Photodiagnosis and Photodynamic Therapy 25 (2019) 275–279

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Evaluation of calculus imaging on root surfaces by spectral-domain optical coherence tomography

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ARTICLE INFO

Keywords:
Optical coherence tomography
Periodontitis
Subgingival calculus
Diagnosis
SD-OCT

ABSTRACT

Objective: The aim of this in vitro study was to evaluate the ability of optical coherence tomography (OCT) to display calculus on root surfaces.

Material and methods: Ten teeth with calculus on the root surface were embedded in resin, omitting the root surface. A region of interest (ROI) was marked by small drill holes coronally and apically of the calculus and imaged by spectral-domain optical coherence tomography ([SD OCT], Telesto SP5, centre wavelength 1310 nm) and light microscopy (LM). To evaluate the impact of different fluids on calculus visualisation, using OCT, root surfaces were covered by a layer of NaCl and blood and displayed by OCT. Subsequently, teeth were completely covered with resin and sectioned for histological evaluation. Within the ROI, lengths of root surface and calculus were measured by LM and OCT, and the ratio [%] was calculated. In addition, at three sites of each ROI, agreement of presence and length of calculus was evaluated. Both methods were compared using Pearson's correlation.

Results: Regarding the presence of calculus, agreement between LM and OCT was strong ($\kappa_i = 0.783$, $p = 0.033$), and measurements regarding the length of the calculus were strongly correlated ($r_i > 0.906$; $p_i < 0.001$). However, the values differed for dry ($p = 0.023$) and NaCl-covered root surfaces ($p = 0.035$).

Conclusion: Calculus on the root surface can be displayed by SD-OCT, which therefore may be suited as imaging technology for subgingival calculus in periodontal pockets.

1. Introduction

Periodontitis is considered a major health burden, and periodontal infection may have important implications for systemic health [1]. As a complex multifactorial disease, it is characterised by destruction and loss of connective tissue attachment [2], and the severe form of periodontitis is found in up to 15% of people worldwide [3], which underlines the importance of improving clinical management of this chronic disease.

Bacterial biofilm attached to tooth surfaces is the main aetiological factor. Therefore, a primary goal in the prevention as well as the treatment of periodontitis is the removal or targeted manipulation of this biofilm. Calculus as the mineralised form of the biofilm is considered a secondary aetiological factor in periodontitis [4]. Subgingival calculus provides an ideal porous niche for biofilm retention and subsequent mineralisation.

Periodontal therapy comprises improvement of oral hygiene and purposeful influencing or removal of calculus and biofilm by means of systematic treatment of all affected root surfaces by using hand-sonic and/or ultrasonic instruments while causing the least damage to the root surface. Complete removal of calculus or even valid assessment of therapeutic intervention might be difficult because the examiner has to rely on tactile sense to judge the morphology of the root surface [5,6]. However, traditional tactile perception of the subgingival environment without visible access before and after treatment is frequently unreliable, and thus may lead to residual calculus, unintentional removal of cementum, or both [7,8]. Therefore, a relevant amount of failure in periodontal treatment depends on remaining calculus and attached microorganisms after therapy. Hence, the correct evaluation of a cleaned surface is key to enable thorough and substance-sparing debridement [9]. To support the clinician's decision either to stop or continue therapy, past years have witnessed the development of several

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https://doi.org/10.1016/j.pdpdt.2019.01.014
Received 7 November 2018; Received in revised form 30 December 2018; Accepted 11 January 2019
Available online 14 January 2019
1572-1000/

calculus detection technologies [9,10] based on fibre-optic endoscopy [11,12], optical spectrometric [13,14], ultrasonic [15,16] and (auto) fluorescence technology [17–19].

In this context, optical coherence tomography (OCT) has the potential to enable calculus assessment on the root surface [20]. OCT was first presented by Huang et al. [21], and in medicine it is an established diagnostic tool in ophthalmology [22–24]. Further medical fields of first application are dermatology [25,26] and cardiology [27]. The use for dental application was reported, such as the imaging of dental structures, carious lesions and the assessment of composite restorations [28–32]. OCT is an imaging method based on low coherence interferometry and allows contact-free, non-destructive, real-time imaging with high, micron-scale resolution by analysing the echo time delay and intensity of light backscattered by internal microstructures in objects. In addition, the method generates data sets for three-dimensional, volumetric images without ionising radiation, making it suitable for in vivo applications.

Few studies focus on imaging dental calculus on the root surface by using OCT [20,33]. However, thus far, OCT imaging of dental calculus was not validated against the reference standard light microscopy (LM) based on cross-sections. Therefore, the aim of the present study was to evaluate the suitability of spectral-domain OCT (SD-OCT) to assess calculus on the root surface, depending on different fluids covering the root surface, and to correlate the findings with light microscopy. We hypothesised that dental calculus on root surfaces can be imaged by SD-OCT.

2. Materials and methods

The study was conducted in accordance with the Declaration of Helsinki, and the protocols were approved by the Ethics Committee of the University of Leipzig. The extracted teeth were used with patients' approval (informed consent, protocol no. 299-10-04102010).

2.1. Preparation of specimens

A total of 10 periodontally involved, freshly extracted human teeth (incisors, premolars) that were partially covered with calculus on the root surface and of various sizes were stored in a 0.5% chloramine solution at 4 °C immediately after extraction. Teeth were embedded in epoxy resin (Stycast, Emerson & Cuming, Westerlo, Belgium), leaving the root surface exposed. Then a region of interest (ROI) was marked by small drill holes, using a diamond bur (957 A M, Komet, Gebr. Brasseler GmbH, Germany), coronally and apically, of the calculus (Fig. 1).

2.2. SD-OCT imaging

The ROIs were imaged two-dimensionally by spectral domain OCT ([SD-OCT], Telesto SP5, Thorlabs GmbH Dachau, Germany) (Fig. 1). The experimental set-up was performed as described elsewhere [17]. At first, imaging was performed under dry conditions, and then, to determine the influence of different fluids occurring in a periodontal pocket on calculus visualisation by OCT during the measurement procedure, teeth were covered with physiological saline solution and, after drying the teeth using a cotton pellet, with human blood. Blood was provided by one of the authors (FK) and stored in a trisodium citrate (4%)–containing collection tube to inhibit the blood coagulation process.

SD-OCT is a variant of Fourier domain OCT, using a broadband, low-coherence light source. The principle upon which it is based has already been well described in numerous publications [30,30,34,35]. The light beam scans the sample surface point by point and line by line. The back-reflected light from the sample and the reference arm interfere with each other, and the resulting coherence signal (spectrum) is recorded. After Fourier transformation of the signal, a depth profile of backscattering along a perpendicular line to the object surface is generated (A-scan). A series of laterally adjacent A-scans generate a two-dimensional (2D), cross-sectional image (B-scan), and a series of 2D images create a three-dimensional (3D) image stack. Image contrast arises in areas with structures of different refractive index and light absorption, such as the dentin–pulp interfaces, gaps, bubbles, material cracks or porous areas [35–37]. The scan field of OCT imaging used was 11 mm x 2 mm, visualised in Fig. 2 by the green box. Table 1 summarises the specifications of the SD-OCT used.

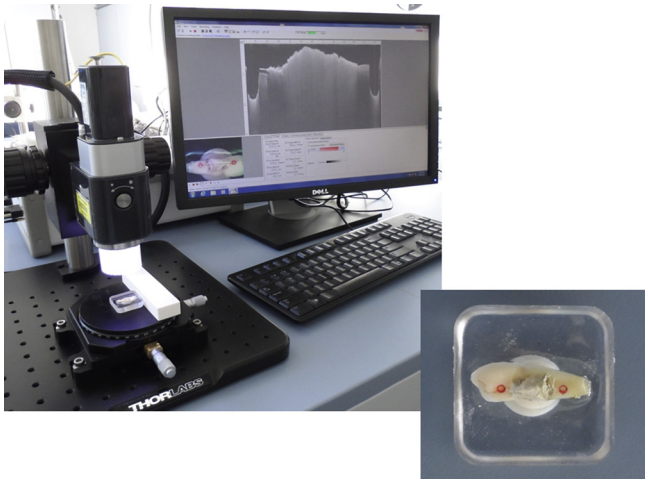

Fig. 1. Experimental setup: Tooth with calculus on the root surface partially embedded in epoxy resin, leaving the surface exposed. Region of interest was marked by drill holes coronal and apical of the calculus and imaged two-dimensionally by spectral-domain OCT (SD-OCT, Telesto SP5, centre wavelength of 1310 nm ± 107 nm).

2.3. Preparation of specimen for histological investigation

After 3D-OCT imaging, the specimens were completely covered with epoxy resin and sectioned along the ROI, using a microtome (thickness of slices: 200 µm; Leitz 1600, Ernst Leitz Wetzlar GmbH, Wetzlar, Germany). The histological sections were imaged with a light microscope (25x; Stemi 2000-C, Carl Zeiss Microscopy GmbH, Germany) to compare histological appearance with the OCT B-scans for ratio of the lengths of root surface and calculus and location of the calculus. Therefore, out of the 3D-OCT image stack, one cross-sectional image, best adapted to the histology of the light microscopic image, was used.

2.4. Analysis of OCT B-scans and histological images

The images obtained by OCT (one B-scan per specimen) and LM (Fig. 3) were analysed using an image editor (ImageJ v1.45, Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). The lengths of the calculus deposits within the ROI were measured by OCT and LM (pixel number in the images) and related to the total lengths of the root surfaces within the ROI; the ratio was expressed as percentage.

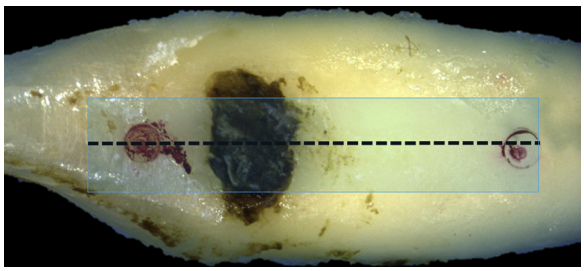

Fig. 2. Region of interest (ROI) was scanned by SD-OCT within the scan field of 11 mm × 2 mm.

Table 1
Technical specification of the used SD-OCT Telesto SP5.

Center wavelength	1310 ± 107 nm
Sensitivity	≤ 106 dB
Axial/lateral resolution	< 7.5 (air)/15 µm
Field of view (max.)	9 mm × 9 mm × 3.5 mm (air, pixel size 700 × 700 × 512 or 1024)
Imaging speed	48-91 kHz
A-scan average	1-5
Spot size	20 µm
Power on sample	3 mW

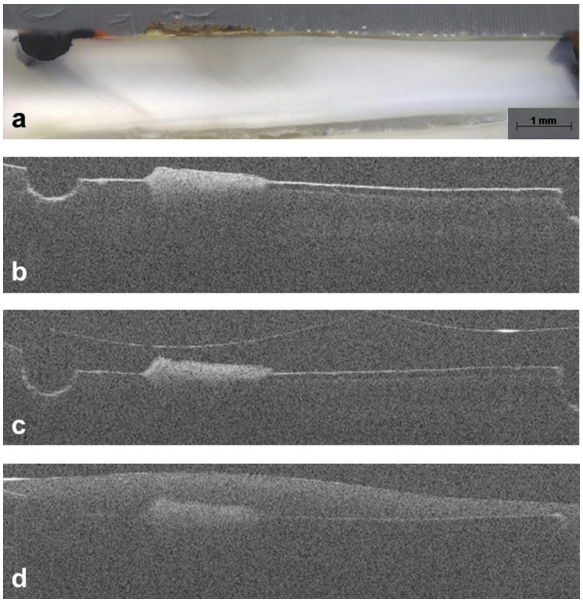

Fig. 3. Visualisation of calculus on the root surface by (a) light microscopy and OCT at (b) dry surface and surfaces covered with (c) NaCl and (d) blood.

In addition, the agreement of presence of calculus (yes/no) was evaluated at three equidistant sites of the ROI (25%, 50% and 75%, measured from coronal surface).

2.5. Statistical analysis

For statistical analysis, the normal distribution of length values for OCT and LM data was analysed (Kolmogorov-Smirnov test). The length was compared with the pairwise *t*-test. Pearson's correlation of both methods was calculated. Agreement of presence of calculus was evaluated by unweighted Cohen's kappa coefficient.

3. Results

Overall, 10 teeth were included and each tooth was imaged by OCT under dry conditions and with a layer of NaCl and blood. Exemplary SD-OCT B-scan images (dry root surface and surface covered with NaCl and blood) with corresponding histological image of the cross-section are shown in Fig. 3. In all cases, histologically apparent calculus on the root surface (Fig. 3a) could be imaged by SD-OCT (Fig. 3b–d).

Regarding presence of the calculus, the agreement between LM and OCT (dry, NaCl, blood) at sites 25% and 75% of the ROI was complete ($\kappa_i = 1.0$, $p_i \leq 0.008$); at the 50% site it was strong ($\kappa_i = 0.783$, $p = 0.033$).

Measurements regarding the length of the calculus were strongly correlated ($r_i > 0.906$; $pi < 0.001$; Fig. 4a–c); however, the values differed for dry ($p = 0.023$) and NaCl-covered root surfaces ($p = 0.035$).

4. Discussion

The aim of this in vitro study was to evaluate the suitability of SD-OCT to image calculus on the root surface, and the results indicated that OCT can display such deposits. Traditionally, the presence of subgingival calculus is tactilely explored by using a probe. However, it has been demonstrated that it might be difficult to define and recognise the endpoint of root-surface instrumentation [5,38] and, therefore, a number of technologies have been developed to identify dental calculus [9]. Numerous potential applications of OCT in dentistry were already part of a recent review article, which discussed the possibilities and limitations of this method in cariology and restorative dentistry [29]. Moreover, the current study focused on non-invasive tooth surface assessment in periodontology, which is gaining increasing interest [39]. Periodontal diseases are of multifactorial character indeed; however, the subgingival establishment of a bacterial biofilm is a key factor in disease development and progression [40]. Accordingly, the detection and removal of these bacterial bearings in the form of dental calculus from the tooth surface is one major task in periodontal therapy [41]. An adequate intervention requires a valid detection and assessment first. The non-invasive assessment of the presence or absence as well as the extent of subgingival calculus has been part of previous studies, which used the application of light-emitting diode (LED), laser-fluorescence or ultrasound for calculus detection [13,15,17,19]. Thereby, especially fluorescence-based techniques could bring a diagnostic benefit. However, they might be unable to detect calculus unequivocally on the root surface, because no subgingival illustration of the situation is possible. In this context, one major benefit of OCT-based diagnostic can be underlined. The application of OCT has been shown to generate high-resolution images of periodontal structures [42]. This allows a clear statement on the presence or absence of calculus. This could be helpful in supportive therapy to avoid instrumentation of calculus-free root surfaces, as an intraoperative control and in the instruction and motivation of patients using the images.

The current study demonstrated that the visual representation of dental calculus with SD-OCT is comparable to light-microscopic examination, which is the laboratory reference standard. Several studies investigated the application of different OCT techniques in subgingival calculus detection. One study used swept-source OCT (SS-OCT) for illustration of dental calculus at one singular tooth in vitro and found a sufficient presentability [20]. These findings are supported by the systematic evaluation of the current study. Another study also differentiated calculus in OCT images, although it exclusively investigated enamel samples [43]. This is in line with findings by Kao et al., which showed that subgingival calculus can be clearly differentiated from sound enamel or enamel pearls by using OCT [33]. Besides pure calculus detection, different studies assessed the detectability of periodontal structures with OCT [42,44,45]. One study examined porcine jaws and demonstrated that OCT imaging at 1325 nm, a wavelength comparable to the current study, could illustrate periodontal structures precisely [44]. Furthermore, a recent study showed the possibility of periodontal imaging and diagnosis by OCT [45]. Similarly, Park et al. [42] concluded that dental OCT could generate high-resolution images of hard- and soft-tissue structures of the periodontium, adding further information to the diagnostic process. Both latter studies also discussed several technical improvements that should be made before routine usage in clinical diagnostic procedures. Accordingly, the application of OCT in periodontal diagnosis could be a diagnostic approach with a high sensitivity as well as two- and three-dimensional imaging of periodontal structures in a non-invasive and ionising, radiation-free way. On the other hand, technical developments to ensure a fast and sufficiently diagnostic tool that is easy to handle and applicable might be a key challenge for further diagnostic approaches [42,45].

The current study systematically investigated the possibility to detect calculus with SD-OCT in vitro. The applied methods were standardised and valid. Furthermore, the comparison to light microscopy as

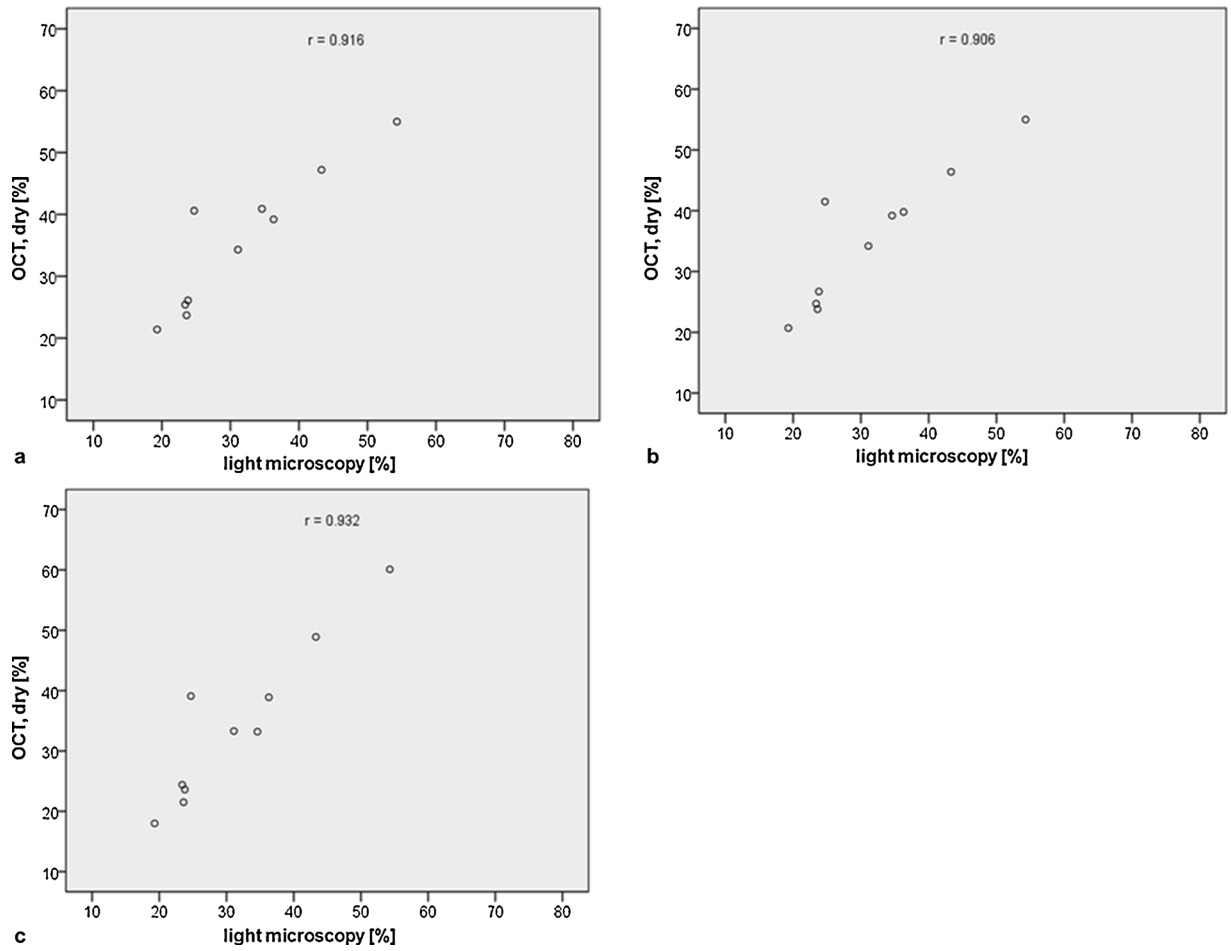

Fig. 4. a-c Correlation of length of calculus (% of ROI) measured by OCT for (a) dry root surface, (b) NaCl, (c) blood and light microscopy (r, Pearson).

the laboratory gold standard strengthens the findings of the study. By using OCT, data sets for three-dimensional volumetric images can be generated. However, for direct comparison of OCT visualisation with two-dimensional light microscopy cross-sections, we used two-dimensional OCT, cross-sectional images, which were best adapted to the light-microscopic images. However, different limitations must be addressed. First, this study is an in vitro study. A perfect simulation of real clinical conditions (gingiva, periodontal tissue, crevicular fluid) was not achieved. Although it was shown that calculus could be identified under gingival tissue [20,42] in our study, we focused systematically only on the calculus visualisation of the root surface because this was not done before. However, in our study surface contamination with NaCl and blood was used. This showed that OCT can work in a less than ideal environment closer to the clinical scenario. In this context, that the intraoral application of OCT depends on technical progress, that is, the development of a clinically usable light-application tool must be considered. The OCT equipment in the current study was only applicable in vitro. Furthermore, the study was limited by the inclusion of only 10 samples. Although this sample size is higher than in comparable literature [20], the results must be regarded as preliminary. Nevertheless, the current study provides new knowledge in the field of periodontal diagnosis by SD-OCT.

To overcome the above-mentioned current limitations of the study, future investigations should focus on the impact of periodontal tissues such as the gingiva (including pathologic areas characterised by swelling of the gingiva) on calculus imaging, depending on various parameters of OCT (e.g., power on sample, centre wavelength), the thickness of the calculus overlapping tissue and in vivo imaging using OCT.

5. Conclusion

Spectral-domain optical coherence tomography can visualise dental calculus validated by comparison with light microscopy as a reference standard. Therefore, this technique may be suited to determine the endpoint of root surface instrumentation and to assess the status of root surfaces during periodontal therapy.

Compliance with ethical standards

None.

Conflicts of interest

The authors declare no conflicts of interest in this manuscript.

Acknowledgements

Thorlabs GmbH, Dachau, Germany, for provision of the OCT equipment and Claudia Rueger for the professional technical assistance.

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