Non-invasive detection of periodontal disease using diffuse reflectance spectroscopy: a clinical study

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ABSTRACT

In clinical diagnostic procedures, gingival inflammation is considered as the initial stage of periodontal breakdown. This is often detected clinically by bleeding on probing as it is an objective measure of inflammation. Since conventional diagnostic procedures have several inherent drawbacks, development of novel non-invasive diagnostic techniques assumes significance. This clinical study was carried out in 15 healthy volunteers and 25 patients to demonstrate the applicability of diffuse reflectance (DR) spectroscopy for quantification and discrimination of various stages of inflammatory conditions in periodontal disease. The DR spectra of diseased lesions recorded using a point monitoring system consisting of a tungsten halogen lamp and a fiber-optic spectrometer showed oxygenated hemoglobin absorption dips at 545 and 575 nm. Mean DR spectra on normalization shows marked differences between healthy and different stages of gingival inflammation. Among the various DR intensity ratios investigated, involving oxy Hb absorption peaks, the R620/R575 ratio was found to be a good parameter of gingival inflammation. In order to screen the entire diseased area and its surroundings instantaneously, DR images were recorded with an EMCCD camera at 620 and 575 nm. We have observed that using the DR image intensity ratio R620/R575 mild inflammatory tissues could be discriminated from healthy with a sensitivity of 92% and specificity of 93%, and from moderate with a sensitivity of 83% and specificity of 96%. The sensitivity and specificity obtained between moderate and severe inflammation are 82% and 76% respectively.

Keywords: Diffuse reflectance spectroscopy, multi-spectral imaging, periodontal disease, gingival inflammation, oxygenated hemoglobin, non-invasive detection of inflammation, EMCCD camera

1. INTRODUCTION

Periodontal diseases are infectious diseases that destroy the periodontal attachment apparatus, resulting in the loss of tooth support. In India, the prevalence of gingivitis is 80-90% and the incidence of chronic periodontitis is 21-28%. Prevalence of chronic periodontitis increases steadily with age from 35.7% for the 30-39 years old age group to 89% for 80-90 years old age group. According to World Health Organization (WHO), advanced disease with deep periodontal pockets (6 mm or more) affects 10-15% of adults worldwide. American Academy of Periodontology (AAP) 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 periodontal diseases. Recent studies reveal that periodontal disease have been associated with adverse pregnancy outcomes, cardiovascular disease, stroke, pulmonary disease, and diabetics.

The diagnosis and classification of periodontal disease is still based almost entirely on traditional clinical assessments. To diagnose these diseases the dentist must rely upon factors such as 1) presence or absence of clinical signs of inflammation; 2) probing depths; 3) extent and pattern of loss of clinical attachment and bone; 4) patient’s medical and dental histories; and 5) presence or absence of miscellaneous signs and symptoms, including pain, ulceration, and amount of observable plaque and calculus. Even though the trained physician’s eye is a powerful tool, such evaluations are subjective and semi-quantitative. Therefore in periodontal diagnosis, uniform criteria have not been yet established.
Skin reactions are events that may be detected by the clinician’s sense of vision, touch and sometimes smell. Primarily, skin reactions such as erythema (redness), blanching (whitening), pigmentation (tanning) and induration are evaluated visually. The severity of the gingival inflammation is assessed, given an index ranging typically from 0 (for no inflammation) to 3 (for severe inflammation). Cutaneous edema and erythema are typically evaluated clinically using a visual analogue scale. Being subjective, this method depends on the experience of the clinician.

Diffuse reflectance spectroscopy (DRS) has been used in various studies to distinguish between healthy and malignant tissues. Subhash et al. proposed that the absorption intensity ratio (R545/R575) of oxygenated hemoglobin in tissues could be used for classification of different grades of oral cancer by studying the DR spectral features of surgically excised tissues. Later, in a clinical study Mallia et al. applied this DR ratio technique for in situ detection and discrimination of oral pre-malignant and cancerous lesions of the oral cavity. Further studies using DR spectral intensity ratio (R545/R575) have convincingly proved that this technique detects oral cancer with high sensitivity and specificity, and it effectively discriminates oral precancers of the tongue and lip that are difficult to diagnose from tissue autofluorescence.

Accurate and objective documentation through imaging is often required to monitor the evolution of the cutaneous inflammation. A longitudinal study with dogs revealed that relative oxygenated and deoxygenated hemoglobin concentrations increased while the oxygenation level reduced during inflammation. These observations were later confirmed by the same group in a cross-sectional study on humans. Taking advantage of the spectral properties of oxyhemoglobin (oxy-Hb) and deoxyhemoglobin (deoxy-Hb) in the visible part of the spectrum Zonios et al evaluated their apparent concentrations in the skin by spectroscopic analysis of the diffusely reflected light from tissue. It was shown that erythema is directly linked to increase in apparent concentration of oxy-Hb, while accumulation of deoxy-Hb depends on blood stasis. Recent advances in digital imaging hardware (optics, detectors, charge coupled devices, etc.) have enabled the development of spectral imaging, which in addition to the spectral information provided by DRS affords 2-D spatial information. More precisely, it provides the means to localize and quantify cutaneous erythema.

Numerous studies were done by various researchers all over the world to find clinically acceptable methods for the accurate non-invasive diagnosis and prognosis of periodontal disease. Objective quantitative evaluation of skin colour reactions using non-invasive instrumentation has been used since the early decades of the 20th century. Optical coherence tomography has been used for visual recording of periodontal tissue contour, sulcular depth and connective tissue attachment. The use of ultrasound as a potential clinical diagnostic device for periodontitis has been investigated by various research groups.

The recent reports by Liu et al. and Zili Ge et al. demonstrate that optical spectroscopy can simultaneously determine multiple inflammatory indices directly in periodontal tissues in vivo. DR imaging studies on periodontal disease, carried out by Zakian et al. based on oxy Hb and deoxy Hb crossovers at 615 and 460 nm demonstrated that the image intensity ratio at these wavelengths could be utilized to discriminate between healthy and diseased sites of periodontium. The images were processed using Matlab software to distinguish between gingiva and tooth.

In this clinical study, in vivo DR images of gingiva were recorded at the oxy Hb absorption dip at 575 nm and other selected wavelengths using a highly sensitive electron multiplying charge coupled device (EMCCD) camera to discriminate various grades of gingival inflammation from healthy gingiva. The results presented show the potential of DR spectral imaging technique employing oxygenated hemoglobin absorption as a screening tool in clinical diagnosis of periodontal inflammation and future disease progression.

2. MATERIALS AND METHODS

2.1 Clinical protocol

The study population consisted of 15 healthy volunteers with no clinical signs of periodontal inflammation and 25 patients with clinical signs of periodontal disease. The study was carried out at the Out Patient (OP) unit of the Government Dental College (GDC), Thiruvananthapuram, Kerala, India. The study protocol was approved by the Institutional Ethical committee of GDC (No. IEC/C/42-A/2011/DCT/dated 18-01-2011). Every volunteer was informed about the nature of the study and was asked to sign a consent form with rights given to withdraw at any point of the study. Patients were in the age group of 25-65 years. This study was conducted during April- November, 2011.
An experienced periodontist selected suitable patients as well as the healthy volunteers for the study. The clinical parameters were recorded for all participants. Both male and female healthy volunteers included in the study were in the age group of 20-35 yrs and maintained good oral hygiene. Patients, both male and female, with gingivitis and periodontitis were categorized based on the classification followed by the AAP. Periodontitis sites were defined as those with pocket depth (PD) ≥5 mm, clinical attachment level (CAL) ≥3 mm, with bleeding on probing (BOP) ≥25. 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. Spectral images of the upper and lower gingival papillae from canine to canine (ten sites in total) were acquired for every volunteer. 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 immunosuppressants) that may interfere with the study; 3) any systemic condition that may interfere with the study, such as inflammatory diseases or diabetes; and 4) volunteers having any previous medical history of cardiovascular problems.

2.2 Instrumentation

2.2.1 Point monitoring system

To obtain site specific spectra of erythema, a portable DR spectroscopic system (DRSS) was used in this clinical study. A tungsten halogen lamp (Model LS1-LL, Ocean Optics, Dundlin, Florida, USA) was used as the white light source to illuminate the gingival tissue through the 200 µm diameter fiber of a specially designed stainless steel hand piece with a diameter of 1 mm at its tip. The scattered light from the tissue was then collected using another fiber of 400 µm diameter, positioned at a distance of 260 microns from the illumination fiber. The DR light emanating from the fiber tip was coupled to the entrance slit of a miniature fiber-optic spectrometer (Model USB 2000 FL VIS-NIR, Ocean Optics, Dunedin, Florida, USA) connected to the USB port of a laptop computer. This spectrometer was fitted with a 500-nm blazed, 600 lines/mm grating and a 2,048-element linear silicon CCD array for recording of spectra in the 360–1000 nm wavelength range with 7 nm resolution. In order to maximize the collection of scattered light from tissue a black PVC sleeve was inserted at the probe tip that maintained an optimum distance between the interrogated tissue and probe tip. Moreover, this black sleeve also prevented the room light from entering into the detection system and being disposable provided extra hygiene. After placing the PVC sleeve at the probe tip in contact with the gingiva the DR spectra were recorded using the OOI Base32 software of Ocean Optics configured to record the spectra, averaged for 40 scans, with a boxcar width of 10 nm and an integration time of 100 milliseconds. DR spectral data were categorized as normal/healthy and inflamed tissue (mild, moderate and severe). 15 sets of measurements were taken from each site and the mean value was used for analysis. The hand piece was sterilized every day and before use on a new patient.

2.2.2 Imaging system

Figure 1: Schematic of the experimental arrangement for in vivo DR imaging measurements.
The diffuse reflectance imaging system (DRIS) shown in Fig. 1 consists of a EMCCD camera (Model: LUCA-S, Andor Technology, UK) with 1024 x 1024 pixels, a Nikkon AF 35-70 zoom camera lens and a liquid crystal tunable filter (LCTF) of 7 nm bandwidth (Model: CRI Inc, USA) that can be tuned to any wavelength between 400-720 nm. However, in this study the LCTF was tuned to coincide with the oxy-Hb absorption peak at 575 nm and the DR spectral intensity maximum at 620 nm. Suitable adapters and roller assemblies were built to couple the camera with the focusing lens and LCTF and facilitate camera lens movement during focusing and zooming. The tungsten halogen lamp (12 V, 55W) of the dental chair (Trichur Surgicals, Trichur, India) was used for white light illumination of oral cavity during imaging. The laptop computer working in Microsoft Windows environment with Andor SOLIS program and LCTF control software was connected to respective instruments to control the image acquisition parameters and compute the ratio image R620/R575 after recording the DR images sequentially at 575 and 620 nm by tuning the LCTF electronically. For image acquisition, the EMCCD-Lens-LCTF assembly was kept at a constant distance of one feet from the diseased site. The camera lens - filter assembly was kept in the instrument tray associated with the dental chair for the easy access of patient mouth and tilt during patient exit and entry. The cheek retractor used to pull the cheeks away from the mouth and hold them in place during camera exposure was sterilized every day and before use on a new patient. The imaging was done in the dark to avoid stray light from entering the camera. While the tungsten halogen lamp was turned on, an eye protective goggle was worn on the patient to ensure comfort. The patients was asked to remain stationary for 20 sec during sequential acquisition of images at 620 and 575 nm. The LCTF control software was used to change the filter wavelength during image acquisition.

The DRIS is portable and easy to manage in clinical settings. Diffuse reflectance imaging was always performed before clinical assessments, because an injury of the blood vessels of the crevicular plexus by paper strips and periodontal probe may affect reflectance spectra of gingiva. Saliva was absorbed each time before taking measurements using point monitoring and imaging techniques to reduce specular reflection, thereby increasing the diffuse reflection from underlying tissues. In our experience, removal of saliva plays an important role in proper diagnosis of tissue using DR spectroscopy.

Andor Solis image capturing software was used to record grayscale images at selected wavelengths. The software performs automatic image alignment with pixel accuracy to compensate for movement during imaging. Arithmetic functions associated with the software were used to take ratio of the images. False coloring of ratio intensity variation was done using the same software to distinguish clearly between healthy and diseased sites. Changes in the diffuse reflectance are calculated for every image pixel, approximately corresponding to the area occupied by a single cell. Properties such as the diffuse reflectance are calculated from the pixel intensity and their spatial distribution was displayed as a Pseudo Colour Map (PCM) overlaid onto the image of the diseased site. The PCM of the ratio-image classify the area of oral lesions into blue (healthy tissue), green (mild inflammation), red (moderate inflammation), and yellow and white (severe inflammation).

3. RESULTS

3.1 DR spectral features on point monitoring

In vivo spectral measurements were taken from 70 healthy sites, 63 mildly inflamed sites and 58 severely inflamed gingival sites (Fig 2). Healthy sites belonged to healthy volunteers. The severe inflammation was observed in periodontitis affected sites having alveolar bone loss, which was evident from radiographs. The spectral features of these three groups only were compared to avoid overlapping of spectra. The oxygenated hemoglobin absorption dips at 545 and 575 nm is prominent in the DR spectra of periodontitis and not in the spectra of healthy gingiva. The DR spectra were recorded by point monitoring to identify the spectral intensity ratio which gives maximum discrimination. Oxy Hb and deoxy Hb have crossovers at 615 and 460 nm wavelengths. Therefore, DR intensity ratios such as R620/R575, R620/R545, R575/R545, R615/R460 were compared. It was observed that ratios involving Oxy Hb absorption gave maximum discrimination between the healthy and inflamed gingiva with the R620/R575 ratio showing the best response to inflammatory conditions of the gingiva.
3.2 Image analysis features

Typical photos of patient’s teeth structure are given along with monochrome and processed ratio images for healthy/mild, moderate, severe periodontitis patients are given respectively in Figs. 3, 4 and 5. Fig. 3(c) shows the false colored ratio image of healthy patient shown in Fig. 3(a), with the corresponding grayscale ratio image in Fig. 3(b). Fig. 3(d) shows the image of a patient with mild inflammation. The grayscale image (Fig. 3(e)) on false coloring shows green color (Fig. 3(f)) in regions of mild inflammation. The false color code given to denote the pixel intensity variation in healthy region is blue. The false coloring on the ratio image with varying pixel intensity helps in identifying the points of maximum oxy Hb absorption. It is observed that the average pixel intensity ratio value for healthy tissue is 4.805 ± 1.04 whereas for mild inflammation it is 8.820 ± 1.79.
Fig. 4(a) shows image of a patient having moderately inflamed gingiva. The portion of inflamed gingiva is shown with yellow outline. On false coloring (Fig. 4(c)) the region inside yellow outline shows red spots that can be clearly seen in Fig. 4(d). Average pixel intensity ratio obtained for moderately inflamed gingiva is $12.917 \pm 2.77$. Fig. 4(b) shows the grayscale image of the moderately inflamed gingiva.

Fig. 5(a) shows the image of a patient having severe periodontal inflammation with the affected site marked by yellow rectangle in both the photo and false colored ratio image (Fig. 5(c)). The enlarged false colored ratio image shown in Fig. 5(d) shows regions of yellow color with average pixel intensity ratio value of $16.641 \pm 2.18$. Fig 5(b) shows the grayscale ratio image of gingiva before false coloring.
Marked differences observed in the pixel intensity ratio values between diseased and normal tissues are associated mainly with oxygenated hemoglobin absorption at 575 nm. The DR image pixel intensity ratios (R620/R575) for different categories of periodontal inflammation were determined and a scatter plot diagram was drawn (Fig. 6). The sensitivity and specificity obtained from the scatter plots of R620/R575 image ratio are given in Table 1.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Healthy-Mild inflammation</th>
<th>Mild-Moderate inflammation</th>
<th>Moderate-Severe inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se</td>
<td>91.6%</td>
<td>83.0%</td>
<td>82.3%</td>
</tr>
<tr>
<td>Sp</td>
<td>93.0%</td>
<td>95.8%</td>
<td>75.9%</td>
</tr>
</tbody>
</table>

Sensitivity (Se) = TP/(TP+FN), Specificity (Sp) = TN/(TN+FP)

The cut-off lines drawn in the scatter plot diagram for group classification are at the mean value of the adjacent groups. The cut-off lines drawn at 6.8123, 10.8681 and 14.7789 in the scatterplot of image ratio R620/R575 ratio (Fig. 6) discriminates the tissue groups as healthy-mild inflammation, mild-moderate inflammation and moderate-severe inflammation respectively. In Fig. 6, out of 96 mild inflammatory cases 8 were misclassified as healthy; giving sensitivity of 91.6%, and out of 77 healthy cases 5 cases were misclassified as mild inflammation giving a specificity of 93%. Also, out of 54 cases of moderate inflammation, 9 cases were misclassified as mild inflammation giving a sensitivity of 83% and out of 96 cases of mild inflammation 4 cases were misclassified as moderate with 95.8%
specificity. Similarly, out of 17 cases of severe inflammation 3 were misclassified as moderate with 82.3% sensitivity and out of 54 cases of moderately inflamed gingiva 13 (Table 1) were classified as severe with 75.9% specificity.

4. DISCUSSION

4.1 Role of inflammation in periodontal disease

In this study gingival erythema is monitored using features of oxy Hb absorption at 575 nm in diffusely reflected spectra of gingiva. It was found that oxy Hb concentration increases with increase in inflammation. This corroborates well with earlier findings. In clinical diagnostic procedures, gingival inflammation is considered in determination of the initial stage of periodontal breakdown. This is often detected clinically by BOP as it is an objective measure of gingival inflammation and is a reliable and verifiable measure. It is very well correlated with histological changes of gingivitis, and is readily interpretable in terms of clinical significance and can be highly correlated with gingival indices (GI). Clinically, active periods show bleeding either spontaneously or with probing. The study results show that sites with BOP correspond well with the false colored image ratio and image pixel intensity ratio value. This study shows the capability of DR image ratio R620/R575 to precisely demarcate areas with increased vascularity, which is known to occur during gingival inflammation (Fig 3, 4 and 5). The other advantages of DR imaging are:

4.2 Location of inflammatory changes in subclinical stage in sites with disease activity

The exact point of inflammation in moderately inflamed and severely inflamed gingiva as seen in the false colored images (Figs. 4 and 5), are not at all observable using conventional diagnostic procedures. The identification of disease location enhances the opportunity for site specific treatments like photodynamic therapy. Periodontal destruction is site specific and does not occur in all parts of the gums and teeth at the same time, but rather on a few teeth at any given time. DR imaging identifies areas with early inflammatory changes in the underlying tissues even when the sites appear clinically healthy. This helps in identifying sites that are prone to disease. It is very common to find sites of periodontal destruction next to sites with little or no destruction. Therefore, the severity of periodontitis increases with the development of new disease sites and increased breakdown of existing sites, or both. Currently used diagnostic procedures cannot distinguish between disease-active and disease-inactive sites at any given point of time and cannot reliably identify susceptible individuals based on disease active and inactive sites. Also, chronic periodontitis is now considered a site-specific disease, but what has continued to puzzle periodontal researchers is the unpredictability of the disease at a patient and site-level. In this context, diffuse reflectance imaging proves to be an effective tool as it can pin point the location of inflammation.

4.3 Early detection of inflammation

Early detection of periodontal disease enhances possibilities for appropriate treatment planning, whether it is prophylactic, medical, or surgical. The DR ratio images obtained in this study reveals that this technique is helpful for physicians to detect early symptoms of gingival inflammation, whether it is due to mild gingivitis or due to attachment bone loss. While radiographs only reveal bone loss only after 30% to 50% of the mineral has been lost, DR imaging technique has the potential to find early stages of mineral loss. Also, early diagnosis and consequent treatment of periodontal disease may have a major impact on the control of other systemic infections. Severe periodontal disease causes attachment loss, alveolar bone loss and tooth loss, extending beyond the local level to produce systemic effects. It exacerbates the inflammatory milieu, increases insulin resistance, and potentially worsens cardiovascular disease. Severe periodontitis is a risk factor for early death due to ischemic heart disease or renal dysfunction independent of diabetes, increasing the risk 3.2 fold as compared with those with none, mild, or moderate periodontitis. Therefore, currently used tools for periodontal examinations like periodontal probing cannot monitor the initiation or progression of periodontal destruction. Thus non-invasive methods that can diagnose early stages of periodontal breakdown are desirable.

4.4 Reproducibility of the results

The DR imaging system used in this study assesses the given area of periodontium as a whole and gives an objective result thereby increasing its reproducibility and reliability. Usually the assessment of periodontal disease, especially those associated with gingival inflammation, is often subjective because evaluation of parameters, such as color and
presence of edema can vary from person to person. Previous studies have demonstrated that DR spectroscopy can be used to assess the degree of erythema and edema taking into consideration the light absorbed by particular chromophores. It has been found that erythema correlates well with the relative concentration of oxygenated hemoglobin. Although these findings were reported for skin, gingival tissues have a similar structure. Gingiva consists of an external layer of epithelium with an underlying layer and connective tissue, where most of the microvasculature is embedded.

4.5 Novel noninvasive technique to diagnose periodontal disease in real time

Image analysis using the scatter plot of R620/R575 (Fig. 6) has shown a sensitivity of 91.6% and specificity of 93% for the discrimination of healthy tissues from mild inflammation and a sensitivity of 83% and specificity of 95.8% for the discrimination between mild inflammation and moderate inflammation. In comparison a sensitivity of 82.3% and specificity of 75.9% was obtained for the discrimination between moderate inflammation and severe inflammation. The misclassified cases cannot be completely attributed to the technique used, but could be due to the limitation in taking the conventional diagnostic technique as our gold standard and also because of the specular reflection from varying amounts of saliva present in the gingiva of patients examined. Currently available methods of clinical diagnosis mainly comprises of periodontal probing, which helps to determine some of critical clinical parameters, such as probing PD and CAL for evaluating the status of periodontal tissue. The poor reliability and reproducibility of these methods could be associated with the non-consistency of measuring CAL. Periodontal probing also evaluates BOP, which is considered as the earliest sign of periodontal breakdown. Another common method of using radiographs in periodontal diagnostic decision making helps to determine the severity of periodontitis and bone-related damage but with limited sensitivity. Radiographs also cannot be taken at each visit due to the hazards associated with radiation exposure to the patient. The attractive aspect about DR imaging is also that it is totally non-invasive, do not impose any discomfort to patients during the procedure and require no tissue to be extracted.

4.6 Patient compliance

The DR imaging system that is described here suits well with the clinical environment as it can be easily mounted onto the instrument tray associated with any dental chair. The fact that the white light source of the dental chair unit can be used as the light source reduces the cost and enhances the applicability of the DR imaging system in clinical environment. The DR imaging system developed is portable, can be handled with ease by non-skilled personnel and is patient compliant as it takes less than a minute for the whole recording procedure to be completed.

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