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On site noninvasive assessment of peri-implant inflammation by optical spectroscopy

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Background and Objective: Optical spectroscopy has been proposed to measure regional tissue hemodynamics in periodontal tissue. The objective of this study was to further evaluate the diagnostic potential of optical spectroscopy in peri-implant inflammation *in vivo* by assessing multiple inflammatory parameters (tissue oxygenation, total tissue hemoglobin, deoxyhemoglobin, oxygenated hemoglobin and tissue edema) simultaneously.

Material and Methods: A cross-sectional study was performed in a total of 64 individuals who presented with dental implants in different stages of inflammation. In brief, visible–near-infrared spectra were obtained, processed and evaluated from healthy ($n = 151$), mucositis ($n = 70$) and peri-implantitis sites ($n = 75$) using a portable spectrometer. A modified Beer–Lambert unmixing model that incorporates a nonparametric scattering loss function was employed to determine the relative contribution of each inflammatory component to the overall spectrum.

Results: Tissue oxygenation at peri-implantitis sites was significantly decreased ($p < 0.05$) when compared with that at healthy sites, which was largely due to an increase in deoxyhemoglobin and a decrease in oxyhemoglobin at the peri-implantitis sites compared with the mucositis and healthy sites. In addition, the tissue hydration index derived from the optical spectra in mucositis was significantly higher than that in other groups ($p < 0.05$).

Conclusion: In summary, the results of this study revealed that hemodynamic alterations can be detected around diseased peri-implant sites by optical spectroscopy, and this method may be considered an alternative and feasible approach for the monitoring and diagnosis of peri-implant diseases.

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Peri-implantitis is a destructive infectious inflammatory disease, which presents with a prevalence ranging from 7 to 20% and affects soft and hard tissue around osseo-integrated dental implants under functional loading. Mucositis is a reversible inflammation confined to peri-implant soft tissues, which may progress to peri-implantitis and has a prevalence of about 65% (1–5). Peri-implant and periodontal diseases present some common clinical and microbiological characteristics, including pockets, bleeding on probing, bone loss, suppuration and a complex pathogenic microbiota (1,6,7). However, some histological and anatomical aspects differ substantially between peri-implant and periodontal tissue, potentially altering the course of the

inflammatory process around implants compared with natural teeth. The peri-mucosal seal around dental implants, for example, is comprised of collagen fibers in a simple nonattached and circular arrangement that differs from the attached and perpendicular arrangement of the fibers around natural teeth (8–11).

To date, the following parameters are considered the only available tools in the differential diagnosis of peri-implant diseases: (i) implant mobility, which is an indicator of lack of osseointegration; (ii) sulcular probing, which is used to monitor marginal bleeding (an indicator of inflammation), probing depth (the distance between the soft tissue margin and the bottom of the peri-implant pocket or sulcus) and suppuration; and (iii) radiography, which is widely employed to detect bone loss around dental implants (1,6,7,12). However, these tools are not sensitive enough to accurately detect early lesions, progressive lesions and sometimes even late lesions, which are important in avoiding further loss of soft and hard peri-implant tissues and eventually implant failure. Therefore, there is an increased interest in finding alternative accurate methods for the monitoring and diagnosis of peri-implant diseases.

As peri-implant diseases are local infections initiated by accumulation of bacterial biofilm (6,7), microbial testing has been proposed for diagnosis and prognosis of peri-implant lesions. In a 2 year longitudinal study, presence of one or more pathogenic species (i.e. *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Porphyromonas gingivalis* and *Treponema denticola*) was reported to enhance the prognostic features of bleeding on probing in predicting disease activity (13). In addition, the presence of specific biomarkers, including cytokines, enzymes and proteases, in peri-implant crevicular fluid or saliva has been suggested as a useful tool in monitoring healthy or diseased peri-implant tissues (14–17). However, considering that such inflammatory biomarkers work more often in a network-like organization than individually, one or two specific markers are very unlikely to stand alone as valuable diagnostic tools for peri-implant diseases.

Based on the optical characteristics of chemical components related to tissue inflammation, it is possible to characterize the inflammatory process by optical infrared spectroscopy. It has been proposed that several absorption bands in the visible and near-infrared spectral region reflect key inflammatory events (18,19). For instance, tissue edema, a clinical sign commonly used as a marker of mucosal inflammation (20,21), can be measured using near-infrared spectroscopy (18,19,22–24). In addition, optical spectroscopy can gauge the relative concentrations of oxygenated hemoglobin (HbO_2) and deoxygenated hemoglobin (Hb) by fitting optical attenuation spectra to the known optical properties (extinction coefficients) of oxygenated hemoglobin and deoxygenated hemoglobin (18,22–26). A previous study by our research group showed that optical spectroscopy could indeed determine various inflammatory indices simultaneously in the periodontal tissues, representing a potential means for diagnosis of inflammation at specific periodontal sites (22). In principle, this newly emerging technology can differentiate periodontitis from gingivitis and healthy periodontal sites based on these optical parameters, specifically, tissue perfusion, oxygenation and hydration (22). Based on the limitations of the currently available methods for the diagnosis of peri-implant diseases, in this study we propose that optical spectroscopy might also be a feasible alternative for the detection of peri-implant inflammation and possibly for the differentiation of peri-implantitis and mucositis from healthy sites.

Material and methods

Subject population

A total of 64 systemically healthy subjects were recruited from the population referred to the Oral Implantology Clinic of Guarulhos University (Guarulhos, São Paulo, Brazil) from June 2009 to July 2009. Medical and dental histories were obtained, and full-mouth periodontal and peri-implant examinations were performed. Subjects who fulfilled the following inclusion/exclusion criteria were invited to participate in the study. The study protocol was explained to each subject, and signed informed consent was obtained. This study protocol was approved by Guarulhos University's Ethics Committee in Clinical Research (CEP no. 113/2008) and National Research Council Research Ethics Board (REB).

Inclusion/exclusion criteria

Inclusion criteria were those subjects who were totally or partly edentulous, and had been treated with at least one screw-shaped machined-surface titanium implant that had been functional for at least 1 year (6). They were non-smokers, not pregnant or lactating, systemically and periodontally healthy or periodontally treated and engaged in supportive periodontal therapy (for partly edentulous subjects). Exclusion criteria were those subjects who had moderate to advanced chronic periodontitis (i.e. bleeding on probing in more than 30% of the subgingival sites or any site with probing depth > 5 mm), had been treated with antibiotics or anti-inflammatory drugs and local antimicrobial agents within the preceding 6 months, and had peri-implant therapy within 6 months. To avoid occlusal interference, those subjects who had an implant-supported prosthesis with mobile abutments and/or screws, as well as fractured prosthetic crowns of ceramic or resin, were also excluded.

Clinical and radiographic examination

Clinical examinations were performed by the same trained examiner after the spectral acquisitions (X.M.X.). The following parameters were assessed at six sites in each implant (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) using a periodontal probe: (i) visible plaque accumulation, assessed as the plaque index, the presence (score 1) or absence (score 0) of plaque along the gingival–mucosal margin (27);

(ii) mucosal marginal bleeding, assessed as the presence (score 1) or absence (score 0) of bleeding obtained by running a probe along the soft tissue margin without probe penetration inside the sulcus or pocket (27); (iii) bleeding on probing, obtained 20 min after recording mucosal marginal bleeding, when the presence (score 1) of bleeding on probing was considered when bleeding occurred up to 15 s after gentle probe penetration into the sulcus or pocket; (iv) suppuration, assessed as the presence (score 1) or absence (score 0) of spontaneous suppuration or suppuration on probing; and (v) probing depth (in millimeters), assessed as the distance between the mucosal margin and the bottom of the sulcus/pocket. The probing depth measurements were recorded to the nearest millimeter using a North Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA).

Standardized intra-oral periapical radiographs were obtained, using a dental X-ray machine equipped with a 35-cm-long cone. Exposure parameters were 70 kV (peak), 15 mA, and 0.25 s at a focus-to-sensor distance of 30 cm. The radiographs were captured with a digital camera and transferred to a personal computer. Image-processing software was used to store the digitized images. Subsequently, the images were displayed on a monitor, and linear measurements were taken using imaging software (Image Pro-Plus 4.5; Media Cybernetics Inc., Silver Spring, MD, USA). The radiographs were analyzed for peri-implant bone loss (i.e. the linear distance in millimeters between the implant shoulder and the first clear bone-to-implant contact).

Definition of diagnostic peri-implant sites

Based on the clinical and radiographic parameters, the peri-implant sites were assigned to one of the following groups: healthy, defined as absence of mucosal marginal bleeding, bleeding on probing, suppuration and radiographic bone loss; mucositis, defined as mucosal marginal bleeding and/or bleeding on probing and absence of radiographic bone loss and suppuration; or peri-implantitis, defined as probing depth ≥5 mm with bleeding on probing and/or suppuration and concomitant radiographic saucer-shaped osseous defects >3 mm (6,7,17). All spectra were collected prior to clinical measurements from 75 peri-implantitis sites, 70 mucositis sites and 151 normal sites, as shown in Table 1.

Table 1. Spectral site distributions

	Buccal	Lingual	Upper	Lower	MB/ML	IP	Total
Peri-implantitis	43	32	47	28	17	58	75
Mucositis	39	31	33	37	38	32	70
Healthy	94	57	72	79	72	79	151

Abbreviations: IP, interproximal; MB, mid-buccal; ML, mid-lingual.

Acquisition of optical spectra

Spectra were collected using a portable PDA512-ISA spectrograph (Control Development Inc., South Bend, IN, USA) interfaced to a customized bifurcated fiberoptic probe designed for use in the oral cavity (Fiberguide Industries, Stirling, NJ, USA). The intra-oral probe was described in detail previously (22). The outer fibers of the probe are coupled to the entrance slit of the spectrograph and collect light subsequently back-scattered 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 (Spectral Products, Putnam, CT, USA) that provides a stable light output. Each reflectance spectrum consisted of 16 co-added scans collected using a 0.03 s integration time. The spectral range between 500 and 1100 nm at 5 nm resolution was used. A 99% Spectralon® reflectance standard (LabSphere, North Sutton, NH, USA) was used as a reference to convert raw data into reflectance spectra. During the collection of the spectra, the subjects were comfortably seated in a relaxed, standard semi-reclined position on a dental chair, and the regular overhead dental operating light was turned off to avoid any potential interference.

Calculation of hemodynamic indices from optical spectra

The derivation of the relative contribution of Hb and HbO_2 to the optical attenuation spectrum obtained from tissue was described in detail previously (28). Briefly, a modified Beer–Lambert unmixing model that incorporates a nonparametric scattering loss function was used to determine the relative contribution of Hb and HbO_2 to the spectrum by using the known absorption coefficients of Hb and HbO_2 to fit the spectrum. The visible region between 510 and 620 nm of the measured tissue attenuation spectrum, A_λ, was modeled as a sum of two parametric terms, Hb and HbO_2, that contribute to the spectrum and a nonparametric term $m(\lambda)$ modeling a vector of covariates, primarily the Rayleigh and Mie scattering losses that contribute to the attenuation of measured light.

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

The concentrations of Hb and HbO_2 per unit photon path length were estimated by solving the equation (28) using a noniterative partially linear method based on kernel smoothing, as first described by Speckman (25). Tissue hemoglobin oxygen saturation, S_tO_2, and a measure of tissue perfusion, tHb, were derived from the predicted Hb and HbO_2 relative concentrations as follows:

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

Statistical analysis

The hemodynamic indices, Hb, HbO_2, S_tO_2 and tHb, derived from the 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 HSD was

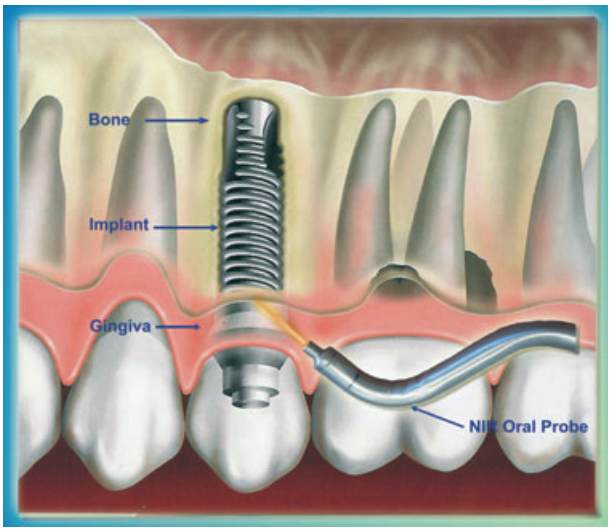

Fig. 1. Illustration of proposed near-infrared (NIR) oral probe as a noninvasive diagnostic tool for the assessment of peri-implantitis in the clinical setting.

used for the *post hoc* pairwise comparisons of mean differences between clinical groups. Pearson product–moment correlation coefficients were calculated between the hemodynamic indices to summarize the linear association between the variables. Statistical calculations were performed with Statistica 7.1 (Statsoft, Tulsa, OK, USA).

Results

Clinical parameters from the study group

The mean age of the recruited patients was 53.6 ± 11.2 years, with a range from 26 to 82 years, and 16 (25.4%) of the subjects were male. The mean pocket depth for the peri-implantitis group was 5.7 (±0.9) mm, while that in the mucositis group was 2.4 (±0.6) mm. Regarding the attachment loss, the mean was 6.2 (±1.5) mm for the peri-implantitis group, with the upper molars being the most affected region, and 3.1 (±1.5) mm for the mucositis group.

Representative pictures of dental implants diagnosed as healthy and peri-implantitis are displayed in Fig. 2. The probing depth of healthy peri-implant sites were <2 mm (Fig. 2A), and radiography revealed no bone loss, since bone tissue filled up the first thread of the implant (indicated by the arrows). In peri-implantitis, the peri-implant tissue exhibited severe inflammation, i.e. bleeding on probing, deep probing depth (7 mm) and suppuration (Fig. 2B). The radiographic examination of this implant showed peri-implant bone loss, extending beyond the fourth thread of the implant (indicated by the arrow).

Peri-implant hemodynamics extrapolated from optical spectra

Based on the information embedded in the spectra, several important local peri-implant hemodynamic indices were calculated for the three groups of sites. The most striking difference was the oxygen saturation of the tissue presented in Fig. 3. There was a significantly lower oxygen saturation in sites diagnosed as peri-implantitis when compared with healthy sites ($p < 0.05$). As mentioned in the material and methods section, the tissue hemoglobin oxygen saturation is derived from the predicted Hb and HbO_2 relative concentrations. Figure 4 presents the mean (±95% confidence interval) relative concentrations of Hb and HbO_2 parameters obtained from the optical attenuation spectra measured *in vivo* from healthy, mucositis and peri-implantitis sites. Although not statistically significant, there was a trend towards a higher relative concentration of Hb and a lower relative concentration of HbO_2 in peri-implantitis sites ($p > 0.05$). Moreover, the tissue hydration index was higher in diseased sites than in healthy sites, as shown in Fig. 5. In particular, the

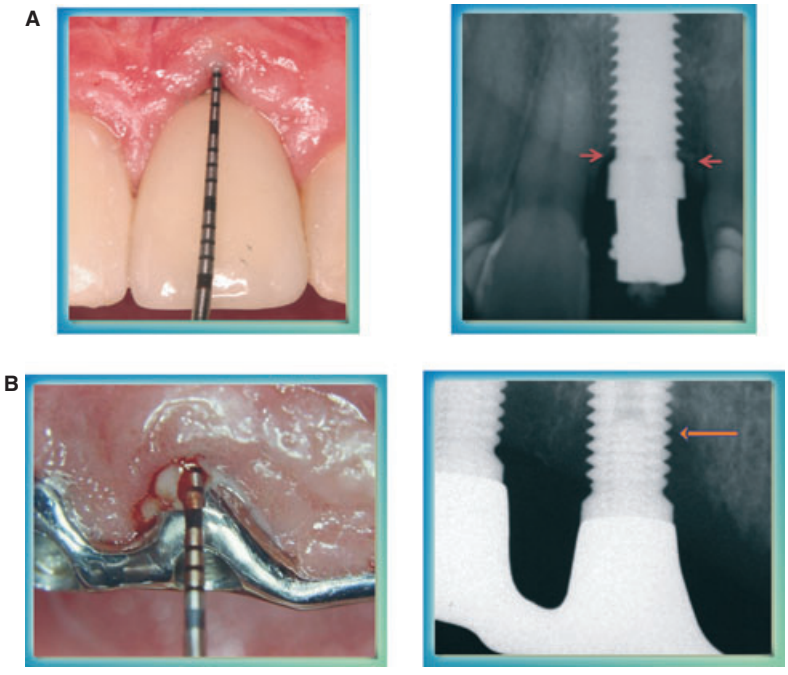

Fig. 2. Representative clinical implant pictures and associated radiographs from healthy control (A) and peri-implantitis groups (B).

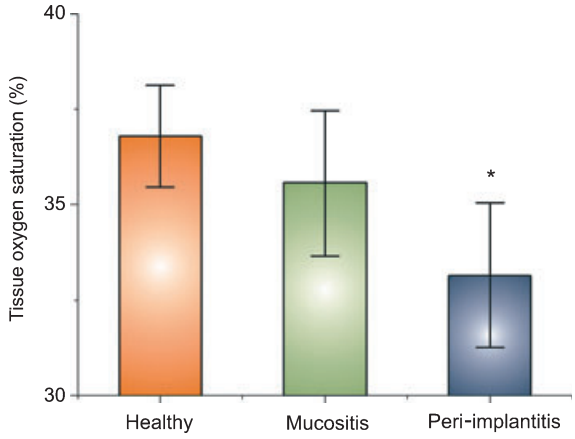

Fig. 3. The percentage of tissue hemoglobin oxygen saturation derived from the relative concentrations of Hb and HbO_2. Indices are compared between healthy, mucositis and peri-implantitis sites. * Significant difference from healthy sites, $p < 0.05$. Vertical bars denote 0.95 confidence intervals.

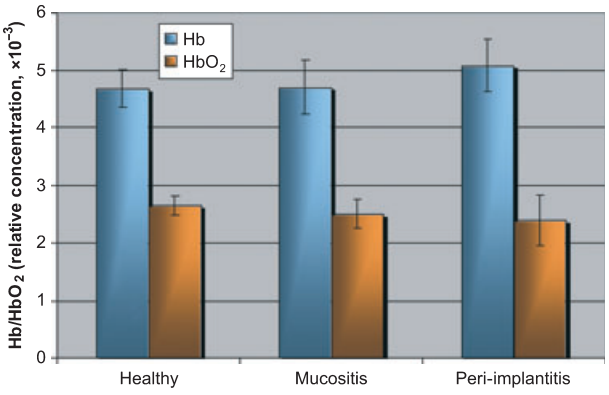

Fig. 4. Relative concentrations of Hb and HbO_2 from healthy, mucositis and peri-implantitis sites. Relative hemoglobin concentrations were calculated using the visible region (510–620 nm) of the reflected light spectrum.

increased tissue hydration in mucositis sites was significant when compared with both healthy ($p < 0.05$) and peri-implantitis sites, whereas no significant difference was seen between healthy and peri-implantitis sites.

Discussion

The development of more accurate methods for diagnosis of peri-implant inflammation is crucial for the prevention of dental implant failures and monitoring of the outcomes of peri-implant therapies. Therefore, this study assessed, for the first time, multiple inflammatory parameters (tissue oxygenation, total tissue hemoglobin, deoxyhemoglobin, oxygenated hemoglobin and tissue edema) around healthy, mucositis and peri-implantitis sites by near-infrared spectroscopy, which has recently been proposed to measure tissue hemodynamics in periodontal tissues (22). In general, the results demonstrated that hemodynamic changes can be clearly detected around diseased peri-implant sites by this convenient noninvasive technique, especially in relation to tissue oxygenation and tissue hydration. The data provide new insights into the diagnosis of peri-implant diseases.

Peri-implant diseases are diagnosed mainly on their clinical and radiographic signs, including bone level, clinical attachment loss, probing depth, bleeding on probing, implant mobility and suppuration (6,7,17,29–34). Although clinical and radiographic parameters are currently well accepted for the diagnosis of peri-implant lesions, these methods inherit some undesirable limitations that might lead to either an under- or overdiagnosis of the presence and severity of the disease, especially when taken alone. Therefore, considerable research has been done in the past on peri-implant inflammation with the intent of developing new alternative diagnostic tools to overcome these limitations. Optical spectroscopy provides a means to assess peri-implant inflammation rapidly, conveniently and without costly consumables or reagents.

As demonstrated in the present study, the spectral profile for the peri-implant sites resembled those observed for periodontal tissues (22), despite some anatomical and histological differences between teeth and dental implants, which include periodontal ligament space and fiber arrangements (9,35,36). For instance, tissue oxygen saturation was significantly lower in the peri-implantitis group than in the healthy and mucositis groups (Fig. 3). However, subtle differences were observed between the previous study (22) that used optical spectroscopy to characterize periodontal inflammation and the present study examining peri-implant inflammation. For example, there is a significant offset between the measured values of tissue oxygen saturation between the two studies. The fact that the two studies used different fiberoptic probes and spectrometers makes absolute comparison of the oxygenation values difficult. In addition, there was not as distinct a difference in oxygenation between the healthy and mucositis sites as observed between healthy and gingivitis sites measured in our previous study. Decreased oxygen saturation may be explained by tissue hypoxia resulting from the infectious inflammatory process in peri-implantitis (37,38). In fact, it has been demonstrated that active inflammation is characterized by dramatic shifts in tissue metabolism, including low levels of oxygen (hypoxia), subsequent accumulation of lactate and metabolic acidosis (39,40). In healthy tissues, the oxygen tension is usually between 20 and 70 mmHg, whereas in

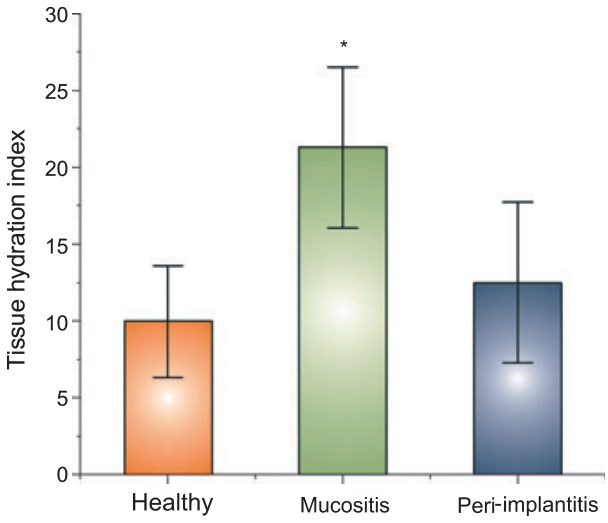

Fig. 5. Comparison of relative tissue hydration index between healthy, mucositis and peri-implantitis sites. * Significant difference from healthy sites, $p < 0.01$. Vertical bars denote 0.95 confidence intervals.

diseased tissues, with transient or chronic areas of hypoxia, the oxygen tension is < 10 mmHg. The microenvironmental metabolic changes (e.g. hypoxia) are related to profound recruitment of inflammatory cells, such as neutrophils and monocytes, that contribute to disease progression (37,38). It has previously been demonstrated that tissue oxygen saturation correlates with oxygen tension in periodontal pockets, because oxygen tension tends to decline as pocket depth increases (39,40). Another possible explanation for the low tissue oxygen saturation observed in the peri-implantitis sites may be the colonization of the pocket by specific pathogens. The peri-implantitis sites are predominately colonized by gram-negative facultative or obligate anaerobic microrganisms (6,7,41,42) that are related to a decrease in the oxygen tension in deep pockets (39,40). Furthermore, the low tissue oxygen saturation observed in the peri-implantitis sites may relate to the fact that loss of osseo-integration is related to decreased vascular proliferation in peri-implant diseases (43,44).

Another important profile detected in this study was the hydration index, which was significantly higher in mucositis sites than that in peri-implantitis and health control sites (Fig. 5). This result was somewhat expected, since mucositis is the first stage of peri-implant disease, characterized by signs of acute inflammation, including vessel dilatation, increased intercellular spacing and accumulations of interstitial fluid and inflammatory exudates (7,32). Our finding parallels the clinical signs of mucositis, which is characterized by swelling and redness due to local edema and inflammation. However, when the disease progresses to peri-implantitis, the primary pathological changes are the destruction of the supporting bone along with a certain degree of inflammatory infiltrate (41,45,46). At this stage, the microbial challenge might change; therefore, the peri-implantitis sites may or may not be inflamed (6,23,24), reflecting the lower level of tissue hydration in our peri-implantitis group.

As neither tissue oxygen saturation nor subtle local edema is readily detectable clinically, our data suggest that optical spectroscopy could be a useful supplementary tool for diagnosis and monitoring of inflammation around dental implants. In principle, optical spectroscopy is an entirely noninvasive technique that uses low-energy radiation; spectra can be captured instantly; no consumables need to be purchased or developed; and minimal training is required to obtain reliable and reproducible data (22–24). This leads us to believe that optical spectroscopy can be an important tool in the near future, to help determine healthy and diseased dental implants, as well as to help distinguish between progressing lesions and lesions that represent the consequences of a previous disease (23,24).

In conclusion, the results of this proof-of-concept study reveal that hemodynamic alterations can be detected around diseased peri-implant sites by optical spectroscopy, offering a feasible approach for the monitoring and diagnosis of peri-implant diseases. Prospective longitudinal clinical studies are warranted to demonstrate the role of this tool in differentiating progressive from stable lesions, and in monitoring the effect of peri-implant therapies.

Acknowledgements

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