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Gingival Crevicular Fluid- An Insight into its Role in Defense

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Introduction

The periodontium is a dynamic structure composed of the tissues supporting and investing the teeth. It comprises of four principle constituents, which have been divided into two parts [1]:

- Gingiva whose main function is protection of the underlying tissues
- Periodontal ligament, cementum, alveolar bone, which comprises the attachment

Gingiva is the part of oral mucosa that covers the alveolar processes of the jaws and surrounds the neck of the teeth [1].

Gingival sulcus is the shallow crevice or space around the tooth bounded by the surface of the tooth on one side and the epithelium lining the free margin of the gingiva on the other side [1].

Gingival Crevicular Fluid {GCF}, a serum transudate or inflammatory exudate, can be collected from the gingival crevice covering the teeth. GCF plays a major part in maintaining the structure of junctional epithelium and the anti-microbial defense of periodontium and GCF indicators that will help in the accurate diagnosis, appropriate treatment of periodontal disease.

History

-Brill et al (1950) laid foundation of understanding the physiology of gingival crevicular fluid formation and its composition.
-Loe et al (1965) used gingival crevicular fluid as indicator of periodontal disease.
-Goodson thoroughly studied major issues in gingival crevicular fluid flow rate and the method of collection.
-Egelberg focused on dentogingival blood vessels and their permeability as they relate to the gingival crevicular fluid flow [2].
The presence of sulcular fluid, has been known since the 19th century, but its composition and its possible role in oral defense mechanisms were elucidated by the pioneering work of Waerhaug and Brill and Krase during the 1950s [1].

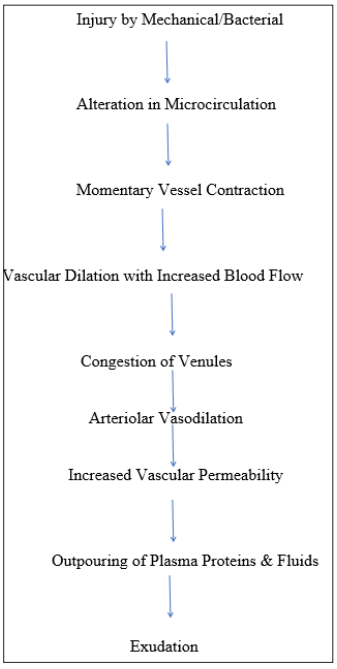
The latter investigators introduced filter paper into the gingival sulci of dogs that had previously been injected intramuscularly with fluorescein, within 3 min; the fluorescent material was recovered on the paper strips. This indicated the passage of fluid from the bloodstream through the tissues and the existing of fluid via the gingival sulcus [1].

In subsequent studies, Brill confirmed the presence of GCF in humans and considered it as a "transudate". However, others demonstrated that GCF is an inflammatory exudate rather than a continuous transudate [1].

Further report describes a theory, which explains the above and other controversies relating to the origin of the gingival fluid. It is based on premise that gingival fluid may formed by two distinct mechanisms:
Two events occurring in the inflammatory process are responsible for molecular sieving [3]:

1. A rise of hydrostatic pressure within the microcirculation
2. unlocking of endothelial cell junctions

Mechanism of GCF Production

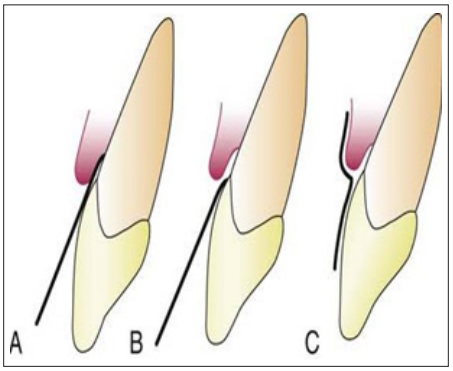


Method of Collection

1) Absorbing Paper Strips

Filter paper strips are used to collect GCF by inserting the strips into the crevice until mild resistance was detected or by inserting the strips at or over the entrance of the pocket to pick up the seeping fluid [4].

A) Intracrevicular - within sulcus B) Extracrevicular - at its entrance



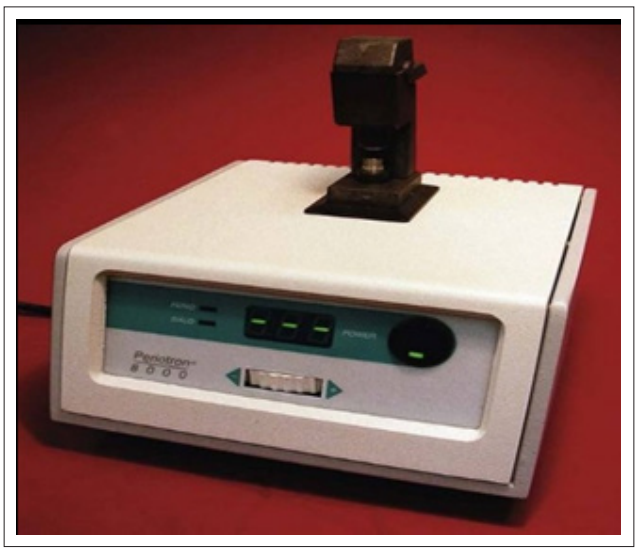
The fluid volume on the strips was quantified by a number of ways:

a. **Staining Method**
Originally, strips are stained with a protein disclosing dye such as ninhydrin at concentration varying between 0.2% and 2%.The stained area can be measured by using a magnifying device like graded microscope.

b. **By Weighing the Strip**
Strips are weighed before collection within a scaled microcentrifugation plastic tube and this weighing was repeated immediately after collection in the same micro tube.

c. **Using Periotron**

An electronic method has been devised for measuring the fluid collected on a blotter (perio paper) using an electronic transducer (periotron).This electronic device measures the changes in capacitance across the wetted strips. These changes are converted into a digital readout that can be correlated to the volume of GCF [4].

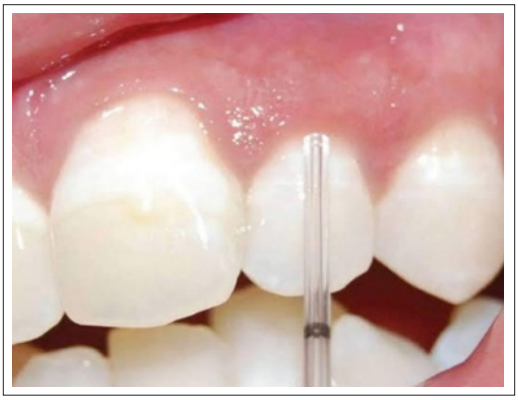


2) Preweighed Twisted Threads

The threads are placed in the gingival crevice surrounding the tooth, and the amount of the fluid collected was estimated by weighing the sample threads [5].

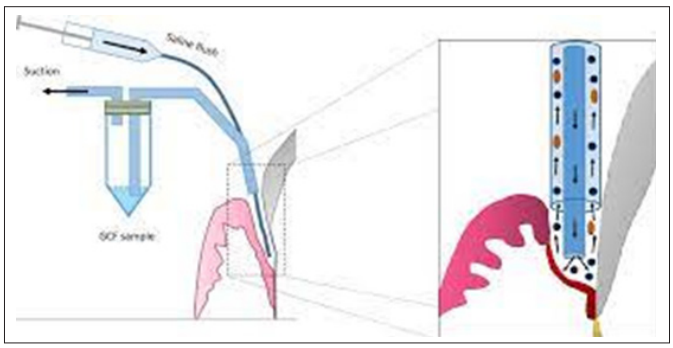
3) Micropipettes

The use of micropipettes permits absorption of fluid through capillarity. Capillary tubes of standardized length and diameter are placed in the pocket and their content was latter centrifuged and are analyzed. The capillary tubes may contaminate the native GCF by influx of serum following disruption of gingival vasculature [4].



4) Crevicular Washings

The appliance designed by Oppenheim permits collection of gingival fluid without disturbing the integrity of marginal gingiva and is modified by Takamori [5]. It consist of a hard acrylic plate covering the maxilla with soft borders and the groove along gingival margin, which is connected to four plastic tubes. Gingival washings are obtained by rinsing sulcular fluid area for a fixed period from one side to another through the palatine and buccal channels with 4 to 6 ml of solution using a peristaltic pump [6].



Composition

Component	Source	Function
Bacteria	Oral biofilm plaque	Initiates the host immune response
Epithelial cells	Oral sulcular and junctional epithelium	Represents the high cell turnover of the gingival sulcus
Leukocytes	Gingival blood vessel plexus	PMNs are involved with innate immunity. Monocytes/macrophages and lymphocytes are involved with cell-mediated immunity.
Erythrocytes	Gingival blood vessels	Results from small blood vessels and capillaries damages.
Alkaline phosphatase	Fibroblasts, osteoblasts, osteoclasts, and neutrophils	Play a role in superoxide generation and in the first line of defense
Cathepsin B	Macrophages	Active enzyme in proteolysis

Collagenase-2 (MMP-8)	Neutrophils	Active enzyme associated with collagenatic activity
Gelatinase (MMP-9)	Neutrophils	Hydrolysis of intercellular matrix
Neutrophil elastase	Neutrophils	Cleavage of elastin, collagen, and proteoglycans
Macrophage elastase (MMP-12)	Macrophages	Cleavage of elastin, collagen, and proteoglycans
ICTP (Collagen telopeptide pyridinoline cross link)	Fragment of bone type I collagen	Highly correlated with bone turnover
Interleukin 1-beta	Macrophages	Regulates immune and inflammatory reactions, stimulates bone resorption
Interleukin 4	Basophils	Anti-inflammatory, macrophage inhibition, Th2 differentiation
Interleukin 6	T cells, macrophages, osteoblasts	Regulator of T- and B-cell growth, stimulate osteoclast formation
Interleukin 8	Macrophages, epithelial cells	Recruitment and activation of neutrophils
Interferon gamma	Leukocytes, lymphocytes	Macrophage activation, suppression of Th2
Immunoglobulin A (IgA)	Plasma B cells	Antigen neutralization
Immunoglobulin G (IgG)	Plasma B cells	Antigen neutralization
Immunoglobulin M (IgM)	Plasma B cells	Antigen neutralization
Lactoferrin	PMNs, acinar cells	Antibacterial, creates iron-limiting environment
Lysozyme	PMNs, macrophages	Hydrolysis of peptidoglycans of bacterial cell walls
Osteoprotegerin (OPG)	Osteoblasts	Decoy receptor for RANK-L, inhibits osteoclast formation
Osteocalcin	Osteoblasts	Calcium binding
Prostaglandin E2 (PGE2)	All cell types	Proinflammatory and immunomodulatory effects
Transforming growth factor-alpha	Macrophages, keratinocytes	Regulation of tissue repair, cell proliferation, chemotaxis, differentiation and matrix synthesis
Transforming growth factor-beta	Macrophages	Modulates proinflammatory cytokine production
TIMPs	Neutrophils, macrophages, fibroblasts, keratinocytes	Inhibits MMPs
Tumor necrosis factor-alpha	Neutrophils, macrophages, lymphocytes	Delays neutrophil apoptosis

Role of GCF in Defense Mechanism of Gingiva

The analysis of GCF has identified cell and humoral response in both healthy individuals and those with periodontal disease. The cellular immune response includes the appearance of cytokines in GCF, but there is no clear evidence of relationship between cytokines and disease. However, 1α and 1L-1β are known to increase the binding of PMNS and monocytes and macrophages to endothelial cells, to stimulate the production of prostaglandins E2 and the release of lysosome enzymes, and to stimulate bone resorption. Preliminary evidence also indicates presence of interferon-α in GCF, which may have a protective role in periodontal disease because of its ability to inhibit the bone resorption activity of 1L-1β [1].

It has been shown that 47% of the cells found in gingival sulcus were WBC, with 98% of these leukocytes being PMNS. Notably absolute number of cells increased in proportional to inflammation severity, while the differential number of PMNS was 95-97%, lymphocytes 1-2% and mononuclear cells represented 2-3% cytomorphometry is routinely used in several diagnostic procedures [7].

Cellular and Humoral Activity in GCF [1]

1L-1α and 1L-1β increase the binding of PMNS and monocyte to endothelial cells and that stimulate the production of PGE-2 and release of lysosomal enzymes and stimulate bone resorption

Interferon-α present on GCF has a protective role in periodontal disease because of its ability to inhibit the bone resorption activity of 1L-1β.

Clinical Significance of GCF [1]

1. GCF plays important part in maintaining the structure of junctional epithelium and anti-microbial defense of periodontium.
2. It have confirmed that GCF is a complex mixture of substances derived from serum leukocytes & structural cells of periodontium& oral bacteria.
3. Circadian periodicity-There is a gradual increase in the amount of GCF from 6.00am to 10.00pm and a decrease thereafter.
4. Sex hormones- Female sex hormones increases the GCF flow by enhancing vascular permeability. Pregnancy, ovulation, oral contraceptive pills increase GCF production.
5. Mechanical stimulation- Chewing and vigorous brushing increases GCF flow.
6. Smoking- Produces immediate transient but marked increase in GCF flow.
7. Periodontal therapy- Increase in GCF during healing period after periodontal therapy.

Drugs that are exerted through the GCF may be used in periodontal therapy very advantageously. Bader and Gold Haber demonstrated in dogs that tetracyclines are excreted through GCF. Metronidazole is another drug excreted in GCF as proved by human studies [1].

GCF as Biomarkers

The markers of connective tissue degradation present in GCF are mainly type I, II, III collagen, proteoglycans, hyaluronan, fibronectin, laminin and bone specific protein.

Protein called carbanyl is seen in this fluid associated with the initial stage and recovered stages of periodontitis [8]. Multiple studies have put forward that MMP-8 and 1L-1β in GCF in periodontitis. Various others have found that MMP-8 is most positive indicator present persistently in periodontits. Another study identified a positive co relation of increased 1L-1β and 1L-6 in GCF with severe BOP and increased pocket depth [9].

Several other proteolytic and hydrolytic enzyme biomarkers also gives useful information to predict, diagnose and monitor periodontal disease. Alkaline phosphatase (ALP) is a chief

indicator of bone formation and its presence in GCF indicates inflammation and destruction of periodontal tissues. The severity of periodontal disease is positively co related to the level of ALP [10]. Lactate dehydrogenase activity is uplifted with increasing probing depth [11]. Aspartate aminotransferase is non-specific marker for cell death and necrosis and its activity is connected with the severity of periodontitis [12]. Finally, cathepsin-β helps in distinguishing periodontitis from gingivitis by serving as a predictor of attachment loss [13].

GCF also holds various bone related biomarkers to reflect the disease status of periodontal tissues [14]. Osteocalcin is the most fixed biomarker of osteoblast function [15]. An increase in Osteocalcin level in GCF is associated with high rate of bone turnover and noticed during increased periodontal activity [16]. Calprotectin changes the immune response by inhibiting the immunoglobulin production and plays a major role in neutrophil recruitment and activation. Higher level of calprotectin are reported in periodontitis patients [16]. Osteopontin is mainly formed by osteoblast and macrophages and its raised levels are found associated with periodontal disease. Similarly, osteonectin is an important biomarker associated with the periodontal disease status and its increasing levels corresponds with the increase in pocket depth [16, 17].

Cell death and tissue breakdown product serve as reliable markers for tissue destruction. Different glycosaminoglycans are found depending on the tissue, chondroitin-4-sulphate is the most favorable marker as it reflect bone degradation [18]. Elevated glycosaminoglycan concentration in GCF illustrate the active destruction of periodontal tissue [19]. Fibrinogen place a vital role in variety of cellular activity and is considered as an active player in inflammation [20]. It is a marker for periodontal disease status as certain fibrinogen fragments are involved in pathogenesis of periodontitis [16].

Profile of Biomarkers Assessed in GCF [21]

Sl.no	Biomarker	Inference
1	AST(Oringer et al 2001)	AST levels are significantly reduced 12 months post treatment in patients with chronic periodontitis.
2	IL- 1β (Engebretson et al., 2002)	High GCF IL- 1β levels is seen in Periodontitis. IL- 1β reduced significantly after NSPT.
3	IL-10 (Goutoudi et al., 2004)	Total amount in GCF rather than the concentration of IL-10 positively correlated with periodontal disease and reduced post treatment.
4	Elastase IL-8 (Isabelle et al., 2006)	Improved clinical parameters with reduced elastase levels and IL-8 levels higher in CP patients which reduced after NSPT.
5	IL-1β PGE-2 (Zhong et al., 2007)	GCF IL-1β, PGE-2 levels positively correlated to PPD, CAL, BOP
6	PGE2 IL-1β (Buduneli et al., 2010)	Clinical parameters reduced, PGE-2 values non-significant, IL-1β-reduced
7.	PGE2 IL-1β (Papova, Mlachkova et al., 2010)	PGE-2 and IL-1β show lower levels of inhibition when gene expression is done post treatment with NSPT and NSAID's together
8	Alkaline Phosphatase (Kunjappu et al 2012)	All parameters reduced, Positive co-relation between PD and ALP
9	IL-6, IL-8 (Gontoudi, Diza, Arvanitidou, 2012)	Il-6 and Il-8 levels Increased post treatment
10	MMP-3,TIMP-1 (Reddy et al 2013)	MMP-3 levels increase and TIMP levels decrease with periodontal disease progression

Chair Side Diagnostic Kit in GCF [22]

- Perio-check (Ac Tech) : Rapid chair side test for neutral protease like collagenase in GCF
- Prognos-stik : It detect the presence of serine protease elastase in GCF sample
- PerioGard TM : it is based on measurement of level of enzyme aspartate aminotransferase in GCF
- Pocket watch : Detects presence of AST

Conclusion

Monitoring periodontal disease is a complicated task. Analysis of GCF constituents in a health and periodontal disease may be extremely useful to monitor periodontal disease because GCF can be easily obtained with non-invasive methods. Thorough knowledge gives better aid for diagnosis. The studies of GCF chemistry has been suggested due to the importance of an exuberant Polymorphonuclear leukocytes (PMN) response to subgingival plaque in the active phases of periodontal destructions. GCF was emerged in the last decade as new domain for improved periodontal diagnosis and therapy.

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