Gingival Crevicular Fluid

Introduction

Gingival crevicular fluid (GCF), also known as sulcular fluid, is defined as physiologic fluid secreted in the gingival crevice that is classified as inflammatory exudate during disease or serum transudate during health. GCF secreted in minute amount in healthy state, which is increased in response to inflammation.

Gingival crevice is a V-shaped space bounded by tooth from one side and epithelium lining the free margin of gingiva on the other side. Waerhaug (1950) studied pathologic transformation of gingival sulcus into periodontal pocket. In the same period, work of Brill et al. established the understanding the physiology of gingival crevicular fluid formation and its composition. Later, Loe et al. (1965) used gingival crevicular fluid as an indicator of periodontal disease. 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.

Formation of GCF

Epithelial lining of the sulcus characterizes by permeability that allows traversing of the molecules through intercellular spaces. Previous studies showed that wide range of molecules could penetrate the epithelial barrier including albumin and endotoxin.

Pashley et al. (1976) suggested a model that explains the flow of fluid into gingival crevice area. This model suggested that GCF production is controlled by the passage of fluid from capillaries into the tissues (capillary filtrate) which is removed by lymphatic system. However, when the role of capillary fluid exceeds that of lymphatic uptake, the fluid accumulate as edema and leave the area as GCF (Fig 1).

According to this model, the flow of GCF can be explained as following:

A- In the absence of inflammation: In health state, 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.

B- 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.

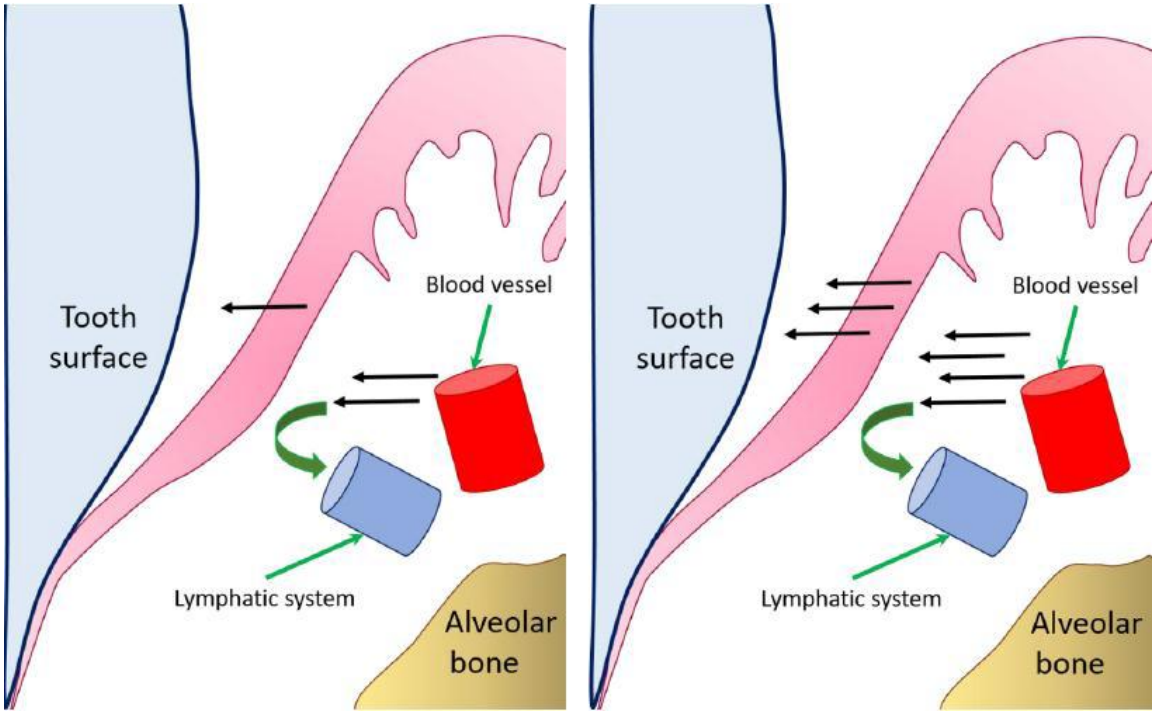

Figure 1. i) In healthy condition, the majority of interstitial fluid is drained by lymphatic system and only small amount leak into gingival crevice forming transudate. ii) During periodontal disease, the amount of leaked fluid from blood vessels is beyond the drainage capacity of lymphatics, leading to formation of inflammatory exudate.

Function

GCF plays protective role and maintain health of gingival tissue through the following:

1. Clearance of dead cells and bacterial molecules from sulcus.
2. Exerts antibacterial and and antibody activity due presence of different immunological factors such as immunoglobulins and lysozyme.
3. Contain plasma protein that potentially improve adhesion of epithelium to tooth .

Composition

The GCF contains range of proteins, antibodies, antigens, enzymes and cellular elements. Until now, more than 40 components were detected in GCF. Most of these components can be used to detect for diagnosis or prediction of patients at risk of periodontal disease. However, the origin of these components is not known whether bacterial or host-derived. Main components of GCF are summarized in table 1.

Cellular elements	Inorganic components	Organic Compounds	Bacterial products	Enzymes
Bacteria, desquamated epithelial cells, PMNs, lymphocytes, monocytes /macrophages	Potassium, Sodium, Calcium, Phosphate, Magnesium	Carbohydrates, Proteins, Lipids, Albumin, Immunoglobulin	Endotoxins, Trypsin like enzyme	Acid phosphatase , Alkaline phosphatase , Prostaglandin E2 , Collagenase , Lysozyme , Lactoferrin

Methods of collection

1- Absorbing paper strip

Paper strips are available in standard sizes that can absorb volume of GCF ranging between 1.2-2 µl.

Absorbing papers can be used in two ways:

a. Intra-crevicular method: the paper strip is placed superficially at the entrance of gingival crevice (Loe and Holm-Pederson technique) to minimize irritation to the sulcular epithelium. The other approach is to insert the paper strip gently within the gingival crevice until minimal resistance is felt (Brill‟s technique). Brill‟s method cause more irritation to the tissue and may stimulate oozing of the fluid (Fig 2).

Typically, absorbing papers are inserted no more than 1–2 mm into the sulcus/ pocket with gentle placement until mild resistance is felt, and held in place for 30 seconds.

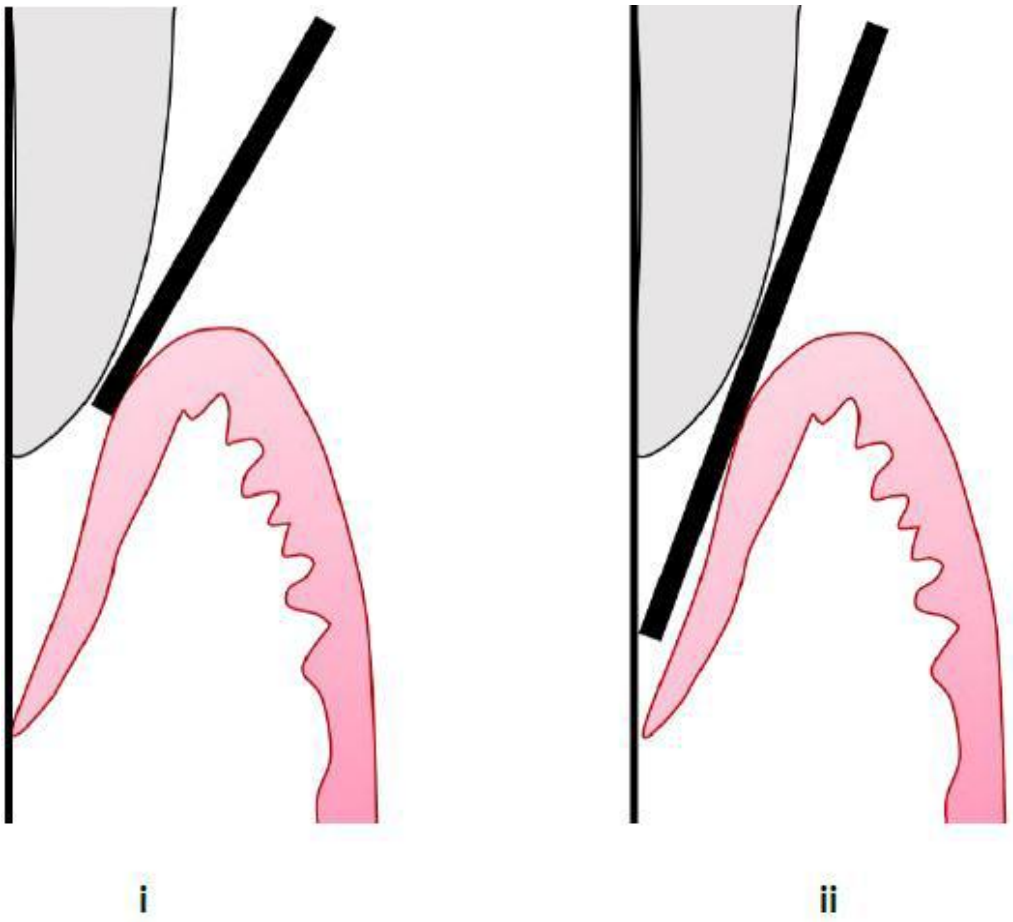

Figure 2. i) Loe and Holm-Pederson technique, positioning of paper strip at gingival margin without penetrating gingival sulcus; (ii) Brill‟s technique, positioning of paper strip into gingival sulcus-P3.

b. Extra-crevicular method: The paper strip is adapted on the gingiva & the hard tissue of the tooth in this region so the paper strip will absorb the seepage of GCF (Fig 3).

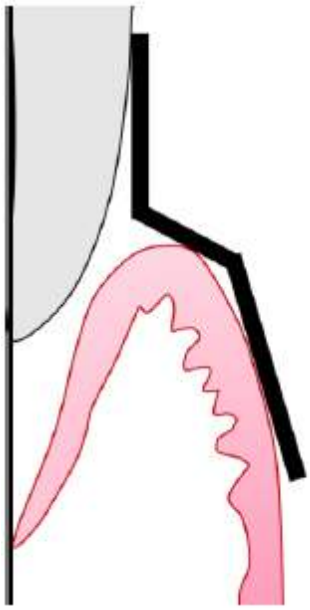

Figure 3. Positioning of paper strip overlying the tooth, gingiva, and alveolar mucosa for obtaining GCF

2- Micropipettes or capillary tubes

This method was first introduced by Krasse and Egelberg (1962). The principle of this technique depends on collection of fluid by capillary action. After isolation and drying of collection site, capillary tubes of known diameter are inserted into the entrance of gingival crevice, gingival crevicular fluid migrates into the tube by capillary action (Fig 4).Since the diameter is known, GCF can be calculated by measuring the distance which the gingival crevicular fluid has migrated.

The advantage of this method is that it provides an undiluted sample of native GCF whose volume can be accurately assessed.

Disadvantages:

• It has a long collection period.

• The collection of fluid is difficult because the viscosity of fluid makes the aspiration difficult.

• It is also difficult to remove the complete sample from the tubing.



Figure 4. Gingival crevicular fluid collection by micro-capillary pipettes

Methods for estimating GCF amount

1-The wetted area of paper strip can be made visible by staining with alcoholic solution of ninhydrin (concentration between 0.2 & 2%); this stain has specificity to for amino acids & gives a blue or purple color. The stained part is then measured on an enlarged photograph or with a magnifying glass or a microscope using ruler or Vernier . The shortcomings of this method include evaporation may affect estimation of GCF volume beside it is not easily applicable chairside.

2- **Electronic transducer (Periotron)** is an electronicinstrument that measures the effect of wetness of filter paper strips on the capacitance .

between the „jaws‟ of the device, between which the filter paper is placed after the sample has been collected. The wetness of the paper strip affects the flow of an electronic current and gives a digital readout . The readings obtained by Periotron can be converted into corresponding clinical conditions and scores recorded by gingival index.

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

Total amount of GCF that can be collected each day ranged between 0.5 to 2.4 µl.

Problems associated with GCF collection

1- Contamination: Usually sample is contaminated with blood, saliva or plaque. Contaminated samples must be discarded

2- Small sample size

3- Sampling time: Prolonged sampling at the site resulted in protein concentrations that may affect the results

4- Volume determination: Evaporation is a significant problem in accurate volume determination of GCF samples

5- Difference in quality of paper strips used from different manufacturers

GCF as diagnostic tool

Different components of GCF have been used to determine progression of periodontal disease by utilizing certain biomarkers. In general, these components can be classified as follow:

A- Inflammatory mediators and host-response modifiers

B- Host-derived enzymes and their inhibitors

C- Tissue breakdown products

The potential source of these components could be from host cells, host tissue, microbial biofilm, and immune response.

Examples of biomarkers used to monitor periodontal disease activity:

- Collagenase-2 (MMP-8) secreted by neutrophils: is a significant enzyme because it appears to be released from fibroblasts and pocket epithelial cells during tissue destruction or may be released by the bacteria.

- Neutrophilic elastase: is a neutrophil-specific granule enzymes involved in tissue destruction during inflammation.

- **Phospholipases:** are lysosomal and cytoplasmic enzymes, but are also produced by the micro organisms

- **Aspartate aminotransferase (AST)**: increases in periodontitis and gingivitis patients and decreases after periodontal therapy

- **Lactoferrin (LF)**: Level of LF significantly decrease following periodontal surgery. Hence, LF levels in GCF could serve as a useful marker for monitoring of periodontal treatment results

- **C-reactive protein (CRP):** the level of CRP increases as the disease progress from gingivitis to periodontitis.

- **Substance-P**: level of this biomarker significantly increase at sites with periodontal destruction and reduce following periodontal treatment.

Clinical significance of GCF:

The amount of GCF is greater when inflammation is present and is sometimes proportional to the severity of inflammation. However, level of GCF may increase due to other reasons. These factors could be summarize as follow:

1- Circadian periodicity: there is gradual increase in GCF amount from 6 AM to 10 PM & decrease afterward.

2- Sex hormones: gingival inflammation increases during pregnancy, menstrual cycle, hormonal contraceptive and puberty. Female sex hormones increase the GCF flow because they enhance the vascular permeability.

3- Mechanical stimulation: GCF production is not increased by trauma from occlusion but is increased by mastication of coarse foods, toothbrushing and gingival massage.

4- Smoking: It produces an immediate transient but marked increase of GCF.

5- Periodontal therapy: there is an increase in GCF production during the healing period after periodontal surgery. This increase was probably the result of inflammatory reaction from gingival trauma and the loss of an intact epithelial barrier.

GCF Composition in Relation with Systemic Diseases

Systemic conditions are associated with changes in the levels of certain biomarkers that could be detected in GCF.

• Increase glucose in diabetic patient.

• Increase urea, alteration of protein in kidney disease.

• Increase lactic acid in liver disease.

• Increase calcium in hyperparathyroidism.

• Increase alkaline phosphatase in bone disease (Ricket's, Paget's disease)

Drugs and GCF

Medications that are excreted through the GCF may be used advantageously in periodontal therapy. **Tetracyclines** are one of these medications & are effective in treating refractory periodontitis because of their concentration in the gingival crevice is 2-10 times than that in serum. This allows a high drug concentration to be delivered into periodontal pockets & inhibit the growth of bacteria. **Metronidazole** is another antibiotic that has been detected in human GCF. **Ampicilin, cephalexin, rifampicin** were also detected.