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Bone Formation in the Goat Maxillary Sinus Induced by Absorbable Collagen Sponge Implants Impregnated With Recombinant Human Bone Morphogenetic Protein-2

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This study assessed the efficacy, safety, and technical feasibility of inducing bone formation in an animal model of maxillary sinus floor augmentation using recombinant human bone morphogenetic protein-2 (rhBMP-2) impregnated on an absorbable collagen sponge (ACS). Bilateral antral maxillary sinus floor elevation procedures were surgically performed in six adult female Alpine-Saanen goats. Bone formation in response to the implant was evaluated using sequential radiographs, computerized tomography, and gross pathologic and histologic analysis performed at necropsy. Computerized tomographic scans documented nonosseous radiopacity in both sinuses postimplantation. Sinuses implanted with rhBMP-2/ACS subsequently demonstrated increasing radiopacity local to the implant site, while radiopacity of the negative control sinuses remained unchanged or decreased. The results demonstrated the ability of an rhBMP-2/ACS implant to induce substantive new bone formation within the maxillary sinus of goats without adverse sequelae. The rhBMP-2/ACS composite implant may represent an acceptable alternative to traditional bone grafts and bone substitutes for maxillary sinus floor augmentation procedures in humans. (Int J Periodont Rest Dent 1996;16:9–19.)

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Patients with edentulous posterior maxillae often present with loss of alveolar bone and increased maxillary sinus pneumatization. As a result, dental restoration is often difficult to achieve due to an inadequate volume of bone to support the placement of endosseous dental implants. The maxillary sinus floor augmentation surgical procedure is meant to increase alveolar bone height by the formation of new bone in the lower portion of the maxillary sinus.[1]

Bone graft and bone graft substitutes have been used in maxillary sinus floor augmentation procedures with varying degrees of success. Success rates for autogenous bone are in the range of 90% to 100%,[2,3] but patients often do not have enough bone that can be readily harvested from the maxillofacial area. Harvesting bone from the iliac crest or the chin is inconvenient, costly, and associated with significant morbidity. Approximately 8% of iliac

crest grafts result in major complications such as infection, blood loss resulting from arterial injury, hematoma formation, nerve injury, short- and long-term pain, and functional deficit.[4]

Compared to autogenous bone, allogeneic bone has lower osteogenic potential, but in combination with a Gore-Tex barrier it has provided predictable results in the maxillary sinus surgical procedure.[5,6] Radiographic and histologic findings support consistent bone fill in the form of a loose trabecular pattern when the lower portion of the maxillary sinus was filled with mineralized allograft bone and covered with the augmentation membrane. Less success was observed with demineralized bone, and greater scarring was evident on gross examination of the osteotomy site where no membranes were utilized.[6]

Another commonly used material, hydroxyapatite (HA), is osteoconductive but nonresorbable. While it has been used successfully to augment autogenous bone for grafting procedures,[7] there is no scientific evidence to support its use alone as a sinus floor grafting material.[2]

Over 30 years ago, Urist[8] demonstrated that protein extracts from bone implanted into animals at nonbony sites were capable of inducing the local formation of new cartilage and bone; hence the term bone morphogenetic proteins (BMPs). Since that time, at least nine BMPs, BMP-1 to BMP-9, have been cloned, and their osteogenic activities have been characterized.[9–16] The results of these experiments demonstrate that human recombinant BMP-2 (rhBMP-2) has the ability to induce bone and repair bony defects at a variety of anatomic sites in many animal models.[11–14]

The aim of the present study was to test the efficacy, safety, and technical feasibility of using an rhBMP-2 absorbable collagen sponge (ACS) implant as a potential alternative to existing bone graft for inducing osteogenesis in an animal model of maxillary sinus floor augmentation.

Method and materials

The study used six adult (age 3 to 7 years) female Alpine-Saanen goats that weighed 55 to 70 kg.

A dry 3 × 4 inch collagen sponge was uniformly soaked with either rhBMP-2 (at a concentration of 0.43 mg/mL, produced and purified by the Genetics Institute) or a control buffer. After soaking, each sponge contained a total of 3.4 mg of rhBMP-2 or control buffer. One half of the composite sponge device was used at each implant site (1.7 mg total protein delivered unilaterally in the sinus for each animal). Each animal received one rhBMP-2/ACS implant and one buffer/ACS implant on the contralateral side.

Implantation

Twelve hours prior to surgery, goats received 11 mg/kg of ampicillin intravenously. Just prior to the induction of anesthesia with ketamine (8 mg/kg, intravenously), goats were pretreated with xylazine (0.05 to 0.1 mg/kg, intravenously) and atropine (0.2 mg/kg administered intramuscularly). Intraoperative anesthesia was maintained with isoflurane, which was delivered along with oxygen in a semi-closed-loop system.

The maxillary region was draped, and an oblique caudodorsal, rostroventral, extraoral incision approximately 5 cm in length was made over the most ventral aspect of the maxillary sinus. Subcutaneous tissue and the masseter muscle were divided to expose the maxillary periosteum, which was incised and elevated dorsally. The lateral wall of the sinus was approached with a surgical rotating orthopedic diamond bur to perform a surface osteotomy. The antral membrane was then gently eased superiorly via a lateral approach through the osteotomy to

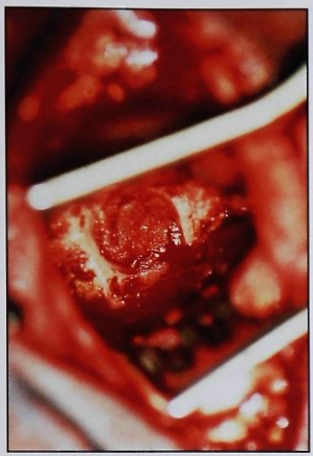


Fig 1 (left) *Recombinant human bone morphogenetic protein-2/absorbable collagen sponge implant after placement onto the floor of the maxillary sinus.*

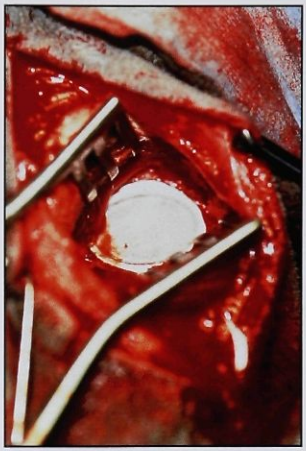


Fig 2 (right) *Gore-Tex membrane placed over the osteotomy.*

expose the floor of the maxillary sinus. Care was taken to avoid perforation of the antral membrane of the sinus.

Exposure of the maxillary sinuses was carried out bilaterally, with one sinus receiving the rhBMP-2/ACS implant and the other receiving an ACS/buffer implant impregnated with control buffer (Fig 1). A Gore-Tex membrane was placed over the osteotomy (Fig 2). The overlying soft tissues were routinely closed with sutures.

All goats were administered 11 mg/kg of ampicillin four times daily for 5 days postsurgery. Buprenorphine (0.005 mg/kg intramuscularly, twice daily) and/or phenylbutazone (4 mg/kg loading dose, then 2 mg/kg) was administered for approximately 48 hours postsurgically or as needed.

Clinical observations

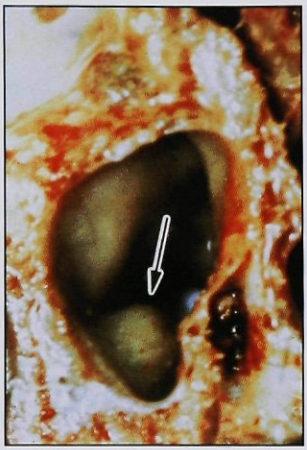
All goats were assessed daily for the duration of the study by means of a complete physical examination. In addition, complete blood counts and blood chemistry profiles were performed once prior to implantation and again prior to sacrifice.

Radiographic assessments

Plain lateral radiographs of each maxillary sinus were made presurgically, immediately postsurgery, and thereafter at 4-week intervals. When necessary, goats were sedated with xylazine (0.1 to 0.5 mg, intravenously) for this procedure. Radiographs were assessed qualitatively for new bone formation.

CT scans

Tomographic assessment of bone formation was completed presurgery (n = 6), postsurgery (n = 6), and at 2 (n = 4), 4 (n = 2), 6 (n = 4), 8 (n = 4), 10 (n = 2), and 12 (n = 2) weeks. With the goats in sternal recumbency, transaxial scans of both maxillary sinuses were completed in a rostrocaudal direction using 5-mm slice widths and a 2-mm overlap with a Shimadzu SCT3000TE (Shimadzu) tomographic scanner.



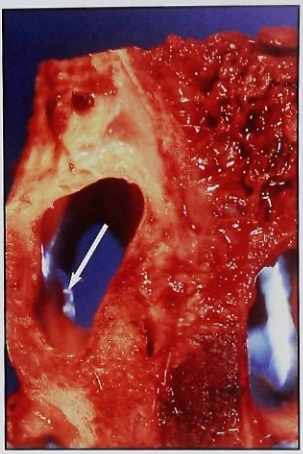


Fig 3a (left) *Twelve-week gross section of the right sinus with recombinant human bone morphogenetic protein-2/absorbable collagen sponge.*

Fig 3b (right) *Twelve-week gross section of the left sinus with absorbable collagen sponge/buffer.*

Animal sacrifice and histologic analysis of tissues

The goats were sacrificed either 4, 8, or 12 weeks after implantation (2 animals at each interval) and were evaluated at necropsy for gross pathology. Soft tissue, the surgical site, and the maxillary sinuses were examined and photographed (Figs 3a and 3b). The maxillary sinuses were then excised and placed in Millonig's fixative for 2 weeks at 4°C. Samples were divided in a transverse plane at the rostrocaudal midpoint of the osteotomy site. One half was decalcified, embedded in paraffin, cut into 5- to 6-μm sections, and stained with hematoxylin-eosin; the other half was embedded in poly(methyl methacrylate) (PMMA), cut into 5-μm sections, and stained in modified von Kossa with safranin O and fast green. A subset of sections were stained with toluidine blue.

Serologic analysis

Serum samples were obtained prior to surgery and at 5 and 14 days postsurgery. The presence of antibovine collagen antibodies was evaluated using an enzyme-linked immunoabsorbent assay (ELISA) specific to bovine collagen compared to both negative and positive controls.

Hematologic analysis

Preoperative and postoperative blood samples were obtained from the jugular vein of each goat, and general hematology, differentials, and serology were determined.

Results

Clinical observations

The clinical course for all animals was uneventful, and no adverse clinical sequelae were encountered. Postsurgical swelling was observed at the incision sites in all animals, which was consistent with the surgical procedure and was resolved by 7 to 10 days postoperatively. Some animals also experienced a transient reduction in body temperature postoperatively, possibly because of the length of general anesthesia. However, all goats had normal body temperatures within 2 days after implantation. Some weight loss was observed in three animals postoperatively, but this was considered to be unrelated to surgery.

CT scans and radiographs

Computerized tomographic scans documented a similar level of radiolucency in both sinuses immediately preimplantation and postimplantation. In sequential biweekly postoperative CT scans, sinuses receiving the rhBMP-2/ACS implant demonstrated increasing osseous radiopacity local to the implant site, while radiopacity of the contralateral control sinuses remained unchanged or decreased (Figs 4a to 4c).

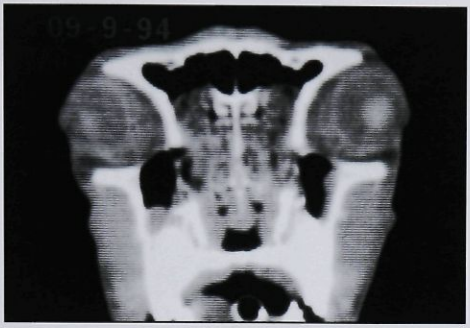


Fig 4a *Four-week CT scan showing nonosseous radiopacity in the rhBMP-2/ACS–treated sinus* (left) *compared to negative control buffer/ACS-treated sinus* (right).

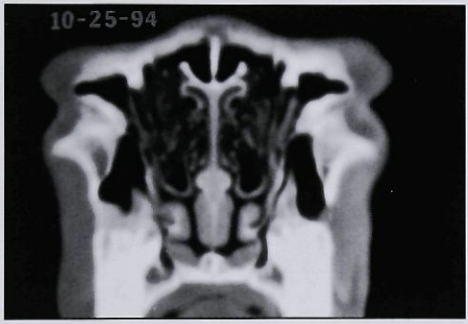


Fig 4b *Eight-week CT scan showing increased radiopacity in the rhBMP-2/ACS–treated sinus* (left) *compared to buffer/ACS-treated sinus* (right).

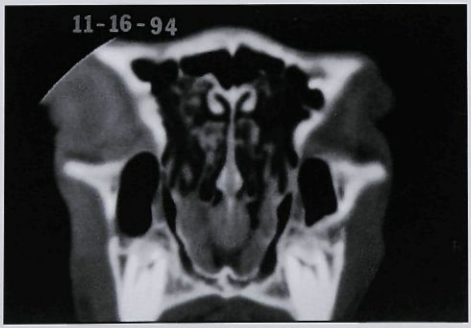
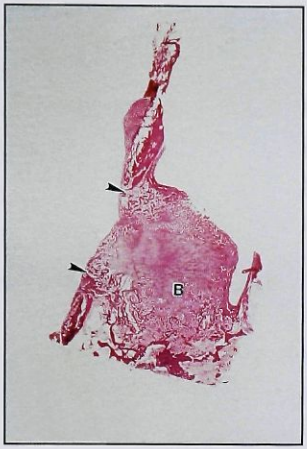


Fig 4c *Twelve-week CT scan with rhBMP-2/ACS–treated sinus* (right) *and buffer/ACS-treated sinus* (left).



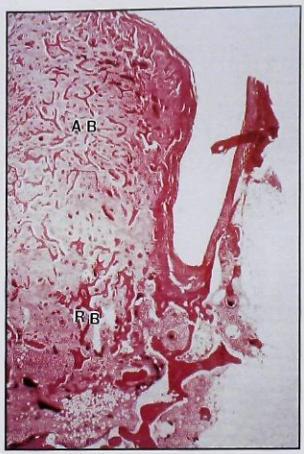


Fig 5a *Hematoxylin-eosin-stained section through the maxillary sinus treated with an rhBMP-2–impregnated implant and harvested at 4 weeks. Arrowheads indicate the lateral edges of the osteotomy and* B *denotes new trabecular bone.*

Fig 5b *Increasing power photomicrograph of the section shown in Fig 5a.* AB *indicates active trabecular bone deposition and* RB *indicates remodeling bone.*

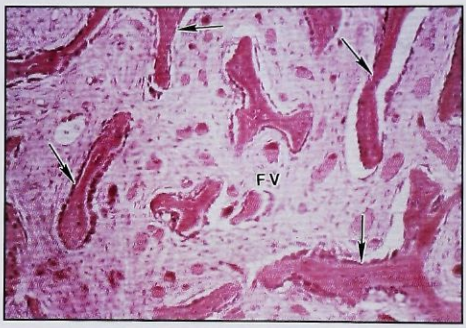


Fig 5c *Hematoxylin and eosin–stained section showing new trabecular bone (arrows) covered by active osteoblasts at 4 weeks. The new bone is embedded in fibrovascular tissue* (FV).

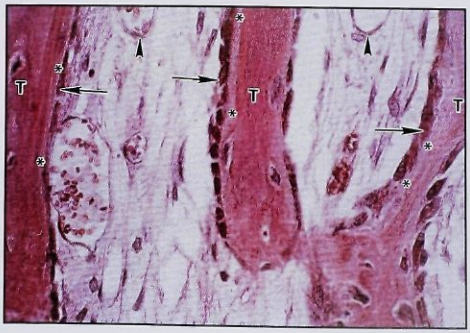


Fig 5d *Increasing power photomicrograph of the trabeculae* (T) *seen in Fig 5c. Cuboidal osteoblasts (arrows) rest on a layer of unmineralized osteoid (**). Arrowheads indicate blood vessels.*

Histologic analysis

Four weeks postimplantation. In sinuses implanted with rhBMP-2/ACS, substantive new cancellous (trabecular) bone was apparent local to the implant sites (Figs 5a to 5d). The trabeculae of the new bone were covered by cuboidal osteoblasts with few osteoclasts present, suggesting that the remodeling was in an early stage. In a small inferiomedial portion of the sinus the trabeculae were covered by flat lining cells, and osteoclasts were essentially absent. This suggests that more complete remodeling had occurred in this region. All trabeculae were embedded in a fine, fibrillar network of connective tissue that was highly vascular, and there was no evidence of bone marrow or fat cells. There was no evidence

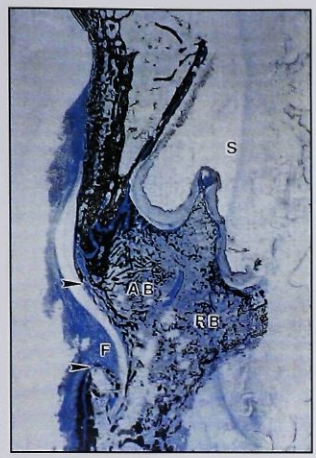


Fig 6a (left) *Von Kossa-stained section through the maxillary sinus treated with an rhBMP-2–impregnated implant and harvested at 8 weeks.* Arrowheads *indicate the lateral edges of the osteotomy;* F = *fibrous tissue;* AB = *active bone deposition;* RB = *remodeling bone; and* S = *the sinus.*

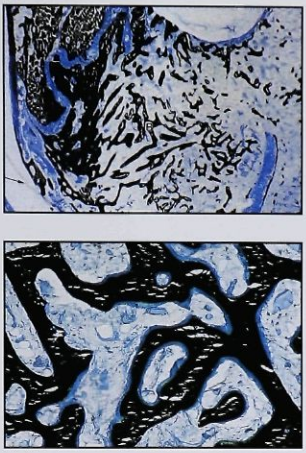


Fig 6b (top) *Increasing power photomicrograph of the section shown in Fig 6a.* B *indicates new trabecular bone and the* arrow *points to the Gore-Tex membrane.*

Fig 6c (bottom) *Higher power photomicrograph of the section shown in Fig 6b.*

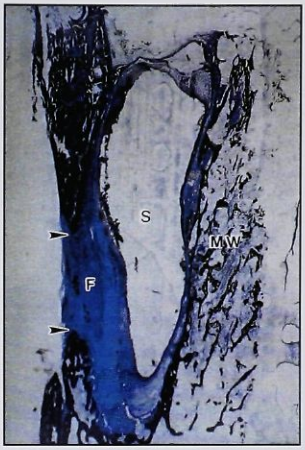


Fig 6d *Von Kossa-stained section through the maxillary sinus treated with a buffer-impregnated implant and harvested at 8 weeks.* Arrowheads *indicate the lateral edges of the osteotomy;* F = *fibrous tissue;* S = *the sinus; and* MW = *the medial wall of the sinus.*

of inflammation, residual ACS, or cartilage. On the sides that received control implants, there was no evidence of new bone formation, residual ACS, or inflammation.

Eight weeks postimplantation. In the sites implanted with rhBMP-2/ACS, new trabecular bone persisted, with architecture varying with location (Figs 6a to 6c). In the superiolateral portion, the trabeculae were spindle-shaped, interconnected, and covered with a comparatively thick layer of osteoid. The presence of osteoid suggests active deposition by osteoblasts, and the absence of osteoclasts on the trabecular surfaces demonstrates that the primary activity in this region was bone deposition. In contrast, bone in the inferiomedial part of the sinus was characterized by little osteoid. Osteoclasts and resorption pits were evident on the surfaces of the trabeculae, suggesting that the bone was in a remodeling phase. The bone marrow in both regions exhibited a few fat cells and a paucity of red and white blood cells. A large number of thin-walled blood vessels were also present.

Sections from the sites that received control implants revealed the osteotomies to be largely healed over with collagenous connective tissue (Fig 6d), and small islands of reactive bone formation were also observed. There was no evidence of any inflammatory response.

Twelve weeks postimplantation. Sections from the sites implanted with rhBMP-2/ACS revealed that dense isolated trabeculae and bone marrow were present within the sinuses. However, cortical bone had not formed by 12 weeks

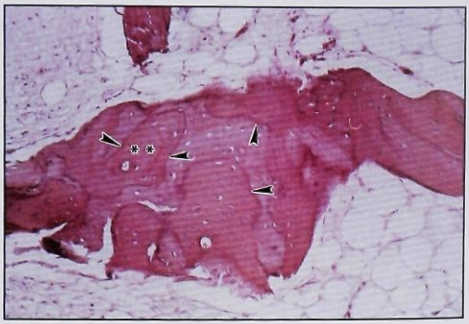


Fig 7a *Hematoxylin-eosin–stained section of a trabecula in fatty marrow at 12 weeks. Cement lines* (arrowheads) *separate bone deposited earlier (dark pink) from more recently deposited bone (light pink). Asterisks indicate the Haversian system.*

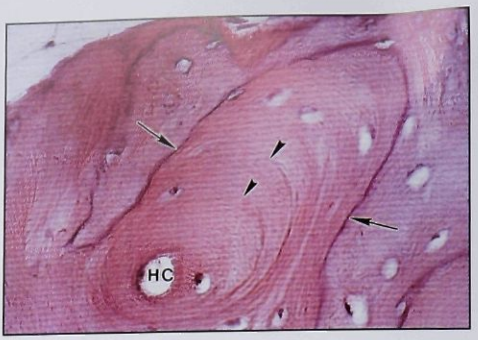


Fig 7b *Higher power of the section shown in Fig 7a showing cement lines* (arrows), *concentric rings of a Haversian system* (arrowheads), *and a Haversian canal* (HC).

postimplantation. Substantial surface areas of the trabeculae were covered with cuboidal osteoblasts, suggestive of continual bone-forming activity. Other surfaces were covered by flattened osteoblasts and osteoclasts with resorption pits, suggesting that a slow remodeling process was taking place. The trabeculae were primarily embedded in fatty marrow with a small central island of fibrovascular connective tissue (Figs 7a and 7b).

As at week 8, sections from the sites that received control implants were largely healed over with collagenous connective tissue. Reactive bone formation was present at one of the two sites. There was still no evidence of either inflammation or neo-osteogenesis.

Hematology and serology

No significant changes were noted in hematologic parameters during the course of the study. There were slight increases in mean corpuscular hemoglobin concentration and decreases in hematocrit for some animals both preoperatively and postoperatively, but they were not considered to be clinically significant.

All animals experienced decreased levels of phosphorus both preoperatively and postoperatively. This was considered to be related to diet. Other slight abnormalities in serum chemistry were not considered to be clinically significant.

Immunology

Serum samples indicated no detectable levels of antibovine collagen antibodies either preoperatively or at 5 or 14 days after implantation.

Discussion

The results of the current study demonstrate that delivery of rhBMP-2 in an ACS results in significant new bone formation in the maxillary sinus of goats, and that this delivery system does not induce any significant immune or other adverse response in these animals. These results are consistent with a number of previous studies[11–14,17] showing that rhBMP-2 can induce significant new

bone growth in a variety of applications.

The bone growth observed with the rhBMP-2/ACS implants in the present study was rapid. New growth was detected within 4 weeks in both CT scans and in microscopic analysis. No cartilage formation was observed, even at this relatively early point in time, suggestive of a direct route of bone formation, as seen in previous BMP studies. The histologic assessments at 4, 8, and 12 weeks postimplantation show the expected normal progression of the bone formation process. This rapid induction of new bone growth with rhBMP-2, followed by normal bone maturation, is consistent with the results of earlier studies. Toriumi et al[18] detected new bone formation across a 3-cm full-thickness mandibular defect in dogs on radiographs taken 21 days after treatment with rhBMP-2. Wang et al[11] observed the development of cartilage 7 days after implantation of rhBMP-2 and bone growth 14 days after implantation in a rat ectopic bone formation assay. Gerhart et al[19] evaluated rhBMP-2 in a sheep model, where bone formation was required across a 2.5-cm gap in the femur. Treatment with rhBMP-2 resulted in new bone formation by 3 to 4 weeks and union across the defect by 12 weeks. Aspenberg et al[17] observed significant bone growth at 6 weeks after placement of rhBMP-2–impregnated implants in superficial pouches in the vastus lateralis muscles of monkeys.

The present results demonstrate that ACS implants impregnated with rhBMP-2 did not provoke an immune, toxic, or otherwise adverse response. These findings are consistent with the results of earlier studies that have demonstrated the absence of an inflammatory response to bovine collagen sponges in both experimental animals and human patients.[20,21] Additionally, previous studies have shown rhBMP-2 to be nonimmunogenetic.[18]

The effects of rhBMP-2 may have further application in the treatment of periodontal defects, since periodontal regeneration includes the formation of new bone, new cementum, and a new periodontal ligament to form a new functional attachment apparatus on a pathologically exposed root surface.[22] This was first noted histologically in 1968, when an infrabony defect was treated with particulate autogenous bone in humans.[23] It has since been demonstrated histologically with other treatment modalities, including intraoral and extraoral autografts, guided tissue regeneration and allogeneic bone grafts, and a variety of other surgical procedures.[22–32] Most recently, rhBMP-2 used in conjunction with a synthetic resorbable delivery system has also offered the potential of rapidly replacing the periodontal attachment apparatus lost as a result of the disease process.[33]

A variety of approaches have been undertaken to promote bone formation in localized edentulous areas with osseous deformities so that dental implants can be placed in prosthetically usable positions. The principles of guided bone regeneration are grounded in earlier orthopedic investigations of the healing of critical osseous defects with exclusionary techniques to regulate the cells populating the damaged area.[34–37] Recent clinical evidence is available to demonstrate success with the use of augmentation membranes in combination with autografts, allografts, and stainless steel pins to support the membranes.[38–44] Most examples demonstrate the horizontal expansion of the damaged alveolar process, with insufficient data supporting the vertical increase of bone that is important to avoid anatomic obstacles. In addition to use of sinus floor augmentation to restore dentition of patients with edentulism of the posterior maxilla, it is hoped that the rapid trabecular formation encountered in this study can be extrapolated to more challenging clinical alveolar ridge defects and enhance the outcome in terms of bone yield.

Conclusion

Recombinant human bone morphogenetic protein-2 impregnated on an absorbable collagen sponge was used successfully to induce bone formation in the floor of the maxillary sinus in a relevant animal model without evidence of any adverse clinical response.

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