinsing with saline could only bring a benefit to treatment.

Regarding root canal temporary medication, triple antibiotic paste used has good concentration that seems to be the most appropriate in order to avoid any problems associated with calcium dihydroxide (weakening dentinal walls, inducing tissue necrosis, and decreasing effectiveness by infectious exudates). Indeed, the three antibiotics cover at best action spectra of root canal bacteria and show minimum stem cells cytotoxicity when used in adequate concentration (0.39 μg/mL).

During the second step of the procedure, the addition of PRF in root canal may be beneficial. PRF provides an additional supply of blood components, such as growth factors and a more solid support (scaffold) allowing growth of the generated tissue.

Biodentine would be proposed for root canal capping because it appears to have the necessary assets for this procedure (same mechanical properties as human dentine expand to entirely fill space by its plasticity that would increase crown-root tightness, absence of cervical area coloration, and very low cytotoxicity).

For the final hermetic filling, the choice of material does not greatly matter but it should be as airtight as possible and sustainable.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Effects of Er:YAG Laser on Mineral Content of Sound Dentin in Primary Teeth

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The aim of the present study was to evaluate the mineral content of sound dentin in primary teeth prepared using an Er:YAG laser at two different power settings. Thirty-six primary second molars were used in this study. Three dentin slabs were obtained from each tooth, and the slabs were randomly divided into three groups: Group A, control; Group B, Er:YAG laser at 3.5 W, 175 mJ, and 20 Hz, short pulse mode; and Group C, Er:YAG laser at 4 W, 200 mJ, and 20 Hz, medium-short pulse mode. One dentin slab per group was used to evaluate the dentinal morphology and surface roughness values using SEM and profilometer, respectively. Mineral content in the dentin slabs were calculated by inductively coupled plasma-atomic emission spectrometry (ICP-AES). The data were analyzed by one-way analysis of variance and Tukey's HSD tests. No significant differences in Ca, K, Mg, Na, and P levels or Ca/P ratio were found among the groups ($P > 0.05$). SEM micrographs showed that surface irregularities increased with a higher power setting. The surface roughness after laser treatment in Group B and Group C was found to be similar, unlike Group A.

1. Introduction

Dental caries can lead to pain, infection, pulp necrosis, and tooth loss; as such, it is still considered the most prevalent oral disease during childhood and adolescence [1–3].

Cavity preparation traditionally can be performed based on mechanical and biological principles using nonrotatory and rotatory instruments. However, mechanical techniques can cause vibration, pressure, noise, and pain. Pain may be reduced by local anesthesia, but needles can also cause fear; thus, these techniques usually cause anxiety and stress in pediatric patients [4–6]. Dental pain and fear may be decreased using lasers in dentistry. It has been reported that using an erbium: yttrium aluminium garnet (Er:YAG) laser for cavity preparation produces minimal vibration and noise, minimal or no need for local analgesia, reduction of stress, and minimal removal of sound tooth structure, as well as providing a better surface for adhesive restorative materials [6–8]. Therefore, the use of lasers in pediatric dentistry has increased recently [9–11].

Laser technology can be used in soft tissue surgery, caries prevention, caries diagnosis, cavity preparation, and endodontic treatment for children. Cavity preparation procedures have used different laser systems such as Er:YAG and erbium, chromium: yttrium scandium gallium garnet (Er,Cr:YSGG). However, changes in the dentinal morphology of primary teeth resulting from laser exposure have been reported in the literature. Zhang et al. [12] reported cracks and microfissures in the dentin surface of primary teeth prepared using a high-powered Er:YAG laser. In a different study, Zhang et al. [13] evaluated the dentinal morphology of primary teeth prepared using the Er:YAG laser with different power parameters, and they reported that there was no smear layer and the dentinal tubules were clear. In addition, they found dentin melting and cracks associated with high-powered Er:YAG lasers [13].

There have been recent reports in the literature regarding the mineral content of dental hard tissue prepared by different laser treatments [14–20]. In addition, Ari and Erdemir [19] reported that the adhesion of dental restorative materials to hard tissue was affected by changes in the mineral content of dentin. Therefore, change in the mineral content of dentin is important to restorative practice, bonding mechanism, and microleakage. In recent studies on the mineral contents of dental hard tissue prepared by different laser treatments, permanent teeth have been used [14–20]. Because the mineral contents of enamel and dentin in primary teeth are different from those of permanent teeth, the effect of laser on the mineral content of primary teeth may be different from that of permanent teeth as well.

There are limited studies related to mineral content of dental hard tissue in primary teeth [21–23]. In addition, there are some reports related to the concentration of trace element in primary teeth of children with healty, different conditions and/or syndromes [24–28]. However, the effect of Er:YAG laser on the mineral content of sound dentin in primary teeth has not been studied yet.

The aim of the present study was to evaluate the mineral content of sound dentin in primary teeth prepared using an Er:YAG laser at two different power settings. The null hypothesis tested was that there would be differences in the mineral content—levels of calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), and phosphorus (P) and Ca/P ratio—of sound dentin in primary teeth prepared using an Er:YAG laser at two different power settings.

2. Material and Methods

2.1. Sample Preparation. All sample preparation was performed by the same operator (V.A.G.) to prevent interoperator variation. The study samples were comprised of 36 human lower primary second molars that were free of dental caries or restoration and extracted for orthodontic reasons. The study was approved by the Faculty of Medicine, Noninvasive Clinical Research Ethic Committee, Ordu University (2013-34). After cleaning, the teeth were mounted 1 mm above of cementoenamel junction, vertically in quadrangular molds with an autopolymerizing acrylic resin (Meliodent; Bayer Dental, Newbury, UK). Tooth enamel was removed with a diamond high speed cylinder bur under water cooling manually because of dentin layer being thin. The cut surface of each tooth to confirm the absence of enamel was digitally observed under light microscope, magnification X20 (Olympus SZ4045 TRPT, Osaka, Japan). Then, the occlusal thirds of the crowns were cut using a slow-speed diamond saw (Isomet; Buehler, Lake Bluff, IL) under water cooling. Three 0.6-mm thick, dentin slabs were obtained from each tooth for the same mineral concentrations [17] and randomly divided into three groups ($n = 36$ per group):

Group A: control group, no treatment.

Group B: dentin irradiated with an Er:YAG laser at 3.5 W, 175 mJ, and 20 Hz, short pulse mode.

Group C: dentin irradiated with an Er:YAG laser at 4 W, 200 mJ, and 20 Hz, medium-short pulse mode.

2.2. Laser Treatment. All laser applications were performed by the same operator (M.B.) to prevent interoperator variation. The laser probe was held in the same position so that it did not move across the tooth surface.

Two standard laser device modes (Fidelis Plus 3; Fotona, Ljubljana, Slovenia) were used for the laser treatment. The first, 3.5 W, 175 mJ, and 20 Hz, short pulse mode, was used for Group B. In this group, the laser was applied with a sapphire probe (1 mm in diameter); the contact handpiece (R14) was placed perpendicular to the surface at a distance of 1 mm, and the entire dentin area was scanned at a speed of 1 mm/sec with water and air cooling. For this application, the device power was set at 3.5 W with short pulse mode (300 μs), and the repetition rate was 20 Hz with 175 mJ pulse energy.

The second, 4 W, 200 mJ, and 20 Hz, medium-short pulse mode, was used for Group C. In this group, the laser was applied with 4 W power (200 mJ pulse energy with 20 Hz repetition rate) and with a medium-short pulse (100 μs).

The average time of laser irradiation in each group was 30 seconds per specimen.

2.3. Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) Technique. ICP-AES experimental procedures were conducted according to Malkoc et al. [14, 15] and Secilmis et al. [16].

All dentin slabs ($n = 34$ per group) were stored at 70°C in cabinet desiccators (Ventisell, Italy) until they reached a constant weight. Then each specimen was weighed on an electronic balance (AX200; Shimadzu Corporation, Kyoto, Japan) and the weight was recorded [14]. Nitric acid (10 mL) and hydrochloric acid (3 mL) were added onto the specimens and they were digested in a microwave reaction system (Mars 5; CEM, Matthews, NC) at 180°C and 180 psi [16].

After calibration of the ICP-AES instrument (Vista AX, Varian, Mulgrave, Australia), 2 mL of solution was taken. The solutions are carried in a nebulizer with the help of a peristaltic pump. The specimen is turned into an aerosol which is carried by an argon spray. The aerosol is heated by conduction and radiation and reaches approximately 10,000°C. Light is transferred to a detector, and every element is evaluated according to its wavelength. Five measurements were performed on each element for each solution, and the means of the measurements were calculated in milligrams per liter (parts per million) [15, 16]. The levels of Ca, K, Mg, Na, and P in each specimen were determined, and the mineral contents were then calculated as percentage by weight.

2.4. Scanning Electron Microscopy (SEM) Examinations. All dentin slabs ($n = 1$ per group) were prepared for SEM (LEO EVO 40 VP; Leo Electron Microscopy Ltd., Cambridge, UK). After surface treatment, the specimens were coated (BAL-TEC SCD 050 sputter coater; BAL-TEC AG, Balzers, Liechtenstein) with gold/palladium, and micrographs were obtained.

2.5. Surface Roughness Examinations. All dentin slabs ($n = 1$ per group) were prepared for surface roughness examinations. The mean surface roughness values (Ra) of study samples were evaluated using a profilometer (Surf Test-402

Table 1: Mean percentage weights of the five elements (mean ± standard deviation) and Ca/P ratio according to groups (n = 34 per group).

Groups	Ca	K	Mg	Na	P	Ca/P
Group A	21.554 ± 1.605	0.054 ± 0.013	0.587 ± 0.165	0.646 ± 0.094	9.302 ± 0.734	2.328 ± 0.220
Group B	22.385 ± 1.442	0.060 ± 0.016	0.647 ± 0.092	0.614 ± 0.060	9.260 ± 0.386	2.415 ± 0.06
Group C	23.075 ± 1.538	0.059 ± 0.014	0.621 ± 0.118	0.685 ± 0.052	9.545 ± 0.417	2.415 ± 0.07
P values	0.103	0.651	0.579	0.100	0.450	0.284

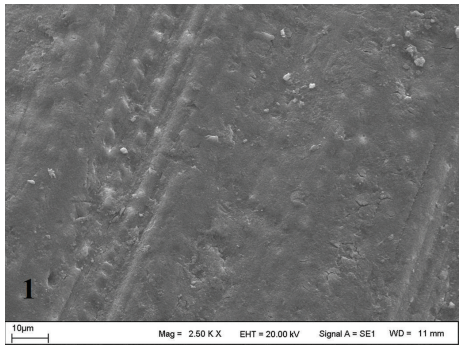

Figure 1: SEM micrograph of dentin surface in control group (original magnification ×2.50 k).

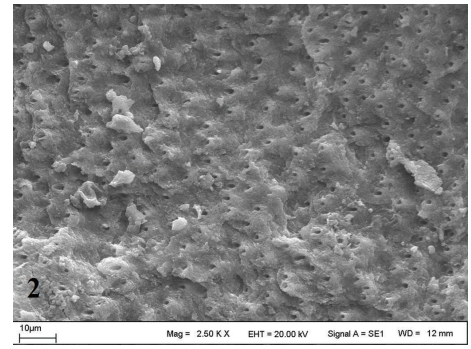

Figure 2: SEM micrograph of dentin surface treated with 3.5 W laser in Group B (original magnification ×2.50 k).

surface roughness tester; Mitutoyo Corp., Tokyo, Japan) in all groups. To measure the roughness value in micrometers, a diamond stylus (tip radius, 5 lm) was moved across the surface under a constant load of 0.75 mN with a speed of 0.5 mm/sec and a range of 350 lm. The instrument was calibrated using a standard precision reference specimen. Three traces were recorded for each specimen at three different locations in different positions (parallel, perpendicular, and oblique) giving nine tracings per sample. The average of these nine mean surface roughness measurements was used as the score for each sample. The scores were entered into a spreadsheet (Excel; Microsoft, Seattle, WA) for calculation of descriptive statistics.

2.6. Statistical Analysis. Statistical analysis of the data was performed using SPSS 16.0 for Windows (SPSS, Chicago, IL). The differences in mineral content between the groups were analyzed with one-way analysis of variance (ANOVA), and the comparison of means was performed with the Tukey HSD multiple comparisons test. Statistical differences were determined at a 95% confidence level (P = 0.05).

3. Results

3.1. ICP-AES Evaluation. The mean percentage weights of the five elements (Ca, K, Mg, Na, and P) in the dentin slabs are shown in Table 1. One-way ANOVA showed that there were no significant differences between Ca, K, Mg, Na, and P levels or Ca/P ratio ($P > 0.05$) among the three groups.

3.2. SEM Evaluation. SEM views of the control (Group A) are shown in Figure 1. Er:YAG laser-treated dentin surfaces (Groups B and C) are shown in Figures 2 and 3. The surface

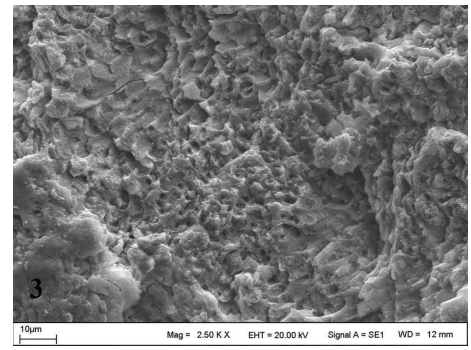

Figure 3: SEM micrograph of dentin surface treated with 4 W laser in Group C (original magnification ×2.50 k).

treated at Group C was rougher than those of Groups A and B. A rough, crystalline irradiated dentin surface could be observed in both slabs treated with the laser. This appearance was more pronounced in the Group C (Figure 3).

3.3. Surface Roughness Evaluation. The mean surface roughness values of all groups are shown in Table 2. The lower mean surface roughness value was found in Group A. The higher mean surface roughness value was found in Group C. However, similar mean surface roughness values were found in laser treatment groups (Groups B and C).

4. Discussion

In this study, the compositional changes (Ca, K, Mg, Na, and P) in the sound dentin in primary teeth prepared by an Er:YAG laser at two different power settings were evaluated

Table 2: Mean surface roughness values according to groups (nine tracings per sample).

Groups	Roughness value (means ± standard deviation)
Group A	0.41 ± 0.2
Group B	1.68 ± 0.3
Group C	1.90 ± 0.2

and the Ca/P ratios of the groups were compared using ICP-AES. The mean percentage weights of Ca, Mg, Na, and P and the Ca/P ratio of the groups were not affected by laser irradiation. Therefore, the null hypothesis was rejected.

To evaluate the mineral content of dental hard tissue, different methods can be used, such as energy dispersive spectrometer (EDS), wavelength dispersive X-ray fluorescence spectrometry (WDXRF), atomic absorption spectrophotometer (AAS), and FT-Raman spectroscopy [18, 29–31]. However, the ICP-AES technique was preferred for this study due to the following advantages: (1) this technique is one the most attractive methods for the measurement of trace elements; (2) trace elements can be measured at the micrograms per liter level; and (3) multiple elements can be measured at the same time [19, 32].

Dentinal morphology is important when a tooth needs to be treated with adhesive restorative materials. The cavity preparation with the Er:YAG laser could be an alternative for fearful children in pediatric dentistry. However, use of the Er:YAG laser in both primary and permanent teeth might cause changes in dentinal morphology. Dentin consists of organic and inorganic components; the major inorganic components of dental hard tissue are Ca and P present in hydroxyapatite crystals. It has been reported that the Ca/P ratio of dentin depends on factors such as hydroxyapatite crystal type, the availability of Ca, the anatomic location, and the technique used [19, 33, 34]. In addition, it has been reported that altering the Ca/P ratio of dentin can change the morphology of dentin and affect the adhesion of restorative materials to dental hard tissues [19, 35]. According to results of this study, the Ca/P ratios in the dentin of primary teeth were not affected by Er:YAG laser irradiation at two different power settings. However, SEM photographs indicated that the surface irregularities increased when the power setting was increased. Secilmis et al. [16] reported that surface irregularities in the dentin of permanent teeth increased when a high-powered laser was used. These results were in accordance with our findings. However, they found that Ca, Mg, Na, and P levels and Ca/P ratio in the dentin of permanent teeth were affected by Er,Cr:YSGG laser treatment. These results were not in accordance with our findings and might be related to the use of permanent teeth and Er,Cr:YSGG laser. Also, in the present study, surface roughness was increased after laser treatment. The higher mean surface roughness value was found in Group C: 4 W, 200 mJ, and 20 Hz, medium-short pulse mode. However, similar mean surface roughness values were found in laser treatment groups (Groups B and C). The increase of surface roughness value may be related to energy density of the Er:YAG laser. However, our surface roughness results should be supported by further studies using more dentin slabs.

A few studies have been conducted on the effect of Er:YAG laser on the dentinal micromorphology of primary teeth. In these laser studies, different power settings of Er:YAG laser in primary teeth were used. Kornblit et al. [36] evaluated the enamel and dentin morphology of primary teeth using an Er:YAG laser with different power parameters. They observed no carbonization or cracks, and the SEM micrographs were similar to those of permanent teeth. Flury et al. [37] compared the dentinal morphology of primary teeth using an Er:YAG laser and a diamond bur, and they reported open dentin tubules for all the laser-treated groups. These results were in accordance with our findings; we observed open dentin tubules for both laser groups when compared to the control group. However, our SEM results should be supported by further studies using more dentin slabs.

The mineral content of sound dentin in primary teeth prepared using "3.5 W, 175 mJ, and 20 Hz, short pulse mode," and "4 W, 200 mJ, and 20 Hz, medium-short pulse mode," of Er:YAG laser was evaluated in this study. Laser parameters used in the study were predetermined by the manufacturer's instructions. The same parameters of Er:YAG laser in primary teeth have not been studied yet. However, similar power setting of Er:YAG laser in primary teeth was used in a few studies [12, 13, 37, 38]. Zhang et al. [13] reported that laser power of less than 4 W at Er:YAG laser should be used for cavity preparation in primary teeth. In addition, they found that the use of the laser at 10 Hz/200 mJ and 10 Hz/300 mJ for cavity preparation in primary teeth is safe and effective. Monghini et al. [39] reported that Er:YAG laser irradiation of dentin in primary teeth adversely affected bond strength. In addition, they found that the increase of laser energy resulted in increasingly cratered surfaces. These results were in accordance with our findings.

Most of the studies on the effect of dental lasers on the mineral content of hard tooth tissues have been carried out on permanent teeth [14–20]. However, there are some differences between primary and permanent tooth dentin, such as thickness, number of dentin tubules, degree of mineralization, and inorganic component. The concentration of Ca and P in peritubular and intertubular dentin is lower in primary teeth than in permanent teeth. In this study, it was found that the mineral content of sound dentin in primary teeth was not affected by laser irradiation. It may be that the two laser power settings of Groups B and C did not lead to different mineral content, but they did vary in surface irregularities. The surface irregularity in Group C was higher than that in Group B. Mineral content of sound dentin in primary teeth may be change with different power settings of Er:YAG laser. Therefore, correlations among Er:YAG laser power settings, mineral content, surface irregularities, and roughness during laser preparation of dentin in primary teeth should be investigated in further studies.

From a clinical viewpoint, there are limitations concerning the correlation between in vitro and in vivo tests and also clinical usage. There are some limitations of this in vitro study. First, only two laser power settings of Er:YAG laser were used

in this study. Mineral content of sound dentin in primary teeth may change using different power settings of Er:YAG laser. Second, only Er:YAG laser was used in this study. Mineral content of sound dentin in primary teeth may change using different laser type. Third, surface irregularities and roughness were evaluated in this study using only one dentin slab per group. The surface irregularities and roughness results may change using more dentin slabs.

5. Conclusion

Laser irradiation makes structural and chemical changes on the dental hard tissues. These changes alter the level of solubility and permeability of dentin. Consequently, the bond strength of adhesive systems on dentine surfaces may be affected in clinical practice. However, laser treatment did not affect the mean percentage weights of Ca, K, Mg, Na, and P or the Ca/P ratio in any group of primary teeth in present study. Therefore, tested power settings of the Er:YAG laser is safe for cavity preparation in primary teeth.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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