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Characterization of the dental pulp using optical coherence tomography

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ABSTRACT

The inner structure of teeth, i.e. the root canal anatomy, is very complex. However a good knowledge of endodontic architecture is the first step towards successful endodontic treatment. Optical coherence tomography (OCT) is a powerful technique to generate images of hard and soft tissue. Its images show dependency on the optical properties of the tissue under analysis. Changes in the scattering and absorption of tissues can be observed through the OCT images. In this work, we used optical coherence tomography to perform *in vitro* studies of the inner structure of the first molar of albino rats (Rattus norvegicus). Focusing on the pulp chamber and in the root canal, we compare the images generated with the OCT technique to the histology. We are analyzing the feasibility of OCT to help on the diagnostic of endodontic diseases.

Keywords: Endodontic, Optical Coherence Tomography.

1. INTRODUCTION

The morphological characteristics of human dental pulp have been extensively analyzed [1, 2], including the fact that dental pulp is almost completely surrounded by hard tissue, and there are few major vessels through the apical foramen supplying the human dental pulp. The microvascular bed arrangement of the dental pulp plays a major role in hard- and soft-tissue physiology, and thus its anatomy and histology have received considerable attention [3, 4].

The dental pulp is the soft tissue of the tooth, which develops from the connective tissue of the dental papilla, and it is encased in a rigid hard tissue. Within the crown is contained the pulp chamber and the coronal pulp. Within the root is the radicular pulp. The dental pulp consists of cells, ground substance, and neural and vascular supplies. The pulp, in conjunction with the dentin that surrounds it, is referred to as the pulp-dentin complex. The primary function of the pulp is formative and defensive. The pulp has also been thought to act as a sensory organ that warns against disease.

The vascular system of the pulp helps it to overcome problems of encapsulation within the rigid tooth. Arterioles from the dental arteries enter through the apical foramina and pass centrally through the pulp, giving off lateral branches, which divide further into capillaries. Smaller vessels reach the odontoblastic layer, where they divide extensively to form a plexus below and within the layer. Venous return is collected by a network of capillaries, which unite to form venules coursing down the central portion of the pulp. The unique feature in this arrangement is the arteriovenous shunt, which prevents build-up of unsustainable pressure in the rigid environment. Lymphatic vessels inside the pulp have not been definitely confirmed. In general, with age, the blood supply diminishes and its architecture becomes simpler. This diminished blood supply may render a pulp more susceptible to irreversible damage.

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Anatomical variability of the tooth is often a complicating factor in root canal treatment and many different methods have been used to investigate tooth morphology. These methods include sectioning of extracted teeth [5], casts of the root canals with Wood's metal [6], celluloid [7] or resin [8], decalcification of the teeth and dye injection [9] and radiographic studies in vitro [10]. Several methods were used to investigate the anatomy of the root canals, such as direct observation with the aid of a microscope [11], macroscopic sections, filling of canals with inert material and then decalcification [12], filling of canals and clearing. But all these methods had serious limitations, as most of the relationship of the external structure to the pulp is lost during preparation of samples. A significant constraint of conventional radiography is the superimposition of overlying structures, which obscures the object of interest.

Several model systems are used to evaluate the biocompatibility of dental materials [13] and animal testing has proved invaluable in preclinical screening [14]. Rodents are among the most preferred supplements to testing in higher animals, both for biological and practical reasons. Studies in rat molars on the response to various pulp-capping agents have been performed for decades [15-18]. Numerous investigators have described morphologic similarities between human and rat molars [19, 20], and Kozlov and Massler [21] have suggested that the physiology of human and rat molar pulps are probably similar. Schour and Massler [22] have stated that human and rat molars are similar histologically and physiologically, as well as in form and function. In a number of histological studies, the rat molar has been used as a test object and analyzed for its reactions to various medicaments and experimental conditions [22-24]. At this time, the rat molar is the preferred biologic test object because of its established similarities to the human molar and its availability in large numbers, and because it can be studied under controlled conditions.

The diagnosis of a diseased dental pulp can be difficult. Conventional diagnostic testing, both thermal and electric, does not accurately correlate with morphological changes [25]. In particular, the correlation between pulpal inflammation and clinical symptoms, and tests results is uncertain. Reliable information of pulp vitality would be important for diagnosis. The methods used at the present are mostly based on the patient's subjective estimation or on indirect signs. Vitality tests performed using ice, heated gutta percha, and electric stimulation require co-operation by the patient and are hence not possible to perform on unconscious, sedated, handicapped, or very young patients. To determine the viability of a tooth, clinicians now rely on the radiographic exam and subjective tests that may induce pain. Improved diagnostic techniques are needed, also as motivation for the development of therapies aimed at preserving tooth vitality. Investigators have considered the efficacy of electric and thermal stimulation, measurement of surface temperature, spectrophotometry, pulse oximetry, transillumination ultraviolet light photography and photoplenthysmography. Each method has been used, achieving different degrees of success. Optical diagnostic methods are particularly attractive because of their potential for noninvasive assessment of parameters related to pulpal blood flow and oxygenation, which primary determinants of pulp circulation [26].

Dental tissue has been subject to Optical Coherence Tomography (OCT) investigations before [27-29]. It has already been shown that OCT has the potential to provide high resolution images containing unparalleled spatial information [30]. Among the potential applications in dentistry we consider of hidden dentinal caries, the quantitative monitoring of de-remineralization of demineralised lesions, the visualization of interproximal surfaces of pre molars and molars, and the investigation of the effectiveness of restorative fillings relevant area of dental OCT as well as early detection of caries.

Up to the present, to our knowledge, there are no OCT studies of the dental pulp. In this work we study the possibility of using this technique as a dental pulp diagnoses tool. Penetration depth and ability to distinguish both dentine and pulp chamber are some of the features we are looking for. This method, if proved to be accurate, could have the ability to visualize both the radicular channels anatomy as the pulp morphology, non-invasively, and together with video rate imaging and Doppler [31, 32], it would be possible to analyze blood flow within the pulp chamber and root.

2. METHODOLOGY

2.1 Animals

Extract molars of male wistar rats from the Bioterism Center (UFPE) weighing 260-320 g were used all through the experiments. This study has been approved by the local Animal Ethics Committee with the number 030/04. The animals were left to adapt to the environment conditions (23-25^{o}C, 12 h dark/light cycle) for 24 h before the beginning of the

experiments while maintained with water and food ad libitum. These animals were sacrificed, and the maxilla was extracted. All animals were cared for with routine husbandry.

2.2 Rat Pulp

As systematic information on the influence of age on the detailed histology of the pulp is limited, it is often difficult to evaluate more subtle changes following long-term experimental interventions in Wistar rat molars [33]. Knowledge of changes in the pulpodentinal organ with age is essential in order to understand the site-specific dentine response, when the pulp is challenged with restorative procedures, exposure, and/or capping agents. It is know that there is a dentine deposition with age and this causes the pulp to become thinner. Our aim, at this point, is to know if we can determine the thickness of the dentine layer and through that evaluate the ageing of the pulp.

2.3 OCT Experiment

OCT is a noninvasive imaging technique for generating high resolution (10-20μm) cross sectional optical images of both hard and soft tissue structures with penetration depths of up to a few millimeters. It has been used clinically in ophthalmic, dermatological, and in endoscopic applications. OCT images are similar to those produced using ultrasound technology with 1000 times better spatial resolution. Near-infrared light rather than sound waves are used, and direct contact with the tissue is not required.

A schematic of the OCT instrumentation is shown in Figure 1. It is based on a Michelson interferometer. Output from a low coherence light source is split by a 50/50 optical beam splitter and directed towards the sample and a reference mirror (delay line). Reflections from the mirror and backscattered light from the tissue are recombined and directed to the detector. Coherence between the reference and sample reflections creates a signal at the detector when the reflections have traveled approximately the same optical group delay. The shorter the coherence length of the source, the more closely the sample and reference arm group delays must be matched for an interference signal to be detected. A Fast Fourier scanning is used in the reference arm to impose a change in the optical delay [34, 35]. Knowledge of the velocity which the path is scanned allows the amplitudes and longitudinal positions of sample reflections to be measured with high accuracy.

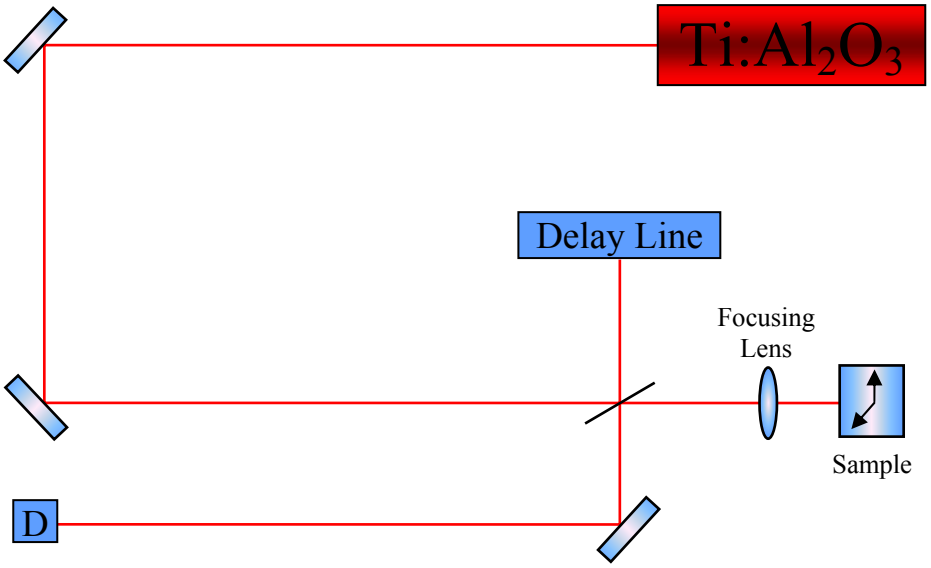

Figure 1: Schematic of the OCT instrumentation.

A cross sectional image is produced by transversely scanning the beam across the sample and collecting a reflectance profile at each point. The reflectance intensities are filtered and recorded digitally on a grayscale image as a function of transverse and axial distances. We use an electronic filter, band pass, and a digital filter to get the normalized signal envelope. The OCT system was designed using a Ti:Sapphire laser centered at 830 nm, its spectral

bandwidth was 20 nm, the repetition rate was 78 MHz, optical power at the sample was 50 μW, and horizontal polarization. The free space axial resolution of the system was 21 μm and the transverse resolution was 10 μm. Our scanning system allows delays up to 3 mm at frequencies up to 200 Hz.

2.4 Experimental Procedure

The sample was place in our experimental setup and a sequel of measurements was performed. Figure 2 shows pictures of one sample in our setup at different moments of the measurement. These are video images taken by a webcam used to site the sample and the OCT probe.

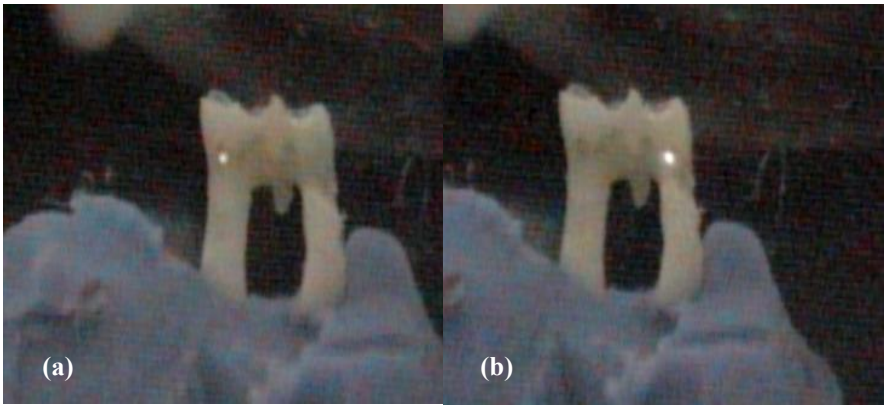

Figure 2: Photo of the rat tooth in our OCT setup. We scan the tooth, and we use these camera images to site the OCT probe and the sample. The bright spot is the OCT probe, at the beginning (a) and at the end (b) of the measurement.

After all the OCT data was acquired, the tooth was immersed in a Policrom crystal Resin, cut and polished. This procedure was necessary prior to cut the sample, since it becomes dry and very fragile. Cutting the sample without the resin layer could easily damage it. Figure 3 shows a microscope picture of a treated sample in the resin.

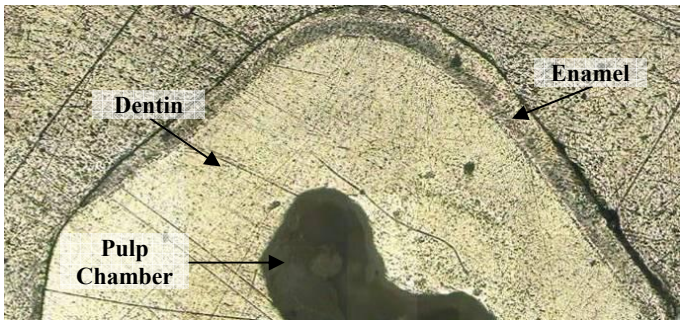

Figure 3: Microscope image of a transversal section of the rat tooth. The tooth was immersed in a Policrom crystal Resin, cut and polished. The arrows indicate the thin layer of enamel, the dentin and the pulp chamber.

3. DATA AND RESULTS

OCT images of the studied teeth are shown below. If enamel is present, there is a first bright surface followed by followed by a less scattering medium (dark layer), which is the enamel. The second layer is the dentin. It is shown as a scattering medium, thicker and granulated. The last one is the pulp chamber. In our case, there was no pulp material inside. The pulp dies very fast and its material dissolves and empties the chamber. The OCT image should show a dark layer indicating the chamber (see figure 4).

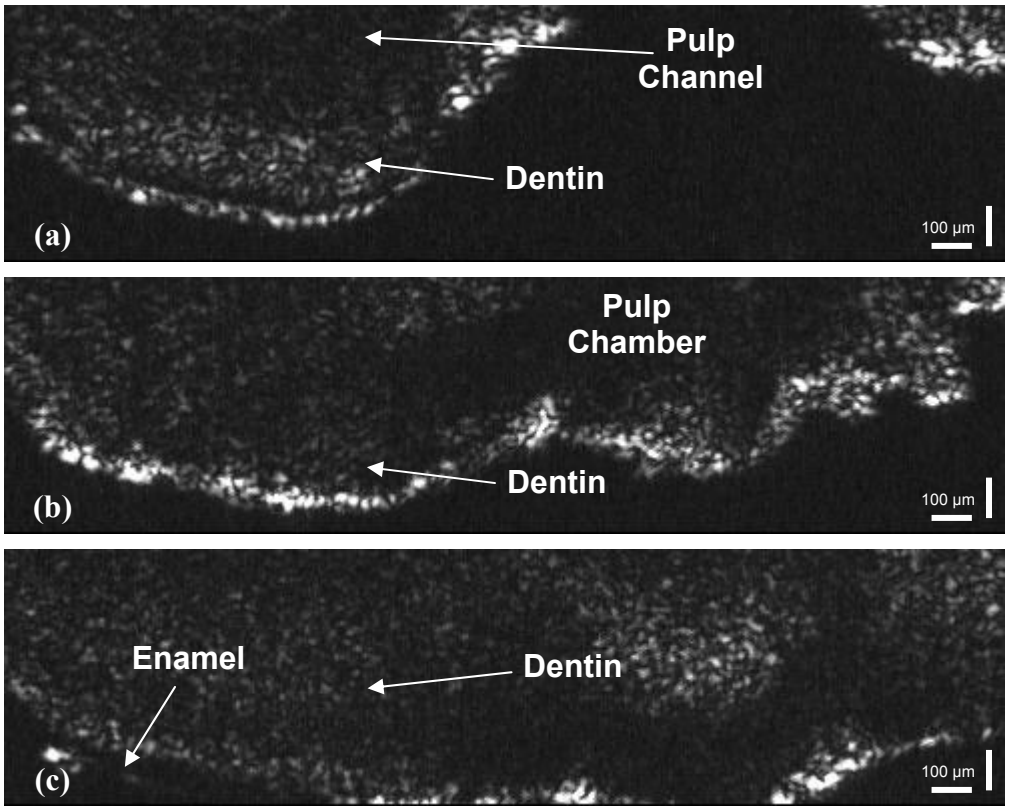

Figure 4: OCT image of the rat tooth. The figures show: (a) one of the roots; (b) an area just below the crown (400 µm above the previous picture); and (c) an area of the crown (800 µm above the fist picture) where the enamel starts to appear.

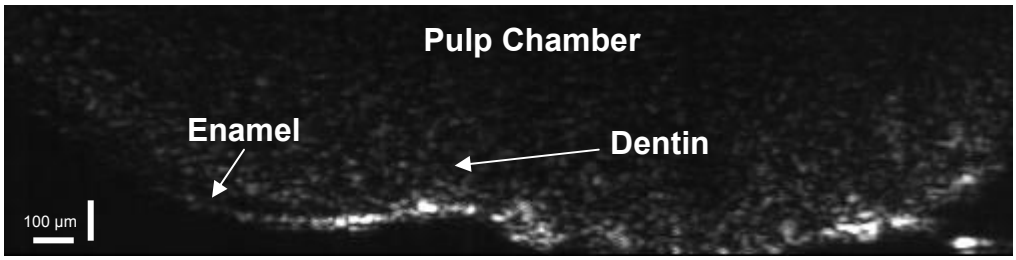

Figure 5: OCT image of the rat tooth crown.

Figure 4 shows one sequence of measurements. The first image (4a) shows one of the roots. In this image is possible to identify the dentin, more scattering layer (this region of the tooth does not have enamel), and the radicular pulp channel, the darker area. The next image (4b) shows an area just below the crown, it is a cross section of a region 400 µm above figure 4a. In this image, the chamber is identified, but there is not enough contrast to draw a limiting line. The last one (4c) shows an area of the crown, 400 µm above figure 4b, where the enamel starts to appear. In this picture, it is not possible to determine the chamber area. Figure 5 shows an area of the crown in a different tooth. The enamel, the dentin and the pulp chamber area can all be identified, but there is not enough contrast to see the limiting line.

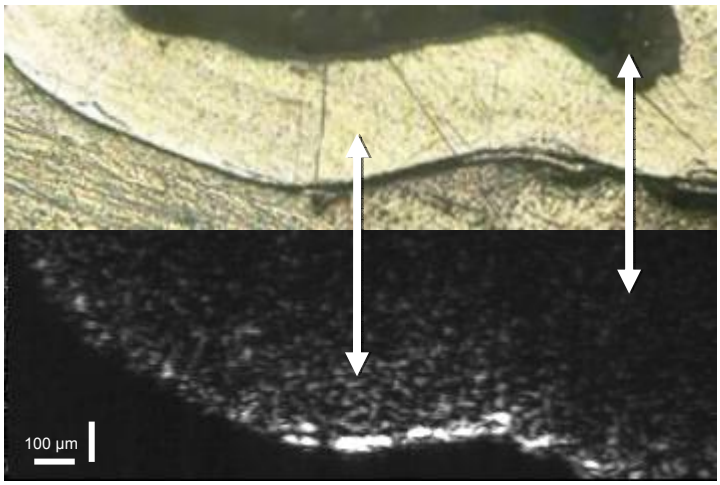

Figure 6: Comparison between the OCT and the microscope images of a cross section of the region below the crown of a rat tooth.

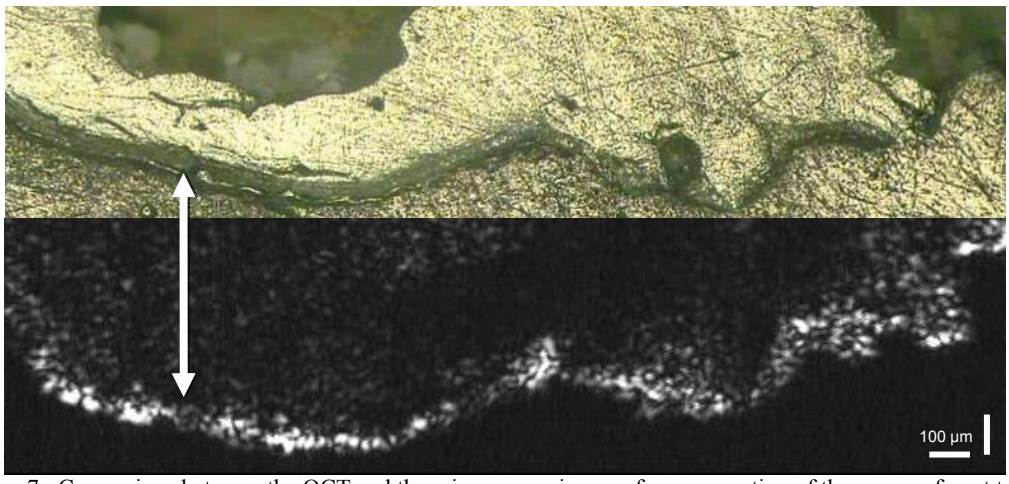

Figure 7: Comparison between the OCT and the microscopes image of a cross section of the crown of a rat tooth.

Figures 6 and 7 show comparisons between the OCT and the microscope images. Figure 6 shows a cross section image of a region below the crown of a rat tooth. We can identify in both pictures, OCT and microscopy, the dentin and the pulp chamber. Figure 7 shows a cross section image of the crown of a rat tooth. Similar features are identified on both pictures, but the contrast is not good enough to identify the pulp chamber. In both cases, the teeth were cut through a section as close to the OCT image plane as possible, after the experiment was performed.

4. CONCLUSIONS

The OCT imaging technique was applied to preliminary studies of the pulp chamber in tooth of extracted molars of male wistar rats. The data was compared with microscopic image for a direct analysis. The measurements were performed using the wavelength of 830 nm and a spatial resolution in the OCT system of ~21 µm was obtained. As the aim of the study was to verify the possibility of observing the features of the inner structure of the tooth, the results shown here are very promising and stimulating. The OCT images obtained can clearly identify the main features, such as the dentin, pulp chamber and root. An improvement in contrast and depth penetration can be obtained by using a more appropriate wavelength, such as 1300 nm instead of 830 nm, and such new experimental setup is under way in our laboratory for further assessment of tooth inner structures. Also, at these longer wavelengths, light sources based on superluminescent diodes are readily available, making the setup more attractive for development of compact OCT

systems. We believe that with further instrumentation, OCT images of the inner tooth structure will unveil more detail and will potentially be useful for diagnostics.

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