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Gingival Crevicular as a Source of Biomarkers for Periodontitis

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Introduction

Easily collected and containing local and systemic-derived biomarkers, oral fluids may offer the basis for patient-specific diagnostic tests for periodontal disease. Gingival crevicular fluid (GCF) is a physiological fluid as well as an inflammatory exudate originating from the gingival plexus of blood vessels in the gingival corium, subjacent to the epithelium lining of the dentogingival space. The presence of fluid in the gingival crevice has been described since the nineteenth century (11, 66). Waerhaug's classic studies further described its composition and flow in response to periodontal diseases (74, 75). Originally, most investigators classified GCF as an inflammatory exudate (18). However, there is evidence to suggest that GCF from clinically normal tissue is an altered serum transudate that only becomes an inflammatory exudate when disease is clinically present (76). The recognition in the last decade that neutrophils migrate into the periodontal crevice even in health tends to obfuscate the characterization of GCF as an inflammatory exudate vs a physiologic transudate. Furthermore, it is clear that the composition of the GCF differs in terms of microbial composition and the concentration and composition of molecular biomarkers when one compares healthy sites from diseased individuals vs healthy sites from periodontally healthy individuals. Additionally, there are clear changes in GCF composition during disease progression and certain mediators can be used to predict future patient-based or site based disease outcomes. Taken together the findings suggest that the composition of GCF can potentially be used to detect subclinical alterations in tissue metabolism, inflammatory cell, recruitment and connective tissue remodeling. Currently, most medical fields are searching for useful biological diagnostic markers that can indicate the presence of a disease process before extensive clinical damage has occurred. GCF is composed of serum and locally generated components such as tissue breakdown products, inflammatory mediators and antibodies in response to oral microorganisms present in the dental biofilm thus it offers great potential to reflect the response that the cells and periodontal tissues promote to attempt regaining homeostasis and also how certain periodontopathogens co-opt these response mechanisms to promote bacterial survival within the gingival crevice and pocket. The aim of this review is to describe the historic evolution of GCF as a diagnostic marker for periodontal disease and its current application to diagnose and predict periodontal disease activity.

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Although the importance of GCF has been recognized for decades, historically the origin and function of this fluid has been a subject of controversy. Most of the controversy relied on whether this fluid is the result of a physiological or pathological process. Early investigations demonstrated that GCF is present in the healthy gingival tissues (6, 15, 22). However, Loe & Holm-Pedersen reported that healthy gingival crevices do not exhibit GCF flow (52). The authors suggested that GCF is an inflammatory exudate, but if it is present prior to clinically detectable signs of inflammation, it would appear to be derived from healthy gingival tissues. Although the GCF has an ionic composition comparable to an inflammatory exudate (45), its protein composition is considerably lower for an inflammatory exudate (76). In 1974, Alfano described a theory related to the origin of GCF (1). The theory is based on the premise that GCF arises from two distinct mechanisms: the generation of a standing osmotic gradient, and the initiation of classical inflammation. The gingival crevicular fluid originates from the vessels of the gingival plexus of blood vessels and flows through the external basement membrane and the junctional epithelium to reach the gingival sulcus. It has been shown that GCF can be isolated from a healthy sulcus, although only in small amounts. In the healthy periodontium, GCF represents the transudate of gingival tissue interstitial fluid produced by an osmotic gradient (figure 1) However, leukocytic infiltrates are seen throughout the junctional epithelium and PMNs can always be found in the sulcus, even in clinically healthy situations where the flow of GCF is relatively low (5).

Transepithelial Fluid Dynamics

During the early investigations about the origin of gingival fluid, several processes were postulated to explain how fluid might be transported across epithelial membranes, including theories of hydrostatic filtration, active transport and classical osmosis (9). Diamond and Tormey proposed a model for fluid transport based on the observation on the morphological changes occurring during in vitro transport of water across bladder gallbladder epithelium (20). Basically, it was noted that in conditions where water transport was maximal, the intercellular spaces were widely distended and conversely when water transport was partially inhibited, intercellular spaces were restricted. Diamond and Tormey postulated in 1966 that there was a standing osmotic gradient within the intercellular spaces and that osmotic equilibrium was reached as water is osmotically pulled across the cell membrane (20).

In the discussion of the osmotic gradient model into intercellular spaces of inflamed gingival tissues, transport fluid would be intensified. Tolo in 1971 suggested that ATPase present in certain segments of epithelial cell membranes could be an indicative of active transport of solute molecules (72). However, transcriptome studies have indicated that many solute transport proteins are expressed in the tissues and many of these are upregulated in inflammation (19, 35, 36).

In search for a model of gingival flow that would incorporate modulation by subgingival plaque, Tolo and also Schwartz, Stinson and Parker performed studies using radiolabeled macromolecules traced by autoradiography through the sulcular epithelium showing that basement membrane could restrict the passage of the label (65, 73). Therefore in the

gingivitis model as bacterial byproducts accumulate at the basement membrane, a standing osmotic gradient is generated and the flow of gingival fluid was initiated. There was also a discussion that this initial process corresponded to osmotically modulated, pre-inflammatory exudate. The progression to a “secondary inflammatory exudate” was also previously described by Weinstein et al. based on the protein/calcium ratio that increases markedly in fluid from normal, mildly inflamed and inflamed gingival tissues (76).

The flow of fluid into the gingival crevice is typical of inflammation and accompanied by an increase in cellular infiltrate. Neutrophils, which are considered to be the major cellular defense system in the gingival crevice, leave the capillary network in the underlying connective tissue and migrate through the junctional epithelium into the crevicular space, where they accumulate at the interface of the subgingival plaque and the gingival epithelium. This migration occurs along with chemotactic gradients established by both bacterial components and activated host messengers. Approximately 75–80% of neutrophils isolated from the GCF are viable cells and it would appear that crevicular neutrophils are capable of ingesting and killing microorganisms and utilize their armamentarium of antibacterial molecules. However the neutrophil functional capability at sulcular/pocket microenvironment is still a controversial topic. Nonetheless, the presence of an IL-8 gradient in health that serves to recruit neutrophils into the crevice coupled with the observation that the junctional epithelium lacks tight junctions suggests that neutrophil egress into the crevice in health is a physiological process that likely results in concomitant fluid flow. One could argue that neutrophils in the crevice are part of the physiology of health maintenance and not truly an inflammatory response.

In evaluating the pathogenesis of periodontal diseases, the initial signs of disease are visible in the marginal gingiva displaying clinical changes indicative of inflammation concurrent with an apical advancement of plaque formation and an increase in plaque mass. As an initial host response to bacterial plaque, an increase in the vascular permeability of the subepithelial blood vessels can be observed, allowing the escape of plasma from the circulation, leading to gingival edema. As this fluid escapes into the gingival crevice, via the junctional epithelium, it results in increased crevicular fluid flow (62). It has been demonstrated that in experimental gingivitis with induced inflammation promoted for a period of 3 weeks, the GCF flow increase was 5.5-fold during the 21 days period (43). Thus, classically the amount of GCF that flows into the crevice or pocket in terms of volume per unit of time is associated with the tissue inflammatory response.

During the inflammatory state, numerous cells and inflammatory factors such as cytokines, proteins, proteinases, phosphatases and local tissue degradation products are released in the crevicular fluid. The GCF content becomes a mixture of molecules originated from the blood, host tissues and subgingival biofilm, including electrolytes, small organic molecules, proteins, cytokines, specific antibodies, bacterial antigens, and enzymes of both host and bacterial origin. This composition clearly indicates that the epithelium lining of the gingival sulcus was permeable to small molecular weight compounds and that the passage of tissue fluid into the sulcus and pockets acts as a possible defense mechanism that may play an important role in the homeostasis of the crevicular environment (44) but also interferes with biofilm colonization as the disease progresses. Many studies have now confirmed that one of

the initial events of tissue inflammation is an increase in fluid filtration from capillaries to interstitium, which may be due to increased Starling forces across the capillary walls and increase in vascular permeability or vasodilation, with consequent net fluid movement and elevation in the interstitial hydraulic pressure (33, 48, 49, 51, 63).

Dynamics of the Gingival Crevicular Fluid composition and development of Periodontal Disease

The development of periodontitis is associated with a rise in pH in the gingival sulcus to around 8.5, and this is thought to occur by the bacterial degradation of proteins supplied by GCF to produce NH_4, assacharolytic subgingival bacteria utilize proteins as primary nutrients and produce NH_4 as a by-product, which serves to promote the precipitation of calcium salts from the GCF or saliva resulting in the promotion of calculus formation subgingivally or right at the free-gingival margin. GCF has also been investigated for its potential function as a growth medium that can support the growth of host cells serving the function of serum, or in support of proliferation of periodontopathogens. Certain proteins, such as immunoglobulin appear to be elevated in GCF relative to serum indicating the local production of IgG by tissue-resident plasma cells. Other proteins, like complement proteins, appear to be depleted; likely due to adsorption by the local biofilm.

In the transition from a ‘healthy’ to a ‘disease-associated’ subgingival microbiota a number of allogenic factors play a role which includes pH, oxygen levels, temperature, osmotic pressure and oxidation–reduction potential and in addition, protein-based nutrients become available within the GCF (70). Takahashi has proposed an order of microbial succession in which the saccharolytic organisms, *Fusobacterium nucleatum* and *Prevotella intermedia*, initially colonize the shallow periodontal pocket (71). During these early stages, the pH of the GCF in the pocket may be acidic but the metabolism of nutrients, which corresponds mainly to host proteins supplied by GCF, leads to the establishment of a neutral environment (10). The shift towards lower pH likely is a consequence of the downward extension of saccharolytic bacteria which are adherent and utilize glucose to produce lactic acid. The pH range of the gingival sulcus has been reported to vary between 6.5 and 8.5, and increased pocket depth and inflammation have been associated with increased alkalinization and the severity of the inflammatory host response (46), likely associated with the shift to assacharolytic subgingival organisms. The rise in localized pH facilitates the emergence of acid-sensitive and more proteolytic species, such as *Porphyromonas gingivalis* to colonize. The destruction of host tissues and increased efflux of GCF caused by the proliferation of the so-called ‘red complex’ group of bacteria (32) leads to an increase in the fermentation of amino acids into organic acids producing the rise in pH above neutrality. In particular, ammonia produced by the metabolism of amino acids found in GCF and released by the breakdown of host tissues, leads to an increase in pH above 8.0, thereby promoting the proliferation of acid-sensitive pathogenic bacteria (10, 46).

Mediators of Periodontal Disease in Gingival Crevicular Fluid

Numerous cytokines are released from cells of the sulcular and junctional epithelia, dendritic cells, connective tissue fibroblasts, macrophages, and neutrophils. In addition, a number of

enzymes such as matrix metalloproteinases (MMPs) are produced by neutrophils, fibroblasts and osteoclasts, leading to the degradation of connective tissue collagen and alveolar bone. To date, more than 90 different components in GCF have been evaluated for periodontal diagnosis (53). Initially, the GCF flow monitoring (volume per unit time collected) was reported to reflect the clinical condition of the periodontium (27). Many studies demonstrated that GCF flow increases with an increase in the severity of gingival inflammation (14, 21, 60). With the advance of laboratory techniques, GCF has been eluted and analyzed extensively for host response factors, including molecules from blood, local host tissue, and plaque biofilm (23, 24, 28, 30, 31, 39, 57).

Matrix metalloproteinases are an important group of proteinases related to collagen degradation during the destructive process of periodontal disease that can be measured in GCF (28, 39, 40). The neutrophils are the major source of matrix metalloproteinases at the infected site, specifically MMP-8 and MMP-9(54). Macrophages and neutrophils, in response to bacterial lipopolysaccharide, are activated to produce important inflammatory mediators, such as TNF-α, IL-6, IL-1, and other cytokines related to the host response and tissue destruction (2, 56).

Among the biochemical markers for the measurement of bone homeostasis, pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP), receptor activator of nuclear factor-kappa β ligand (RANKL), osteoprotegerin (OPG), and osteocalcin are the most studied in GCF. ICTP is a 12- to 20-kd fragment of bone type I collagen released by digestion with trypsin or bacterial collagenase (64). GCF pyridinoline cross-linked carboxyterminal telopeptide of type I collagen has been shown to be a good predictor of future alveolar bone and attachment loss, is strongly correlated with clinical parameters and putative periodontal pathogens, and demonstrates significant reductions after periodontal therapy (26). Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen and osteoprotegerin have also been linked with alveolar bone loss around dental implants, suggesting that their local levels may help to distinguish diseased and healthy sites (4).

Gingival Crevicular Fluid as a Diagnostic Tool

Collection of GCF is non-invasive and therefore this approach has been extensively explored in the search for potential diagnostic biomarkers of periodontal disease (13, 17, 25, 50, 53). As a result of the interaction between the bacterial biofilm and the cells of the periodontal tissues (16), GCF appears as an attractive oral fluid due to its ease of collection and allowing for sampling of multiple sites within the oral cavity simultaneously.

Various methods of GCF sampling that most often include collection of GCF on paper strips to measure specific analytes have been reported. Most studies collect GCF using standardized strips of filter paper [e.g. Periopaper]. Some studies collect GCF for specific amounts of time; typically 30 seconds and measure the total amount of mediator collected on the strip by elution followed by a biomarker-specific assay, such as an enzyme linked immunoassay. These investigations express the level as a total mass of biomarker present. Multiplexing methods using immunobeads now permit the analyses of up to 100 analytes in a single GCF sample, which can be only 0.5 to a few microliters in total volume (67). Other

reports collect GCF on paper strips and measure the amount of fluid collected, typically using a Periotron® instrument that measures the volume of GCF collected to enable the computation of mediator concentration within the sample (Figure 2). Although the utility of either method can be argued, some discussion of the differences are noteworthy since many studies have looked at the association between the total amount (e.g. total level of mediator) or concentration of different GCF constituents, often expressed as ng/mL or pg/mL) as related to periodontal health status (7, 12, 29, 59). To understand the difference between the two methods the compartments that are sampled should be considered. First, the GCF volume collected reflects the sum of two compartments- the GCF void volume which is a resting GCF volume that is independent of flow, and the GCF flow contribution that is dependent upon the collection time and flow rate. The GCF resting void volume is highly dependent upon the pocket depth. For example as a pocket increases from 3mm to 6mm there is a 50% increase in the resting pocket void volume. Using a total amount method, one would naturally expect that in disease there is an increase in pocket depth and a concomitant increase in total amount of biomarker. It may or may not reflect any tissue inflammatory contribution, but an increase in total amount of biomarker is expected as a consequence of an increase in pocket void volume. Typically this void volume wicks within the first few seconds of collection and is therefore adsorbed quickly and depleted during the first sampling, if repeat samples are taken. In addition, depending on the length of time the sample is collected, the relative contribution of the void volume to the total volume can vary and therefore it will change the total amounts of mediator present. Furthermore, the pocked void volume will usually contain neutrophils (typically in the range of 10^4–10^6 cells), which are present in health and disease that can contribute to the biomarker measurement, if the marker is associated with neutrophils. As an example, if one samples GCF multiple times at the same site, the total amount of beta glucuronidase decreases rapidly with each repeated sample. This is consistent with the collection of fewer neutrophils on the paper strip as the pocket is depleted of neutrophils until the total amount reaches a lower plateau. This suggests that the level of beta glucouronidase present is highly dependent upon the number of neutrophils entering the pocket. By contrast, if one measures levels of PGE_2 within the GCF, which is not produced by neutrophils or other cells within the pocket, the PGE_2 level remains rather stable with repeat sampling, as it is produced within the tissues, pointing to the second source of GCF biomarkers- the flow of mediator from adjacent tissues. Thus, the level of mediator present is a function of both the resting pocket volume, which is dependent upon the pocket depth, and the flow from the tissues into the crevice. If one is interested in tissue markers of inflammation, the concentration method is preferred by some investigators, as it is independent of pocket depth and by comparison with adjacent tissue biopsy samples – seems to reflect tissue levels by diffusion from a high level within tissues to a lower level within the GCF compartment. This difference in sampling and data reporting should be considered when examining studies when values are expressed as total level of mediator versus concentration. The collection and storage of GCF samples are shown in Figure 2. The storage of GCF in liquid nitrogen has several advantages over −80C storage. Liquid nitrogen is colder (−196C), but more importantly it displaces dissolved oxygen in the fluid phase. This prevents the oxidation of the biological sample and the reaction with molecular H_2O. As a consequence samples can be stored for decades in liquid nitrogen and remain stable, ungraded, unoxidized, unmodified by enzymes and available for molecular analysis.

Host susceptibility is a critical determinant in periodontal disease pathogenesis giving the inflammatory mediator levels present in GCF an important value for evaluating risk for disease activity. In a molecular epidemiologic study, new periodontal health status represented by distinct biological phenotype was described based on clinical, microbial, inflammatory, and host-response measures (59). In a sample size of over six thousand patients, GCF IL-1β and IL-6 were significantly associated with deep pocket depths and severe gingival inflammation. The proteomic analysis of GCF in different periodontal conditions also demonstrates marked differences according to disease profile (69). Studies report that up to 432 different proteins have been identified in GCF samples (7, 38, 69). Among these proteins, there are proteins exclusively associated with both healthy and diseased sites. However, further studies with larger sample populations are needed to validate the role of the identified proteins in the pathogenesis of periodontal disease. The use of GCF alone or clustered with periodontal pathogens, serum, and saliva biomarkers suggests potential diagnostic value to identify periodontal disease activity and response to therapy. In an experimental gingivitis model, Offenbacher and co-authors reported the expression of 33 GCF biomarkers, including cytokines, MMPs, and adipokines, during the inflammatory induction and resolution phase (58). It was demonstrated that the stent-induced gingivitis model was associated with marked, but reversible increases in IL-1α and IL-1β with suppression of multiple chemokines as well as selected MMPs. Silva and collaborators evaluated the level of GCF biomarkers, inflammatory cells, and periodontal pathogens associated with sites demonstrating ≥2mm attachment loss (68). The authors evaluated fifty-six patients with moderate or severe chronic periodontitis. Higher RANK-L, IL-1β, and MMP-13 were found at sites exhibiting active periodontal disease. In addition, the proportion of periodontal pathogens and the number of CD4 (+) T cytokines were higher in active sites. In another longitudinal study, Kinney and co-authors evaluated the sensitivity/specificity and positive predictive value/negative predictive value (PPV/NPV) of GCF biomarkers to detect sites with disease activity (40). In this specific cohort study examining 100 patients over a 12-month period, GCF alone provided low sensitivity and high specificity values, 23% and 95% respectively. Although GCF IL-1β demonstrated a significant difference at baseline between progressing and stable patients, it was not a good predictor of periodontal disease progression. Combined with plaque pathogens, GCF biomarkers demonstrated the highest PPV and NPV, 73% and 70% respectively.

TGF-β1 levels were also investigated by Khalaf and collaborators as potential makers for periodontal disease in GCF samples from 60 patients, 30 with chronic periodontal disease and 30 healthy subjects. The investigators found that TGF-β1 levels were significantly elevated in serum, saliva and GCF in patients with periodontal disease compared to controls (37).

More recently, with the objective to investigate potential markers in patients with diabetes mellitus, Kajiura et al. analyzed 78 patients to examine the levels of glycated albumin and calprotectin in GCF samples from individuals with or without periodontitis and DM. They examined the possibility that glycated albumin and calprotectin in GCF can serve as markers that can predict periodontitis in patients with diabetes mellitus (34). Diabetes Mellitus is medically diagnosed by determining glycated hemoglobin (HbA1c), glycated albumin (GA), and blood glucose levels. HbA1c and GA are intermediate products called Amadori

compounds, involved in the non-enzymatic glycation reactions of blood proteins, which finally change to AGEs (3). Data indicate that HbA1c and GA levels, which reflect the status of glycemic control, are positively correlated, and that the level of glycated albumin has been shown to be significantly associated with plasma glucose levels in individuals with and without DM in epidemiologic research (77). The authors argue that HbA1c level represents the mean glycemic status over the previous 2 to 3 months while GA indicates the glycemic condition in the short term (2 to 3 weeks) because the half-life of albumin is approximately 17 days. GA values have been used to evaluate postprandial glycemic status and glycemic control after medication for DM treatment. They accurately reflect the status of glycemic control in patients with DM with certain diseases, including anemia, hemoglobin disorder, and renal failure, as well as in pregnancy, suggesting that GA can be a reliable marker to evaluate glycemic status in patients with DM (41). The authors report that the cutoff values of GA amount and concentration for a prediction of DM were 6.00 mg/site, with a sensitivity of 82.5% and a specificity of 66.9%, and 5.13 mg/mL GCF, with a sensitivity of 75.2% and a specificity of 71.1%. The median GCF GA amounts in DM and non-DM groups were 16.12 and 3.00 mg/site, respectively, and the concentrations were 9.57 and 2.00 mg/mL GCF suggesting that glycated albumin in GCF can serve as a marker associated with disease progression in diabetic patients.

Calprotectin levels were also measured in this study since it is known that neutrophils, monocytes/macrophages, and oral epithelial cells secrete calprotectin and its expression is also increased by inflammatory cytokines and *Porphyromonas gingivalis* LPS (47). Data collected from GCF samples, calibrated and diluted, reflected the infectious and inflammatory levels in disease showing more than a two fold increase in samples from patients with periodontal disease in comparison to periodontally health subjects. Based on these reported results, signature of GCF biomarkers in association with pathogens and clinical measures can provide a sensitive measure for discrimination of periodontal disease progression. Table 1 shows the cellular and molecular components of GCF.

Proteomic analysis of gingival crevicular fluid in search for periodontal disease markers

Currently, proteomic analysis of GCF is still in its infancy with limited data available in the literature. With the understanding that the protein composition of GCF may reflect the pathophysiology in the monitoring of periodontal diseases establishment and progression, protein profiles of GCF obtained from apparently healthy individuals are starting to be explored as standard GCF proteomic patterns to potentially serve as a reference to identify biomarkers of periodontal diseases by proteome analyses.

Zelko and collaborators analyzed GCF proteins in the GCF of periodontally healthy individuals using a gel free method and described proteins broken down into small peptide fragments and analyzed directly by LC-MS/MS analysis (78). The authors found a total 327 GCF proteins and suggested these may be used as a reference for future proteomic studies searching for GCF biomarkers of periodontal diseases. The authors also reported eight protein spots found to be significantly more intense in GCF including superoxide dismutase

1 (SOD1), apolipoprotein A-I; (ApoA-I), and dermcidin (DCD) (78). The superoxide dismutases (SOD) are the most important line of antioxidant enzyme defense system against reactive oxygen species (ROS). SOD1, one of the human SOD, is also known as superoxide dismutase [Cu-Zn]. DCD was identified as a gene for an antimicrobial peptide DCD-1 in human sweat glands (55). This peptide has antimicrobial function and a range of biological functions and ApoA-I is the major protein component of high-densitylipoprotein (HDL) in plasma. ApoA-I has a specific role in lipid metabolism (42).

In search of markers to predict health or disease, Bostanci and investigators used quantitative proteomic analysis by LC/MS to analyze 10 GCF samples 5 healthy subjects and 5 patients with aggressive periodontitis. They reported that GCF proteins Cystatin-B and defensins were detected only in the healthy control samples and also Annexin-1 was detected in 5-fold higher levels in samples from periodontally health individuals. L-plastin was detected only in aggressive periodontitis GCFs (15.6 +/− 12.1 fmol) (12). L-plastin is an actin-bundling protein, exclusively expressed in leukocytes and plays a crucial role in immune-mediated events. This protein has been shown to be elevated also in chronic periodontitis. Its value as a marker for periodontal disease still needs to be further evaluated (61).

Baliban et al. aiming to use biomarker combinations to predict health or disease from GCF samples using high throughput proteomic analysis, described G3P_HUMAN, TYPH_HUMAN and KV101_HUMAN selected as human protein biomarkers for periodontally healthy status. G3P_HUMAN (Glyceraldehyde 3-phosphate dehydrogenase) is an enzyme that participates in glycolysis and serves to break down glucose for energy and carbon molecules. TYPH_HUMAN (Thymidine phosphorylase) is an enzyme that participates in purine metabolism pathway and pyrimidine metabolism pathway and is only found in periodontally healthy GCF samples tested. The protein KV101_HUMAN (Ig kappa chain V-I region AG) was observed in most of periodontally healthy samples but few of the periodontitis samples. The authors recommend further investigation in the functional role of these proteins (8). The combination of human protein biomarkers and bacterial protein biomarkers needs to be further validated in experiments exploring different periodontal conditions, as well as dynamic changes during and after periodontal treatment. So far, the findings indicate that advances in proteomics technology and optimization-based models for analysis and prediction of biomarkers can potentially contribute in developing tools to help predict the periodontal status of individuals based on analysis of gingival crevicular fluid (GCF) samples.

Concluding Remarks

GCF as a diagnostic and prognostic tool has been explored since the initial studies on GCF which aimed to demonstrate that the flow of gingival fluid was sufficiently indicative of the inflammatory state of the periodontal tissues. Research methods have evolved to enable the assessment of the transition phase between health and inflammation at the gingival level to disease progression. More recently, metabolomic analyses that measure small degradation molecules associated with host and bacterial metabolism show promise. There may be many different cytokine inflammatory pathways or microbial stimuli that are associated with the causal pathway of periodontal pathogenesis. However, these pathways likely converge to

similar profiles of metabolites. For example, one microbial community structure may induce specific inflammatory signatures whereas another dysbiotic microbial community might induce a different inflammatory signature. Differences in host genetics may also dictate microbial colonization or inflammatory signatures adding to the heterogeneity of the potential causal pathways. However, small molecules that reflect the virulence of the dysbiotic community and the intermediary or final end-points of host tissue breakdown are likely to be common effector pathways of disease pathogenesis. Thus, metabolomic analyses of GCF that measures microbial and host interactions associated with the onset and progression of periodontal disease has the potential utility to expand our understanding and improve the landscape for the discovery of diagnostic, prognostic and therapeutic markers.

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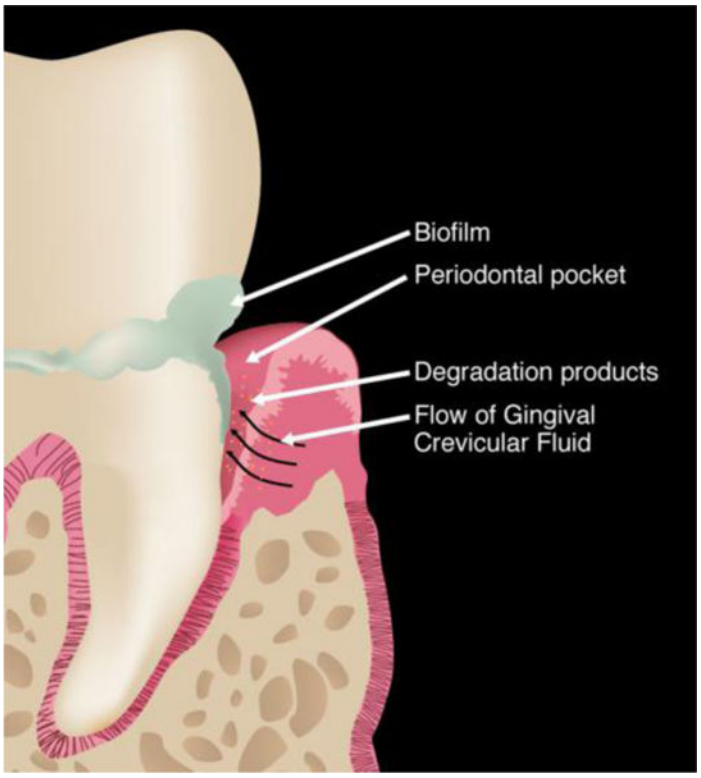

Figure 1.

Schematic figure indicating the flux of gingival crevicular fluid through epithelial cells in the presence of a biofilm.

In this microenvironment the exudate contributes to reorganization of subgingival biofilm and also carries inflammatory mediators and inflammatory cells to the periodontal pocket while harboring bacterial antigens, and enzymes of both host and bacterial origin.

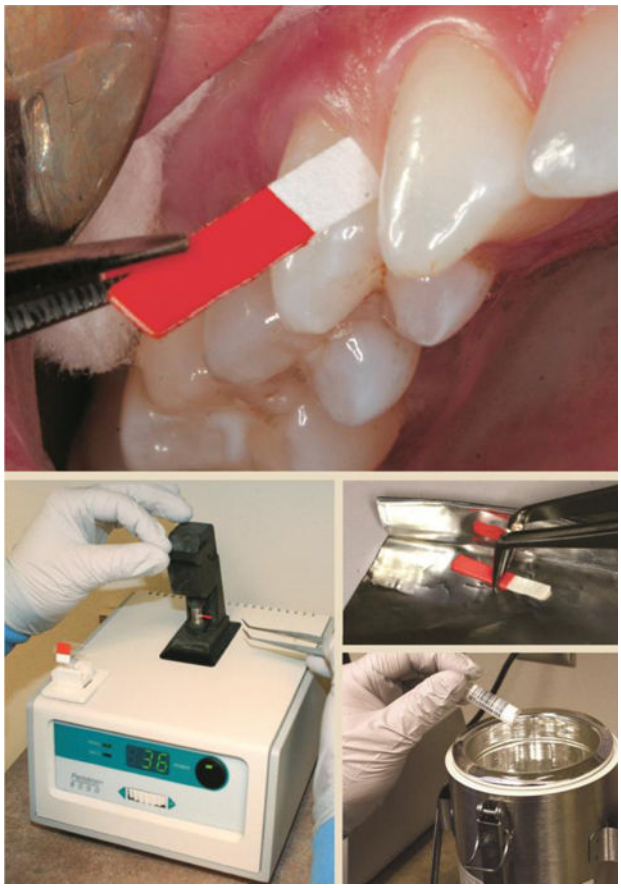

Figure 2.

Ilustration of GCF sampling sequence and use of Periotron®. Once the sites are isolated with cotton rolls and gently air-dried, the Perio® paper strips are inserted in the gingival sulcus for 30 seconds. The paper strips are then inserted in the Periotron 8000® device (Harco, Tustin, CA, USA), previously calibrated to each individual sample to obtain the fluid volume. Paper strips are then wrapped in aluminum foil, transferred to liquid nitrogen and stored at −80°C until assayed.

Table 1

The major cellular and molecular components of gingival crevicular fluid

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