Assessment of Gingival Tissue Hemodynamics by Optical Spectroscopy in Diagnosis of Periodontal Disease - Multicenter Clinical Trials

Xiaoming Xiang

Abstract

Background: Currently used diagnostic methods in periodontics are unable to identify disease activity and progression until significant attachment loss has happened. New diagnostic modalities and parameters are needed to monitor disease progression and detect disease activity at an early stage.

Aims: To determine the features of the in vivo optical spectra characteristic of periodontitis, gingivitis and healthy gingival tissue and the potential to use these spectral signatures to differentiate periodontitis from gingivitis and healthy gingiva and determine the risk of progression from gingivitis to periodontitis.

Materials and Methods: 12 cross-sectional clinical trials were conducted at 6 university based dental clinics or research centers in Canada, China, Italy and Brazil from 2007 to 2014 including 562 patients with moderate to severe chronic periodontitis. Optical spectra were obtained at the chair-side using a custom designed optical probe from 705 periodontitis, 1306 gingivitis and 1691 healthy sites in situ. A modified Beer-Lambert unmixing model was used to determine tissue oxygen saturation and relative contribution of oxygenated and deoxygenated hemoglobin components.

Results: Multiple hemodynamic parameters were simultaneously derived from the optical spectra of gingival tissue including tissue oxygen saturation, oxygenated hemoglobin, deoxygenated hemoglobin and total hemoglobin indices. The tissue oxygen saturation and oxygenated hemoglobin index in periodontitis was significantly lower than gingivitis and healthy gingiva ($p < 0.0001$) but no significant difference in oxygenated hemoglobin between gingivitis and healthy gingiva ($p > 0.05$). On the other hand, deoxygenated hemoglobin in periodontitis was significantly increased compared to gingivitis and healthy gingiva ($p < 0.0001$). A classification model was established to predict the risk level of gingivitis based on the features of the optical spectra of characteristic of health gum and periodontitis.

Conclusions: 1) multiple local hemodynamic profiles such as tissue oxygenation and perfusion can be simultaneously generated by optical spectroscopy to reflect subclinical gingival inflammation. 2) Decreased tissue oxygenation saturation in periodontitis and gingivitis was mainly due to increased concentration of deoxygenated hemoglobin. 3) Optical spectroscopy has the potential to diagnose periodontal disease and monitor disease progression at an early stage. More longitudinal studies are needed to validate this potential.

Introduction

Chronic periodontitis is an inflammatory disease of the tooth supporting apparatus resulting in the destruction of alveolar bone and the loss of teeth. The etiopathogenesis of periodontitis has yet to be fully established, but most evidence indicates a strong relationship between periodontitis and a group of periodontopathogens in subgingival bacterial biofilm, predominantly gram-negative, anaerobic bacteria (Page & Kornman 1997). Chronic periodontitis is one of the two most common dental diseases affecting populations in all regions of the world. According to most recent Nation Health and Nutrition Examination Survey (NHANES), chronic periodontitis affected 47.2% of American adults aged 30 years or older, 8.5% with severe form, 30% with moderate and 8.7% with mild chronic periodontitis (NHANS 2009-2010). Furthermore, periodontal disease increases with age; 70.1% of seniors 65 years or older have some form of periodontal disease. It has become a significant socioeconomic burden with aging populations worldwide.

The commonly used parameters for periodontal diagnosis include periodontal pocket depth (PD), bleeding on probing (BOP), radiographic evidence of bone loss, clinical signs of tooth mobility, gingival erythema and suppuration. Periodontal probing is the most common clinical examination for the diagnosis of periodontal disease. It provides clinicians valuable information about the extent and severity of disease such as bleeding on probing, pocket depth and clinical attachment level (CAL). However, it is also well recognized that clinical probing is not an accurate or reliable measurement. Measurement errors arise from a wide range of sources. For instance, bleeding on probing is closely related to probing pressure. Lang et al (1991) evaluated the

relationship between probing forces of 0.25, 0.5, 0.75 and 1.0 N and BOP on the healthy periodontium using a probe with diameter of 0.4 mm. The results indicate a strong positive, linear relationship between BOP value and probing pressure. The accuracy of periodontal pocket depth measurement and clinical attachment level is also influenced by a number of other factors, such as angulation of probe placement and access for probing. More importantly, the penetration of the probe tip is affected by the inflammatory status of the soft tissue at the base of pocket, which is often invisible. An animal study using mongrel dogs by Anderson et al. 1991 revealed a strong correlation between both the gingival index and depth of probe penetration as well as number of inflammatory cells on the microscopic section and depth of probe penetration. Histologic probe position in treated and untreated human periodontal tissues was also compared between untreated and treated teeth (Fowler et al.1982). The probe tips penetrated beyond the junctional epithelium on untreated teeth by a mean of 0.45 mm, whereas in treated teeth, the probe stopped coronal to the apical end of junctional epithelium by a mean of 0.73 mm. A difference of 1.2 mm in probing penetration was found between untreated and treated sites at a probing force of 0.5N. Clinical probing using manual probes, which has been by far the most commonly used diagnostic tool in both clinical practice and clinical trials, is therefore not considered a reliable measurement.

New generations of probes are emerging to address the shortcomings of conventional manual probes. For instance, electronic probes have gained increasing popularity in periodontal practice due to their measurement accuracy, probing force control and computerized data storage. For instance, second generation probes are pressure sensitive and allow for standarized probing pressure. The third generation of automatic

probes are featured by standarized pressure, digital readouts and computer storage of data. The fourth generation of probes allow for 3-D pocket examination and the fifth generation of probes are non invasive by identifying the attachment level without penetrating it. However, these advantages were not a consistent finding in clinical studies. Khocht et al. (1998) compared the measurements of an electronic controlled-force probe to that of a manual controlled-force probe and a conventional probe. The study found that the conventional probe and the manual-controlled force probe yielded more reproducible results than the electronic-controlled force probe and none of these probes completely eliminated probing errors.

An ideal periodontal diagnostic tool is not only able to determine presence of disease, more importantly identify disease activity characterized by ongoing tissue destruction and loss of clinical attachment. A number of clinical parameters were examined in longitudinal studies to determine their predictive value for disease activity, including clinical probing depth, bleeding on probing, suppuration on probing, plaque score. Unfortunately, none of the currently used clinical tests could determine disease activity or predict further clinical attachment loss (Greenstein et al. 1990). For instance, in a 60-month longitudinal study of the effect of non-surgical therapy on nonmolar teeth, the maximal positive predictive value of plaque score or bleeding on probing was about 30% and about 50% for a residual probing depth ≥ 7 mm (Badersten et al. 1990). Similar results were confirmed in other longitudinal clinical trials (Claffey et al. 1990, Kaldahl et al. 1990, Lang et al. 1986). Periodontal diseases are therefore either undertreated or overtreated due to the limited reliability and accuracy of clinical probing based diagnosis.

Initiation of periodontal disease is triggered by colonization of periodontal pathogens on biofilm formed on root surface. Additionally, transition of reversible gingivitis to irreversible periodontitis and progression of destructive periodontitis once established depend on the interaction of host immunoinflammatory response and virulent factors of the bacterial pathogens. Detection of subgingival microflora and the analysis of biomarkers in gingival crevicular fluids or saliva have been extensively investigated for periodontal diagnosis to monitor disease progression. More than 700 bacteria have been identified in the oral cavity and 500 species in dental plaque (Aas et al. 2005). The presence and levels of 40 subgingival taxa were analyzed in 13261 subgingival plaque sample from 185 subjects and 5 major complexes were identified (Socransky et al, 1998). Among the 5 complexes, the red complex species were closely related to pocket depth and bleeding on probing. The species within a complex were associated with one another while the complexes themselves had specific relationship with each other. For instance, orange complex was more associated with red complex, but yellow and green complexes were less associated with members of red and orange complexes. In spite of great effort and advancement made in using microbial diagnosis, it has little value in the routine management of periodontal disease mainly due to ploymicrobial nature of periodontal infection and complex interaction with host immunoinflammatory response (Brochut et al. 2005).

Gingival crevicular fluid is an inflammatory exudate in the gingival crevice or periodontal pockets (Cimasoni 1983) which is composed of tissue breakdown products, inflammatory mediators, cytokines, antibodies against putative periodontal pathogens, bacteria and their products. It is a rich source of biomarkers for monitoring periodontal

disease. Qualitative and quantitative analysis of GCF may provide valuable information regarding the dynamic immunoinflammatory process in periodontal tissue. Indeed, significant progresses have been made in identifying biomarkers in GCF for diagnosis of disease activity and monitoring disease progression. There have been more than 65 GCF components examined as potential periodontal diagnostic markers, such as matrix metalloproteinases (MMPs) - 1, 2, 3, 8, 9 and 13 (Tuter et al. 2005, Alpagot et al. 2001, Buduneli et al. 2002, Kiili et al. 2002), elastases (Jin et al. 2003, Lamster et al. 2007) and cathepsins (Mogi et al. 2007). Unfortunately, none of these components have been established as a reliable diagnostic test due to limited sensitivity and specificity.

Seeking new diagnostic markers to establish more reliable and accurate diagnostic methods is ongoing. Non invasive in vivo diagnostic means are of particular use for long term disease follow up as repeated measurements are necessary to identify disease activity. Recently, measurement of tissue oxygenation using optical spectroscopy has emerged as a novel diagnostic tool for periodontal diagnosis. Hanioka et al first used tissue reflectance spectrophotometry to measure gingiva tissue hemoglobin concentration and oxygen saturation in 10 healthy subjects and 25 patients with mild to moderate gingivitis (Hanioka et al. 1990). Higher hemoglobin concentration and lower oxygen saturation were observed in inflamed gingiva suggesting increased blood supply could not meet the increased demand of oxygen in gingival inflammation. Similar results were observed in experimental periodontitis in dogs induced with silk ligatures (Hanioka et al. 1989). The deoxygenated hemoglobin after ligation increased up to 2 times that observed before ligation while the increase of oxygenated hemoglobin after ligation was relatively small reflecting imbalance of oxygen supply and consumption in periodontitis.

The potential of using tissue oxygen saturation as a new diagnostic marker was further investigated in patients with chronic periodontitis. Tissue oxygenation showed a consistently decreasing trend from healthy sites to sites exhibiting gingivitis and periodontitis. There was a significant difference in oxygenation between healthy sites and sites with periodontitis (Liu et al. 2009, Ge et al.2011). A probabilistic classification model that was established using the spectral data from healthy sites and sites with periodontitis could be used to predict the sites with gingivitis that have optical properties or oxygenation values that are more indicative of periodontitis (Liu et al, 2011).

The purposes of this multicenter clinical trial were therefore to: 1) To determine the optical characteristic of periodontitis, gingivitis and healthy gingival tissue and 2) to investigate the potential of using these spectral signatures to differentiate periodontitis from gingivitis and healthy gingiva and 3) to use these patterns to determine the risk of progression from gingivitis to periodontitis.

Materials and methods

Study Locations and Subjects

Twelve separate cross-sectional studies were carried out at five clinical locations between 2007 and 2014. A total of 562 patients were examined; 108 patients were recruited by the Graduate Periodontics Clinics at the University of Manitoba (Winnipeg, Canada), 65 patients in 2 separate studies at the First Affiliated Hospital of Soochow University (Soochow, China), 38 patients at the Second Affiliated Hospital of Harbin Medical University (Harbin, China), 76 patients at the Second Affiliated Hospital of Qingdao University 63, 252 patients in four separate studies carried out at the Periodontal Research Center of Guarulhos University (Guarulhos, São Paulo, Brazil) as well as 23 patients at Magna Gracia University (Catanzaro, Italy). The research protocol was approved by the research ethics boards of each participating Institution as well as that of the National Research Council of Canada. Informed written consent was obtained from each individual prior to collection of spectra.

Periodontitis sites were defined as those with a periodontal probing depth ≥ 5 mm, clinical attachment loss ≥3 mm and with bleeding on probing. Gingivitis sites were defined as those with periodontal probing depths ≤ 3 mm and bleeding on probing. Healthy sites were defined as those with periodontal probing depths ≤ 3 mm and no bleeding on probing. Spectra were obtained from suspicious sites as well as from healthy sites contralateral to the site suspected of being diseased. All spectra were collected prior to clinical examination and measurements. A total of 3978 periodontal sites were examined from 562 patients; 1750 of the sites that were measured using reflectance spectroscopy were diagnosed as healthy, 1308 were clinically assessed as

sites of gingivitis while 747 clinically scored as sites of periodontitis. Exclusion criteria were: 1) use of anti-inflammatory, antibiotic and immunosuppressant medications within the past 6 months; 2) systemic conditions that may interfere with the progression of periodontal diseases (e.g. diabetes mellitus and immunological diseases); 3) gingival lesions unrelated to plaque accumulation; 4) use of orthodontic appliances; 5) pregnancy and lactation; 6) periodontal treatment within the past 12 months; 7) continuous use of mouthrinses containing antimicrobials within the past 2 months.cquisition of optical spectra

Spectra were collected using portable PDA512-ISA spectrograph interfaced to a customized bifurcated fiber optic probe designed for use in the oral cavity (Liu et al, 2009). The intraoral probe was a 180 mm long stainless steel shaft, 5 mm diameter, housing 29 optical fibers arranged as presented in Figure 1. The central fiber area, which delivers light to the oral cavity, was 0.8 mm in diameter. The outer ring of fibers ~0.2 mm wide was located 1 mm away from the central fiber area (Figure 1a). The outer fibers of the probe were coupled to the entrance slit of the spectrograph and collected light subsequently back-scattered from the tissue. The inner fibers at the bifurcated end of the probe were coupled to a 5-watt tungsten halogen light source that provided a stable light output. Each reflectance spectrum consisted of 16 co-added scans collected using a 0.03 s integration time. The spectral range between 500 and 1100 nm at 5 nm resolution was used. A 99% Spectralon® reflectance standard was used as a reference to correct for the instrument response function convert raw reflectance measurements into effective reflectance spectra of the gingiva.

$$R_{sample}(\lambda) = \log_{10}\left(\frac{I_{ref}(\lambda)}{I_{sample}(\lambda)}\right)$$

Prior to measuring the gum the fiber optic probe was covered with a disposable transparent polyurethane sheath (Protek Medical Products, Iowa City, IA) to provide a barrier for effective infection control. During the collection of gum spectra, subjects were comfortably seated in a relaxed, standard semi-reclined position on a dental chair.

Calculation of hemodynamic indices from optical spectra

The derivation of the relative contribution of Hb and HbO_2 to the optical attenuation spectrum obtained from tissue was described in detail previously. (Sowa MG et al. 2002) Briefly, a modified Beer-Lambert unmixing model that incorporates a nonparametric scattering loss function was used to determine the relative contribution of Hb and HbO_2 to the spectrum by using the known absorption coefficients of Hb and HbO_2 to fit the spectrum. The visible region between 510 – 620 nm of the measured tissue attenuation spectrum, A_λ, was modeled as a sum of 2 parametric terms, Hb and HbO_2, that contribute to the spectrum and a nonparametric term m (λ) modeling a vector of covariates, primarily the Rayleigh and Mie scattering losses that contribute to the attenuation of measured light.

$$A(\lambda) = \sum_{i=1}^{3} \xi_i(\lambda)\ c_i L + m(\lambda) + error$$

The concentrations of Hb and HbO_2 per unit photon pathlength were estimated by solving equation using a noniterative partially linear method based on kernel smoothing, as first described by Speckman.[20] (Speckman P 1988) Tissue hemoglobin oxygen saturation, StO_2, and a measure of tissue perfusion, tHb, were derived from the predicted Hb and HbO_2 relative concentrations as follows:

$$S_tO_2 = \frac{[HbO_2]}{[HbO_2]+[Hb]} \qquad \text{and} \qquad tHb = [HbO_2]+[Hb]$$

Inter-operator and inter-instrument comparisons

We have examined the variability potentially generated from different operators or instruments (Ge et al, 2011). To this end, we have employed a double matched pair study (*see* Figure 2*)*, matched reflectance spectra were collected on two instruments with two different operators. Each user acquired measurements from 10 subjects using two instruments. The 10 subjects used for the inter-operator, inter-instrument test were not part of the patient population used in the subsequent periodontitis study.

Bland – Altman plots provide a convenient graphical method to help visualize the agreement between two methods of measurement when matched pairs of data are available. The differences of the matched pairs are plotted against the mean of the matched pairs. The Bland-Altman plot is used to visually inspect whether the difference and its variance is constant as a function of the average

The Bradley – Blackwood (BB) procedure tests whether the regression coefficients in the regression of the pairwise difference versus the pairwise means are significantly different from zero. This test is equivalent to simultaneously testing the equality of the means and variances of the paired measurements.

Bland-Altman plots and accompanying statistical BB tests were made for the paired S_tO_2 parameters derived from measured optical reflectance spectra to determine the inter-operator and inter-instrument variation of near infrared measures of tissue inflammation.

Calibration of gingivitis risk index

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 (Liu et al, 2011). 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. 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.

Statistical analysis

The hemodynamic indices, Hb, HbO_2, StO_2, and tHb, derived from optical spectral were analyzed separately using a one-way analysis of variance (ANOVA) to test the hypothesis that the indices from the three groups of sites, healthy, gingivitis and periodontitis would differ significantly. The unequal Tukey HSD was used for the post-hoc pairwise comparisons of mean differences between the clinical groups. Pearson product moment correlation coefficients were calculated between the hemodynamic indices to summarize the linear association between the variables. Statistical calculations were performed with Statistica 10.0[‖].

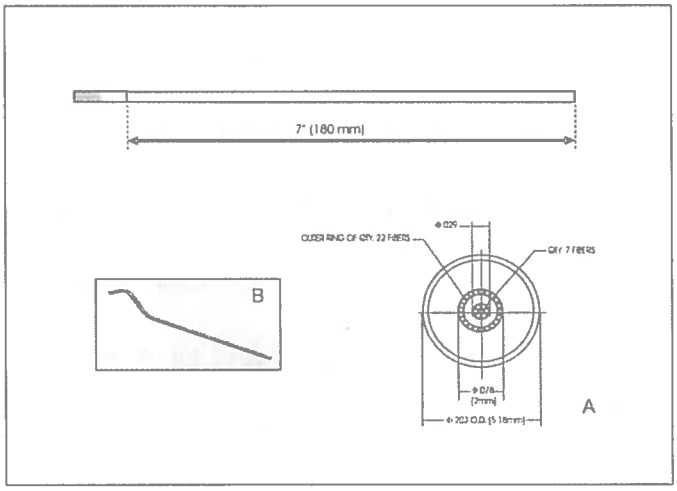

Figure 1. Design of the intraoral probe for the acquisition of NIR spectra from periodontal tissues. The intraoral probe consists of a 180 mm long stainless steel shaft, 5 mm diameter, housing 29 fibre optic bundles (A). Amended probe shape (B).

‖ Statsoft, Tulsa, OK

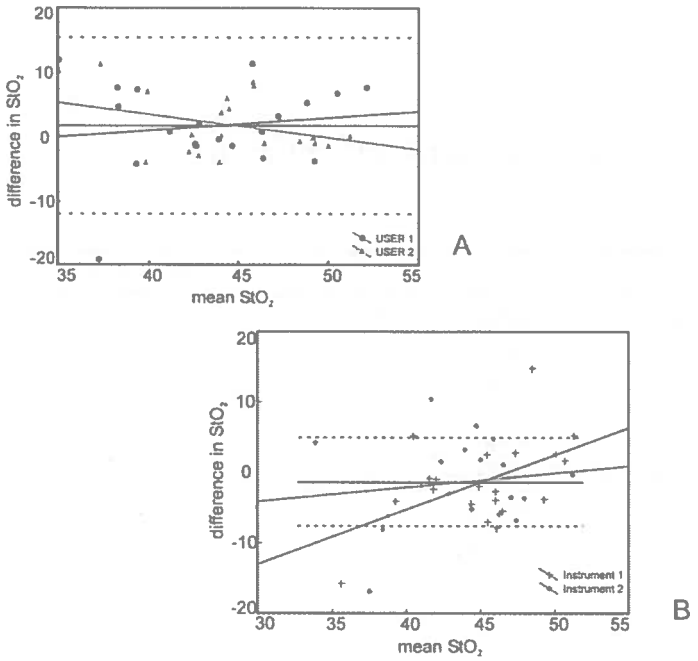

Figure 2. Inter-operator Bland-Altman plot (A) and inter-instrument Bland-Altman plot (B)

Results

1. Study locations and spectral sites distributions

Study No.	Location	Year	Subjects	Periodontitis	Gingivitis	Healthy
1	Winnipeg, Canada	2007	51	60	76	92
2	Soochow, China	2008	51	45	95	62
3	Sao Paulo, Brazil	2009	63	73	69	146
4	Sao Paulo, Brazil	2010	57	59	146	174
5	Soochow, China	2011	14	19	87	6
6	Catanzaro, Italy	2011	23	18	75	246
7	Qingdao & Harbin, China	2011	38	44	91	91
8	Sao Paulo, Brazil	2011	78	109	118	277
9	Sao Paulo, Brazil	2011	54	80	99	108
10	Qingdao, China	2012	76	91	282	286
11	Winnipeg, Canada	2013	15	60	76	92
12	Winnipeg, Canada	2014	42	47	92	111
Total			562	705	1306	1691

In total, 12 studies completed in Canada, China, Brazil and Italy from 2007 to 2014 were included for final analysis including the spectra from 705 periodontitis, 1306 gingivitis and 1691 healthy periodontal sites from 562 subjects with moderate to advanced periodontitis.

2. Tissue oxygen saturation of periodontitis, gingivitis and healthy gingiva

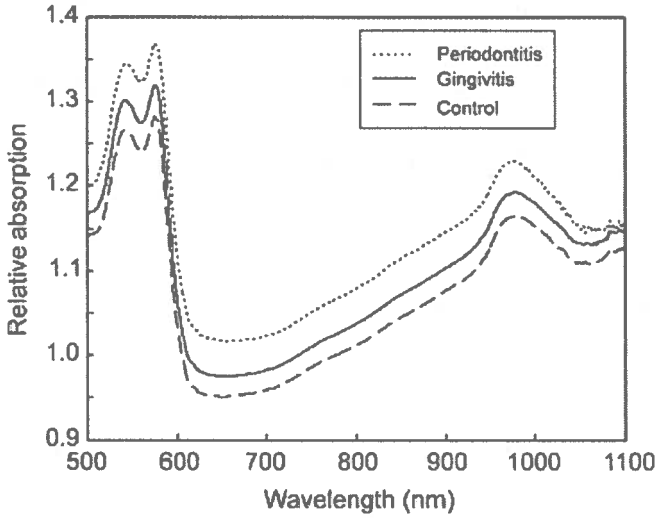

Figure 3. Mean near-infrared spectra from healthy, gingivitis and periodontitis sites.

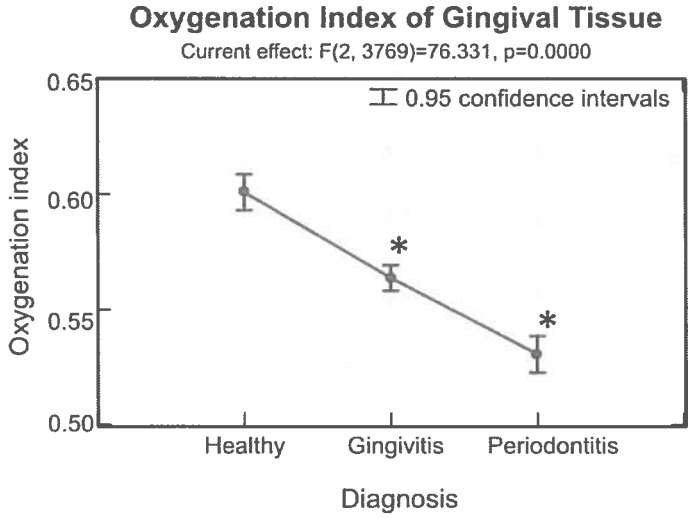

Figure 4. Percent tissue haemoglobin oxygen saturation compared between healthy, gingivitis, and periodontitis sites. * represents $p < 0.001$ compared with healthy group.

Figure 3 plots the representative reflectance spectra of periodontitis, gingivitis and healthy gingiva in vivo and in situ. Light absorption by oxygenated and deoxygenated hemoglobin in the gingival capillary bed dominates the measured light reflectance in the short wavelength region from 500 to 600 nm. The patterns of the spectra from diseased and healthy sites were virtually indistinguishable., The main difference results from the relative contribution of oxygenated and deoxygenated hemoglobin reflecting the balance between oxygen supply and consumption. The mean tissue oxygenation of gingiva in periodontitis as shown in Figure 4 was significantly decreased compared to gingivitis which in turn was significantly lower than healthy group.

3. Oxygenated and de-oxygenated hemoglobin indices in periodontitis, gingivitis and healthy gingiva

In contrast to the oxygen saturation in gingival tissue, the deoxygenated hemoglobin in periodontitis was significantly higher than gingivitis which was significantly increased compared to healthy group as shown in Figure 5. On the other hand, the oxygenated hemoglobin in gingivitis and healthy group was not significantly different but significantly higher than periodontitis as seen in Figure 6.

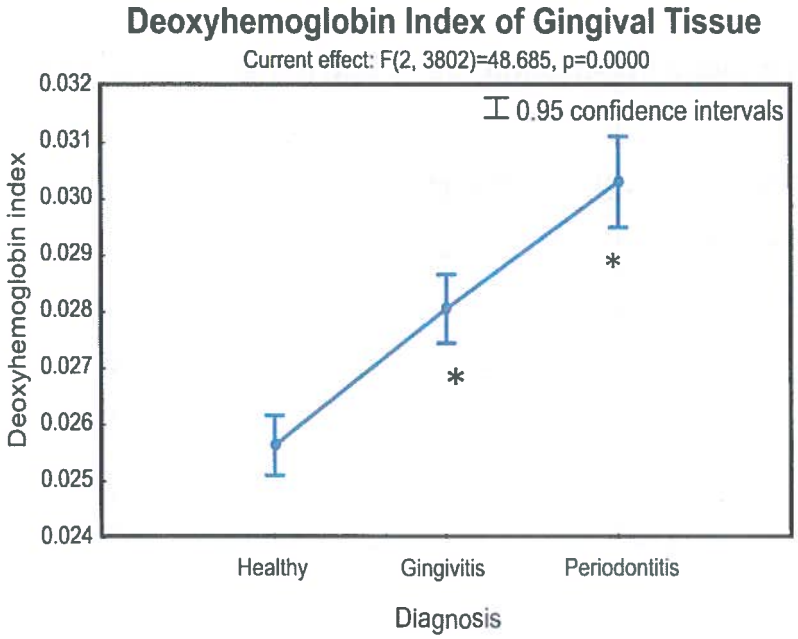

Figure 5. Relative concentrations of deoxygenated hemoglobin calculated using the visible region (510–620 nm) of the reflected light spectrum. * $p < 0.001$ compared with healthy group.

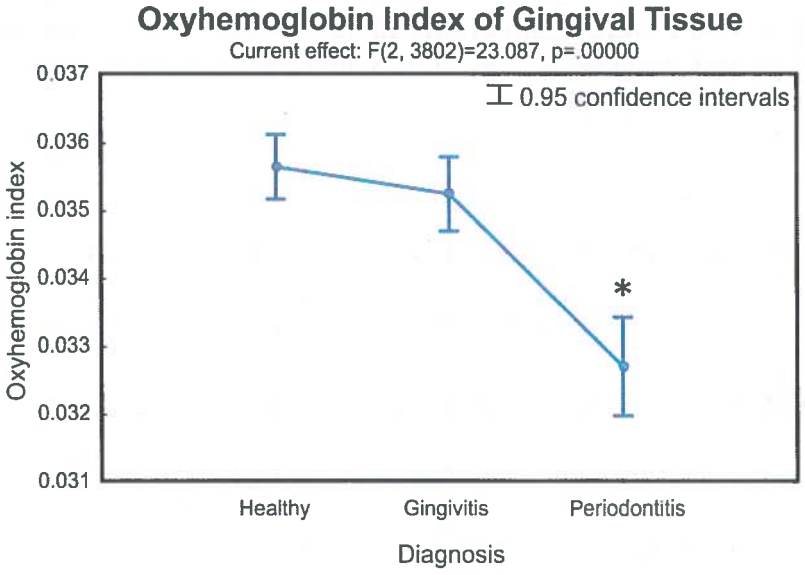

Figure 6. Relative concentrations of oxygenated hemoglobin calculated using the visible region (510 nm of the reflected light spectrum). * $p < 0.0001$ compared with healthy group.

4. Total hemoglobin index in periodontitis, gingivitis and healthy gingiva

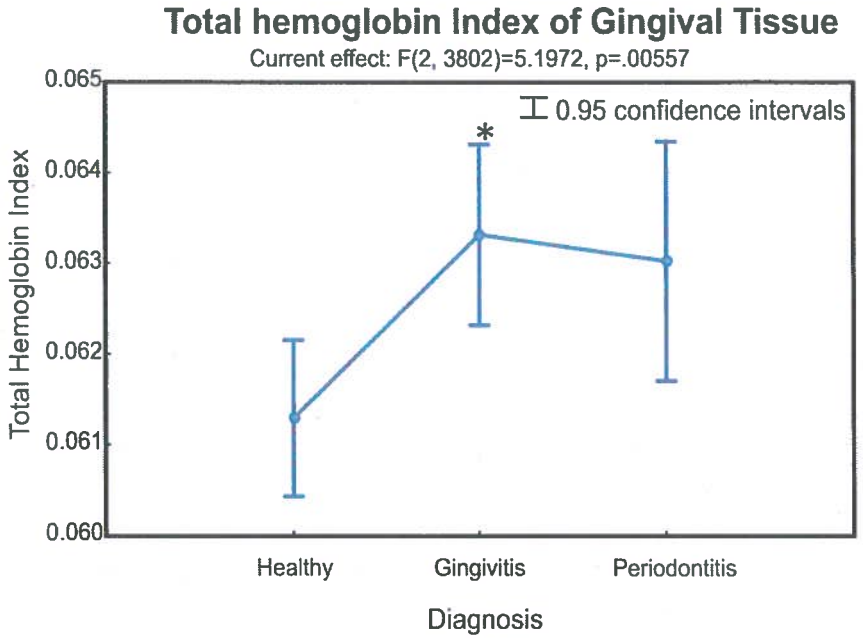

Figure 7. Total hemoglobin indices derived from the relative concentrations of deoxygenated hemoglobin and oxygenated hemoglobin. Indices are compared among healthy, gingivitis and periodontitis sites. * $p < 0.05$ compared with healthy group.

Total hemoglobin index reflect gingival tissue blood supply including both oxygenated and deoxygenated hemoglobin. As expected, the total hemoglobin index for inflamed gingival tissue, gingivitis and periodontitis, was higher than healthy gingiva (Figure 7) with the sites with gingivitis being statistically significantly high than healthy sites. The difference between gingivitis and periodontitis was not statistically significant.

5. Logistic regression model of the risk index of gingivitis

A probabilistic classification model using partial least squares with penalized logistic regression was developed based on two studies (Winnipeg, 2007 and Soochow, 2008) to stratify gingivitis sites into high risk with spectral features that are more indicative of periodontitis or low risk with spectra similar to healthy gingiva (Figure 8). The positive values indicate higher risk level of gingivitis and negative values indicating lower risk.

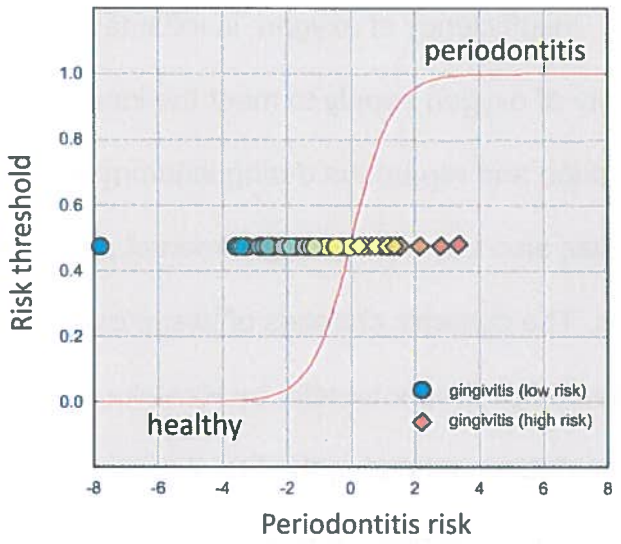

Figure 8. Logistic regression model that weights sites exhibiting signs of gingivitis towards healthy sites (negative periodontal risk values) or periodontitis (positive risk values).

Discussion

Oxygenation of tissue reflects the balance between oxygen supply and consumption. Decreased oxygen saturation in inflamed gingival tissue found in this study was in consistence with the results from other clinical reports (Liu et al. 2009, Ge et al. 2011, Hanioka et al. 1990). Oxygen supply in gingival tissue depends on blood flow and volume (Lindbom et al. 1980, Kim et al. 1984, Kim 1985) and is affected by oxygen metabolism (Chien et al. 1985). Insufficiency of oxygen in inflamed gingiva of gingivitis and periodontitis reflects inability of oxygen supply to meet the increased oxygen consumption for tissue proliferation and reparation during inflammation (Glickman et al. 1949). Reduced oxygenation was also observed in experimental periodontitis induced by placing silk ligatures in dogs. The dynamic changes of tissue oxygenation were monitored using tissue reflectance spectrophotometry by Hanioka (Hanioka et al. 1989). The apparent oxygen saturation decreased during the first 7 days after ligation and remained relatively stable at lower level during the rest of 16 weeks. Furthermore, the apparent oxygenation saturation was negatively correlated with gingival index, plaque index, periotron score, probing depth and attachment loss (Hanioka et al. 1989).

Oxygenation index of gingival tissue in periodontitis was significantly lower than that found in gingivitis in this study. A number of factors may contribute to the further decrease of oxygen saturation in periodontitis, such as involvement of deep tissue including alveolar bone, decreasing oxygenation tension as the depth of periodontal pockets increases. Tanaka et al. (1998) studied the relationship between gingival oxygenation and pocket oxygen tension measured with a polarographic method and

found significant negative correlation between gingival oxygenation and probing depth (R = -0.57) and a positive correlation between gingival oxygenation and pocket oxygen tension (r = 0.69). Periodontal pathogens, such as red and orange complex species, in subgingival biofilm are dominated by anaerobic bacteria. Decreased gingival oxygenation measured with optical spectroscopy is in favor of periodontal pathogens.

Based on the distinction in oxygenation of gingival tissue among healthy, gingivitis and periodontitis sites, a risk model using the spectral data from healthy sites and sites with periodontitis could be established to predict the risk of progression of gingivitis into periodontitis (Figure 8). Similarly, the same risk assessment model can be used to identify periodontal sites with ongoing breaking down which require immediate intervention to prevent further tissue destruction. Longitudinal studies indicated disease activity is an infrequent event and occurs only in small percentage of periodontal sites in patients with periodontal disease. Lindhe et al. (1983) monitored 64 Swedish subjects with mild to moderate periodontal disease for a period of 6 years with no periodontal treatment, only 1.6% of sites had more than 2 mm attachment loss. In another group of American subjects with more advanced periodontal disease, only 3.2% of sites exhibited more than 2 mm attachment loss over 1-year period. Identification of disease activity will allow treatment to be delivered to specific sites with active disease and avoid overtreatment of vast majority of sites with stable periodontal condition. Therefore, well controlled longitudinal clinical studies are needed to validate the sensitivity and specificity of the risk model. Once established, the risk model calibrated with non-invasive optical spectroscopy would allow us to monitor periodontal disease progression at the chair side, in a real time site specific manner.

The decreased oxygenation in gingiva of gingivitis and periodontitis was mainly contributed by increased content of deoxygenated hemoglobin. As shown in figure 4, the deoxygenation of periodontitis was significantly elevated compared to gingivitis which is in turn higher than that in healthy gingiva. On the other hand, no significant difference in total hemoglobin is evident between gingivitis and periodontitis in spite of increased total hemoglobin contents in both gingivitis and periodontitis compared to healthy gingiva (Figure 7). Similar results were observed in experimental periodontitis induced with silk ligatures whereby the maximum deoxygenated hemoglobin concentration was elevated two times as that at the baseline measurement prior to ligature placement. In contrast, the increase in oxygenated hemoglobin was relatively small (Hanioka et al. 1989).

The increased total hemoglobin index in both gingivitis and periodontitis was in consistence to the findings in human gingivitis (Hanioka et al. 1990) and in ligature induced periodontitis in animals (Hanioka et al. 1989). Histological analysis of inflamed gingiva indicated dilated venules and elongated blood vessels (Sonderholm et al. 1973). Vascular endothelium growth factor (VEGF) is an important mediators to promote angiogenesis (Ferrara N et al. 2003), vasodilation and permeability (Olsson AK et al. 2006, Jin ZG et al. 2003), therefore increasing the blood and oxygen supply. Angiogenesis is usually initiated by tissue hypoxia to maintain oxygen supply and increased expression of VEGF may indicate insufficient oxygen supply in gingival tissue. In a recent study using Wistar rat animal model, Gyurkovics et al. found increased production of VEGF in gingivitis and application of VEGF receptor 2 antagonist caused significant vasoconstriction in inflamed gingiva measured by vital microscopy

(Gyurkovics et al. 2013). Decreased gingival oxygenation in spite of increased blood supply indicated the increased oxygen consumption in inflamed gingiva can not be met by increased blood supply (Hanioka et al. 1990). Therefore, measurement of tissue oxygenation may provide an sensitive indicator of initiation and progression of periodontal disease before the clinical signs of gingival inflammation become visible.

One of the weaknesses of this study is the relatively large standard errors of the optical parameters except tissue oxygenation. A number of factors may contribute to the variation of the measurement, such as the angulation and distance of the optical probe placement, severity and extent of gingiva inflammation, local or systemic factors that may influence the gingival microcirculation including smoking and diabetes. For instance, in one of the studies, the tissue oxygenation in healthy gingiva was compared between 63 patients with poorly controlled type 2 diabetes and 15 non-diabetic subjects. The ginigiva oxygen saturation was significantly lower in diabetic group (PM Duarte et al, 2014). However, it is unclear the impact of well controlled diabetes on the change of gingiva blood and oxygen supply. Similarly, oxygen saturation of hemoglobin of healthy gingiva in smokers was significantly lower than nonsmokers (Hanioka et al. 2000). The variation in angulation and distance of the optical probe to gingival surface will affect the light reflection from gingival surface and light scattering within gingiva tissue, therefore change the absolute values of oxygenated, deoxygenated and total hemoglobin indices. However, at a given angle and distance, these measurements will increase or decrease simultaneously. The angulation and distance of the optical probe placement were not standarized in this study, which may explain the relatively smaller standard error of tissue oxygenation than each individual parameters as it is the ratio of oxygenated

hemoglobin to total hemoglobin. The volume of tissue assessed by optical spectroscopy depends on the diameter of the probe and the penetration of the light. The variation of gingiva thickness in different subjects and in different areas of the teeth may affect the measurements. Hanioka et al. (1990) evaluated the hemogobin concentration and oxygen saturation of healthy gingiva using an optical probe with diameter of 2 mm and light penetration of 2.5 mm (Hanioka et al. 1989) and found no significant difference among papillary, marginal and attached gingiva suggesting the inflammatory status of gingiva is the primary factor that affects the tissue hemoglobin and tissue oxygenation.

In conclusion: 1) multiple local inflammatory hemodynamic profiles can be simultaneously generated by optical spectroscopy to reflect subclinical gingival inflammation such as tissue oxygenation, perfusion and hydration. 2) Decreased tissue oxygenation saturation in periodontitis and gingivitis was mainly due to increased concentration of deoxygenated hemoglobin. 3) Optical spectroscopy has the potential to diagnose periodontal disease and monitor disease progression at an early stage. More longitudinal studies are needed to validate this potential.

References

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