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

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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 study periodontal inflammation in a non-invasive manner. The study objective was to evaluate the ability of optical spectroscopy to determine simultaneously 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 generate complex inflammatory profiles of periodontal tissues. Tissue oxygenation at periodontitis sites was significantly decreased ($p < 0.05$) compared to sites with gingivitis and healthy controls. This was largely the result of an increase in deoxyhemoglobin in the periodontitis sites compared with 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 significantly between periodontitis sites and both healthy and gingivitis sites ($p < 0.03$).

Conclusion: This study established 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, chairside, site-specific, diagnostic and prognostic test for periodontitis.

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Gingivitis is a reversible infectious disease of the periodontia characterized by an inflamed periodontium that bleeds readily. Periodontitis is an infectious, destructive inflammatory disease of the soft and hard tissues surrounding the teeth and is the leading cause of edentulism. Moderate periodontitis occurs in a majority of adults, whereas severe, generalized disease occurs in approximately 10% of adults (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 [chronic obstructive pulmonary disease (COPD) and pneumonia] and preterm 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 loss 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). Therefore, 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 that 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 E_2 and interleukin-1) (12–14). To date, however, the predictive value of all potential markers has not been high enough to permit their routine use in clinical practice. In addition, most diagnostic methods that have been investigated are too complicated for routine chairside use. Furthermore, because of 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 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 near-infrared 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 noninvasive 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 and deoxygenated hemoglobin by fitting optical attenuation spectra to the known optical properties (extinction coefficients) of oxygenated hemoglobin and deoxygenated hemoglobin (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 noninvasive diagnostic technique for periodontitis. Therefore, we hypothesized that optical spectroscopy can be used to monitor in a noninvasive manner a combination of hemodynamic and edema-based markers, which should provide a reliable and robust *in vivo* indicator of periodontal inflammation.

Material and methods

Subjects

Thirty patients [17 men and 14 women; 54.4 ± 16.6 years of age (mean ± standard deviation)] with chronic moderate to severe periodontitis and attending the Graduate Periodontics Clinics at the University of Manitoba, Canada, participated in the study. 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 a periodontal probing depth of ≥ 5 mm and clinical attachment loss of ≥ 3 mm. Gingivitis sites were defined as those with a periodontal probing depth of < 3 mm and bleeding on probing. Healthy sites were defined as those with a periodontal probing depth of < 3 mm and no bleeding on probing, contralateral, or nearest to contralateral, to the diseased site. The spectral measurements were acquired at midfacial, midlingual, mesiofacial, mesiolingual, distofacial or distolingual sites. All spectra were collected prior to taking 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 (X. M. X./A. M.).

Acquisition of optical spectra

Spectra were collected using a portable PDA512-ISA spectrograph system (Control Development Inc., South Bend, IN, USA) with a customized bifurcated fiber optic bundle, or 'probe', designed for use in the oral cavity (Fiberguide Industries, Stirling, NJ, USA). The intra-oral probe consisted of a 180-mm-long stainless steel shaft, 5 mm in diameter, housing 29 fiberoptic bundles, arranged as presented in Fig. 1. The central fiber

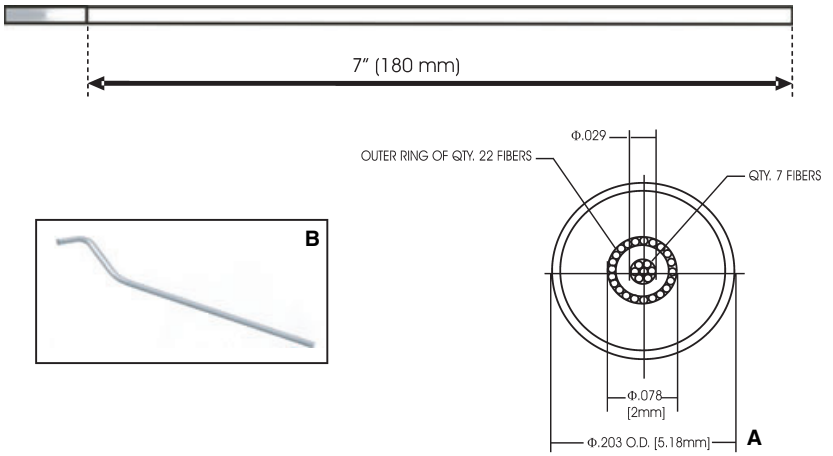

Fig. 1. Design of the intra-oral probe for the acquisition of near-infrared spectra from periodontal tissues. The intra-oral probe consists of a 180 mm long stainless steel shaft, 5 mm in diameter, housing 29 fibreoptic bundles (A). Amended probe shape (B).

area, which delivers light to the oral cavity, is 0.8 mm in diameter. The outer ring of fibers is $\sim$ 0.2 mm wide and located 1 mm away from the central fiber area (Fig. 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, USA) 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 semireclined position on a dental chair. A test–retest repeatability study was also carried out to determine the reliability and calibration stability of this system, and the data are discussed in the Results section.

Testing of infection control sheaths

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

Calculation of inflammatory indices from optical, near-infrared spectra

Reference spectra generated in our laboratories for water, deoxygenated hemoglobin and oxygenated hemoglobin are presented in Fig. 2. To calculate the clinically relevant parameters of O_2 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 which 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 deoxygenated hemoglobin, oxygenated hemoglobin and H_2O 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 and 620 nm, where deoxygenated hemoglobin and oxygenated hemoglobin dominate the spectrum, the measured attenuation spectrum, A_λ, is modeled as the sum of two parametric terms – deoxygenated hemoglobin and oxygenated hemoglobin, which contribute to the spectrum – and a nonparametric term, m (λ), 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(λ) to account for the Rayleigh and Mie scattering losses that contribute to the attenuation of measured light. Deoxygenated hemoglobin and oxygenated hemoglobin concentrations per unit photon pathlength were recovered by using the noniterative method for estimation in partially linear models based on

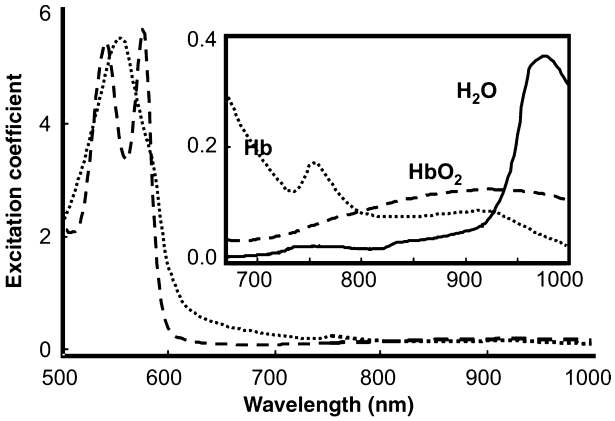

Fig. 2. Near-infrared reference spectra (500–1000 nm) for water, deoxygenated hemoglobin and oxygenated hemoglobin. The extinction coefficient data for water have been multiplied by a scaling factor of 10. Hb, deoxygenated haemoglobin; HbO_2, oxygenated haemoglobin.

kernel smoothing, as first described by Speckman (22). The combined spectral signatures of oxygenated hemoglobin and deoxygenated hemoglobin measure total hemoglobin, an indicator of tissue perfusion, whereas the ratio of oxygenated hemoglobin to total hemoglobin represents the oxygen saturation of the tissue, as reported recently (15,16,21,23). Therefore, tissue oxygen saturation and tissue perfusion (total hemoglobin) were derived from the predicted deoxygenated hemoglobin and oxygenated hemoglobin concentrations, where:

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

The visible, near-infrared spectrum of water is well characterized (24) and dominates the near-infrared region between 900 and 1050 nm of tissue. The zero-order 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, and 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 spectra were analyzed separately using a one-way analysis of variance 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 using STATISTICA 7.1 (Statsoft, Tulsa, OK, USA).

Results

Testing of infection-control sheaths

Several different types of sterile, disposable sheaths for use with the intra-oral optical 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, USA) 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 Fig. 3. The short-wavelength region, 500–600 nm, was dominated by the absorption from oxygenated hemoglobin and deoxygenated hemoglobin in the capillary bed gingival tissue, whereas the absorption from water resulted in an increased attenuation at longer wavelengths in the 900–1100 nm region. The spectral readings between the gingivitis and healthy sites were virtually indistinguishable; however, the mean spectrum from the periodontitis group showed subtle deviations in both the hemoglobin and water regions of the spectrum.

Test–retest reliability

Analysis of the test–retest reliability indicated that the variance in the measurements between healthy sites was 22–40% of the repeatability (test–retest within site) variance for all the spectroscopic indices (tissue oxygen saturation, total hemoglobin, deoxygenated hemoglobin and oxygenated hemoglobin). No significant differences ($p > 0.05$) were observed between the measurements made at the 24 distinct healthy sites for the total hemoglobin and deoxygenated hemoglobin indices, whereas the tissue oxygen saturation and oxygenated hemoglobin indices showed a statistically significant difference ($p < 0.05$). *Post hoc* analysis showed that site 36 db and 44 mb had distinctly lower measured oxygenated hemoglobin and tissue oxygen satura-

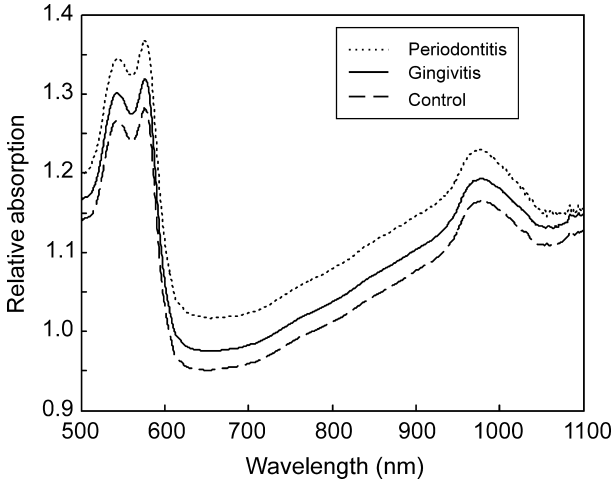

Fig. 3. Mean near-infrared spectra from healthy, gingivitis and periodontitis sites.

tion respectively compared with the remaining sites. The test–retest reliability study showed that the repositioning error of replacing the probe on the gum contributed substantially to the variance of the measurements and outweighed the variability in the measurements between healthy sites. This has implications for longitudinal studies using this method to follow disease progression at specific sites. Improvements in probe design are underway to ameliorate the effects of probe positioning error.

Periodontal inflammatory indices extrapolated from spectra

Figure 4 presents the relative concentrations of deoxygenated hemoglobin and oxygenated hemoglobin 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, such as tissue oxygen saturation and total hemoglobin (which is closely related to tissue blood volume), are presented in Fig. 5. There was no difference in tissue water content *per se* between sites, but a water index associated with tissue electrolyte levels and temperature differed significantly in periodontitis sites when compared with 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 was significantly decreased compared with controls ($p < 0.05$, Fig. 5A). In addition, we found a statistically significant increase in deoxygenated hemoglobin in the periodontitis sites compared with healthy ($p < 0.01$) and gingivitis ($p = 0.05$) sites (see Fig. 4A). The level of total hemoglobin (Fig. 5B) showed an increasing trend, proceeding from healthy to gingivitis and periodontitis sites ($p = 0.07$). Although no significant 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 in both healthy and gingivitis sites ($p < 0.03$).

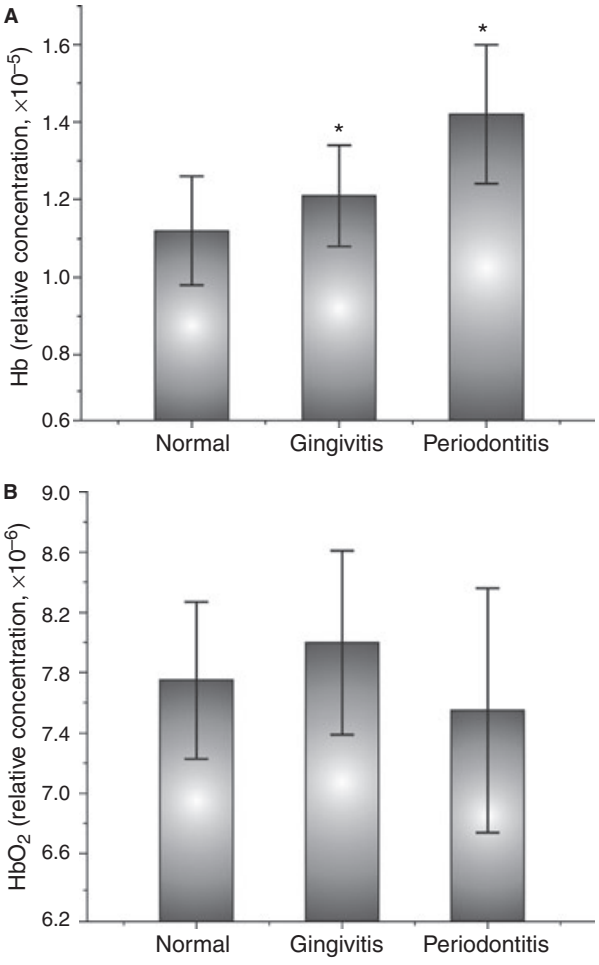

Fig. 4. Relative concentrations of deoxygenated hemoglobin (A) and oxygenated hemoglobin from healthy, gingivitis and periodontitis sites (B). Relative hemoglobin concentrations were calculated using the visible region (510–620 nm) of the reflected light spectrum. *Represents a significant difference from healthy sites, $p < 0.01$. Vertical bars denote 0.95 confidence intervals. Hb, deoxygenated haemoglobin; HbO_2, oxygenated haemoglobin.

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 tissue oxygen saturation 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 total hemoglobin 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 ($r_S = -0.18$). Whereas 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.

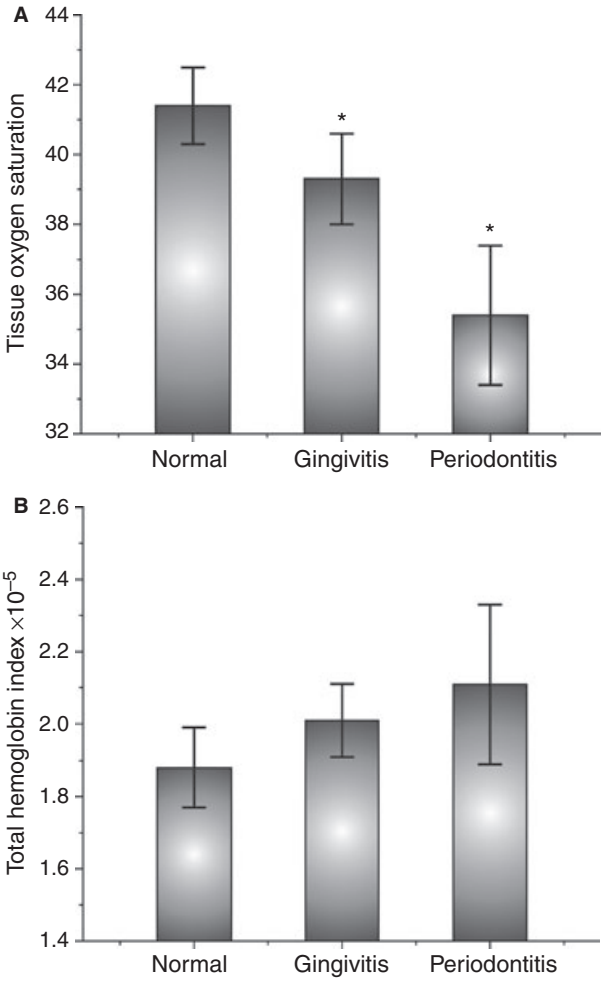

Fig. 5. Per cent tissue hemoglobin oxygen saturation (A) and total hemoglobin indices (B) derived from the relative concentrations of deoxygenated hemoglobin and oxygenated hemoglobin. Indices are compared among healthy, gingivitis and periodontitis sites. *Represents a significant difference from healthy sites, $p < 0.01$. Vertical bars denote 0.95 confidence intervals.

Discussion

The measurement of individual inflammatory indices, such as pocket oxygen tension, oxygen saturation of hemoglobin and temperature, has for some time been posited to represent a potential diagnostic approach to inflammatory periodontal diseases (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 intra-oral optical spectroscopy system and to evaluate the ability of this system to determine simultaneously 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 operatory. This presented some unique problems, including the need to design and develop an intra-oral 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 intra-oral 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 (Figs. 4 and 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 with control sites (Fig. 5A). Such decreased oxygen saturation probably reflects tissue hypoxia resulting from an ongoing inflammatory response, leading to increased oxygen consumption (32). It is well known that in destructive periodontal diseases, anerobic microorganisms predominate in the periodontal pocket, and diminished oxygen tension in deep pockets would be expected to promote the growth of anerobic 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 be useful to the periodontist. In other words, after future studies the intra-oral 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 anerobicity 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 deoxygenated hemoglobin signals and altered tissue oxygen saturation observed in the optical spectra from periodontitis sites (Figs. 4 and 5) probably 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 with gingivitis sites. As gingivitis is defined by an active inflammatory process, where as 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 centre of gravity index alone shows modest diagnostic accuracy, but is only weakly correlated with the tissue oxygen saturation 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 (the diagnosis is positive if either tissue oxygen saturation or the centre of gravity 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 (the diagnosis is negative if either tissue oxygen saturation or centre of gravity 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 diagnose diseased and healthy periodontal sites accurately. 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. healthy 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 intra-oral 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 indices needs to be addressed. However, it should be noted that a further advantage of optical spectroscopy is that because carbon monoxygenated 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 disease indices. Indeed, we have recently observed multiple alterations to the molecular and biochemical profile of serum from smokers and nonsmokers (39). There are a number of other factors that could influence the spectra of periodontal tissues. These include nonperiodontitis-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 subclinical inflammation, suggesting that 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 reports are sensitive to subjective components (40). Indeed, Rosin *et al.* (40) point out that noninvasive 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 for application 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 noninvasive technique that uses low-energy radiation. Future studies are warranted that will establish the spectral characteristics of healthy and diseased periodontal sites in individual subjects with gingivitis and/or periodontitis.

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