Non-Invasive Periodontal Probing Through Fourier-Domain Optical Coherence Tomography

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Background: Periodontitis is a multifactorial and infectious disease that may result in significant debilitation. The aim of this study is to exploit two optical coherence tomography (OCT) systems operating in the Fourier domain at different wavelengths, 930 and 1,325 nm, for structural analysis of periodontal tissue in porcine jaws.

Methods: Five fresh porcine jaws were sectioned and stored in formalin before OCT analysis. Two- and three-dimensional OCT images of the tooth/gingiva interface were performed, and measurements of the gingival structures were obtained. The 930-nm OCT system operates in the spectral domain, whereas the 1,325-nm system is a swept-source model. Stereomicroscope images, the gold standard, were used for direct comparison.

Results: Through image analysis, it is possible to identify the free gingiva and the attached gingiva, the calculus deposition over tooth surfaces, and the subgingival calculus that enables the enlargement of the gingival sulcus. In addition, the gingival thickness and the gingival sulcus depth can be non-invasively measured, varying from 0.8 to 4 mm.

Conclusions: Regarding the ability of the two OCT systems to visualize periodontal structures, the system operating at 1,325 nm shows a better performance, owing to a longer central wavelength that allows deeper tissue penetration. The results with the system at 930 nm can also be used, but some features could not be observed due to its lower penetration depth in the tissue. J Periodontol 2015;86:1087-1094.

KEY WORDS
Diagnostic imaging; gingiva; periodontal index; periodontics; periodontium; tomography, optical coherence.

Early diagnosis in health care is desirable and necessary since it implies a better patient prognosis. Imaging methods are widely used in diagnostic medicine, as reviewed by Dhawan et al.1 Among the diversity of imaging modalities, optical coherence tomography (OCT), first introduced by Huang et al.,2 is now commercially available and considered the most innovative imaging technique used in ophthalmology;3 it is also used in several other clinical applications such as dermatology,4,5 gastroenterology,6 cardiology,7 gynecology,8 oncology,9 dentistry,10,11 and non-medical applications, for instance, painting12 and evaluation of materials’ porosity.13

In dentistry, OCT studies in hard and soft tissues have been performed since 1998,10 and they have been proposed to be an effective tool to evaluate early caries and caries-related issues14-19 and oral cancer.20 Some studies analyzed periodontal structures, suggesting the possibility of applying OCT to periodontal diagnostics,21-24 but evidence is not yet sufficient, especially related to the gingival sulcus, which is an important parameter of periodontal health and disease. Furthermore, OCT has been applied to evaluate dental materials,25-28 with the advantages of being a non-destructive imaging technology and rendering three-dimensional (3D) images in real-time acquisition mode.

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According to Petersen and Ogawa,29,30 using data from the World Health Organization, gingival bleeding is highly prevalent among adult populations in all regions of the world; advanced disease with deep periodontal pockets (≥6 mm) affects 10% to 15% of adults worldwide. Those authors also concluded that periodontal disease is one of the two most important oral diseases contributing to the global burden of chronic disease.30

In a recent article by Hsieh et al.,22 it was discussed that microbial dental plaque is an etiologic factor of periodontitis, and dental calculus is a type of mineralized plaque from deposited microorganisms that promotes more plaque retention. The traditional diagnosis of subgingival calculus is based on clinical examination using periodontal probing and radiographs. Periodontal probing has the drawback of being an uncomfortable and painful technique from the patient point of view. On the other hand, radiography can determine the level of bone-related breakdown and identification of subgingival calculus located only on the proximal surface of the teeth, since the superposition of facial and lingual surfaces does not allow proper imaging.22 In addition, radiographs underestimate bone loss by ≈30%.31 Within the past 10 years, several novel methods have been developed for dental calculus detection, such as a smart ultrasonic device, light-emitting diode–based optical probe, and laser fluorescence.32,33 In Hsieh et al.,22 a table is presented in which several available methods of calculus detection are compared, including radiography, dental computed tomography, intraoral digital camera, periodontal probe, OCT, Raman spectroscopy, and laser fluorescence spectroscopy. The main conclusion that can be drawn is that OCT is certainly a promising tool to be considered in dental clinics, since it is non-invasive, non-destructive, and non-radioactive and a real-time monitoring method of imaging diagnostics that is able to give quantitative results.

In this study, the authors exploit the different technical characteristics of two available OCT systems operating in the Fourier domain at different wavelengths, 930 and 1,325 nm, for identification and differentiation between free and attached gingiva in porcine jaws. In addition, the efficiency for identifying subgingival calculus is evaluated. Quantitative measurements of the gingival sulcus are obtained, as well as the thickness of the free gingiva, which is one of the predictors of gingival phenotype.34-36 Stereomicroscope images, the gold standard, are used for direct comparison.

**MATERIALS AND METHODS**

**Ethical Aspects and Sample Preparation**

This ex vivo study was approved by the Ethical Committee on Animal Experiments (Center of Biologic Sciences, Universidade Federal de Pernambuco) under process number 23076057216/2013-91, in accordance with guidelines approved by the Council of the American Psychologic Society for use of animal experiments.37

Five fresh porcine jaws obtained from a local slaughterhouse were dissected and sectioned, aiming to preserve the dental structure and corresponding bone and periodontal tissues. Subsequently, they were stored in formalin solution at 10%§ for 24 hours before analysis by OCT and stereomicroscope. Images were obtained of mandibular incisors, premolars, and molars and their periodontal structures.

**FourierDomain-OCT**

OCT systems are interferometric devices exploiting the low coherence of broadband optical sources, which when associated with a Michelson interferometer and a detection system forms the basic setup. OCT systems can operate in the temporal (TD-OCT) or Fourier (FD-OCT) domain.38,39 In both cases, the source bandwidth determines the axial resolution, whereas the transverse resolution is determined by the geometric optics characteristic of the focusing beam. One of the advantages of the FD-OCT system is that it has no movable parts (apart from the lateral scanning mirror that provides the so-called B-scan), in contrast with TD-OCT, where a mirror displacement is necessary in the interferometer’s reference arm.38 Depending on the specific method, FD-OCT can be classified as spectral-domain OCT (SD-OCT), whereby the spatial Fourier transform process occurs at the detection system, formed by a grating and charged-couple device (CCD) arrangement. The other method is known as swept-source OCT (SS-OCT), and in this case the optical source has its narrowband frequency swept to achieve a broad spectral band. The detection is made by a single photodetector.39

The SD-OCT and SS-OCT systems used in this work are both commercially available, and their working principle and theoretic background have been well described.38,39 For the SD-OCT¶ system, the light source used is a superluminescent diode with a central wavelength of 930 nm, spectral bandwidth of 100 nm, and maximum output power of 5 mW. Images generated by this system present an axial resolution of 7/5.3 µm (air/water), lateral resolution of 8 µm, and maximum imaging depth at 1.6 mm. The axial scan rate of the system is 1.2 kHz, capturing two frames per second with 105 dB of sensitivity.

The SS-OCT§ uses a fast-tuned narrowband source to illuminate the interferometer and record the

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§ Sigma-Aldrich, St. Louis, MO.
¶ Callisto Spectral Domain OCT System, Thorlabs, Newton, NJ.
¶ OCS1300SS SS-OCT System, Thorlabs.
information with a single photodetector. The swept laser source tunes the lasing wavelength across a broad wavelength range at a repetition rate of tens of kilohertz. Each sweep on the laser wavelength provides a depth scan at a sample surface point that yields a detailed depth-dependent reflectivity profile along the direction of the laser illumination path. The swept laser source central wavelength is 1,325 nm, with spectral bandwidth of >100 nm and instantaneous bandwidth of 0.13 nm (0.5 GHz). The axial scan rate is 16 kHz, average output power ∼10 mW, and sensitivity 100 dB. Regarding imaging capability, the system captures 25 frames per second, with axial resolution in air/water of 12/9 μm and lateral resolution of 25 μm.

Two-dimensional (2D) and 3D images are obtained by both 930-nm SD-OCT and 1,325-nm SS-OCT. In this study, 2D images present 2,000 pixels per column at the x-axis, corresponding to maximum 6-mm scanning, and 512 pixels per column at the y-axis, corresponding to maximum 1.3-mm depth (in air). A complete mapping of the buccal surface was performed for each tooth. Cross-sectional images were obtained from the start of one of the proximal margins to the next. For SD-OCT, in which 2D image capture was a manual process, the interval between images was 250 μm. For SS-OCT, the scanning was controlled by the computer, so 73 cross-sectional images were captured over an extension of 1 mm. SD-OCT provides volumetric 3D images of the sample surface, each one composed of 100 A-scans per millimeter at the volume width and volume length (x- and y-axis, respectively) and 512 pixels of volume height. The SS-OCT 3D image is a conjugate of several image slices, in which it is possible to visualize each B-scan along the XY, XZ, and ZY planes, composed of 2,000 pixels at the x-axis and 512 pixels at the y- and z-axes.

After OCT analysis of the samples, the teeth and their corresponding periodontal and bone tissues were sectioned along the sagittal axis, from buccal to lingual surface, using flexible diamond disks under water refrigeration for further stereomicroscopy analysis (Fig. 1).

**Stereomicroscopy**

Stereomicroscopic images** of the samples were obtained before and after the OCT analysis, with a ×10 eyepiece providing magnification from ×6.5 to ×50, 35.4- to 4.6-mm field of view, and 92-mm working distance, coupled to a CCD camera with nominal resolution of 1.3 μm.

Initially, images were obtained from the buccal surface of the samples. Then, after OCT analysis and sectioning of subsequent samples, stereomicroscopic images were obtained directly from the sectioned surface.

**RESULTS**

Figure 2 shows a representative image set obtained from the porcine jaw sample. In Fig. 2A, a ×6.5-magnified image of the porcine mandibular incisors is shown; the black line shows the position where the OCT images were obtained, scanning through hard and soft tissue. In Fig. 2A, it is possible to observe the amount of calculus deposition over the teeth surfaces. In Figs. 2B and 2C, SD-OCT and SS-OCT images, respectively, are shown with the main observed features: calculus deposition, dentin-enamel junction, and gingival tissues. The arrows indicate the location of the gingiva/tooth interface, where a faint white line is seen. Figure 2D shows an expanded image of Fig. 2C, highlighting the gingival sulcus, where the white dashed line was drawn as a guide.

During analysis of the generated images, it is possible to visualize distinct periodontal structures in different areas of the same tooth. Figure 3 shows a sequence of images at the buccal surface of a mandibular molar captured by SD-OCT. In Fig. 3A, a normal periodontal condition is shown, in which free gingiva is very close to the tooth surface; Fig. 3B shows an initial, but discrete, enlargement of gingival sulcus, making it difficult to differentiate the gingiva/air and air/tooth surface interfaces, because the space is still less than the OCT resolution. Finally, Fig. 3C shows the increase of the distance between tooth and epithelium caused by the presence of calculus in this area, and a large free space can be clearly identified at the gingival sulcus. Also in Fig. 3, the relative homogeneity of light propagation through gingival tissue can be observed, whereas at enamel and dentin the light propagation depends on the pattern and intensity of scattering through calculus at the tooth surface. Using software,**†† the gingival thickness and gingival sulcus depth were measured. The value directly obtained is the so-called optical path length (OPL), which is given by the product of the physical length times the refractive index, n, which in turn can also be measured with OCT. The free gingiva OPLs measured from Figures 3A, 3B, and 3C are 1.53, 1.71, and 1.82 mm, respectively, measured at their thicker portion. Using the method described by the American Psychological Association,** 40 refractive index values of 1.432–0.047 and 1.27–0.05 mm.

# 7075, KG Sorensen, São Paulo, Brazil.

** Stermi 2000-C Stereomicroscope, Zeiss, Oberkochen, Germany.

†† ImageJ, v.1.48, including ImageJ64, National Institutes of Health, Bethesda, MD.
Measurements of gingival sulcus depth are 3.14 ± 0.25, 3.42 ± 0.27, and 3.44 ± 0.27 mm for Figures 3A, 3B, and 3C, respectively. As this value is in air \((n = 1)\), the OPL is equal to the real depth. The results are consistent with the stereomicroscopy measurements. The gingival sulcus depth was also measured for other samples analyzed in this study, and the values varied from 0.8 to 4 mm.

After OCT analysis, all samples were sectioned along the sagittal axis, and stereomicroscopic images were performed, as seen in Fig. 4, which shows images of a mandibular molar under the two OCT systems and stereomicroscopy. Figures 4A and 4B were obtained with the SS-OCT, and Figs. 4C and 4D used the SD-OCT. Figure 4E shows the stereomicroscopic image, and the arrows in all figures indicate the analyzed region. The images in Figs. 4A and 4C show a clear interface between gingival tissues and tooth surface, regardless of the OCT system. On the other hand, the images observed in Figs. 4B and 4D clearly show the presence of a larger distance between free gingiva and tooth surface, suggesting an increase of gingival sulcus thickness, probably due to the presence of calculus. An alternative possible cause of the gingival sulcus enlargement in the present case is tissue retraction caused by dehydration after immersion in formalin. Figure 4 also indicates the limit between free gingiva and attached gingiva. Special attention should be given to Figs. 4A and 4B, in which the gingival epithelium and connective tissue interface are shown; observe that this distinction is seen only in 1,325-nm SS-OCT images due to their higher penetration depth compared with the 930-nm SD-OCT.

Both SD-OCT and SS-OCT are able to obtain 2D and 3D images. Figure 5A is a volumetric 3D image reconstruction performed by SD-OCT, in which it is possible to observe superficial details, such as the free gingiva close to the tooth and the continuity with the attached gingiva. On the other hand, the SS-OCT performs 3D reconstruction associated with en face imaging, and it is possible to visualize internal B-scans of the sample through several slices on the XY, XZ, and YZ planes of imaging (Figs. 5B through 5H).

**DISCUSSION**

Periodontitis is a multifactorial and endemic infectious disease of the tissues surrounding the teeth and may result in significant debilitation for about half of affected persons. A porcine model was used in this study because it is considered a large animal model for periodontal research owing to the similarity between porcine teeth and periodontal tissues and the same anatomic structures in humans.

The diagnosis of periodontal disease is mainly dependent on clinical measurements and radiographs, whereas frequent monitoring of the gingival sulcus will provide valuable information for judging the presence and severity of periodontal disease. One of the most widely used periodontal examinations is periodontal probing, but there are some unavoidable limitations: the extent of probe penetration is influenced by the inflammatory status of the tissue, and the reliability and reproducibility limitation is reflected in the difficulty of precisely duplicating insertion force, probe placement, and angulation. Another important point to consider is pain, which may be present during manual probing owing to instrument penetration into sulcus or pocket with gingival inflammation, overpressure of the probe, or probe dimensions and shape. Periodontal probing is considered an invasive technique because of the risk of its active tip penetrating periodontal tissues, thus leading to trauma. Lack of pressure control during this step can lead to bleeding from excess pressure of probe penetration and subsequent diagnostic error about the presence of disease. Another measurement, clinical attachment level (CAL), consists of measuring the distance from the cemento-enamel junction to the bottom of the periodontal pocket. CAL and periodontal probing constitute the main diagnostic parameters of periodontal disease, as digitally measured by Schneider et al.
OCT, on the other hand, reveals microstructural details of the periodontal soft tissues, providing information for controlling the volume of attached keratinized gingiva, as well as enabling the observation of periodontal structures that might be associated with inflammatory processes. The enlargement of the gingival sulcus is an important predictor of the presence or absence of periodontal disease: in healthy conditions, free gingiva is very close to the tooth surface. Therefore, it offers the potential for accurately identifying active periodontitis before significant alveolar bone involvement occurs. Those features are confirmed by the experimental results obtained in this ex vivo study, indicating that the OCT technique can be well suited to a clinical environment for early and non-invasive periodontal diagnostics, also yielding quantitative information on pocket formation (depth and transverse dimension).

As an additional and important result, the present authors were able to measure the gingival thickness, which is one of the predictors of the gingival phenotype. Its variation has direct implications on the results of periodontal therapy, root coverage procedures, implant placement, and surgeries for maxillary sinus elevation owing to the strong correlation between the gingival phenotype and the Schneiderian mucosa thickness. Several methods have been used, both invasive and non-invasive, to evaluate gingival thickness, such as histologic sections, injection needles, transgingival probing, cephalometric radiographs, probe transparency, ultrasonic devices, and cone beam computed tomography. Another way is visual evaluation, but that cannot be considered a reliable method to determine the degree of gingival thickness because of its qualitative nature. The main advantage of OCT compared with these techniques is its real-time and quantitative high-resolution pattern. The results obtained in the porcine gingiva are within the instrument capability, which can measure thicknesses up to 2 mm with resolution of <20 μm, which can also be taken as the inferior limit for thickness measurement.

The 1,325-nm SS-OCT used in this study shows some advantages compared with the 930-nm SD-OCT: its longer wavelength enables deeper penetration into the sample. However, the method being...
based on spectral domain or swept source is not relevant. Depending on OCT characteristics, acquisition image rates can differ, which affect spatial resolution. In the system used here, the SS-OCT has a faster image acquisition rate, an important factor in a clinical environment. On the other hand, the fast imaging processing of SS-OCT, associated with limitations inherent to the light source and physical instrumentation, makes lower-resolution images compared with the SD-OCT used here. Given that all technical characteristics are similar, the only important issue is the source wavelength, and 1,325 nm gives a deeper penetration in soft tissue than 930 nm.

Regarding light interaction with the sample, this study shows that in gingival tissues, there is a relative homogeneity of scattering pattern, probably due to the constant water content and other constituents of the tissue. But the same behavior was not observed in hard tissues, specifically dentin and enamel, because of calculus deposition on the tooth: the irregular and aleatory deposition of calcium phosphate promotes a strong scattering of incident light that, according to Hsieh et al.,\textsuperscript{22} is due to a high refractive index (2.097 ± 0.094).

Despite positive ex vivo results of OCT, there are some limitations on the clinical popularization of dental OCT applications. One of them is the insufficient scanning range of OCT, which can necessitate several pictures for a whole lesion. Another limitation is the lack of a suitable and inexpensive handpiece compatible with the oral cavity to allow obtaining OCT images of all teeth (especially premolars and molars) and other anatomic areas with difficult access. Both require technologic developments.

**CONCLUSIONS**

This study shows that OCT can evaluate the periodontal environment in porcine jaws. Two- and three-dimensional images of the tooth/gingiva interface are shown using SD-OCT at 930 nm and SS-OCT at 1,325 nm, as well as quantitative measurements of the gingival sulcus from 0.8 to 4 mm. The 930-nm SD-OCT system has a better axial and transversal resolution than the 1,325-nm SS-OCT, but overall the latter shows a better performance, due to a longer central wavelength that allows deeper penetration. Furthermore, the model used in this work has faster image acquisition than the SD-OCT system, an essential factor for the clinical setting. The ability to measure gingival thickness shows important potential for measuring it in patients, which can lead to a quantitative standard for this phenotype parameter.
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REFERENCES


Figure 5.

Three-dimensional OCT images obtained from a mandibular central incisor. A) Volumetric 3D reconstruction of the tooth surface using SD-OCT at 930 nm. B through D) Images from the YZ plane along the axis from cervical to incisal. E through H) Images from the YZ plane displaced along the XZ plane, also from cervical to incisal. Images B through H were obtained with the SS-OCT system. The static lines in these figures are due to an artifact of the OCT scanning system, which did not alter the results.


