The effectiveness of photodynamic therapy in endodontic sealer adhesion

D. HRAB[a], O. PASTRAV[a], R.CHISNOIU[a], D. PRODAN[b], M.E. BADEA[c], A. CHISNOIU[c*]

[a]*Department of Odontology and Oral Pathology, Faculty of Dental Medicine, " Iuliu Hațieganu" University of Medicine and Pharmacy Cluj-Napoca, 33 Motilor Street, Cluj-Napoca, Romania.*

[b]*"Raluca Ripan" Institute for Research in Chemistry, "Babes-Bolyai" University, 29-30 Fantanele Street, Cluj-Napoca, Romania.*

[c]*Department of Preventive Dentistry, Faculty of Dental Medicine, " Iuliu Hațieganu" University of Medicine and Pharmacy Cluj-Napoca, 32 Clinicilor Street, Cluj-Napoca, Romania*

[d]*Department of Prosthetic Dentistry, Faculty of Dental Medicine, " Iuliu Hațieganu" University of Medicine and Pharmacy Cluj-Napoca, 32 Clinicilor Street, Cluj-Napoca, Romania*

The objective of the current study is the comparative assessment of physico-chemical properties of two bioceramic-based root canal filling materials (the conventional TotalFill BC sealer and an experimental sealer (ICCRR Cluj-Napoca)) using different storage media. Forty monoradicular teeth were included in the study. The root canals were mechanical and antiseptically treated (using photodynamic therapy or classic irrigants), obturated using one of the two sealers and then stored in different media (saliva or bovine blood). Samples were embedded in resin, sectioned and analyzed using Fourier Infrared Transmission Spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM). The interaction between TotalFill and saliva was weaker, while the experimental bioceramic sealer had a similar behavior in the two storage media, however, the interaction with saliva was stronger than the interaction with blood for the experimental sealer, but the results were not significant. Storage media can influence sealer adhesion to root dentin. Photodynamic therapy improves the adhesion of the root canal filling material.

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1. Introduction

The tightness of root fillings is a prerequisite for the success of the endodontic treatment [1]. Appropriate root filling is achieved by using a sealer that is able to fill the spaces between gutta-percha cones and dentin walls, where these fail to make direct and very close contact [2]. Successful root canal treatment depends on the adhesive properties on dental hard tissues (dentine) that must be possessed by root canal filling materials [3,20] .

There is a great variety of materials used in endodontic practice and the newly developed bioceramic-based root canal sealers have improved physicochemical properties (biocompatibility, lack of toxicity and grip contraction, chemical stability). In endodontics, bioceramic-based materials do not cause a significant inflammatory response in case of excessive apical extrusion. Another advantage is the ability to form hydroxyapatite (during adhesion) and bond to dental tissues (dentin). Superior adjustment to the upper walls is also due to its hydrophilic nature. The adhesion of bioceramic root canal sealers consists in hydrated calcium silicate powder that results in calcium silicate hydrate gel and calcium hydroxide [4]. SEM analysis was used to evaluate the characteristics of the interface between dentin and endodontic sealer. Comparing to optical microscopy, SEM has a higher resolution and are therefore is able of a higher magnification, offering ultra-structural information on the ability of sealer adhesion and penetration in dentinal tubules [5].

Infrared spectra represent an appropriate option for obtaining valuable information regarding coupling vibrations that are characteristic for bioceramic materials. Molecules, due to their specific frequencies, may vibrate or rotate depending on the strength of chemical coupling [6,7].

The objective of the current study is the comparative assessment of the physico-chemical properties of two bioceramic-based root canal filling materials (the conventional TotalFill BC sealer (FKG Swiss Endo) and an experimental sealer (ICCRR Cluj-Napoca) using different storage media.

2. Experimental

The study included 40 monoradicular teeth extracted for periodontal reasons 4 weeks prior to the study. Inclusion criteria: no caries, presence of rectilinear tooth root and a mature apex, no pathological changes. For batch uniformity, digital radiographs were performed from two incidences, thus eliminating teeth undergoing internal root resorption, calcifications and fractures.

After decontamination and autoclave sterilization, teeth were further sectioned in the coronal level using a diamond disc attached to the straight hand piece, so as to obtain a working length of 16 mm, identical in the entire study group. Sectioned teeth were kept in saline solution before mechanical and antiseptic treatment, aiming at avoiding their desiccation. Working length was determined visually by introducing a K-file instrument (MM® ISO, diameter 10/100 mm) into the canal until the tip was visible at the apex. Working length was determined by pulling out the instrument from the canal 0.5 mm.

Tooth preparation

Teeth were prepared to working length using ProTaper® system (Dentsply Maillefer). For 20 teeth, the mechanical and antiseptic treatment assumed the use of a chelating gel - MM Etilendiaminotetraacetic acid (EDTA) ® (Micromega, France) and the continuous irrigation using sodium hypochlorite (NaOCl) at a concentration of 2% (5 ml for each batch).

For the other 20 teeth, antiseptic treatment involved the use of a laser diode (HELBO TheraLite Laser) with a wavelength of 660 nm (group L). The laser tip (HELBO 3D Endo Probe, Helbo Photo-Dynamic Systems GmbH & Co., Germany) was placed in the apical region and irradiation was performed for 1 minute. The laser beam was directed using a 60-degree flexible optical fiber. The design of the optical fiber allows a three-dimensional image of the root canal, ensuring a uniform horizontal and vertical distribution [8].

Each tooth underwent root canal sealing. The filling material used consisted of a combination of gutta-percha and sealer (TotalFill or experimental sealer) (Fig. 1). Root canal filling was performed using the warm vertical condensation technique in the apical third and the technique of injection-molded thermoplasticized gutta-percha in the two coronary thirds.

In order to enable the final adhesion of filling materials, the teeth were stored in saline solution and further, each five teeth from each group were stored in artificial saliva or bovine blood medium at 100% humidity and 4°C for 14 days.

After the complete setting of the filling material, the samples were placed in distilled water for 10 minutes, rinsed with pure ethanol for 15 minutes and then placed in an oven at a constant temperature of 37° C for 24 hours to dry. After proper drying, samples were introduced into resin blocks and left for 24 hours until the complete adhesion reaction of the resin.

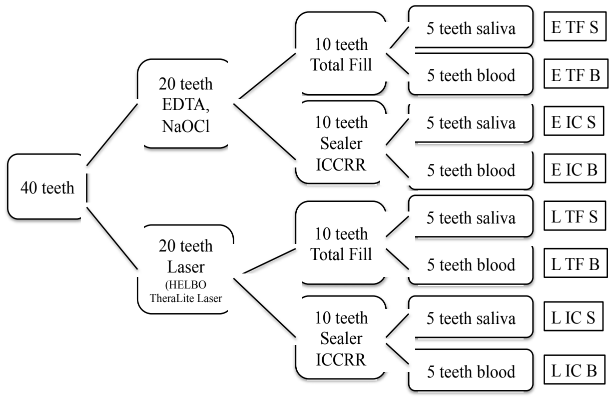

Fig. 1. Sample distribution

Fourier Transform Infrared Spectroscopy

Teeth embedded in resin blocks were sectioned into 1 mm slices using a microtome (Buehler-Isomet 1000), from apical to coronal level. Tooth slices from the apical third that did not show damage after cutting (the separation of one of the materials, gaps, etc.) were selected. Tooth slices thus obtained were prepared for Fourier Transform Infrared Spectroscopy using the FT-IR spectrometer Spectrum BX and an attenuated total reflection (ATR) device. To this end, background spectrum was recorded, followed by sample spectrum between two diamond windows, pressing using the screw mechanism provided with a stress control function. Measurements were repeated on several portions for each sample, keeping the spectra with the highest intensity and lowest noise. No baseline or smoothing corrections were applied for the obtained spectra.

The morphology and the formation of the hybrid layer were studied using scanning electron microscopy, SEM (Inspect F, FEI Company) under vacuum pressure of $1.33 \cdot 10^{-3}$ Pa and voltage of 20 kV.

3. Results

Fig. 2 shows that absorbance peaks are the same in the four samples analyzed for tooth restorations performed using TotalFill sealer, which suggests that the processes in the two storage media (blood or saliva) are different, with a change in the ratio of absorbance peaks.

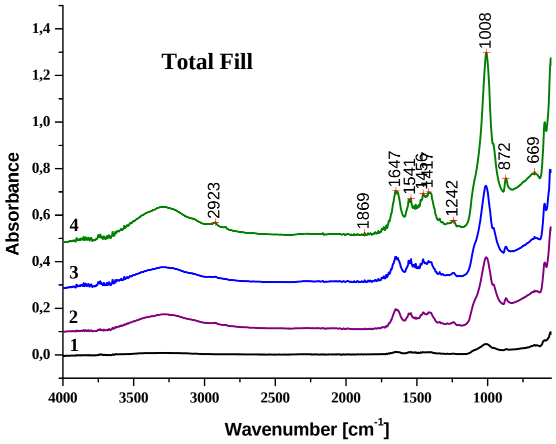

Fig. 2. FT-IR spectra of TotalFill samples
1 – TotalFill EDTA saliva (E TF S)
2 – TotalFill EDTA blood (E TF B)
3 – TotalFill Laser saliva (L TF S)
4 - TotalFill Laser blood (L TF B)

Thus, samples kept in blood show a decrease in maximum absorbance at 1008 cm^{-1} (corresponding to the C=O carbonyl group) when compared with samples immersed in saliva, suggesting that the interactions between the blood and TotalFill took place via carbonyl groups. Considering the double bond of the aromatic nuclei as an internal standard, because it does not participate in reactions, could help estimate the quantitative changes that occur in the spectra based on the ratio between the height of absorbance peaks for the study group and the height of the peak corresponding to the bonds between aromatic nuclei. Therefore, the ratio between C=O maximum intensity at around 1008 cm^{-1} and the value determined for the C=C bond (aromatic) at around 1242 cm^{-1} showed lower values for samples stored in blood medium compared to those stored in saliva medium.

In conclusion, the interaction between the TotalFill material and saliva was weaker, leading to changes in a smaller number of bonds within the filling material.

The results obtained for the experimental cement (CEM ICCRR, 'ICCRR' = "Raluca Ripan" Institute for Research in Chemistry) and infrared spectrum determinations are shown in Fig 3.

The difference between spectra is not significant for these samples; the relative intensities of absorbance peaks are close for the samples stored in saliva and those stored in blood. There is a little difference in sample behavior regarding the intensity of absorbance peaks. The ratio between the intensity of the C=O peak and the value determined for the C=C bond (aromatic) in samples stored in blood triggered a value of 4.22, while the value obtained for samples stored in saliva was lower. Thus, the experimental bioceramic sealer has a similar behavior in the two storage media, however, the interaction with saliva was stronger than the interaction with blood for the experimental sealer, but the results were not significant. On the other hand, the absorbance peaks tended to shift towards longer wavelengths in the case of samples stored in saliva.

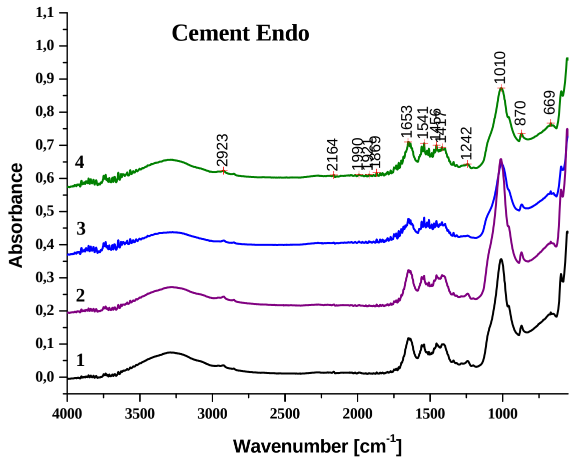

Fig. 3. FT-IR spectra of Experimental Bioceramic Sealer samples
1 – Cem ICCRR EDTA saliva (E IC S)
2 – Cem ICCRR EDTA blood (E IC B)
3 – Cem ICCRR Laser saliva (L IC S)
4 - Cem ICCRR Laser blood (L IC B)

Infrared absorbance spectra present the same absorbance peaks due to characteristic vibrations. Spectra corresponding to the intertubular region of adhesive/dentin interfaces highlight along with apatite characteristic bands at ~ 870 cm^{-1} and dentin collagen bands at 1653 cm^{-1} (C-C= aromatic group), 1417 cm^{-1}, specific bands of the monomer mixture at 1722 cm^{-1} (specific to urethane carbonyl), 1185 cm^{-1} (dimethyl in isophorone structure); 2870-2965 cm^{-1} (CH_2= methylene in urethane monomer structure). The band at 1242cm^{-1} (amide IV band) is characteristic to urethane and it is the result of the coupling of C-N (carbon- nitrogen) and C-O (carbon-oxygen) lengthening vibrations. The bands at 1455, 1510 cm^{-1} are vibrations of the aromatic ring ($\gamma_{C=C}$), and bands at 800-875cm^{-1} are characteristic of the substitution of the benzene ring, due to outside vibrations of C-H (carbon-hydrogen) bonds in the benzene ring.

The intensity of the bands associated with urethane monomers indicate a good diffusion of the root canal sealer in laser-treated samples compared to those undergoing EDTA irrigation to remove the smear layer at the interface with root canal dentin and forming a hybrid layer, which is an indication of the affinity to tooth structure in these conditions, therefore justifying the choice of photodynamic laser therapy as potentially favoring the adhesion of the root canal filling material.

The evaluation of the experimental endodontic sealer using SEM images showed a homogenous layer in the apical region, with extensions that intersects the hybrid layer. We observed an electronic density similar with the upper layer, which demonstrates a continuity of bioceramic nanoparticles concentration in depth. We also noticed the hybridization of the periphery of dentinal tubules and the hybrid layers extensions to the depth for samples immersed in saliva (Fig. 4).

The use of photodynamic therapy favors the removal of the smear layer, the exposure of the dentinal tubules and allows a better penetration of the sealer, comparing to the samples irrigated with EDTA/NaOCl (Fig. 5).

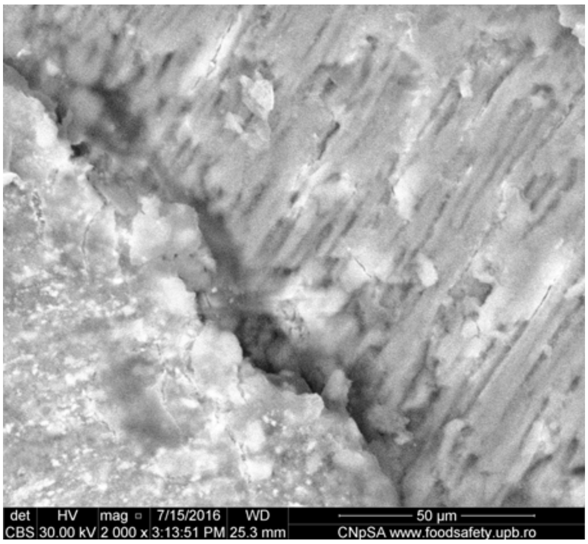

Fig. 4. SEM image of hybrid layer for experimental sealer (samples immersed in saliva)

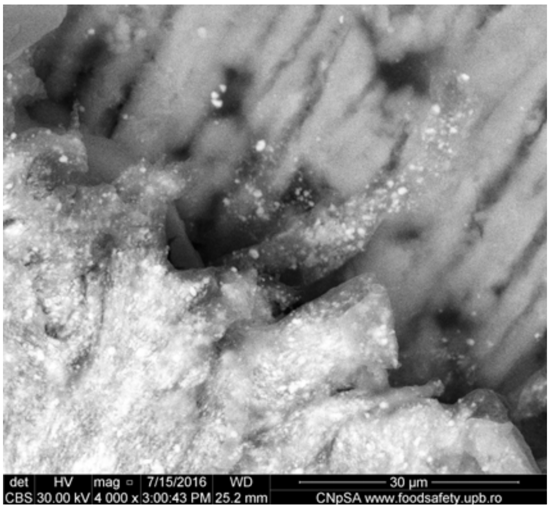

Fig. 5. Bioceramic extensions after photodynamic therapy for experimental sealer (samples immersed in blood).

4. Discussions

One of the major goals of root canal filling materials is to ensure optimum apical sealing. Due to its hydrophilic / synthetic hydroxyapatite formula and excellent fluidity, TotalFill® BC Sealer TM enables an increased adhesion to dentin. A recent study showed that the sealing capacity of Total Fill BC sealer is superior to that of other root canal filling materials available in the market. Adhesive properties of root canal sealers were tested under various conditions of humidity. The adhesion of TotalFill BC sealer was superior to other materials regardless of humidity [9].

Apical sealing after root canal treatment may be compromised by root contact. A study conducted by Hasheminia et al. [10] regarding the adhesion of sealing materials under ideal conditions, compared to blood and saliva media, showed that the calcium-enriched mixture cement behaved better in saliva storage medium than a bioceramic sealer (MTA= mineral trioxide aggregate). In our study, the experimental bioceramic material presented satisfactory adhesion, similar in the two storage media, while the conventional bioceramic sealer showed superior adhesion in blood medium.

Root canal instrumentation produces the smear layer (a 1-5 micrometer thick layer of organic and inorganic material composed of dentine debris, necrotic pulp debris and bacteria) [11]. Smear layer removal before root canal treatment remains controversial. On the one hand, it can promote bacterial micro-infiltration and a nutritious substrate for residual bacterial populations [12,13]. Other studies support that this smear layer can block dentinal tubules, causing a reduction in permeability and thus limiting bacterial penetration. After root canal disinfection and instrumentation, residual bacteria can also be buried in dentin tubules via the smear layer and the filling material. The adhesion of filling materials can be influenced by the presence/absence of the smear layer [14,15].

The use of chelation agents such as EDTA and NaOCI ensures the removal of the smear layer and root canal disinfection [16].

Laser photodynamic therapy is more effective in root canal disinfection than NaOCl due to the production of reactive oxygen species that have a bactericidal effect, destroying bacterial cell membranes [17,18]. Endodontic irradiation with Erbium: YAG laser determines the removal of the smear layer and the creation of uneven surfaces in the root. The presence of these irregularities can improve sealer adhesion to dentin [19]. Our results are consistent with the literature, observing a superior adhesion and the formation of a hybrid layer that is more substantial for samples where root canal disinfection was performed using photodynamic therapy.

5. Conclusions

Storage media can influence sealer adhesion to root dentin, the conventional bioceramic sealer (TotalFill BC) showing superior adhesion in saliva. However, the differences were not significant when it comes to the experimental bioceramic sealer.

Photodynamic therapy improves the adhesion of the root canal filling material through more efficient disinfection and removal of the smear layer from the root canal.

Acknowledgements

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*Correspondent author: dr.chisnoiu@yahoo.com