# 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. Brill and Krasse (1958) had first demonstrated the seepage of GCF. They introduced filter paper into the gingival sulci of dogs previously 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 exiting via the gingival sulcus. Later, other studies showed little or no fluid can be collected from the gingival crevice in health condition. 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.

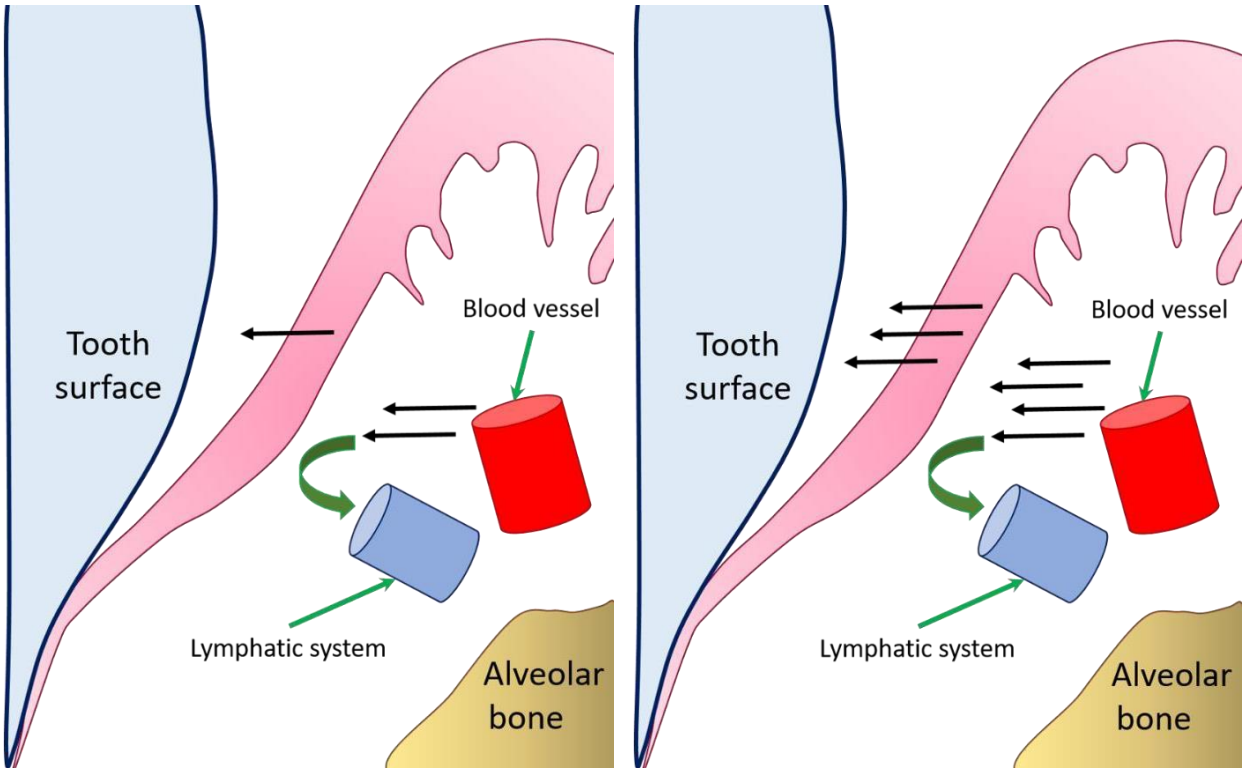


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**Figure 1. i) In healthy condition, the majority of interstitial fluid is drained by lymphatic system and only small amount leak into gingival crevice forming transudate. ii) During periodontal disease, the amount of leaked fluid from blood vessels 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:

- Clearance of dead cells and bacterial molecules from the sulcus
- Exert antibacterial and antibody activity due to presence of different immunological factors such as immunoglobulins, lysozymes, and inflammatory cells
- Contain plasma proteins that potentially improve adhesion of epithelium to the 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.

## Methods of collection

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

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.

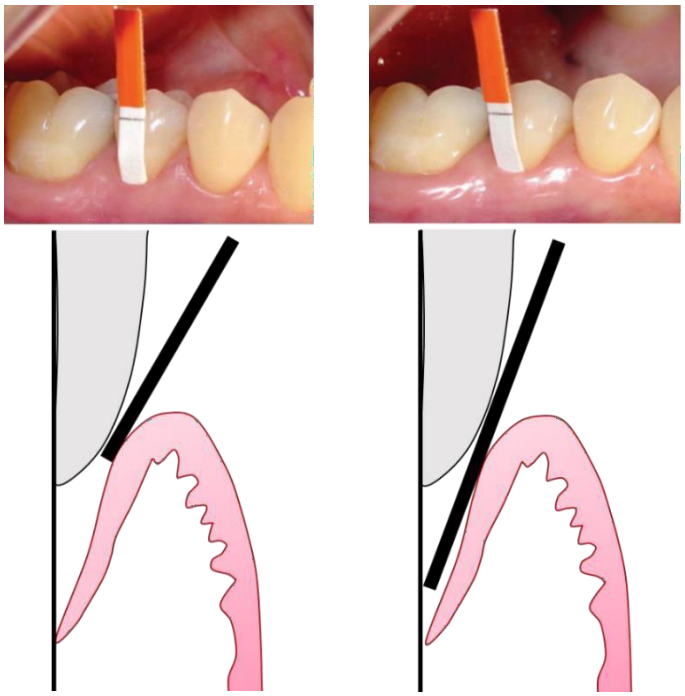


**Figure 2. i) Loe and Holm-Pederson technique, positioning of paper strip at gingival margin without penetrating gingival sulcus; (ii) Brill's technique, positioning of paper strip into gingival sulcus-P3.**

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- Crevicular washing

The Gingival sulcus is perfused with an isotonic solution of fixed volume. Three methods of gingival washing are available:

a- Instillation and re-aspiration of 10 µl of Hank's balanced salt solution at the interdental papilla. It is repeated several times to allow thorough mixing of transport solution and GCF. The fluid collected represents a dilution of GCF that contains both cells and soluble constituents such as plasma proteins.

b- Second method is more complicated which involves the construction of a customized acrylic stent that isolates the gingival tissue from the rest of the mouth. The tissues are irrigated with a saline solution, using a peristaltic pump and the diluted GCF is removed. The stent is made of hard acrylic plate covering the maxilla with soft borders and a groove following the gingival margin, connected to four collection tubes. This method lacks accuracy, technically demanding and limited to use for maxillary arch only.

c- Third method is a modification that uses two injection needles fitted one within the other such that during sampling, the inner needle (or ejection) is at the bottom of the pocket and the outside, or collecting, one is at the gingival margin. The collection needle is drained into the sample tube by continuous suction (Fig 4). The advantage of this method that it can be used in case of clinically healthy gingiva; however, dilution factor cannot be determined.

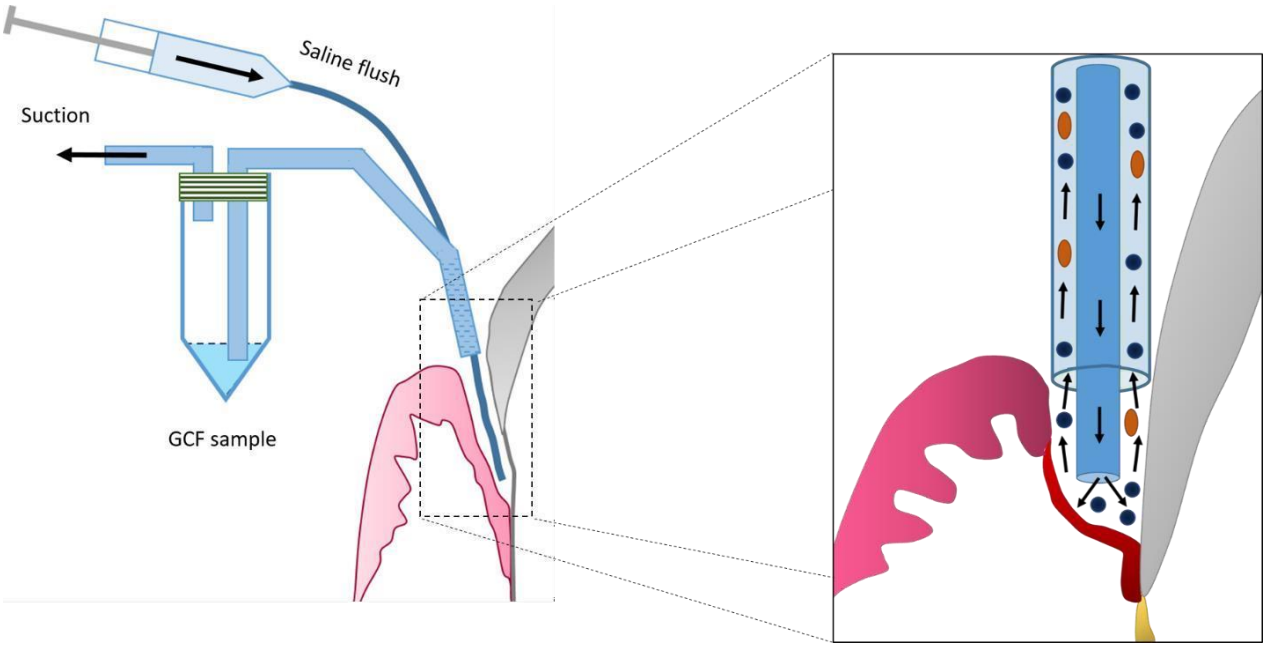


**Figure 4. Schematic illustration of modified capillary technique**

3- 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 5). Since the diameter is known, GCF can be calculated by measuring the distance which the gingival crevicular fluid has migrated. Both fluid and cellular components can be investigated by collecting crevicular fluid by the micropipette technique but this is not practicable in subjects with clinically normal gingiva.

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.

• Difficult to hold capillary tube at the entrance of gingival crevice for such lengthy periods

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



**Figure 5. 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 (Fig 6). The shortcomings of this method include evaporation may affect estimation of GCF volume beside it is not easily applicable chairside.

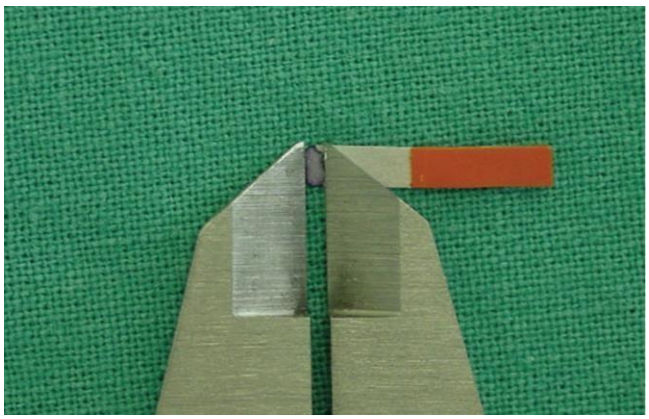

**Figure 6. Linear measurement of paper strip area stained by ninhydrin solution.**

2- An electronic method has been devised for measuring the fluid collected on a "blotter" (Periopaper), employing an electronic transducer (Periotron). Periotron is an electronic instrument 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 (Fig 7).



**Figure 7. Electronic device (Periotron) for measuring the amount of fluid collected on filter paper.**

3- Weighting the strip: pre-weighted strip is inserted into the gingival crevice & then determined the amount of fluid collected by weighting the sample.
4- Isotope dilution method to measure extremely small amount of GCF present in a particular space at any given time.

## 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

## Factors affecting amount 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 summarized 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 as diagnostic tool for periodontal disease

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.

To overcome the limitations of conventional diagnostic methods, there is an aim to incorporate biomarkers into the new classification system of periodontal disease. One of the most notable biomarkers available in GCF is active-matrix metalloproteinase 8 (aMMP8). The level of aMMP8 is markedly upregulated in proportion to the severity of disease, which potentially makes it possible candidate to accurately reflect periodontal health/disease status.

The main source of this collagenase is degranulated polymorphonuclear leukocytes (PMN) cells, which release up to 20% of their content as MMP8. In addition, fibroblasts, epithelial cells, endothelial cells, macrophages, and smooth muscle cells can also contribute to the production of this enzyme.

Currently, aMMP8-based assays are available as chair-side kits that are sensitive, time-saving, specific and accurate in discriminating periodontal health and disease. Current literature suggest that MMP-8 can be a helpful adjunct tool in enhancing the prediction, diagnosis and prognosis of periodontal diseases.

Other examples of biomarkers that can be used to predict, diagnose, and monitor progression of periodontal disease include IL-6, IL-1β, lactoferrin, and phospholipases.

### Drugs and GCF

Medications that are excreted through the GCF may be used advantageously in periodontal therapy. Spiramycin was found at higher concentrations in GCF than in blood .Another example, tetracyclines which 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 *Aggregatibacter actinomycetem comitans* and other anaerobic bacteria. Metronidazole is another antibiotic that has been detected in human GCF. Ampicilin, cephalexin, rifampicin was also detected.