NRC Publications Archive Archives des publications du CNRC

Assessment of local hemodynamics in periodontal inflammation using optical spectroscopy

Ge, Zili; Liu, Kan-Zhi; Xiang, Xiaoming; Yang, Qing; Hui, Jianhua; Kohlenberg, Elicia; Sowa, Michael G.

This publication could be one of several versions: author's original, accepted manuscript or the publisher's version. / La version de cette publication peut être l'une des suivantes : la version prépublication de l'auteur, la version acceptée du manuscrit ou la version de l'éditeur.

For the publisher's version, please access the DOI link below. / Pour consulter la version de l'éditeur, utilisez le lien DOI ci-dessous.

Publisher's version / Version de l'éditeur:

<https://doi.org/10.1902/jop.2011.100632>

Journal of Periodontology, 82, 8, pp. 1161-1168, 2011-08

NRC Publications Record / Notice d'Archives des publications de CNRC:

<https://nrc-publications.canada.ca/eng/view/object/?id=9085087e-1c28-4dbf-bd8a-8a91e92823ab>

<https://publications-cnrc.canada.ca/fra/voir/objet/?id=9085087e-1c28-4dbf-bd8a-8a91e92823ab>

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at

<https://nrc-publications.canada.ca/eng/copyright>

READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site

<https://publications-cnrc.canada.ca/fra/droits>

LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

Questions? Contact the NRC Publications Archive team at

PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the first page of the publication for their contact information.

Vous avez des questions? Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.

Assessment of Local Hemodynamics in Periodontal Inflammation Using Optical Spectroscopy

Zili Ge,* Kan-Zhi Liu,†‡ Xiaoming Xiang,† Qing Yang,* Jianhua Hui,* Elicia Kohlenberg,† and Michael G. Sowa†

Abstract

Background: Among the newly emerging diagnostic approaches for periodontitis, optical spectroscopy is a promising complementary diagnostic tool. The objective of this study is to verify the reproducibility of this method at a geographically distinct location (Suzhou, China) to a broader patient population using similar instrumentation to that in a previous report.

Methods: Using a portable optical near-infrared spectrometer, optical spectra were obtained, processed, and evaluated from healthy ($n = 62$), gingivitis ($n = 98$), and periodontitis ($n = 47$) sites from a total of 51 patients. A modified Beer-Lambert unmixing model that incorporates a non-parametric scattering loss function was used to determine the relative contribution of oxyhemoglobin and deoxyhemoglobin to the overall spectrum. The balance between tissue oxygen delivery and oxygen use in periodontal tissues was then assessed.

Results: Tissue oxygenation decreased significantly from healthy sites to sites with gingivitis ($P < 0.01$) and between gingivitis and periodontitis ($P = 0.015$). This is largely caused by a significant increase in deoxyhemoglobin between normal and gingivitis ($P < 0.01$) and a concomitant decrease in oxyhemoglobin between gingivitis and periodontitis ($P = 0.02$).

Conclusion: This study supports previous findings that tissue oxygenation as measured by optical spectroscopy is significantly decreased in periodontitis and that optical spectroscopy can simultaneously determine multiple inflammatory indices related to periodontal disease directly in gingival tissues in vivo.

KEYWORDS

Diagnosis; inflammation; periodontitis; spectroscopy, near-infrared

Periodontitis, the leading cause of edentulism, is an endemic infectious disease of the tissues surrounding the teeth. It occurs in about 50% of the population and may result in significant debilitation for about half of these persons.¹ Currently, this disease is almost entirely diagnosed based on visual assessment of the tissues and a physical examination using the periodontal probe, providing indices of disease, such as signs of gingival inflammation (e.g., redness and swelling), probing depth (PD), and periodontal attachment or alveolar bone loss.² Unfortunately, these clinical diagnostic methods are largely descriptive and most are subjective measures of historical disease that have been used for >50 years without significant improvement.

Therefore, major diagnostic and prognostic problems exist for periodontitis. To this end, the American Academy of Periodontology, in partnership with the Centers for Disease Control and Prevention, has recently acknowledged that the development of enhanced surveillance and prognostic tools is essential to the improvement of periodontics.³⁻⁵

There is little doubt that vast research in the past on periodontitis has dramatically improved the understanding of the disease and has influenced clinical practice. Diagnostic methods for periodontal diseases can generally be classified into three categories based on their features and clinical aspects.⁶ Clinical examination is the mainstream of current practice or the gold standard that primarily measures clinical parameters, such as bleeding on probing (BOP), PD, and clinical attachment loss (AL). The second group consists of genetic analysis and laboratory-type tests that measure aspects of oral biochemistry, microbiology, or radiography. The last group consists of newly emerging non-invasive techniques, such as molecular fingerprinting with infrared spectroscopy, and morphologic imaging using optical coherence tomography or ultrasound that do not use ionizing radiation and are being explored as complementary diagnostic tools in periodontal diagnostics.

Indeed, a simple, reagent-free, user friendly, chairside, site-specific diagnostic test for periodontitis would be an invaluable addition to the dental clinic. The visible near-infrared (NIR) spectral region of the electromagnetic spectrum covers the range from 400 to 2,500 nm and conveys information on local tissue hemodynamic changes of the disease, such as edema and oxygenation.^{7,8} For instance, the short wavelength region, 500 to 600 nm, is dominated by the absorption from oxygenated hemoglobin (HbO₂) and deoxygenated hemoglobin (Hb) in the capillary bed of gingival tissue, whereas the absorption from water results in an increased attenuation at longer wavelengths in the 900 to 1,100 nm region.^{9,10} Recently, we have shown that periodontal tissue edema, an index that is commonly used as a marker of gingival inflammation,^{10,11} can be measured in vivo using NIR spectroscopy (NIRS).¹² Monitoring the intensity of the water absorption bands in the reflectance spectrum of gingival tissues provides an index of tissue hydration representing a simple indicator of inflammation at specific periodontal sites, and can be readily detected at subclinical levels. The increase in blood supply is often insufficient to meet the oxygen demand in inflamed gingivae.¹³ Therefore, in addition to measuring tissue water content, we have successfully used NIRS to assess the balance between tissue oxygen delivery and oxygen use in periodontal tissues¹² by fitting optical attenuation spectra to the known optical properties (extinction coefficients) of HbO₂ and Hb.

Based on the same principle, NIRS has been widely applied in biomedical problems, including cancer diagnostics; the early prediction of inflammation related treatment failures in burn victims;^{8,9,14} and monitoring ischemic conditions in urology, such as testicular tissue perfusion and oxygenation of testicular torsion.^{15,16} Commercially, some NIR-based cerebral oximetry monitors[§] have been used in the clinical setting for the surveillance of the cerebral oxygen balance under CO₂ challenge.¹⁷

Focusing on the development of non-invasive technology, recently our group has examined the use of optical spectroscopy as a rapid and simple noninvasive method to determine the inflammatory status of gingival tissue.¹² In that study, based on a population of 31 patients, we demonstrated that tissue oxygenation as measured by optical spectroscopy was significantly lower ($P < 0.05$) at sites with periodontitis compared to sites with gingivitis or healthy sites. To further validate this newly established periodontal diagnostic tool, we therefore investigated the reproducibility of this method by examining a different patient population in a geographically different location with a similar instrument. It is known that genetic polymorphisms have been claimed to play a role in the predisposition to and the progression of aggressive and chronic periodontitis, whereas the racial and ethnic backgrounds of both cases and controls are important for critical analysis of polymorphisms.¹⁸ Therefore, our study might also reveal whether racial and ethnic background is an important factor in this newly developed technology.

MATERIALS AND METHODS

Study Sites and Patients

The study was carried out at the First Affiliated Hospital of Soochow University, Suzhou, China. A total of 51 patients with moderate to severe chronic periodontitis were recruited in October 2008. The research protocol was approved by each of the Research Ethics Boards of the First Affiliated Hospital of Soochow University and the Institute for Biodiagnostics, National Research Council of Canada, Winnipeg, Manitoba. Informed, written consent was obtained from each individual before collection of spectra. Periodontitis sites were defined as those with PD ≥ 5 mm, clinical AL ≥ 3 mm, with BOP.2 Gingivitis sites were defined as those with PD < 3 mm and BOP. Healthy sites were defined as those with PD < 3 mm and no BOP. Exclusion criteria were: 1) tobacco smoking; 2) anti-inflammatory medications within the past 3 months (e.g., non-steroidal anti-inflammatory drugs, steroids, antibiotics, or immune suppressants) that may interfere with the study; 3) any systemic condition that may interfere with the study, such as inflammatory diseases or diabetes; and 4) lesions of the gingiva unrelated to plaque-induced periodontal disease. Spectra were obtained from healthy sites contralateral, or nearest to contralateral, to the diseased site. A total of 207 spectral measurements were acquired at sites of mid-buccal, mid-lingual, mesio-buccal, mesio-lingual, disto-buccal, or disto-lingual. All spectra were collected before clinical measurements from 47 periodontitis sites, 98 gingivitis sites, and 62 healthy sites as shown in Table 1. All clinical parameters and spectra were assessed and obtained by the same calibrated examiner (XX).

Table 1.
Spectral Site Distributions

Group	Buccal	Lingual	Upper	Lower	MB/ML	IP	Total
Periodontitis	41	6	24	23	1	46	47
Gingivitis	91	7	46	52	23	75	98
Healthy	59	3	39	23	45	17	62

MB = mid-buccal; ML = mid-lingual; IP = interproximal.

Acquisition of Optical Spectra

Spectra were collected using a portable spectrograph interfaced to a customized bifurcated fiber-optic probe designed for use in the oral cavity. The intraoral probe was described in detail previously.¹² The outer fibers of the probe were coupled to the entrance slit of the spectrograph and collected light subsequently backscattered from the tissue (Fig. 1). The inner fibers at the bifurcated end of the probe were coupled to a 5-W tungsten halogen light source** that provided a stable light output. Each reflectance spectrum consisted of 16 coadded scans collected using a 0.03-second integration time. The spectral range between 500 and 1,100 nm at 5-nm resolution was used. A 99% reflectance standard^{††} was used as a reference to convert raw data into reflectance spectra. During the collection of the spectra, the patients were comfortably seated in a relaxed, standard semi-reclined position on a dental chair.

Calculation of Hemodynamic Indices From Optical Spectra

Derivation of the relative contribution of Hb and HbO₂ to the optical attenuation spectrum obtained from tissue was described in detail previously.¹⁹ Briefly, a modified Beer-Lambert unmixing model that incorporates a non-parametric scattering loss function was used to determine the relative contribution of Hb and HbO₂ to the spectrum by using the known absorption coefficients of Hb and HbO₂ to fit the spectrum. The visible region between 510 and 620 nm of the measured tissue attenuation spectrum, A_λ , was modeled as a sum of two parametric terms, Hb and HbO₂, that contribute to the spectrum and a non-parametric term $m(\lambda)$ 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 HbO₂ per unit photon pathlength were estimated by solving an equation using a non-iterative partially linear method based on kernel smoothing, as first described by Speckman.²⁰ Tissue hemoglobin oxygen saturation (StO₂) and total hemoglobin (tHb), a measure of tissue perfusion, were derived from the predicted Hb and HbO₂ relative concentrations as discussed next.

Interoperator and Interinstrument Comparisons

We have examined the variability potentially generated from different operators or instruments. To this end, we used a double-matched pair study (Fig. 2). Matched reflectance spectra were collected on two instruments with two different operators. Each user acquired measurements from 10 patients using two instruments. The 10 patients used for the interoperator, interinstrument test were not part of the patient population used in the subsequent periodontitis study.

Bland–Altman plots provide a convenient graphic 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 inspect visually whether the difference and its variance are 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 testing simultaneously the equality of the means and variances of the paired measurements.

Bland–Altman plots and accompanying statistical BB tests were made for the paired StO₂ parameters derived from measured optical reflectance spectra to determine the interoperator and interinstrument variation of NIR measures of tissue inflammation.

Statistical Analyses

The hemodynamic indices Hb, HbO₂, StO₂, and tHb 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. The unequal Tukey honestly significant difference 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 a software program.

RESULTS

Interoperator and Interinstrument Plots

Initially, we assessed the variability generated from different operators and instruments by the matched pair experiments depicted in Figure 3. First, we used the interinstrument Bland–Altman plot to compare the pairwise difference in StO₂ to the pairwise mean StO₂ measurement from two identical instruments (Fig. 3A). The plot divides measurements made by the two different users. The blue circles mark the 20 measurements made by User 1 on each instrument, whereas the red triangles indicate the 20 measurements made by User 2 on each instrument. The random scatter suggests no systematic bias and good agreement between the two operators regardless of which user made the measurement. A separate regression analysis was performed on the data from each user. The Bland–Altman plot shows no obvious trend in either the difference or variance as a function of the average. Regression coefficients were not significantly different from zero. No obvious difference was observed between the measurements made between users. Visual inspection of the plot and the BB tests suggest the difference and its variance are constant as a function of the average.

Figure 1. Block diagram of in situ optical oral diagnostic equipment.

Figure 2. Experimental design for the interoperator and interinstrument comparisons.

The interoperator Bland–Altman plot, as shown in Figure 3B, was used to analyze the pairwise difference in StO₂ versus the pairwise mean StO₂ measurement from two different instruments. Each user made 20 paired measurements on two instruments. The plot divides the measurements made on the different instruments. Again, the random scatter in the Bland–Altman plot suggests good agreement and no systematic bias between two identical instruments. The plot also indicates that the agreement between operators is consistent between the two instruments that were investigated. The BB test shows that the regression coefficients are not significantly different from zero, thus corroborating the visual assessment of the Bland–Altman plot.

Clinical Parameters From the Study Group

The mean age of the recruited patients was 46.5 years, with a range from 22 to 82 years, and 23 (45.1%) of the patients were male patients. Based on the PD, clinical AL, and dental presence as shown in Table 2, the most frequent kind of periodontitis detected was of the chronic form. The mean PD for the periodontitis group was 5.81 – 1.48 mm, whereas that in the gingivitis group was 2.48 – 0.65 mm. Regarding the clinical AL, the mean was 6.40 – 2.28 mm for the periodontitis group, with the upper molars being the most affected teeth, and 3.29 – 1.34 mm in the gingivitis group.

Periodontal Hemodynamics Extrapolated From Optical Spectra

Table 3 lists the Pearson product moment correlation coefficients between the hemodynamic indices. Given the definition of the tHb parameter, it is expected to be highly correlated with Hb and HbO₂ parameters extracted from the spectra. StO₂ shows a modest negative correlation ($r = -0.41$) with Hb with a similar magnitude but positive correlation ($r = 0.45$) with HbO₂, whereas being poorly correlated ($r = -0.12$) with tHb. The relative concentrations of Hb and HbO₂ extracted from the visible region of the 207 optical attenuation spectra collected from 51 patients display a modest positive correlation ($r = 0.54$).

Figure 4 presents the mean (– 95% confidence interval) relative concentrations of Hb and HbO₂ parameters obtained from the optical attenuation spectra of periodontal tissues measured in vivo for the three groups. The relative concentrations of Hb in periodontal tissues increase from healthy sites to gingivitis to periodontitis, whereas the relative concentrations of HbO₂ show a decreasing trend, respectively. The study shows significant increases in Hb in sites with gingivitis and periodontitis ($P < 0.01$) compared to healthy sites, but the Hb parameter was not significantly different between sites with gingivitis and periodontitis ($P = 0.2$). The concentration of HbO₂ in sites with periodontitis shows a significant decrease compared to healthy sites ($P < 0.01$) and sites with gingivitis and periodontitis ($P = 0.02$). However, the decrease in mean HbO₂ between healthy and gingivitis sites is not considered significant ($P = 0.6$).

The StO₂ and tHb (closely related to tissue blood volume) are presented in Figure 5. StO₂ significantly decreases from healthy sites to sites identified as having gingivitis ($P < 0.01$), and the sites diagnosed as periodontitis have significantly lower oxygenation ($P = 0.015$) compared to sites with gingivitis (Fig. 5A). The tHb parameter, an indicator of perfusion, shows only a modest difference between healthy and inflamed gums (Fig. 5B).

Table 2.
Clinical Measurements From Each Group

Group	Clinical Parameters	
	PD	Clinical AL
Healthy (n = 62)	1.81 ± 0.76	3.18 ± 1.32
Gingivitis (n = 98)	2.48 ± 0.65	3.29 ± 1.34
Periodontitis (n = 47)	5.81 ± 1.48	6.40 ± 2.28

Table 3.
Correlations Between Hemodynamic Parameters Derived From Optical Spectra

Hemodynamic Parameter	StO ₂	tHb	Hb	HbO ₂
StO ₂		–0.12	–0.41	0.45
tHb	–0.12		0.94	0.79
Hb	–0.41	0.94		0.54
HbO ₂	0.45	0.79	0.54	

N = 207 spectra from 51 patients.

DISCUSSION

Because destructive periodontitis is an inflammation-mediated disease, alterations related to the local inflammatory response of tissue are the key to periodontal diagnosis and prognosis. Signs of clinical inflammation have long been used to determine the clinical status of the periodontium. For instance, BOP remains one of the most commonly used diagnostic parameters in periodontal diagnosis. However, it is not a sensitive and reliable measurement because of many confounding factors that can significantly affect its diagnostic accuracy. For these reasons, a number of new inflammatory parameters have been advocated as potential periodontal diagnostic indicators, such as pocket oxygen tension, oxygen saturation of hemoglobin, and temperature.²¹⁻²⁶ However, the methodologies available have been limited, and those available are often inconvenient or impractical for routine usage.

Nevertheless, as demonstrated in this study, the optical spectroscopy system designed and built for use in the oral cavity can readily acquire spectra from periodontal tissues, and the data extracted from these spectra provide detailed site-specific information on multiple aspects of periodontal inflammation. Among the various disease-specific variations derived from the raw spectra, the tissue oxygenation in patients with gingivitis and periodontitis was significantly decreased compared to healthy controls (Fig. 5). This was largely caused by the significant increase in deoxygenated hemoglobin in the periodontitis sites compared to sites exhibiting gingivitis (Fig. 4). The concentration of tHb showed an increasing trend, proceeding from healthy to gingivitis and periodontitis sites. These results generated from a Chinese ethnic group are in full concordance with the findings from our previous study in a mixed ethnic population.¹²

Vascular dilation and increased permeability are among the most consistent pathohistologic findings in gingival inflammation, which result in changes in the color and consistency of gingiva and increased gingival crevicular fluid.²⁷ Therefore, gingival blood flow, which is intimately associated with inflammation, may serve as a diagnostic and prognostic marker for periodontal disease. Our data with increased tHb index in inflammatory periodontal sites (both gingivitis and periodontitis) probably reflects such altered vascularity in the periodontal inflammation.

Our study also revealed that StO₂ was significantly decreased in both gingivitis and periodontitis sites, especially in destructive periodontitis, compared with control sites. Such decreased StO₂ likely reflects tissue hypoxia resulting from an ongoing inflammatory response, leading to increased oxygen consumption.²⁸ 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.^{29,30} It has also been demonstrated previously that StO₂ correlates well with oxygen tension in periodontal pockets because oxygen tension tends to decline as PD increases.²¹ Because of the fact that the StO₂ is not measurable clinically, optical spectroscopy can serve as a supplementary tool for monitoring local oxygenation and provide essential information about local inflammation.

Figure 3. Assessment of interoperator and interinstrument errors. A) Int

Figure 4. Relative concentrations of Hb and HbO₂ from healthy, gingivitis, and periodontitis sites. Relative hemoglobin concentrations were calculated using the visible region (510 to 620 nm) of the reflected light spectrum. * Represents a significant difference from healthy sites, P < 0.01. Vertical bars denote 0.95 confidence intervals.

Figure 5. Percent StO₂ (A) tHb indices (B) derived from the relative concentrations of Hb and HbO₂. 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.

Furthermore, because periodontal inflammation largely manifests a complex interaction between pathogenic bacteria and host defense mechanisms of local tissues, multiple indices generated from NIR measurement would nicely reveal various facets of the dynamic inflammatory process. Combining these indices can be a great advantage in the clinic and may provide a robust diagnostic algorithm with increased sensitivity and specificity.

It was almost a decade ago that the scope for an ideal diagnostic test for periodontal inflammation was tentatively proposed.³¹ An ideal diagnostic technology should be quantitative; a highly sensitive method capable of analyzing a single periodontal site in health and disease; reproducible; highly specific; simple to perform; a rapid, one- or two stage procedure; non-invasive; versatile in terms of sample handling, storage, and transport; amendable to chairside use; economical; and dependent on simple and robust instrumentation. Among other attractive initiatives in this field, the approach with optic spectroscopy is one recently developed means that fits most of the features of an ideal diagnostic tool for periodontitis. For example, this method can pinpoint the inflamed status of a single periodontal site, is rapid and simple to perform, and is capable of providing instantaneous site-specific results at the chairside. More important, the measurement is noninvasive because it uses non-ionizing radiation or light and is economic with no reagents or contrast agents needed. Furthermore, the results generated from the current study and those from previous publications at entirely distinct geographic locations prove its high reproducibility.¹² Both studies revealed a consistently decreasing trend in tissue oxygenation from healthy sites to sites exhibiting gingivitis and periodontitis. There was a significant difference in oxygenation between healthy sites and sites with periodontitis. This is further proven by the favorable comparison of interoperator and intraoperator variability carried out in this study (Fig. 3).

CONCLUSIONS

The present results generated from this much larger patient group with a different ethnic background at a different location further confirm the findings of the preliminary cross-sectional clinical study.¹² It clearly demonstrates that based on these optical parameters, one can readily differentiate inflammatory periodontal sites from healthy sites and distinguish two different types of inflammatory periodontal conditions: periodontitis and gingivitis. The philosophy behind the development of such a test is that the earlier active disease is diagnosed, the less invasive, time consuming, and costly the required treatment, and the better the long-term prognosis for patients with destructive disease. Furthermore, with the recognition that risk groups and unpredictable disease patterns exist, the benefits of objective testing for initial diagnosis and for the long-term maintenance of periodontal patients become clear. Optical spectroscopy represents an attractive device for periodontal diagnosis and monitoring by measuring multiple subclinical inflammatory indices. Compared to current clinical means, such as manual and electric probes, the optical measurement is painless, non-invasive, and instant. Once the equipment is in place, it is very inexpensive to operate and maintain, and easy to fit in a confined dental office at chairside. Future studies

are warranted to establish a large database for identifying optical classifiers to distinguish different types of periodontal diseases and determine disease activity, essential steps for the anticipated automation.

ACKNOWLEDGMENTS

This study was generously supported by the Natural Sciences and Engineering Research Council of Canada and the Canadian Institutes for Health Research, Ottawa (KZL, MGS), and Johnson & Johnson, New Brunswick, NJ (KZL, MGS). The authors report no conflicts of interest related to this study.

REFERENCES

1. Burt B; Research, Science and Therapy Committee of the American Academy of Periodontology. Position paper: Epidemiology of periodontal diseases. *J Periodontol* 2005;76:1406-1419.
2. Armitage GC; Research, Science and Therapy Committee of the American Academy of Periodontology. Diagnosis of periodontal diseases. *J Periodontol* 2003; 74:1237-1247.
3. Kwok V, Caton JG. Commentary: Prognosis revisited: A system for assigning periodontal prognosis. *J Periodontol* 2007;78:2063-2071.
4. Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. *J Periodontol* 2007;78(Suppl. 7):1387-1399.
5. Tomar SL. Public health perspectives on surveillance for periodontal diseases. *J Periodontol* 2007;78(Suppl. 7):1380-1386.
6. Xiang XM, Sowa MG, Iacopino AM, et al. An update on novel non-invasive approaches for periodontal diagnosis. *J Periodontol* 2010;81:186-198.
7. Sowa MG, Leonardi L, Payette JR, Cross KM, Gomez M, Fish JS. Classification of burn injuries using nearinfrared spectroscopy. *J Biomed Opt* 2006;11:054002.
8. 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.
9. Sowa MG, Payette JR, Mantsch HH. Near-infrared spectroscopic assessment of tissue hydration following surgery. *J Surg Res* 1999;86:62-69.
10. Hanioka T, Shizukuishi S, Tsunemitsu A. Hemoglobin concentration and oxygen saturation of clinically healthy and inflamed gingiva in human subjects. *J Periodontal Res* 1990;25:93-98.
11. Scott DA, Singer DL. Suppression of overt gingival inflammation in tobacco smokers: Clinical and mechanistic considerations. *Int J Dent Hyg* 2004;2:104-110.
12. Liu KZ, Xiang XM, Man A, et al. In vivo determination of multiple indices of periodontal inflammation by optical spectroscopy. *J Periodontal Res* 2009;44: 117-124.
13. Otal EH, In'ō'n FA, Andrade FJ. Monitoring the temperature of dilute aqueous solutions using near-infrared water absorption. *Appl Spectrosc* 2003;57:661-666.
14. Liu KZ, Shi MH, Mantsch HH. Molecular and chemical characterization of blood cells by infrared spectroscopy: A new optical tool in hematology. *Blood Cells Mol Dis* 2005;35:404-412.

15. Capraro GA, Mader TJ, Coughlin BF, et al. Feasibility of using near-infrared spectroscopy to diagnose testicular torsion: An experimental study in sheep. *Ann Emerg Med* 2007;49:520-525.
16. Stothers L, Shadgan B, Macnab A. Urological applications of near infrared spectroscopy. *Can J Urol* 2008;15:4399-4409.
17. Yoshitani K, Kawaguchi M, Tatsumi K, Kitaguchi K, Furuya H. A comparison of the INVOS 4100 and the NIRO 300 near-infrared spectrophotometers. *Anesth Analg* 2002;94:586-590.
18. Yoshie H, Galicia JC, Kobayashi T, Tai H. Genetic polymorphisms and periodontitis. *Int Congr Ser* 2005; 1284:131-139.
19. 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.
20. Speckman P. Kernel smoothing in partial linear models. *J Royal Statistical Society: Series B* 1988;50:413-436.
21. 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.
22. 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.
23. 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.
24. 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.
25. 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.
26. Baab DA, Oberg A, Lundstro"m A. Gingival blood flow and temperature changes in young humans with a history of periodontitis. *Arch Oral Biol* 1990;35:95-101.
27. Zoellner H, Chapple CC, Hunter N. Microvasculature in gingivitis and chronic periodontitis: Disruption of vascular networks with protracted inflammation. *Microsc Res Tech* 2002;56:15-31.
28. 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.
29. 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.
30. Loesche WJ. Oxygen sensitivity of various anaerobic bacteria. *Appl Microbiol* 1969;18:723-727.
31. Chapple ILC. Periodontal disease diagnosis: Current status and future developments. *J Dent* 1997;25:3-15.