## Defense Mechanism of the Gingiva

The gingival tissue is continuously subjected to challenge, influencing the host response type. **The Crevicular Fluid, Saliva, Epithelial Surface and Immune Response (leukocyte in dento-gingival area),** provide active responses to maintain gingival health.

The gingival sulcus which is a V- shaped groove around the tooth structure ranged in depth from (0.5-2) mm is normally filled with a fluid called gingival crevicular fluid (GCF). Sulcular fluid, or *gingival crevicular fluid* (GCF), contains an array of biologic mediators, cells, and bacteria (Fig-1).

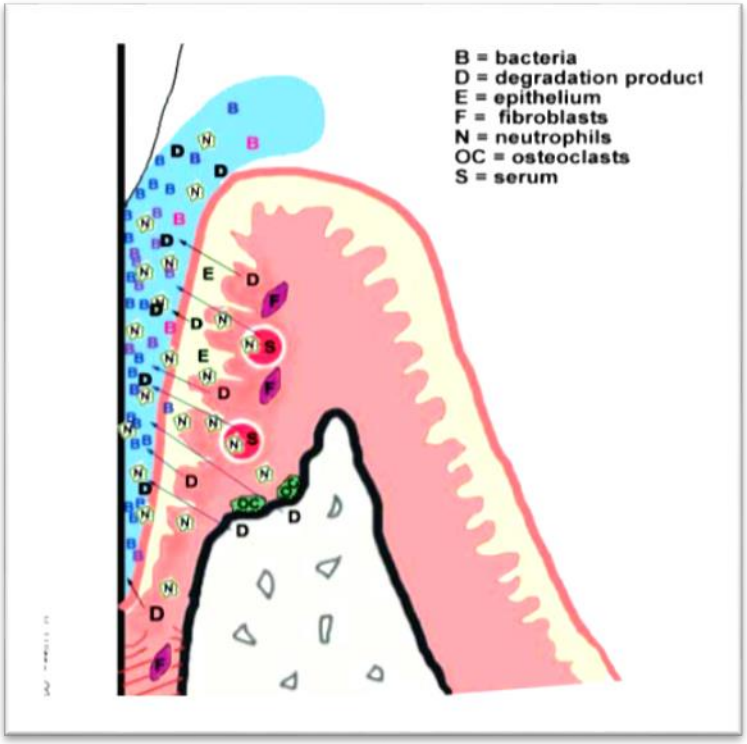


**Fig. 1. Gingival Crevicular Fluid – a window to periodontal disease**

Recognized since the 19th century, its possible role in oral defense was first elucidated by the pioneering work of Waerhaug and Brill and Krasse during the 1950s. The latter investigators applied filter paper to the gingival sulci of dogs that had previously been injected intramuscularly with fluorescein; within 3 minutes, the fluorescent material was recovered on the paper strips. This indicated the passage of fluid from the bloodstream through the tissues and the exiting of fluid via the gingival sulcus. In subsequent studies, Brill confirmed the presence of GCF in humans and considered it as "transudate." However, others demonstrated that GCF is an inflammatory exudate rather than a continuous transudate. In strictly normal gingiva, little or no fluid can be collected.

Recently, it is agreed that GCF is secreted as *transudate* due to osmotic gradient in health and it changes into *exudate* upon in response to inflammation during periodontal disease.

## Mechanism of GCF Production:

When we have injury to the blood vessels (chemical, bacterial, etc…), this irritation induces temporary blood vessels contraction. After that the blood flow will increased leading to local hyperemia in that area due to the blood vessels dilation (arteriolar, capillary and venular) & seepage of the GCF through the blood vessel wall. This dilation will increase the vascular permeability & then seepage of the plasma fluid & proteins of high & low molecular weight through the blood vessel. This phenomenon (injury, dilation & increase in the permeability & seepage of plasma fluid & proteins) is called **exudation**, which is one of the most important features of any inflammatory reaction.

GCF originates from the postcapillary venules of the gingival plexus (capillary filtration) and by the removal of this interstitial fluid by lymphatics of gingiva (lymphatic uptake). When the production of the fluid from capillaries is greater than lymphatic uptake, fluid will accumulated as edema or leave as gingival fluid. According to this model, the flow of GCF can be explained as following:

i. In health state (absence of inflammation): there is a low vascular pressure and low permeability of basement membrane. Subsequently, this will reduce the flow of GCF associated with increased fluid uptake by lymphatics.

ii. During Inflammation: Presence of dental biofilm leads to an increase in osmotic gradient, which is followed by increased leakage of proteins. This will cause increase in hydrostatic pressure and vascular permeability thereby exceeding the capacity of lymphatics to drain fluids leading to upregulation of GCF flow.

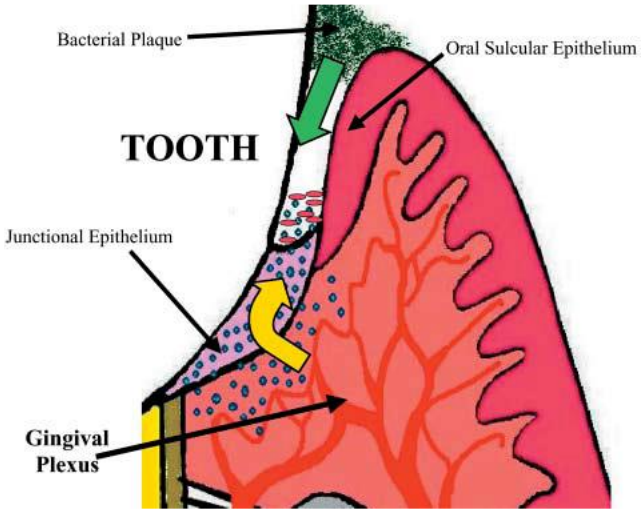


**Fig. 2. The flow of GCF increases (yellow arrow) with the accumulation of bacterial plaque in the vicinity of the gingival sulcus**

## Function:

1. It has a flushing action in the gingival crevice, (clearance of dead cells and bacterial molecules from the sulcus).

2. Functions to bring the blood components (e.g., neutrophils, antibodies, complement components) of the host defenses into the sulcus, so exert antibacterial and antibody activity.

3. Contain plasma proteins that improve adhesion of epithelium to the tooth surface.

## Compositions of GCF

- ***Cellular Elements.*** Cellular elements found in GCF include:
  - Bacteria.
  - Desquamated epithelial cells, and leukocytes (i.e. PMNs, lymphocytes, and monocytes/macrophages).
- ***Electrolytes(inorganic components)***
  - Potassium, sodium, and calcium
- ***Organic Compounds.***
  - Carbohydrates.
  - Glucose (Glucose concentration in GCF is **three to four times greater** than that in serum).
  - Proteins (The total protein content of GCF is **much less** than that of serum).
  - Metabolic and bacterial products identified in GCF include lactic acid, urea, hydroxyproline, endotoxins, cytotoxic substances, hydrogen sulfide.

- ***Enzymes***
    - Acid phosphatase.
    - Alkaline phosphatase.
    - Prostaglandin E2.
    - Collagenase.
    - Lysozyme and Lactoferrin.

## Methods of GCF Collection:

The most difficult hurdle to overcome when collecting GCF is **the scarcity** of fluid that can be obtained from the sulcus. Total amount of GCF that can be collected each day ranged between **0.5 to 2.4 µl**. Many collection methods have been tried. These methods include:

1. Absorbing paper strips:
    a) Intra- sulcular method (The Brill technique) inserting the absorbing strip into the pocket until resistance is encountered (Figure 3, *A*). This method produces some degree of irritation of the sulcular epithelium that by itself can trigger the flow of fluid.
    b) Extra- sulcular method: by (Löe and Holm-Pedersen) to minimize the irritation of intrasulcular method; the filter paper strip placed just at the entrance of the pocket or over the pocket entrance (Figure 3, B & C). In this way, fluid that seeps out is picked up by the strip, but the sulcular epithelium is not in contact with the paper.

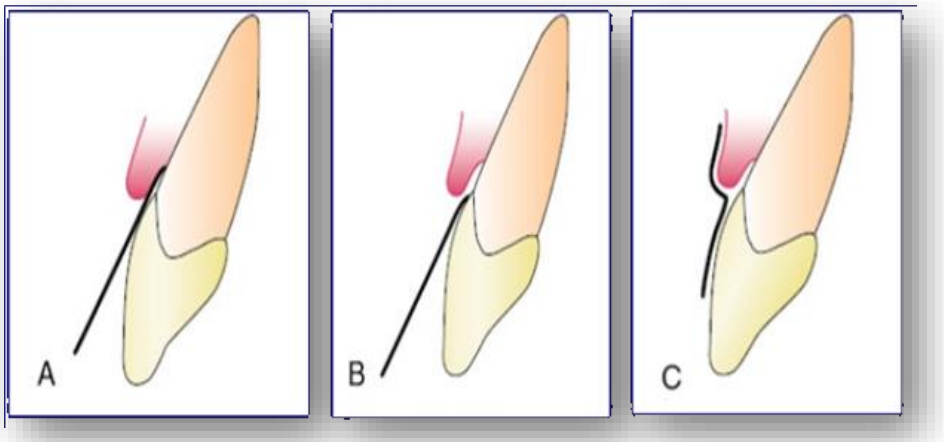


**Fig -3: Placement of a filter strip in the gingival sulcus for the collection of fluid. A, Intrasulcular method. B and C, Extrasulcular methods**

2. Preweighed twisted threads were used by Weinstein and colleagues. The threads were placed in the gingival crevice around the tooth, and the amount of fluid collected was estimated by weighing the sample thread.
3. The use of micropipettes permits the collection of fluid by capillarity. Capillary tubes of standardized length and diameter are placed in the pocket, and their content is later centrifuged and analyzed.
4. Crevicular washings can be used to study GCF from clinically normal gingiva. One method involves the use of an appliance that consists of a hard acrylic plate that covers the maxilla, with soft borders and a groove that follows the gingival margins; it is connected to four collection tubes. The washings are obtained by rinsing the crevicular areas from one side to the other with the use of a peristaltic pump.

A modification of the previous method involves the use of two injection needles that have been fitted one within the other so that, during sampling, the inside (ejection) needle is at the bottom of the pocket and the outside (collecting)

needle is at the gingival margin. The collection needle is drained into a sample tube via continuous suction.

## Methods of GCF Measurement:

1. The wetted area can be made more visible by staining with Ninhydrin 0.2 & 2%); it is then measured planimetrically on an enlarged photograph or with a magnifying glass or a microscope. The shortcomings of this method include evaporation may affect estimation of GCF volume beside it is not easily applicable chairside.
2. An electronic method has been devised for measuring the fluid collected on a "blotter" (Periopaper) with the use of an electronic transducer (Periotron) (Fig-4). The wetness of the paper strip affects the flow of an electric current and provides a digital readout. The readings obtained by Periotron can be converted into corresponding clinical conditions and scores recorded by gingival index (Table 2).

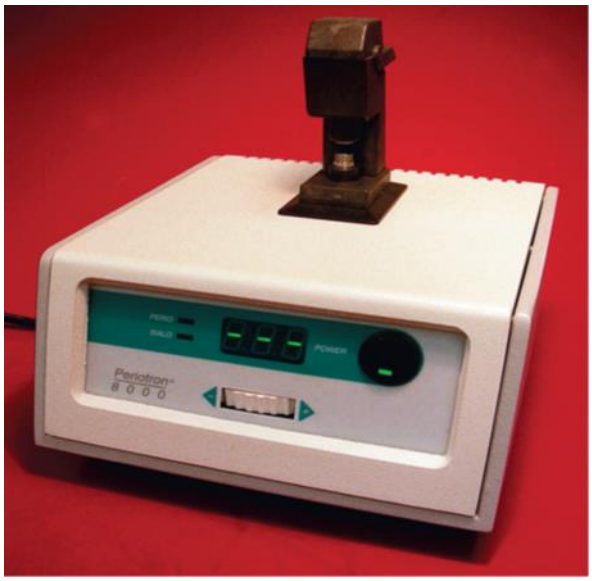


**Fig-4:** Electronic Device (Periotron)

**Table 2.** Translation of Periotron value into associated clinical conditions and gingival index

| Periotron Reading | Level of Gingival Inflammation | Gingival Index |
|---|---|---|
| **0 – 20** | Healthy | 0 |
| **21- 40** | Mild | 1 |
| **41- 80** | Moderate | 2 |
| **81 - 200** | Severe | 3 |

3. Weighting the strip: pre-weighted strip is inserted into the gingival crevice & then determined the amount of fluid collected by weighting the sample.

## Clinical Significance of GCF:

The amount of GCF is greater when inflammation is present, and it is sometimes proportional to the severity of inflammation. GCF production is not increased by trauma from occlusion, the level of GCF may increase due to other reasons. These could be summarize as follow:

- **Circadian Periodicity:** There is a gradual increase in the amount of GCF from 6 a.m. to 10 p.m. and a decrease thereafter.
- **Sex Hormones:** Female sex hormones increase GCF flow, probably because they enhance vascular permeability. Pregnancy, ovulation and hormonal contraceptives all increase GCF production.
- **Mechanical Stimulation:** Chewing and vigorous gingival brushing stimulate the flow of GCF. Even minor stimuli represented by intrasulcular placement of paper strips increases the production of fluid.
- **Smoking:** Smoking produces an immediate transient but marked increase in GCF flow but, in the long term, a decrease of salivary and GCF flow.

- **Periodontal Therapy** There is an increase in GCF production during the healing period after periodontal surgery.
- 

## GCF Composition in Relation with Systemic Diseases

- Increase glucose level in diabetic patient.
- Increase urea, due to alteration of protein in kidney (function) disease.
- Increase lactic acid level in liver diseases.
- Increase calcium level in lyperparathyrodism.
- Increase alkaline phosphatase enzyme level in bone disease (Ricket's, Paget's disease).
- Altered acid phosphatase enzyme level during certain forms of carcinoma.

## Drugs in Sulcular Fluid

Gingival fluid may also serve as a vehicle for antibacterial drugs. The gingival fluid levels of some drugs (e.g., tetracycline) may exceed their blood level by two-to ten-folds than that in serum.

Drugs that are excreted through the gingival fluid may be used advantageously in periodontal therapy. Studies performed in dogs demonstrated that **tetracycline** are excreted through the gingival fluid, this finding triggered extensive research. Metronidazole is another drug that has been detected in human gingival fluid, also ampicilin, cephalexin, rifampicin were detected too.

## ➢ Saliva

Saliva is a complex fluid, which influences oral health through specific and nonspecific physical and chemical properties. It’s produced and secreted from

salivary glands. Salivary secretions are protective in nature because they maintain the oral tissues in a physiologic state (Table -2). Saliva exerts major influences:

Table-2: **Role of Saliva in Oral Health**

| Function | Salivary components | Probable Mechanism |
|---|---|---|
| **Lubrication & Physical protection** | Glycoproteins, mucoids | Coating similar to gastric mucin |
| **Cleansing** | Physical flow | Clearance of debris and Bacteria |
| **Buffering** | Bicarbonate and phosphate | Antacids |
| **Tooth integrity maintenance** | Minerals | Maturation, Remineralization |
| | Glycoprotein pellicle | Mechanical protection |
| **Antibacterial action** | Immunoglobulin A | Control of bacterial colonization |
| | Lysozyme | Breaks bacterial cell walls |
| | Lactoperoxidase | Oxidation of susceptible bacteria |

## Saliva contains:

1. **Antibacterial factors**: Saliva contains lysozymes, myeloperoxidase, lactoperoxidase, glucoproteins, mucins & antibodies etc.

a) Lysozymes: is a hydrolytic enzyme that cleaves the linkage between structural components of the glycopeptide muramic acid– containing region of the cell wall of certain bacteria. Lysozyme works on both gram-negative and gram-positive organisms its targets include *Veillonella* species and *Actinobacillus actinomycetemcomitans.*

b) lactoperoxidase–thiocyanate system in saliva has been shown to be bactericidal to some strains of *Lactobacillus* and *Streptococcu* by preventing the accumulation of lysine and glutamic acid, both of which are essential for bacterial growth. Another antibacterial finding is lactoferrin, which is effective against *Actinobacillus* species.

c) Myeloperoxidase is released by leukocytes; it is bactericidal for *Actinobacillus,* but it has the added effect of inhibiting the attachment of *Actinomyces* strains to hydroxyapatite.

d) Antibodies: As with GCF, saliva contains antibodies that are reactive with indigenous oral bacterial species causes opsonization. Although immunoglobulins G (IgG) and M (IgM) are present, the preponderant immunoglobulin found in saliva is **immunoglobulin A (IgA)**. However, IgG is more prevalent in GCF.

2. **Buffers & coagulation factor:** Salivary buffer bicarbonate – carbonic acid system maintain the physiologic pH at the mucosal epithelial cell surface and the tooth surface. Saliva contains coagulation factors- VIII, IX, and X; plasma thromboplastin antecedent (PTA); that hasten blood coagulation & protect wound from bacterial invasion.
3. **Leukocytes:** Leukocytes reach the oral cavity migrating through the gingival sulcus.

## Role of saliva in Periodontal Pathology

Saliva exerts a major influence on plaque initiation, maturation, and metabolism. Salivary flow and composition also influence calculus formation, periodontal disease, and caries. The removal of the salivary glands in experimental animals

significantly increases the incidence of dental caries and periodontal disease in addition to delaying wound healing.

In humans, an increase in inflammatory gingival diseases, dental caries, and rapid tooth destruction is partially a consequence of decreased salivary gland secretion (xerostomia). Xerostomia may result from sialolithiasis, sarcoidosis, Sjögren syndrome, Mikulicz disease, irradiation, the surgical removal of the salivary glands, and other factors.

## ➢ Leukocytes in Dentogingival Area:

PMNs are the most common leukocytes present in the gingival sulcus. Neutrophils are the first line of defense in the dentogingival area. They travel across the epithelium, to the gingival sulcus, where they are expelled into the oral cavity. Leukocytes are present in gingival sulcus even when histologic area are free of inflammatory infiltrate. Differential count of leukocytes from clinically healthy human gingival sulci have shown 91.2% to 91.5% PMNs and 8.5 - 8.8 % mononuclear cells. Mononuclear cells have 58% B cells, 24% T cells and 18% mononuclear phagocytes. The ratio of T-lymphocytes to B- lymphocytes is reversed from normal ratio of about 3:1 in peripheral blood to 1:3 in GCF.

**References:**


1. Newman MG, Takei H, Klokkevold PR, Carranza FA. (2018). Carranza's clinical periodontology-e-book: Expert consult: Online: Elsevier health sciences.
2. Delima, A. J., & Van Dyke, T. E. (2003). Origin and function of the cellular components in gingival crevice fluid. Periodontology 2000, 31(1), 55–76.
3. Uitto, V.J. (2003). Gingival crevice fluid - an introduction. Periodontology 2000, 31(1), 9–11.