Chapter from the book *Gingival Diseases - Their Aetiology, Prevention and Treatment*
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1. Introduction

Plaque-induced gingivitis is a localized inflammation affecting marginal periodontal soft tissue (Armitage, 1999). It is considered to be a reversible periodontal disease. In contrast, periodontitis is an irreversible destructive periodontal condition, which is usually preceded by gingivitis although not all gingivitis develops into periodontitis. Why some gingivitis sites transition to periodontitis sites is not well understood, although there are some indications of “at risk” populations such as smokers and poorly controlled diabetics (Burt, 2005). It is also understood that development of chronic periodontitis only occurs in areas of long-standing gingivitis and furthermore that teeth with consistently inflamed gingival tissues are at a significantly higher risk of attachment and tooth loss (Lang et al, 2009). Consequently being able to non-invasively and closely monitor gingivitis sites would be very helpful in the prevention of periodontal disease. The basic clinical measures for periodontitis are gingival bleeding, radiographic bone loss, clinical attachment loss and clinical probing depths (Burt, 2005). Current clinical diagnostic measures are unable to identify gingivitis with high risk of transition to periodontitis since not all sites with gingivitis actually progress to periodontitis (Armitage, 1996). Therefore, the search for more accurate periodontal diagnostic instruments is continuing and a number of non-invasive diagnostic modalities such as optical and infrared spectroscopy, optical coherence tomography (OCT) and ultrasound have been evaluated for their potential in periodontal diagnosis. An illustration to better visualize the overall features of currently used clinical methods and emerging optical and infrared based diagnostic methods for periodontal diseases including gingivitis is presented in Figure 1. In principle, these diagnostic methods can be classified into three categories based on their features and clinical aspects. Clinical examination which is the mainstream of current practice and the gold standard, primarily measures clinical parameters such as bleeding on probing (BOP), probing depth (PD), and clinical attachment loss (CAL) as well as bone loss with the use of dental radiographs.
The second group consists of molecular finger printing or finding molecular markers in gingival crevicular fluid (GCF). In addition to regular genetic analysis and laboratory type tests that measure fundamental aspects of oral biochemistry and microbiology, mid-infrared spectroscopy (MIRS) has shown some promise in providing molecular profiles of GCF related to periodontal disease. The last group consists of methods suited to non-invasive in vivo monitoring such as optical spectroscopy, OCT and ultrasound imaging. OCT and ultrasound are generally used to delineate anatomical features of the gingival and surrounding tissues which are affected by disease, whereas optical spectroscopy can simultaneously detect local alterations in tissue hemodynamics and thereby accurately differentiate inflamed periodontal sites from healthy sites.

Non-invasive diagnostic methods that do not employ ionizing radiation are of particular interest for routine use in the diagnosis and monitoring of gingivitis as well as in predicting disease progression. Therefore, methods based on optical and infrared spectroscopy that are being explored as complementary diagnostic tools in periodontal diagnostics will be primarily reviewed in this chapter.
2. Clinical diagnostic criteria for gingivitis and their limitations

Gingivitis is defined as gingival inflammation in the absence of clinical attachment loss or in the presence of reduced but stable attachment levels (Mariotti, 1999). It is one of the most common human diseases and occurs in all ages of populations. The prevalence of gingivitis is high in both high income developed countries and low and middle income developing societies, affecting 50 – 90 % of adults worldwide (Albandar & Rams, 2002). For instance, only 6.1% of American adults showed mean gingival index (GI) <0.50; most (93.9%) were > or = 0.50 (Li et al, 2010). The average GI in 97.9% of Chinese adults was 0.5 or higher, and only 2.1% of them had a GI lower than 0.5 (Zhang et al, 2010). Most people have clinical signs of gingival inflammation, such as redness, edema and bleeding on gentle probing, but the extent and severity of inflammation vary from one population to another and are closely related to bacterial dental plaque.

In gingivitis, inflammation is confined to the periodontal soft tissues and diagnosis of most gingivitis can be readily made on clinical presentation and visual examination. Common signs of gingival inflammation include redness, partly due to the aggregation and enlargement of blood vessels, swelling and loss of texture and bleeding on gentle probing or sweeping on gingival margin (Lang et al, 2009). However, for clinicians, these key clinical parameters are largely subjective observations and difficult to stage gingivitis. Thus, assessment of disease progression and the effect of treatment are often inaccurate and subjective since it relies on clinical monitoring and comparing of these clinical parameters. Some local and systemic factors may further complicate the precise measurement of gingival inflammation. For instance, cigarette smoking is a well established risk factor for periodontal diseases, but clinical signs of periodontal inflammation are reduced in a dose dependent manner in smokers (Scott & Singer, 2004; Dietrich et al, 2004; Erdemir et al, 2004)

Unlike many other infections, painless bleeding often presents as an early, easily recognizable sign of gingivitis, in particular at its early stage when it is easy to treat and maintain. If left untreated, however, some gingivitis will develop into a more destructive irreversible form of periodontal disease, i.e., chronic periodontitis, leaving permanent damage to tooth supporting tissues. Longitudinal studies showed that teeth with chronically inflamed gingiva had 70% more attachment loss than healthy sites and a much higher risk of tooth loss as well (Heitz-Mayfield et al, 2003; Schatzle et al, 2003). Once chronic periodontitis has established, more invasive treatment approaches and life long professional maintenance are required for periodontal health. Therefore, inaccurate diagnosis of periodontal diseases can result in either under-treatment, if one fails to identify progressing gingivitis or over-treatment if treatment is delivered to stable sites. It is thus important to identify the sites and subjects at risk of progression in their earliest stage of development, particularly in cases with high risk of progression. Unfortunately, currently used periodontal diagnostic methods, such as periodontal probing and radiography, are not sensitive measurements in this regard. Neither method is able to differentiate between reversible gingivitis and early but irreversible periodontitis, nor identify progressing periodontitis until significant periodontal tissue has been lost. For instance, the standard deviation for the measurement of attachment level with conventional periodontal probes is reported to range from 0.62 to 1.17 (Glavind & Lo, 1967; Goodson et al, 1982; Aeppli et al, 1985). Error of this magnitude requires a measured change of 2 to 3 mm in order to safely conclude that a change did occur. A more sensitive means is needed to precisely identify disease progression at the early stage (Haffajee et al, 1983, Ranney, 1991). As an adjunct to
periodontal probing, our group has recently explored the potential of using optical - near infrared spectroscopy to measure site specific hemodynamics in relation to periodontal diseases, including gingivitis. The previously published results are elaborated in the following sections, which clearly demonstrate that optical spectroscopy is emerging as a powerful diagnostic tool for inflammatory periodontal diseases.

3. Diagnosis of gingivitis by optical spectroscopy

Indeed, a simple, user friendly, chair-side, diagnostic test for periodontal inflammation would be an invaluable addition to the dental clinic. To this end, optical spectroscopy has been extensively explored as noninvasive modality for the diagnosis of periodontal diseases including gingivitis. The most attractive feature of a fiber optic optical spectroscopy measurement of periodontal inflammation is that it offers a rapid, non-invasive means of assessing the balance between tissue oxygen delivery and utilization. In the methodology pursued by our group, the measurement is made by positioning a fiber optic probe over the area of tissue under investigation but does not require a measurement within the periodontal pocket, unlike conventional periodontal probing. This poses less discomfort for the patient with measurement times on the order of a few seconds; one can envision optical spectroscopy as a practical chair-side tool for the practitioner.

It is generally known that the visible - near infrared spectral region of the electromagnetic spectrum covering the wavelength range from 400 to 2500 nm, conveys information on a few key inflammatory markers of periodontal disease (Sowa et al, 2006; Sowa et al, 2001). The electronic transitions stemming from the heme ring and central metal iron ion of hemoglobin are particularly strong absorbers of visible light as well as absorbing light in the near infrared region of the spectrum. For instance, the short wavelength region, 500 – 600 nm is dominated by the absorption from oxygenated hemoglobin (HbO2) and deoxygenated hemoglobin (Hb) in the capillary bed of gingival tissue while the absorption from water results in an increased attenuation at longer wavelengths in the 900 – 1100 nm region (Fig. 2) (Sowa et al, 1999; Hanioka et al, 1990). By fitting optical attenuation spectra to the known optical properties (extinction coefficients) of HbO2 and Hb, optical spectroscopy can measure relative concentrations of HbO2 and Hb (Hanioka et al, 1990; Attas et al, 2001). Furthermore, the 960 nm water band is known to shift with tissue temperature and changes in electrolyte concentration (Otal et al, 2003). Thus, optical spectroscopy provides a measure of the hemoglobin oxygen saturation of tissues and the degree of tissue perfusion as well as a measure of tissue edema.

Based upon these principles, visible - near infrared spectroscopy has been widely applied to biomedical problems, including cancer diagnostics, the early prediction of inflammation-related treatment failures in burn victims (Sowa et al, 2001; Liu et al, 2005; Sowa et al, 1999), and monitoring ischemic conditions in urology such as testicular tissue perfusion and oxygenation of testicular torsion (Capraro et al, 2007; Stothers et al, 2008). Commercially, some near infrared based cerebral oximetry monitors, i.e., NIRO and INVOS, have been employed in the clinical settings for the surveillance of the cerebral oxygen balance under CO2 challenge (Yoshitani et al, 2002).
Likewise, hemoglobin and oxygenation indices have also been previously measured in periodontal tissues with the data suggesting that the increase in blood supply during inflammation is insufficient to meet the oxygen demand in inflamed gingivae (Hanioka et al, 1990). In addition, tissue edema, an index that is commonly used as a marker of gingival inflammation (Loe et al, 1963; Scott et al, 2004) can also be measured using near infrared spectroscopy (Liu et al, 2009; Sowa et al, 2001). Consequently, monitoring the intensity of the water bands in gingival tissues should provide an index of tissue hydration representing a simple indicator of inflammation at specific periodontal sites. Furthermore, we have recently demonstrated, using optical spectroscopy, that tissue oxygenation at gingivitis sites was significantly decreased (p<0.05) compared to healthy controls as shown in Figure 3 (Liu et al, 2009). Such decreased oxygen saturation likely reflects tissue hypoxia resulting from an ongoing inflammatory response leading to increased oxygen consumption (Hanioka et al, 2000). It is well known that in destructive periodontal diseases, anaerobic microorganisms predominate in the periodontal pocket and diminished oxygen tension in deep pockets would be expected to promote growth of anaerobic bacteria (Amano et al, 1988; Loesche et al, 1969). Interestingly, it has been shown previously that tissue oxygen saturation correlates well with oxygen tension in periodontal pockets (Hanioka et al, 1990). In particular, in chronic gingivitis (stage III), the blood vessels become engorged and congested, venous return is impaired, and the blood flow becomes sluggish. The result is localized gingival anoxemia, which superimposes a somewhat bluish hue on the reddened gingiva (Hanioka et al, 1991).
We have recently attempted to establish a model to predict risk index of gingivitis based on spectral data from several independent studies (Liu et al, 2009; Ge et al, 2011; Nogueira et al, 2011). The method of Fort and Lambert-Lacroix, using partial least squares with penalized logistic regression was applied directly to the measured visible reflectance spectrum (510 – 620 nm) of the gum with a subject-out bootstrap cross validation approach to select classifier parameters. The probabilistic classification model was calibrated using the spectral data from healthy sites and sites with periodontitis and the model was then used to predict the sites with gingivitis that have optical properties that are more indicative of periodontitis. Figure 4 shows a risk index applied to cases that were deemed to be gingivitis based on clinical assessment. This method would allow us to stratify the gingivitis cases into those that have spectroscopic characteristics closer to healthy sites and those that were similar to periodontitis.

Comparing the risk score between sites with or without plaque (Figure 5) revealed that the risk score of the gingivitis sites with plaque were on average higher than the risk score of gingivitis sites without plaque (p=0.02). Both results (Fig. 4&5) strongly indicate that based on the hemodynamic information embedded in the optical spectra, one can readily develop prediction models or risk scores to further stratify gingivitis.
Fig. 4. Logistic regression model that weights sites exhibiting signs of gingivitis towards healthy sites (negative periodontal risk values) or periodontitis (positive risk values). Model: Logistic regression. $Y=\frac{\exp(-0.07661+(1.57564)*x)}{1+\exp(-0.07661+(1.57564)*x)}$

Fig. 5. The risk scores of gingivitis sites with or without plaque. T-test indicates a significantly higher risk score for gingivitis sites with plaque compared to gingivitis sites without plaque ($p=0.02$).
As tissue oxygen saturation is not measurable clinically, optical spectroscopy can provide a further index of inflammation that may prove useful to the periodontist. In other words, after future studies, the intra-oral optical probe may be able to determine sites at which disease has not yet progressed clinically, but whose biochemically-defined profile suggests that a particular site has pathogenic potential, such as the anaerobicity required to establish a pathogenic micro flora.

4. Molecular fingerprinting of gingivitis GCF by MIR spectroscopy

Another important aspect in evaluating gingivitis is to fully utilize the molecular and biochemical information embedded in GCF. In fact, studies on GCF have extended over a period of about 60 years. Originally proposed by Alfano (Alfano, 1974), GCF represents the transudate of gingival tissue interstitial fluid but in the course of gingivitis and periodontitis, GCF is transformed into a true inflammatory exudate (Veli-Jukka, 2003). The composition of GCF is the result of the interplay between the bacterial biofilm adherent to the tooth surfaces and the cells of the periodontal tissues. GCF contains several cellular and molecular components of the immunologic response present in serum, as well as mediators and by-products of tissue destruction generated within the tissues. These substances possess a great potential to serve as indicators of periodontal disease, the healing process after therapy or as a window to periodontal disease. Therefore, GCF provides an easily collected fluid containing inflammatory mediators released during disease processes that affect periodontal tissues (Champagne et al, 2003).

Gingivitis is a form of periodontal disease in which gingival tissues present with inflammation but in which tissue destruction is mild and reversible. Gingivitis affects more than 90% of the population, but only 7-15% of the adult population is affected by a more severe form of the disease, chronic periodontitis (Brown & Löe, 1993). The histological presentation of gingivitis includes vascular changes with increased vasopermeability and vasodilatation, and the presence of an exudate of polymorphonuclear neutrophils, migrating from the tissue into the gingival crevice (Tsai et al, 1998; Page et al, 1976). Gingivitis is thought to be a neutrophil-dominated response, as mostly neutrophil mediators are identified in GCF, including leukotriene B₄, platelet activating factor, prostaglandin E₂, interleukin-1, thromboxane B₂, elastase and collagenase (matrix metalloproteinases-8) (D’Ercole et al, 2008; Munjal et al, 2007; Lamster et al, 2007; Kinane & Mark, 2007). Thus, inflammatory cytokines can be detected within the GCF and serve as an indicator of local immuno-regulatory and inflammatory status. Although in gingivitis the tissue destruction is mild and reversible, the tissue damage products like hydroxyproline/collagen fragments, have also been identified as biomarkers (Bowers et al, 1989; Huynh et al, 2002). Therefore, it is obvious that GCF provides a unique window for analysis of periodontal condition.

Several tests have been developed that are aimed at specifically and sensitively revealing the pathologic and metabolic status of periodontal tissues (Armitage, 2003). Some of them have shown good specificity and sensitivity values as well as potential for predicting disease progression (Jeffcoat & Reddy, 1991; Jeffcoat, 1992; Magnusson et al, 1996; Bader & Boyd 1999; Teles et al, 2009). Unfortunately only a handful of GCF tests have made their way into clinical practice. Clinicians are still missing a practical test based on enzymes,
tissue degradation products or cytokines that accurately indicates the initial periodontitis process, active disease periods or effective healing. However, despite the complex nature of periodontal diseases which involves a multifaceted immune and inflammatory reaction to a polymicrobial flora, and inter-individual variation in inflammatory response, such potential biomarkers are generally studied individually or rarely in small numbers (Kinane & Mark, 2007). This may explain why the predictive value of potential biomarkers studied to date has not been sufficient for effective routine clinical use (Lamster et al, 2007).

Different from analyzing one or more particular biomarkers in tissue or body fluid, IR spectroscopy analyzes complex biological systems by capturing the entire IR spectrum which represents the sum of the contributions of the biomolecules present such as proteins, lipids, sugars and nucleic acids (Petibois & Déléris, 2006). Essentially, the IR spectrum of a tissue or cell sample can be regarded as molecular fingerprint of the tissue or cells. If this molecular fingerprint is modified by a disease process, which is normally the case, then IR spectroscopy can be used to detect and monitor the disease process.

Therefore, IR spectroscopy can distinguish differences in the characteristics of diverse molecules by probing vibrations of chemical bonds and using these molecular and sub-molecular profiles to define and differentiate “diseased” and “healthy” tissues (Jackson et al, 1997). As covalent bonds vibrate, they absorb energy in the form of IR light (Hynes et al, 2005; Liu et al, 2006). The wavelength of light that is absorbed depends on the nature of the covalent bond (e.g., C=O, N-H), the type of vibration (bending, stretching, etc.), and the environment of the bond. In the last fifteen years, IR spectroscopists have taken advantage of this molecular information, in combination with pattern recognition/classification methods, to explore its potential as a powerful tool for the diagnoses of various diseases based upon the spectra of biological fluids, including amniotic fluid, lipid profiles, synovial fluid, saliva and gingival crevicular fluid to predict fetal lung maturity (Liu et al, 1998), diagnose heart disease (Liu et al, 2002) and rheumatoid arthritis (Eysel et al, 1997), assess global diabetes-associated alterations (Scott et al, 2010) and evaluate periodontal inflammations (Xiang et al, 2010), respectively.

The IR spectrum of saliva and GCF is a rich source of information regarding the oral cavity and associated inflammation. In a recent study by Scott et al, they have assessed global, diabetes-associated alterations to saliva at the molecular and sub-molecular levels by using infrared spectroscopy (Scott et al, 2010). For instance, by evaluating the difference spectrum a great deal of molecular information embedded in the saliva from diabetic patients can be distilled as shown in Figure 6. Following Fourier self-deconvolution (FSD), the most striking difference between the spectrum of diabetic saliva and that of control were vibrations arising from sugar moieties and/or glycosylation products, such as AGEs (advanced glycation end products). This can be visualized by examining the spectral range 950-1180 cm\(^{-1}\) that originated from various C-C/C-O stretching vibrations in sugar moieties. The 1020 cm\(^{-1}\) band is usually attributed to the C-O stretch vibration in glycogen while the bands at 1070 and 1169 cm\(^{-1}\) can be assigned as C-O-C symmetric and asymmetric vibrations of sugar moieties and phospholipids. Obviously, therefore, the contribution of AGEs and ALE’s (advanced lipoxidation end products) to diabetic spectra may be large. This is consistent with previous reports that found that stimulated or unstimulated salivary glucose concentrations are higher in diabetic patients than in control subjects (Garay-Sevilla et al, [www.intechopen.com](http://www.intechopen.com)).
These findings are also in keeping with numerous studies that have shown increased salivary AGE content in the development of diabetes complications (Bilous, 2007).

More relevant to gingivitis, our group recently has employed IR spectroscopy to characterize GCF from healthy, gingivitis and periodontitis sites and determined specific spectral signatures that clearly demarcate healthy and diseased tissues (Xiang et al, 2010). With the FSD method which can narrow effective bandwidths, enhance resolution, and increase available discriminatory data (Surewicz et al, 1988), we were able to reveal subtle differences in spectral band intensity and positions arising from the three major
components, i.e., lipid, protein and DNA observed in GCF from healthy, gingivitis and periodontitis groups. For instance, by integrating the three major DNA sensitive bands - the bands at 1087 and 1240 cm\(^{-1}\) arising from symmetric and asymmetric PO\(_2\) stretching vibrations of phosphodiester groups in DNA and the 1713 cm\(^{-1}\) band - we can see that GCF DNA concentrations in diseased subjects are increased compared to healthy subjects (Fig. 7). GCF contains a diverse population of cells, which include bacteria, desquamated epithelia and transmigrating leukocytes (Delima et al, 2003; Palmer et al, 2005). The increased DNA component in GCF from gingivitis and periodontitis sites, relative to healthy controls, is likely due to a combination of an inflammation-driven increase in leukocyte migration into the GCF, particularly neutrophils; an increase in epithelial turnover, reflecting ongoing tissue remodeling; and of the inflammatory stimulus itself, i.e., plaque bacteria.

Fig. 7. Relative DNA contributions are increased in diseased GCF groups. The shade areas highlights DNA-specific signals in GCF. The enlarged area of another important DNA band, 1713 cm\(^{-1}\), arising from DNA pair base vibration after Fourier self-deconvolution (FSD). The histograms representing the integrated area (relative DNA content) in the spectra from the three groups. (Reproduced from J Perio Res, 2010; 45: 345-352 with permission).
Increased protein (Amide I at 1652 cm\(^{-1}\)) and lipid (symmetric CH\(_2\) stretching vibration at 2853 cm\(^{-1}\) from the fatty acyl chains) signals are also evident at diseased sites (Figure 8). In particular, disease-specific cellular and molecular alterations to the composition of GCF are clear, most obviously the increased intensity of the 1652 cm\(^{-1}\) Amide I band at inflammatory sites (gingivitis and periodontitis) compared to healthy sulci. This indicates that the protein concentrations in both disease groups were significantly higher than in controls, in agreement with prior reports of increased total protein levels in periodontitis GCF (Akalin et al, 1993); and a significant correlation between total GCF protein concentration and disease severity (Baltacioglu et al, 2008). Many GCF proteins have been extensively explored as potential diagnostic markers that define periodontal inflammation. They include inflammatory mediators, particularly cytokines and matrix metalloproteinases, and tissue breakdown products, such as, fibronectin, collagen fragments and hydroxyproline, which should reflect the extent of underlying tissue destruction.

In addition, the integrated area of the =CH band at 3012 cm\(^{-1}\) has been used as an index of the relative concentration of double bonds in lipid structures from unsaturated fatty acyl chains (e.g. linolenic, arachidonic, etc.) arising from lipid peroxidation (Severcan et al, 2005; Liu et al, 2002). Interestingly, lipid oxidation is increased in the inflammatory groups, as evidenced by the olefinic =CH band at 3012 cm\(^{-1}\) providing further evidence of the importance of lipid peroxidation in periodontal disease pathogenesis (Tsai et al, 2005; Sheikh et al, 2001).

Fig. 8. Relative concentration of protein and lipid components derived from GCF MIR spectra after FSD procedure. The histograms representing the integrated area (relative protein, lipid and lipid peroxidation content) in the spectra from the three groups. Clear differences in protein and lipid content of GCF from diseased and healthy sites are apparent. (Reproduced from J Perio Res, 2010; 45: 345-352 with permission).
Besides the unique capability of IR for capturing the composite molecular content of GCF, it may also provide qualitative diagnosis of periodontal inflammatory status. This could be achieved by using linear discriminant analysis (LDA), to correlate observed spectral differences of GCF from inflammatory conditions (gingivitis and periodontitis) and normal healthy status. This is primarily due to the fact that periodontal disease is clearly multifactorial and our LDA analyses consider multiple components in the GCF as the basis to designate individual spectra as healthy or diseased. As shown in Table 1, LDA could classify GCF from gingivitis and healthy control sites that the overall accuracy for the classification of GCF samples as controls or gingivitis was 91.4% for the training set and 72.4%, in the validation set. Comparing to the better overall accuracy for the classification of GCF samples in periodontitis, 98.4% for the training set and 93.1% for the test set, this would suggest that the gingivitis-specific molecular alterations to GCF are less profound than in periodontitis.

In a nutshell, there are several advantages to using IR spectroscopy of GCF for screening and diagnosis of periodontal inflammation. Namely, IR spectroscopy is reagent-free requiring only small sample volumes; GCF samples are essentially unprocessed; the process is readily automated; IR spectroscopy is straightforward requiring minimal training for operators; and GCF samples are easily collected by clinicians with sample collection targeted to specific sites or to a representative set of teeth.

<table>
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<th>PPV (%)</th>
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Diagnosis of gingivitis was determined by linear discriminant analysis of the infrared spectra. Overall accuracy was 91.4% on the training set and 72.4% on the test set. Bold numbers indicate accurate classifications. SP=specificity; PPV=positive predictive value.

Table 1. Diagnostic accuracy of gingivitis based on IR spectra of GCF
5. References


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Gingival diseases are a family of distinct pathological entities that involve the gingival tissues. These signs and symptoms of these diseases are so prevalent in populations around the world that they are often considered to be 'normal' features. The diseases are now classified into two main groups namely: Plaque-Induced and Non-Plaque Induced Gingival Diseases. This book provides dentists, dental hygienists, dental therapists and students with a comprehensive review of gingival diseases, their etiology and treatment.

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