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***In vivo* determination of multiple indices of periodontal inflammation by optical spectroscopy**

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Abstract

Background and Objective—Visible – near infrared (optical) spectroscopy can be used to measure regional tissue hemodynamics and edema and, therefore, may represent an ideal tool with which to non-invasively study periodontal inflammation. The study objective was to evaluate the ability of optical spectroscopy to simultaneously determine multiple inflammatory indices (tissue oxygenation, total tissue hemoglobin, deoxyhemoglobin, oxygenated hemoglobin, and tissue edema) in periodontal tissues *in vivo*.

Material and Methods—Spectra were obtained, processed, and evaluated from healthy, gingivitis, and periodontitis sites ($n = 133$) using a portable optical – near infrared spectrometer. A modified Beer-Lambert unmixing model that incorporates a nonparametric scattering loss function was used to determine the relative contribution of each inflammatory component to the overall spectrum.

Results—Optical spectroscopy was harnessed to successfully generate complex inflammatory profiles in periodontal tissues. Tissue oxygenation at periodontitis sites was significantly decreased ($p < 0.05$) compared to gingivitis and healthy controls. This is largely due to an increase in deoxyhemoglobin in the periodontitis sites compared to healthy ($p < 0.01$) and gingivitis ($p = 0.05$) sites. Tissue water content *per se* showed no significant difference between the sites but a water index associated with tissue electrolyte levels and temperature differed was significantly between periodontitis sites when compared to both healthy and gingivitis sites ($p < 0.03$).

Conclusion—This study establishes that optical spectroscopy can simultaneously determine multiple inflammatory indices directly in the periodontal tissues *in vivo*. Visible - near infrared spectroscopy has the potential to be developed into a simple, reagent-free, user friendly, chair-side, site-specific, diagnostic and prognostic test for periodontitis.

Keywords

optical spectroscopy; edema; inflammation; periodontitis; tissue oxygenation

Gingivitis is a reversible infectious disease of the periodontia characterized by an inflamed periodontia that bleeds readily. Periodontitis is an infectious, destructive inflammatory disease

of the soft and hard tissues surrounding the teeth and the leading cause of edentulism. Moderate periodontitis occurs in a majority of adults, while the prevalence of severe, generalized disease is in the region of 10% (1). Increasing evidence suggests that periodontitis may be associated with increased risk of vascular diseases (including coronary artery disease and stroke), diabetes mellitus, lung diseases (COPD and pneumonia), and pre-term delivery (2-4).

Periodontitis is currently diagnosed on gross clinical manifestations, i.e. signs of gingival inflammation (e.g. redness, swelling), periodontal pocketing, and periodontal attachment or alveolar bone loss (5). Such manifestations are measured by periodontal probing and radiographs. Alveolar bone and periodontal attachment loss represent the results of the destructive aspects of host defense mechanisms responding to the microbial plaque biofilm present in the gingival sulcus, as well as the direct effects of virulence factors of periodontal pathogens. Longitudinal studies have indicated that progression of chronic periodontitis, in terms of loss of periodontal attachment, is infrequent and episodic, and most progression occurs in a smaller proportion of highly susceptible individuals (1,6). Furthermore, traditionally used diagnostic procedures (clinical signs) do not distinguish between disease-active and disease-inactive sites. In other words, without long-term longitudinal assessment, it cannot be determined if periodontal destruction is current or a reflection of past disease (5,7). Thus, major diagnostic and prognostic problems exist for periodontitis.

Potential diagnostic tests studied to date include (a) the presence of specific bacteria (including *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*) (8,9), or (b) their products (e.g. volatile sulfur compounds and proteolytic enzymes)(10,11), (c) biomarkers which are involved in the disease process but produced by the host (e.g. matrix metalloproteinase-8; neutrophil elastase; aspartate aminotransferase; glucuronidase; and alkaline phosphatase (12), (d) biomarkers that occur as a consequence of tissue damage (e.g. collagen fragments) (13) and (e) other markers of the inflammatory process such as subgingival temperature probes; prostaglandin E₂; and interleukin-1 (12-14). To date, however, the predictive value of all potential markers has not been high enough for routine use in clinical practice. In addition, most diagnostic methods that have been investigated are too complicated for routine chair-side use. Furthermore, due to the complex nature of periodontitis – which involves a multifaceted immune and inflammatory reaction to a polymicrobial flora - a composite analytical approach to tissue and/or gingival crevicular fluid analysis is much more likely to prove successful than analysis of one or, at best, a few individual biomolecules.

Several absorption bands in the visible and near infrared (NIR) spectral region reflect key inflammatory events (15,16). For instance, tissue edema - an index that is commonly used as a marker of gingival inflammation (17,18) - can be measured using NIR spectroscopy (15, 16). Thus, monitoring the intensity of the water bands in gingival tissues will provide an index of tissue hydration representing a simple indicator of inflammation at specific periodontal sites. Furthermore, the 960 nm water band is known to shift with tissue temperature and changes in electrolyte concentration (19). In addition to measuring tissue water signals, optical spectroscopy offers a non-invasive means of assessing the balance between tissue oxygen delivery and oxygen utilization. Hemoglobin and oxygenation indices have 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 (20). The electronic transitions stemming from the heme ring and central metal ion of hemoglobin are particularly strong in the visible region. Optical spectroscopy can measure relative concentrations of oxygenated hemoglobin (HbO₂) and deoxygenated (Hb) hemoglobin by fitting optical attenuation spectra to the known optical properties (extinction coefficients) of HbO₂ and Hb (16,21,22). Thus, optical spectroscopy provides a measure of the hemoglobin oxygen saturation of tissues and the degree of tissue perfusion.

Given the inadequacies of current techniques, there is an urgent need for the development of a non-invasive diagnostic technique for periodontitis. Therefore, we hypothesized that optical spectroscopy can be used to non-invasively monitor a combination of hemodynamic and edema-based markers, which should provide a reliable and robust *in vivo* indicator of periodontal inflammation.

Materials and Methods

Subjects

Thirty patients with chronic moderate to severe periodontitis attending the Graduate Periodontics Clinics at the University of Manitoba, Canada participated in the study. There were 17 males and 13 females, aged (mean, s.d.) 54.4 ± 16.6 years. The research protocol was approved by each of the Research Ethics Boards of the University of Manitoba and the Institute for Biodiagnostics, NRC. Informed, written consent was obtained from each individual prior to collection of spectra. Periodontitis sites were defined as those with periodontal probing depth ≥ 5 mm, clinical attachment loss ≥ 3 mm. 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 healthy sites contralateral, or nearest to contralateral, to the diseased site. The spectral measurement were acquired at mid-facial, mid-lingual, mesio-facial, mesio-lingual, disto-facial or disto-lingual sites. All spectra were collected prior to clinical measurements from 26 periodontitis sites; 50 gingivitis sites; and 57 normal sites. All clinical parameters and spectra were assessed / obtained by the same calibrated examiners (XMX/AM).

Acquisition of optical spectra

Spectra were collected using a portable PDA512-ISA spectrograph system (Control Development Inc., South Bend, IN) with a customized bifurcated fiber optic bundle, or “probe”, designed for use in the oral cavity (Fiberguide Industries, Stirling, NJ). The intraoral probe consisted of a 180 mm long stainless steel shaft, 5 mm diameter, housing 29 fiber optic bundles, arranged as presented in Figure 1. The central fiber area, which delivers light to the oral cavity, is 0.8 mm in diameter. The outer ring of fibers is ~ 0.2 mm wide and located 1 mm away from the central fiber area (Figure 1A). These outer fibers are coupled to the entrance slit of the spectrograph and collect light subsequently emitted from the tissue. The emitter fibers at the bifurcated end of the bundle were coupled to a 5-watt tungsten halogen light source (Spectral Products, Putnam, CT, USA) that provides a highly 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 (LabSphere, North Sutton, NH) was used as a reference to convert raw data into reflectance spectra. During all spectral collections, the subjects were comfortably seated and relaxed in the standard semi-reclined position on a dental chair. To determine the reliability and calibration stability of this system, a set of experiments to examine the error between intra- or inter-operator were carried out. No statistically significant differences were found in acquired infrared spectral data (data not shown).

Testing of infection control sheaths

Multiple disposable latex and non-latex infection control covers for the intraoral NIRS probe were evaluated. Spectra obtained from the centre of the fingerprint of the index finger with the various sheaths were compared to control spectra (no cover). Once the most suitable material was found, i.e. did not interfere with spectral acquisition, custom covers were produced and tested for *in vivo* use.

Calculation of inflammatory indices from optical - NIR spectra

Reference spectra generated in our laboratories for water, Hb, and HbO₂ are presented in Figure 2. To calculate the clinically relevant parameters of O₂ saturation, blood volume and hydration from the measured attenuation spectrum, the known absorption coefficients of the major chromophores present in the tissue that give rise to the spectral features that are observed in the spectrum were used, as described below. All data presented are qualitative. A modified Beer-Lambert unmixing model that incorporates a nonparametric scattering loss function was used to determine the relative contribution of Hb, HbO₂ and H₂O to the overall spectrum. We have recently demonstrated that such partial linear models show superior performance over parametric regression methods in the face of omitted variable bias or model mis-specification (23).

For the visible region between 510 – 620 nm, where Hb and HbO₂ dominate the spectrum, the measured attenuation spectrum, A_λ , is modeled as a sum of 2 parametric terms, Hb and HbO₂, that contribute to the spectrum and a nonparametric term $m(\lambda)$ modeling a vector of covariates that have an unspecified functional form on the composite spectrum.

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

In our case we used $m(\lambda)$ to account for the Rayleigh and Mie scattering losses that contribute to the attenuation of measured light. Hb and HbO₂ concentrations per unit photon pathlength are recovered by using the noniterative method for estimation in partially linear models based on kernel smoothing, as first described by Speckman (22). The combined spectral signatures of HbO₂ and Hb measure cutaneous blood volume, total hemoglobin (tHb), an indicator of tissue perfusion, whereas the ratio of HbO₂ to tHb represents the oxygen saturation of the tissue (S_tO_2), as we have reported recently (15,16,21,23). Therefore, S_tO_2 and tissue perfusion (tHb) were derived from the predicted Hb and HbO₂ concentrations, where:

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

The visible – NIR spectrum of water is well-characterized (24) and dominates the NIR region between 900 – 1050 nm of tissue. The zero and first order moments were calculated for this strong absorption band of water centered at 970 nm. The zero order term or integrated band intensity is water concentration-dependent (16,21,25) and is used as a tissue hydration index. The first moment corresponds to the centre of gravity of the absorption band and has been shown to shift with tissue temperature and changes in electrolyte concentration, therefore, provides an index for the physical-chemical environment of the tissue.

Statistical analysis

Spearman rank order correlation coefficients were determined to test for the association between the ordinal clinical scores, periodontal probing depth, bleeding on probing, clinical attachment loss, plaque and tooth mobility, and the inflammatory indices determined from the reflectance spectra of periodontal tissues. The null hypothesis of chance correlation was tested to determine if the spectroscopic indices were associated with one or more of the clinical scores.

Inflammatory indices 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. Pairwise comparisons of mean differences between clinical groups were made using separate pooled variance estimates for each pair of means with a Bonferroni adjustment for the total number of multiple comparisons. Statistical calculations were performed with Statistica 7.1 (Statsoft, Tulsa, OK).

Results

Testing of infection control sheaths

Several different types of sterile, disposable sheaths for use with the intraoral NIRS probe were tested. Transparent polyurethane sheaths, which are latex-free, met the project requirements of (i) being an effective infection control barrier that (ii) does not interfere with either the acquisition of spectra at the relevant wavelength region, or (iii) interfere with *in situ* probe handling (data not shown). Thus, custom-produced, sterile probe covers (Protek Medical Products, Iowa City, IA) were used throughout this study.

Overall optical spectra in healthy periodontal tissues and in gingivitis and periodontitis

The mean area normalized spectra generated from the three groups, i.e., gingivitis, periodontitis, and healthy sites, are presented in Figure 3. The short wavelength region, 500 – 600 nm are dominated by the absorption from HbO₂ and Hb in the capillary bed gingival tissue while the absorption from water results in an increased attenuation at longer wavelengths in the 900 – 1100 nm region. The spectral readings between the gingivitis and healthy sites are virtually indistinguishable; however the mean spectrum from the periodontitis group shows subtle deviations in both the hemoglobin and water regions of the spectrum.

Periodontal inflammatory indices extrapolated from spectra

Figure 4 presents the relative concentrations of Hb and HbO₂ extracted from the visible region of the optical reflectance spectrum from periodontal tissues *in vivo* for the three groups of sites. Additional inflammatory indices derived from these measures, S_tO₂ (oxygen saturation of the tissue) and tHb (total hemoglobin which is closely related to tissue blood volume), are presented in Figure 5. Tissue water content *per se* showed no significant difference between sites but a water index associated with tissue electrolyte levels and temperature differed significantly in periodontitis sites when compared to both healthy and gingivitis sites ($p < 0.03$). Clearly, optical spectroscopy can provide detailed, site-specific information on multiple aspects of periodontal inflammation. Disease-specific variations were observed in a number of the inflammatory indices measured. For example, the tissue oxygenation in subjects with gingivitis and periodontitis are significantly decreased compared to controls ($p < 0.05$, Figure 5a). Similarly, we have found a statistically significant increase in Hb in the periodontitis sites compared to healthy ($p < 0.01$) and gingivitis ($p = 0.05$) sites (see Figure 4a). tHb (Figure 5b) shows an increasing trend proceeding from healthy to gingivitis and periodontitis sites ($p = 0.07$). While no difference was observed in the hydration index between the three groups of sites, the centre of mass of the water band was significantly different in the spectra from periodontitis sites than both healthy and gingivitis sites ($p < 0.03$).

Association between inflammatory indices and clinical assessment

Nonparametric correlation analysis rejected the null hypothesis of chance correlation ($r = 0$) at $p < 0.05$ for the association between S_tO₂ and the ordinal clinical parameters of bleeding on probing, clinical attachment loss, pocket depth, plaque and tooth mobility, all showing a modest negative correlation. Only the association between the tHb index and bleeding on probing could be rejected as a chance correlation with the two indices exhibiting a positive correlation. Deoxyhemoglobin showed a positive ($r_s = 0.26$) correlation with bleeding on probing that was above the threshold where it could not be discounted as a random association. Oxyhemoglobin

showed a modest negative correlation with probing depth ($r_s = -0.18$). While these associations could not be discounted as random, none of the correlations were strikingly high indicating that the spectroscopic indices do not closely track any single clinical score used to evaluate the disease. Therefore, a composite approach to data analysis will be adopted in future studies.

Discussion

The measurement of individual inflammatory indices, such as pocket oxygen tension, oxygen saturation of hemoglobin and temperature, has been posited to represent a potential diagnostic approach to inflammatory periodontal diseases for some time now (20,26-31). However, the methodology has been scarce and often inconvenient and / or impractical for routine use. In this initial study, our aim was to design and build an intraoral optical spectroscopy system and to evaluate the ability of this system to simultaneously determine multiple inflammatory indices (tissue oxygenation, total tissue hemoglobin, deoxyhemoglobin, oxygenated hemoglobin; and tissue edema) in periodontal tissues *in vivo* within the confines of a periodontal operator. This presented some unique problems, including the need to design and develop an intraoral optical probe that handles well in the confines of the oral cavity and that is familiar to dental professionals, and the design and development of suitable infection control measures for the intraoral probe.

We have clearly demonstrated that the optical spectroscopy system can be used to acquire spectra from periodontal tissues and that the data extracted from these spectra provide detailed site-specific information on multiple aspects of periodontal inflammation (Figures 4-5). Site-specific variations in a number of inflammatory indices measured (oxygen saturation; deoxygenated hemoglobin; and water band centre of gravity) were observed. In particular, tissue oxygen saturation was significantly decreased in both gingivitis and periodontitis sites compared to control sites (Figure 5A). Such decreased oxygen saturation likely reflects tissue hypoxia resulting from an ongoing inflammatory response leading to increased oxygen consumption(32). 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(33, 34). Site-specific variations in a number of inflammatory indices measured (oxygen saturation; deoxygenated hemoglobin; and water band centre of gravity) were observed.

It is well known that in destructive periodontal diseases, anaerobic microorganisms predominate in the periodontal pocket and that, diminished oxygen tension in deep pockets would be expected to promote growth of anaerobic bacteria (33,34). Interestingly, it has been shown previously that tissue oxygen saturation correlates well with oxygen tension in periodontal pockets (20). 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 intraoral 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 microflora.

Furthermore, gingival blood flow, which is altered by the vascular dilation and angiogenesis intimately associated with inflammation, may serve as a further prognostic marker for periodontal disease. The decreased Hb signals and altered tissue oxygen saturation observed in the optical spectra from periodontitis sites (Figures 4 and 5) likely reflect such altered vascularity.

Periodontal edema (water content, as interpreted by optical spectra) results from an increase in vascular permeability in response to infecting bacteria leading to interstitial fluid

accumulation and, subsequently, the release of a variety of this inflammatory exudate in the gingival crevice (35). Clinically, this appears as swelling at the affected periodontal area. Although tissue water content *per se* did not differ between diseased and control sites, the water index (associated with tissue electrolyte levels and temperature) was significantly depressed at periodontitis sites compared to gingivitis sites. As gingivitis is defined by an active inflammatory process, whereas sites with periodontitis may or may not be inflamed at any given point of cross-sectional analysis, this result is not entirely unexpected.

The above analyses have considered using only a single index as a diagnostic marker. Optical spectroscopy measures multiple parameters or indices simultaneously offering a potential increase in accuracy if multiple indices or features extracted from the spectra were used to establish diagnostic criteria. For example, the COG index alone shows modest diagnostic accuracy but is only weakly correlated with the S_tO_2 index ($\rho=0.24$) that shows good diagnostic accuracy. One simple means of increasing the sensitivity would be to interpret these diagnostic tests in parallel by invoking an OR rule (diagnosis is positive if either S_tO_2 or COG is positive). The sensitivity of the combined result would be higher than either test individually but the specificity would be lower than that conveyed by either individual test. Combining the tests using the AND rule (diagnosis is negative if either S_tO_2 or COG is negative) enhances specificity relative to the individual tests at the expense of sensitivity. Perhaps more intriguing than interpreting these univariate tests in parallel is explicitly combining these indices or other indices and features derived from the optical spectra into a multivariate classification model aimed to accurately diagnose diseased and healthy periodontal sites. However, this extension to multivariate classification requires a larger sample size than is currently available to us. These initial univariate analyses show the diagnostic potential of optical spectroscopy to distinguish periodontitis sites. Future studies with increased sample sizes will develop diagnostic algorithms based on multiple indices or spectral features and test their ability to separate the clinically-defined classes (i.e. health vs gingivitis vs periodontitis).

Nevertheless, our data clearly demonstrate that optical spectroscopy can monitor multiple inflammatory indices in periodontal tissues and provide a complex inflammatory profile. However, in order to make it generally applicable, there are two immediate issues that require attention. The first is the issue of mucosal pigmentation. In highly pigmented skin the resolution of inflammatory indices by using optical spectroscopy is more complex because of the light absorption properties of melanin. This should not present a methodological barrier as inflammatory indices may still be readily measured in persons with heavy pigmentation, as we have recently addressed in skin (23). However, further development is planned in order to address mucosal pigmentation by compensating for melanin in the intraoral environment. The second issue is that of tobacco smoking. Smoking is known to cause key vascular changes and compromise overt inflammation in periodontal tissues (18,36,37). Thus, the confounding influence of tobacco smoke exposure on inflammatory induced needs to be addressed. However, it should be noted that a further advantage of optical spectroscopy is that because carbon monoxxygenated hemoglobin has a distinct visible spectrum (38); the analysis of periodontal spectra may also be able to define the smoking status of individuals concurrently with diseases indices. Indeed, we have recently observed multiple alterations to the molecular and biochemical profile of serum from smokers and non-smokers (39). There are a number of other factors that could influence the spectra of periodontal tissues. These include non-periodontitis-related inflammation and injury, such as trauma-induced recession; vascular pathologies including diabetic complications; and multiple pharmaceuticals, particularly antipyretic medications.

In addition to diagnostic considerations, infrared spectroscopy might also play an important role in risk assessment in periodontal practice. For instance, infrared spectroscopy could be employed to define sub-clinical inflammation suggesting preventive therapy may be

appropriate. Infrared spectroscopy may also allow for assessment of the efficacy of maintenance treatment. The successful application of optical spectroscopy to the study of periodontitis could also provide great potential research benefits. It is generally accepted that the accuracy, reproducibility, and validity of the clinical index reporting are sensitive to subjective components (40). Indeed, Rosin et al (40) point out that non-invasive inflammatory indices (redness and edema) are even more susceptible to the negative influence of subjectivity than invasive measures. At present, there are no technologies in use that can standardize the measurement and report periodontal inflammatory indices. Thus, we propose that optical spectroscopy could conceivably represent a means of standardization of clinical measures of inflammation in periodontal tissues. Such standardized methods would have obvious benefits to both clinical studies and mechanistic studies.

In conclusion, optical spectroscopy represents an attractive technology to apply to the study of periodontal inflammation and periodontal diseases because spectra can be captured instantly; no consumables need be purchased or developed (such as antibodies, substrates, or molecular probes); once the equipment is in place, it is very inexpensive to operate; and minimal training is required to obtain reliable and reproducible data. Furthermore, commercially available portable spectrometers are small, they do not require cryogenic cooling, and therefore, can be readily utilized in small clinical environments, such as a periodontal operatory. Additionally, it is an entirely non-invasive technique that uses low energy radiation. Future studies are warranted that will establish the spectral characteristic of healthy and diseased periodontal sites in individual subjects with gingivitis and/or periodontitis.

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References

1. Burt B. Position paper: Epidemiology of periodontal diseases. *J Periodontol* 2005;76:1406–1419. [PubMed: 16101377]
2. Chun YH, Chun KR, Olguin D, Wang HL. Biological foundation for periodontitis as a potential risk factor for atherosclerosis. *J Periodontol Res* 2005;40:87–95. [PubMed: 15613084]
3. Teng YT, Taylor GW, Scannapieco F, et al. Periodontal health and systemic disorders. *J Can Dent Assoc* 2002;68:188–192. [PubMed: 11911816]
4. Moutsopoulos NM, Madianos PN. Low-grade inflammation in chronic infectious diseases: paradigm of periodontal infections. *Ann N Y Acad Sci* 2006;1088:251–264. [PubMed: 17192571]
5. Armitage GC. Diagnosis of periodontal diseases. *J Periodontol* 2003;74:1237–1247. [PubMed: 14514240]
6. Socransky SS, Haffajee AD, Goodson JM, Lindhe J. New concepts of destructive periodontal disease. *J Clin Periodontol* 1984;11:21–32. [PubMed: 6582072]
7. Greenstein G. Commentary on periodontal diagnostic testing. *J Periodontol* 1995;66:531–535. [PubMed: 7562343]
8. Schacher B, Baron F, Rossberg M, Wohlfeil M, Arndt R, Eickholz P. Aggregatibacter actinomycetemcomitans as indicator for aggressive periodontitis by two analysing strategies. *J Clin Periodontol* 2007;34:566–573. [PubMed: 17433043]
9. Nozaki T, Kusumoto Y, Kitamura M, et al. A sensitive method for detecting Porphyromonas gingivalis by polymerase chain reaction and its possible clinical application. *J Periodontol* 2001;72:1228–1235. [PubMed: 11577955]
10. Pavolotskaya A, McCombs G, Darby M, Marinak K, Dayanand NN. Sulcular sulfide monitoring: an indicator of early dental plaque-induced gingival disease. *J Dent Hyg* 2006;80:11. [PubMed: 16451765]

11. Takaishi Y, Morii H, Miki T. The benzoyl-DL arginine-naphthylamide (BANA) test and polymerase chain reaction measurement of pathogenic bacteria can assess the severity of periodontal disease. *Int J Tissue React* 2003;25:19–24. [PubMed: 12854883]
12. 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. [PubMed: 17435131]
13. Taba M Jr, Kinney J, Kim AS, Giannobile WV. Diagnostic biomarkers for oral and periodontal diseases. *Dent Clin North Am* 2005;49:551–571. vi. [PubMed: 15978241]
14. Maiden MF, Tanner AC, Macuch PJ, Murray L, Kent RL Jr. Subgingival temperature and microbiota in initial periodontitis. *J Clin Periodontol* 1998;25:786–793. [PubMed: 9797050]
15. 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. [PubMed: 17092151]
16. 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. [PubMed: 11311517]
17. Loe H, Silness J. Periodontal Disease in Pregnancy. I. Prevalence and Severity. *Acta Odontol Scand* 1963;21:533–551. [PubMed: 14121956]
18. Scott DA, Singer DL. Suppression of overt gingival inflammation in tobacco smokers - clinical and mechanistic considerations. *Int J Dent Hyg* 2004;2:104–110. [PubMed: 16451473]
19. Otal EH, Inon FA, Andrade FJ. Monitoring the temperature of dilute aqueous solutions using near-infrared water absorption. *Appl Spectrosc* 2003;57:661–666. [PubMed: 14658699]
20. Hanioka T, Shizukuishi S, Tsunemitsu A. Hemoglobin concentration and oxygen saturation of clinically healthy and inflamed gingiva in human subjects. *J Periodontol Res* 1990;25:93–98. [PubMed: 2139123]
21. 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. [PubMed: 11737819]
22. Speckman P. Kernel smoothing in partial linear models. *J R Statist Soc B* 1988;50:413–436.
23. Sowa MG, Matas A, Schattka BJ, Mantsch HH. Spectroscopic assessment of cutaneous hemodynamics in the presence of high epidermal melanin concentration. *Clin Chim Acta* 2002;317:203–212. [PubMed: 11814477]
24. Martin K. In vivo measurements of water in skin by near-infrared reflectance. *Applied Spectroscopy* 1998;52:1001–1007.
25. Brown CD, Davis HT. Receiver operating characteristics curves and related decision measures. *Chemometrics Intellig Lab Systems* 2006;80:24–38.
26. Hanioka T, Amano A, Tamagawa H, Shizukuishi S. Evaluation of the depth sensitivity of tissue reflectance spectrophotometry in gingival measurement. *J Osaka Univ Dent Sch* 1990;30:1–7. [PubMed: 2130163]
27. Hanioka T, Shizukuishi S, Tsunemitsu A, Joh S, Sugiyama K, Sato N. Haemoglobin concentration and oxygen saturation in dog gingiva with experimentally induced periodontitis. *Arch Oral Biol* 1989;34:657–663. [PubMed: 2597056]
28. Meekin TN, Wilson RF, Scott DA, Ide M, Palmer RM. Laser Doppler flowmeter measurement of relative gingival and forehead skin blood flow in light and heavy smokers during and after smoking. *J Clin Periodontol* 2000;27:236–242. [PubMed: 10783836]
29. Tanaka M, Hanioka T, Takaya K, Shizukuishi S. Association of oxygen tension in human periodontal pockets with gingival inflammation. *J Periodontol* 1998;69:1127–1130. [PubMed: 9802712]
30. Niederman R, Naleway C, Lu BY, Buyle-Bodin Y, Robinson P. Subgingival temperature as a gingival inflammatory indicator. *J Clin Periodontol* 1995;22:804–809. [PubMed: 8682928]
31. Baab DA, Oberg A, Lundstrom A. Gingival blood flow and temperature changes in young humans with a history of periodontitis. *Arch Oral Biol* 1990;35:95–101. [PubMed: 2344293]
32. 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. [PubMed: 11156041]

33. 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. [PubMed: 3166001]
34. Loesche WJ. Oxygen sensitivity of various anaerobic bacteria. *Appl Microbiol* 1969;18:723–727. [PubMed: 5370458]
35. Bartold PM, Walsh LJ, Narayanan AS. Molecular and cell biology of the gingiva. *Periodontol* 2000 2000;24:28–55. [PubMed: 11276872]
36. Palmer RM, Wilson RF, Hasan AS, Scott DA. Mechanisms of action of environmental factors--tobacco smoking. *J Clin Periodontol* 2005;32:180–195. [PubMed: 16128837]
37. Mirbod SM, Ahing SI, Pruthi VK. Immunohistochemical study of vestibular gingival blood vessel density and internal circumference in smokers and non-smokers. *J Periodontol* 2001;72:1318–1323. [PubMed: 11699472]
38. Araki R, Nashimoto I, Takano T. The effect of hyperbaric oxygen on cerebral hemoglobin oxygenation and dissociation rate of carboxyhemoglobin in anesthetized rats: spectroscopic approach. *Adv Exp Med Biol* 1988;222:375–381. [PubMed: 3364262]
39. Borden JT, Man A, Scott DA, Liu KZ. Tobacco-induced alterations to the Fourier-transform infrared spectrum of serum. *J Mol Med* 2003;81:788–794. [PubMed: 14556052]
40. Rosin M, Splieth C, Hessler M, Gartner C, Kordass B, Kocher T. Quantification of gingival edema using a new 3-D laser scanning method. *J Clin Periodontol* 2002;29:240–246. [PubMed: 11940144]

Alphabetical list of abbreviations

COG	centre of gravity
Hb	deoxygenated hemoglobin
HbO₂	oxygenated hemoglobin
NIR	near infrared
StO₂	tissue oxygen saturation
tHb	total hemoglobin

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).

Figure 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.

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

Fig. 4A

Fig. 4B

Figure 4.

Relative concentrations of Hb (A) and HbO₂ from healthy, gingivitis and periodontitis sites (B). Relative hemoglobin concentrations were calculated using the visible region (510 – 620 nm) of the reflected light spectrum. * Represents a significant difference from healthy sites, $p < 0.01$. Vertical bars denote 0.95 confidence intervals.

Fig. 5A

Figure 5.

Percent tissue hemoglobin oxygen saturation (A) and THb indices (B) derived from the relative concentrations of Hb and HbO₂. 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.