The use of confocal laser scanning microscopy in endodontic research: sealer/dentin interfaces.


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Confocal laser scanning microscopy can be used in laboratory research to obtain a series of optical XY images through the thickness of the dentin of endodontically treated teeth. With this information the study of the resin/dentin interface of root canal fillings is possible. In comparison to SEM, confocal microscopy has the advantage of providing accurate information and a simple method to determine the adaptation and distribution of sealers inside dentinal tubules in non-dehydrated samples through the use of Rhodamine-marked sealers. 3D reconstructions can also be generated with the digital data. In this work, some examples related to the sealer/dentin interface of different endodontic materials that includes epoxy and methacrylate resin sealers are given.

Keywords dentin, endodontic materials, sealers, dental adhesives.

1. Introduction

The first step of endodontic treatment is the microbial control stage or cleaning and shaping process. This step is performed with endodontic instruments and sodium hypochlorite. The next stage is the filling of the cleaned root canal. The root canal filling has 3 functions: to prevent periapical or inflammatory fluids from tracking into the canal to feed remaining bacteria, to entomb remaining bacteria that is usually present in dentinal tubules and to inhibit coronal leakage of oral bacteria present in saliva [1]. Most obturation techniques use a solid core material such as gutta-percha in conjunction with a sealer. Although gutta-percha can be reasonably adapted to the root canal walls, because of the canal irregularities as isthmuses and fins a sealer is fundamental to fill irregular spaces and to increase the seal during compaction [2].

In general, the sealers used in endodontics are divided into different groups according to their main component, such as zinc oxide-eugenol, silicone, epoxy resin, and methacrylate resin. Epoxy resin based sealers are hydrophobic whilst methacrylate resin based sealers are hydrophilic. Several studies have shown that a common failure of the root canal obturation process is the presence of gaps and porosities at the sealer/dentin interface [3-5]. The presence of gaps can allow infiltration of fluids in the filled root canal disturbing the healing process. Also, the deposition of saliva if the root filling is exposed to the oral environment could be a decisive factor to allow the subsequent bacterial colonization of the treated root canal. Several studies have shown that filled root canals can allow the recolonization of microorganisms [6]. The presence of microorganisms and their products in the treated root canal can lead to failure of the root canal treatment and the necessity of retreatment.

The analysis of the dentin/sealer interface allows the determination of which materials and filling techniques could obturate the root canals with less gaps and voids. Several microscopy techniques are currently used to evaluate the sealer/dentin interface, including stereomicroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM) [4-7]. In comparison to conventional SEM, confocal microscopy has the advantage of providing detailed information about the presence and distribution of sealers or dental adhesives inside dentinal tubules in the total circumference of the root canal walls at relative low magnification as 100× through the use of fluorescent Rhodamine–marked sealers [8]. The aim of this work is to describe the current use of the confocal laser scanning microscope for the study of the sealer/dentin interface.

2. Materials and Methods

Initially the root canals of the teeth studied were cleaned and shaped using endodontic instruments and 2.5% sodium hypochlorite (NaOCl). Because of the complexity of root canal systems and the current inability to instrument mechanically all aspects of the root canal walls surfaces, an important procedure during root canal treatment is the irrigation. The irrigant solutions can reach areas not initially instrumented, i.e., isthmuses or fins. Acoustic and hydrodynamic activation of the irrigant can be used to contribute to the cleaning efficiency and is highly recommended [10]. At the end of the cleaning and shaping procedure, the canals were irrigated with the aid of an ultrasonic unit with 2 mL of 2.5% NaOCl for 1 minute, this procedure was repeated three times. Then, the root canals receive a final flush of 2 mL of 17% EDTA (pH 7.7) for 3 min. This procedure is essential to improve the adaptation of the filling materials to the root canal walls and to allow the penetration of the sealer into dentinal tubules (Fig.1). Finally, the canals were washed with a final rinse of 2 mL distilled water and dried with paper points (Dentsply Maillefer, Ballaigues, Switzerland).
One important stage to facilitate fluorescence under the CLSM is the mixture of the sealer with the fluorescent Rhodamine-B dye to an approximate concentration of 0.1% [11]. The Rhodamine-sealer mixture was thoroughly applied into the root canal with a size 25 lentulo spiral (Dentsply Maillefer, Ballaigues, Switzerland) keeping the instrument 3 mm from the apex. The root canals were filled using an epoxy resin based sealer and gutta-percha cones (ThermaSeal plus, Tulsa, OK, USA) or using a methacrylate resin based sealer and Resilon cones (RealSeal SE, SybronEndo, Orange, CA, USA).

Seven days after the obturation process, the teeth were horizontally sectioned at 2, 4 and 6 mm from the apex using a 0.3 mm Isomet saw (Isomet, Buehler, Illinois, USA) at 200 rpm and continuous water-cooling. Then, the surfaces were polished using sandpapers number 500, 700 and 1200 under running water to eliminate debris product of the cutting procedure (Politriz, Arotec, Cotia, SP, Brazil). The samples submitted to confocal laser microscopy had 2 mm in thick and were previously verified using a stereomicroscope.

CLSM images were recorded at 100×, 400× and 630× using a Leica TCS-SPE confocal microscope (Leica Microsystems GmbH, Mannheim, Germany). The pictures were obtained by using pictures of 0.3µm step-size in a format of 1024 X 1024 pixels. The images were acquired using the Leica Application Suite-Advanced Fluorescence software (LAS AF, Leica Mannheim, Germany). Confocal images were used to identify gaps between the filling material and the dentin.

Figure 1 – SEM of the dentin surface after the use of NaOCl and EDTA, the dentin is clean and the dentinal tubules are visible and patent (A, B). In C, it is possible to visualize in the 3D CLSM picture showing the penetration of a Rhodamine-B labeled sealer inside the dentinal tubules.

3. Results and discussion

In overall, two kinds of failures can be identified: porosities, which are usually found inside the sealer but are not in contact with the dentin, and gaps that is a void between the dentin and the sealer layer. Confocal pictures acquired at 100X magnification showed that it is possible to distinguish the distribution of the sealer around the root canal walls. Large voids and porosities can be also easily visible. In addition, gaps >5µm can be able to be identified at the interface using this magnification. Using this magnification the percentage of free gap extension can be calculated. See Fig. 2. At 400X is possible to measure the width and area of gaps in the interface.

The main observations found by the authors after observe several sections of root canals filled using epoxy resin based sealer or methacrylate sealers were the difference related to greater or lower amount of gaps or porosities. See Figures 3 and 4. Epoxy resin based sealers have a tendency to present a greater incidence of porosities especially in irregular canals. See Fig.4B. On the other hand, methacrylate sealers showed a higher incidence of gaps between the sealer and the dentin. See Fig. 3D. The polymerization process can explain the difference. Epoxy resin sealers for endodontic use have a setting time between 10-14 hours [12]. In contrast, laboratory studies that used methacrylate resin based sealers have reported setting time of 30 min in an anaerobic environment [13]. The difference in setting time can be related to the shrinkage of the material and the high incidence of gaps in methacrylate based sealer [14]. Failure in epoxy resin based sealers appears to be more related to the inability to flow in the irregularities found in the root canals since failures were more presented as porosities inside the root filling. In fact, one important point during the evaluation of the sealer/dentin interface is the geometrical configuration of the root canal that can influence the results and performance of the filling techniques. Irregular anatomy including isthmuses is usually found in all types of roots in which two canals are present, as roots of mandibular molars. These irregularities can be improperly cleaned decreasing the quality and adaptation of root canal fillings [15].
Figure 2. The figure in (A) shows an x-ray of a mandibular molar showing two filled root canals. The filling material can be identified by its radiopacity. A stereomicroscope picture showing a horizontal section. The filling materials can be identified inside the dentin by its orange appearance. (C) A confocal picture of a filled root canal that includes a Rhodamine-B marked sealer. The absence of the sealer at the interface can be observed by the absence of fluorescence (arrows). The arrows indicate the region of interest showing the extension of the gap. This failure indicates a lack of adaptation of the filling material to the dentin. Bar represent 100µm. To calculate the percentage of the gap extension around the root canal, the image can be imported into the Image Tool 3.0 software (UTHSCSA, Texas, USA) and the circumference of the root canal wall can be measured. Next, the area between the arrows can be outlined. Subsequently, the percentage of the root canal wall with gap in that section can be established.

Figure 3. A root canal obturated using a methacrylate resin based sealer. 100X Confocal image shows a thin layer of sealer between the dentin and the filling material. Sealer inside the dentine structure is also evident. Bar represent 100µm. (A) A detail of the interface is showed in (B) and (C) a thin layer of sealer can be observed, penetration of the hydrophilic methacrylate resin based sealer into dentinal tubules is evident. Bar in detail in (B) represent 5µm. Bar in detail in (C) represents 10µm. Another sample is shown in (D) despite the reasonable adaptation of the material in some parts of the root canal wall, it is possible to visualize an area with a gap at the interface even at 100X. The detail at 400X magnification show the gap in detail, the failure has between 8 and 18µm.
Fig 4. A root canal obturated using an epoxy resin based sealer. Confocal image 100X shows a thin layer of sealer and sealer tags inside the dentine, close adaptation of the solid material to the dentin is evident, the sealer has been forced to the dentinal tubules, no failure or void can be seen. Bar represent 100µm (A). A mesial canal of a mandibular molar filled with gutta-percha (GP) and an epoxy resin based sealer is shown in (B). A large porosity is visible at the isthmus level (*). An oval canal filled with gutta-percha (GP) and a Rhodamine labeled epoxy resin sealer (C) The 400X magnification is shown in (D) the presence of a large gap (arrow) is evident.

As shown by previous studies, the use of confocal laser scanning microscope has shown to be a simple method to test the adaptation of dental materials to the dentin [7, 9, 16, 17]. The use of non-decalcified or hard tissue samples that does not require a specific section technique is a clear advantage of confocal analysis [7]. In spite of the SEM has been used extensively for analysis of dental material interfaces, the preparation of the specimens, as the sputter-coating procedure, may increase the possibility of artifacts. The use of confocal not only enables all of these problems but also has the additional advantage that a rapid sampling is available.

Microbial leakage continues to be a major reason for failure of root canal treatment. Ideally, a root canal filling material should provide a barrier that prevents bacterial ingress from the oral cavity until the final restoration of the teeth is completed. However, as shown by our observations, current materials show limitations to provide an adequate adaptation at the dentin interface because the tested material cannot show the absence of failures [5]. By these reasons, it must be recommended that the coronal restoration needs to be completed immediately after the root canal filling to prevent coronal leakage and to avoid the posterior re-colonization of the treated root canals [6-18]. The constant evolution of the materials used in endodontics is necessary to provide clinical success. Thus, microscopic investigations addressing the adaptation of filling materials are necessary. An ideal material provides an interface free of gaps and voids in the entire dentin/sealer junction.

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References