We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Diagnosis and Monitoring of Gingivitis in vivo Using Non-Invasive Technology - Infrared Spectroscopy

Kan-Zhi Liu^{1,2}, Xiaoming Xiang¹,
Anastasia Cholakakis² and Michael G. Sowa¹

¹*Institute for Biodiagnostics, National
Research Council of Canada*

²*Department of Dental Diagnostics and Surgical
Sciences, University of Manitoba
Canada*

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.

Fig. 1. Summary of current and proposed infrared spectroscopy based diagnostic methods for gingivitis.

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 ≥ 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 & Loe, 1967; Goodson et al, 1982; Aepli 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).

Fig. 2. Near infrared reference spectra (500–1000 nm) for water, deoxygenated hemoglobin (Hb) and oxygenated hemoglobin (HbO₂). The extinction coefficient data for water have been multiplied by a scaling factor of 10. (Reproduced from J Perio Res, 2009;44:117-24 with permission).

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).

Fig. 3. Percent tissue hemoglobin oxygen saturation derived from the relative concentrations of Hb and HbO₂ from distinct two locations. Indices are compared between healthy, gingivitis and periodontitis sites. * Represents a significant difference from healthy sites, $p<0.01$. Vertical bars denote 0.95 confidence intervals.

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. $Y = \exp(-.07661 + (1.57564) \cdot x) / (1 + \exp(-.07661 + (1.57564) \cdot x))$

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 & Délérís, 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,

2005; Sola-Penna, 2008). These findings are also in keeping with numerous studies that have shown increased salivary AGE content in the development of diabetes complications (Bilous, 2007).

Fig. 6. General features of FSD-processed mean IR spectra of control and diabetes (bottom) subjects and the difference spectrum (diabetes minus control, top). Note: Although some non-highlighted bands exhibit pronounced differences, they do not convey significant meaning in terms of biological significance. (Reproduced from *Diabetology & Metabolic Syndrome* 2010, 2:48 (1-9) with permission).

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 $=\text{CH}$ 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 $=\text{CH}$ band at 3012 cm^{-1} providing further evidence of the importance of lipid peroxidation in periodontal disease pathogenesis (Tsai et al, 2005; Sheikhi 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 multi-factorial 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.

Classes			Accuracy(%)	SP (%)	PPV (%)
<i>Training Set</i>					
Control	32	<u>1</u>	97.0	84.0	88.9
Gingivitis	<u>4</u>	21	84.0	97.4	95.5
<i>Validation Set</i>					
Control	12	<u>2</u>	85.7	60.0	66.7
Gingivitis	<u>6</u>	9	60.0	85.7	81.8

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

- Aeppli DM, Boen JR, Bandt CL. Measuring and interpreting increases in probing depth and attachment loss. *J Periodontol* 1985; 56: 262-4.
- Akalin FA, Sengun D, Eratalay K, Renda N, Caglayan G. Hydroxyproline and total protein levels in gingiva and gingival crevicular fluid in patients with juvenile, rapidly progressive, and adult periodontitis. *J Periodontol* 1993; 64: 323-329.
- Albandar JM, Rams TE. Global epidemiology of periodontal diseases: an overview. *Periodontology* 2000 2002;29:7-10
- Alfano MC. The origin of gingival fluid. *J Theor Biol* 1974; 47: 127-136.
- Amano A, Tamagawa H, Takagaki M, Murakami Y, Shizukuishi S, Tsunemitsu A. Relationship between enzyme activities involved in oxygen metabolism and oxygen tolerance in black-pigmented *Bacteroides*. *J Dent Res* 1988; 67: 1196-1199.
- Armitage GC. Periodontal diseases: diagnosis. *Ann Periodontol* 1996; 1:37-215.
- Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999; 4: 1-6.
- Armitage GC. Research, Science and Therapy Committee of the American Academy of Periodontology. Diagnosis of periodontal diseases. *J Periodontol* 2003; 74: 1237-47.
- Attas M, Hewko M, Payette J, Posthumus T, Sowa M, Mantsch H. Visualization of cutaneous hemoglobin oxygenation and skin hydration using near-infrared spectroscopic imaging. *Skin Res Technol* 2001; 7: 238-245.
- Bader HI, Boyd RL. Long-term monitoring of adult periodontitis patients in supportive periodontal therapy: correlation of gingival crevicular fluid proteases with probing attachment loss. *J Clin Periodontol* 1999; 26: 99-105.
- Baltacioglu E, Akalin FA, Alver A, Deger O, Karabulut E. Protein carbonyl levels in serum and gingival crevicular fluid in patients with chronic periodontitis. *Arch Oral Biol* 2008; 53: 716-722.
- Bilous, R. The management of proteinuria in diabetes. *Clin Med* 2007; 7: 116-117.
- Bowers MR, Fisher LW, Termine JD, Somerman MJ. Connective tissue-associated proteins in crevicular fluid: potential markers for periodontal diseases. *J Periodontol* 1989; 60: 448-451.
- Brown LJ, Le H. Prevalence, extent, severity and progression of periodontal disease. *Periodontol* 2000 1993; 2: 57-71.
- Burt B. Research, Science and Therapy Committee of the American Academy of Periodontology. Position paper: epidemiology of periodontal diseases. *J Periodontol* 2005; 76: 1406-1419.
- Capraro GA, Mader TJ, Coughlin BF, Lovewell C, St Louis MR, Tirabassi M, Wadie G, Smithline HA. Feasibility of using near-infrared spectroscopy to diagnose testicular torsion: an experimental study in sheep. *Ann Emerg Med* 2007; 49: 520-5.
- Champagne CM, Buchanan W, Reddy MS, Preisser JS, Beck JD, Offenbacher S. Potential for gingival crevice fluid measures as predictors of risk for periodontal diseases. *Periodontol* 2000 2003; 31: 167-180.
- Delima AJ, Van Dyke TE. Origin and function of the cellular components in gingival crevice fluid. *Periodontol* 2000 2003; 31: 55-76.

- D'Ercole S, Catamo G, Piccolomini R. Diagnosis in periodontology: a further aid through microbiological tests. *Crit Rev Microbiol* 2008; 34: 33-41.
- Dietrich T, Bernimoulin JP, Glynn RJ. The effect of cigarette smoking on gingival bleeding. *J Periodontol* 2004; 75: 16-22.
- Erdemir EO, Duran I, Haliloglu S. Effects of smoking on clinical parameters and the gingival crevicular fluid levels of IL-6 and TNF-alpha in patients with chronic periodontitis. *J Clin Periodontol* 2004; 31: 99-104.
- Eysel HH, Jackson M, Nikulin A, Somorjai RL, Thomson GTD, Mantsch HH. A novel diagnostic test for arthritis: Multivariate analysis of infrared spectra of synovial fluid. *Biospectrosc* 1997; 3: 161-167.
- Garay-Sevilla ME, Regalado JC, Malacara JM, Nava LE, Wrobel-Zasada K, Castro-Rivas A, Wrobel K. Advanced glycosylation end products in skin, serum, saliva and urine and its association with complications of patients with type 2 diabetes mellitus. *J Endo Invest* 2005; 28: 223-230.
- Ge ZL, Liu KZ, Xiang XM, Yang Q, Hui JH, Kohlenberg E and Sowa MG. Classification of periodontal inflammations based on local hemodynamic changes using near infrared spectroscopy. *J Periodontol* 2011; (In press).
- Glavind L, Löe H. Errors in the clinical assessment of periodontal destruction. *J Periodontal Res* 1967; 2: 180-4.
- Goodson JM, Tanner AC, Haffajee AD, Sornberger GC, Socransky SS. Patterns of progression and regression of advanced destructive periodontal disease. *J Clin Periodontol* 1982; 9: 472-81.
- Haffajee AD, Socransky SS, Goodson JM. Comparison of different data analyses for detecting changes in attachment level. *J Clin Periodontol* 1983; 10: 298-310.
- Hanioka T, Shizukuishi S, Tsunemitsu A. Hemoglobin concentration and oxygen saturation of clinically healthy and inflamed gingiva in human subjects. *J Periodontal Res.* 1990; 25:93-98.
- Hanioka T, Shizukuishi S, Tsunemitsu A. Changes in hemoglobin concentration and oxygen saturation in human gingiva with decreasing inflammation. *J Periodontol* 1991; 62: 366-369.
- Hanioka T, Tanaka M, Ojima M, Takaya K, Matsumori Y, Shizukuishi S. Oxygen sufficiency in the gingiva of smokers and non-smokers with periodontal disease. *J Periodontol* 2000; 71: 1846-1851.
- Heitz-Mayfield LJ, Schätzle M, Löe H, Bürgin W, Anerud A, Boysen H, Lang NP. Clinical course of chronic periodontitis. II. Incidence, characteristics and time of occurrence of the initial periodontal lesion. *J Clin Periodontol* 2003; 30: 902-8.
- Huynh QN, Wang S, Tafolla E, et al. Specific fibronectin fragments as markers of periodontal disease status. *J Periodontol* 2002; 73: 1101-1110.
- Hynes A, Scott DA, Man A, Singer DL, Sowa MG, Liu KZ. Molecular mapping of periodontal tissues using infrared microspectroscopy. *BMC Med Imaging* 2005; 5: 2-11.
- Jackson M, Sowa MG, Mantsch HH. Infrared spectroscopy: a new frontier in medicine. *Biophys Chem* 1997; 68: 109-25.

- Jeffcoat MK, Reddy MS. A comparison of probing and radiographic methods for detection of periodontal disease progression. *Curr Opin Dent* 1991; 1: 45-51.
- Jeffcoat MK. Radiographic methods for the detection of progressive alveolar bone loss. *J Periodontol*. 1992; 63: 367-72.
- Kinane DF, Mark BP. Clinical relevance of the host responses of periodontitis. *Periodontol* 2000 2007; 43: 278-293.
- Lamster IB, Ahlo JK. Analysis of gingival crevicular fluid as applied to the diagnosis of oral and systemic diseases. *Ann N Y Acad Sci* 2007; 1098: 216-229.
- Lang NP, Schätzle MA, Löe H. Gingivitis as a risk factor in periodontal disease. *J Clin Periodontol* 2009; 36: 3-8.
- Li Y, Lee S, Hujoel P, Su M, Zhang W, Kim J, Zhang YP, DeVizio W. Prevalence and severity of gingivitis in American adults. *Am J Dent* 2010; 23: 9-13.
- Liu KZ, Dembinski TC, Mantsch HH. Prediction of RDS from amniotic fluid analysis: a comparison of the prognostic value of TLC and infra-red spectroscopy. *Prenat Diagn* 1998; 18: 1267-75.
- Liu KZ, Man A, Shaw RA, Liang B, Xu Z, Gong Y. Molecular determination of liver fibrosis by synchrotron infrared microspectroscopy. *Biochim Biophys Acta* 2006; 1758: 960-7.
- Liu KZ, Shaw RA, Man A, Dembinski TC, Mantsch HH. Reagent-free, simultaneous determination of serum cholesterol in HDL and LDL by infrared spectroscopy. *Clin Chem* 2002; 48: 499-506.
- Liu KZ, Shi MH, Mantsch HH. Molecular and chemical characterization of blood cells by infrared spectroscopy: a new optical tool in hematology. *Blood Cells Mol Dis* 2005; 35: 404-12.
- Liu KZ, Xiang XM, Man A, et al. In vivo determination of multiple indices of periodontal inflammation by optical spectroscopy. *J Periodontal Res* 2009; 44: 117-24.
- Loe H, Silness J. Periodontal Disease in Pregnancy. I. Prevalence and Severity. *Acta Odontol Scand* 1963; 21: 533-551.
- Loesche WJ. Oxygen sensitivity of various anaerobic bacteria. *Appl Microbiol* 1969; 18: 723-727.
- Mariotti A. Dental plaque-induced gingival diseases. *Ann Periodontol*, 1999;4, 7-19.
- Magnusson I, Persson RG, Page RC, DeRouen TA, Crawford JM, Cohen RL, Chambers DA, Alves ME, Clark WB. A multi-center clinical trial of a new chairside test in distinguishing between diseased and healthy periodontal sites. II. Association between site type and test outcome before and after therapy. *J Periodontol* 1996; 67: 589-96.
- Munjal SK, Prescher N, Struck F, Sorsa T, Maier K, Netuschil L. Evaluation of immunoassay-based MMP-8 detection in gingival crevicular fluid on a point-of-care platform. *Ann N Y Acad Sci* 2007; 1098: 490-492.
- Nogueira G, Shibli JA, Duarte PM, Xiang XM, Sowa MG, Ferrari DS., Onuma T, Cardoso LG, Liu KZ. On Site Non-Invasive Assessment Of Peri-Implant inflammation By Near Infrared Spectroscopy. *J Periodontal Res* 2011; 46: 382-388.
- Otal EH, Inon FA, Andrade FJ. Monitoring the temperature of dilute aqueous solutions using near-infrared water absorption. *Appl Spectrosc* 2003; 57: 661-666.

- Page RC, Schoeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. *Lab Invest* 1976; 33: 235 – 249.
- Palmer RM, Wilson RF, Hasan AS, Scott DA. Mechanisms of action of environmental factors--tobacco smoking. *J Clin Periodontol* 2005; 32: 180-195.
- Petibois C, Délérís G. Chemical mapping of tumor progression by FT-IR imaging: towards molecular histopathology. *Trends Biotechnol* 2006; 24: 455-62.
- Ranney RR. Diagnosis of periodontal diseases. *Adv Dent Res* 1991; 5: 21-36.
- Schätzle M, Löe H, Bürgin W, Anerud A, Boysen H, Lang NP. Clinical course of chronic periodontitis. I. Role of gingivitis. *J Clin Periodontol* 2003; 30: 887-901.
- Scott DA, Renaud DE, Krishnasamy S, Meriç P, Buduneli N, Çetinkalp Ş, Liu KZ. Diabetes-related molecular signatures in infrared spectra of human saliva. *Diabet & Metab Syn* 2010; 2: 48(1-9).
- Scott DA, Singer DL. Suppression of overt gingival inflammation in tobacco smokers - clinical and mechanistic considerations. *Int J Dent Hyg* 2004; 2: 104-110.
- Severcan F, Gorgulu G, Gorgulu ST, Guray T. Rapid monitoring of diabetes-induced lipid peroxidation by Fourier transform infrared spectroscopy: evidence from rat liver microsomal membranes. *Anal Biochem* 2005; 339: 36-40.
- Sheikhi M, Bouhafs RK, Hammarstrom KJ, Jarstrand C. Lipid peroxidation caused by oxygen radicals from *Fusobacterium*-stimulated neutrophils as a possible model for the emergence of periodontitis. *Oral Dis* 2001; 7: 41-46.
- Sola-Penna M. Metabolic regulation by lactate. *IUBMB Life* 2008; 60:605-608.
- Sowa MG, Leonardi L, Payette JR, Cross KM, Gomez M, Fish JS. Classification of burn injuries using near-infrared spectroscopy. *J Biomed Opt* 2006; 11: 054002.
- Sowa MG, Leonardi L, Payette JR, Fish JS, Mantsch HH. Near infrared spectroscopic assessment of hemodynamic changes in the early post-burn period. *Burns* 2001; 27: 241-249.
- Sowa MG, Payette JR, Mantsch HH. Near-infrared spectroscopic assessment of tissue hydration following surgery. *J Surg Res* 1999; 86: 62-9.
- Stothers L, Shadgan B, Macnab A. Urological applications of near infrared spectroscopy. *Can J Urol* 2008; 15: 4399-409.
- Surewicz WK, Mantsch HH. New insight into protein secondary structure from resolution-enhanced infrared spectra. *Biochim Biophys Acta* 1988; 952: 115-130.
- Teles RP, Sakellari D, Konstantinidis A, Socransky SS, Haffajee AD. Application of the checkerboard immunoblotting technique to the quantification of host biomarkers in gingival crevicular fluid. *J Periodontol* 2009; 80: 447-56.
- Tsai CC, Chen HS, Chen SL, et al. Lipid peroxidation: a possible role in the induction and progression of chronic periodontitis. *J Periodontal Res* 2005; 40: 378-384.
- Tsai CC, Hong YC, Chen CC, Wu YM. Measurement of prostaglandin E₂ and leukotriene B₄ in the gingival crevicular fluid. *J Dent* 1998; 26: 97-103.
- Veli-Jukka Uitto. Gingival crevice fluid – an introduction. *Periodontol 2000* 2003; 31: 9-11.
- Xiang XM, Liu KZ, Man A, Ghiabi E, Cholakis A, Scott DA. Periodontitis-specific molecular signatures in gingival crevicular fluid. *J Periodontal Res* 2010; 45: 345-352.

- Yoshitani K, Kawaguchi M, Tatsumi K, Kitaguchi K, Furuya H. A comparison of the INVOS 4100 and the NIRO 300 near-infrared spectrophotometers. *Anesth Analg* 2002; 94: 586-90.
- Zhang J, Xuan D, Fan W, Zhang X, Dibart S, De Vizio W, Panagakos F, Zhang YP. Severity and prevalence of plaque-induced gingivitis in the Chinese population. *Compend Contin Educ Dent* 2010; 31: 624-9.

IntechOpen

IntechOpen

Gingival Diseases - Their Aetiology, Prevention and Treatment

Edited by Dr. Fotinos Panagakos

ISBN 978-953-307-376-7

Hard cover, 230 pages

Publisher InTech

Published online 22, September, 2011

Published in print edition September, 2011

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 aetiology and treatment.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Kan-Zhi Liu, Xiaoming Xiang, Anastasia Cholakakis and Michael G. Sowa (2011). Diagnosis and Monitoring of Gingivitis in vivo Using Non-Invasive Technology - Infrared Spectroscopy, *Gingival Diseases - Their Aetiology, Prevention and Treatment*, Dr. Fotinos Panagakos (Ed.), ISBN: 978-953-307-376-7, InTech, Available from: <http://www.intechopen.com/books/gingival-diseases-their-aetiology-prevention-and-treatment/diagnosis-and-monitoring-of-gingivitis-in-vivo-using-non-invasive-technology-infrared-spectroscopy>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License](https://creativecommons.org/licenses/by-nc-sa/3.0/), which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen