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Analysis of Stability in Time of Marginal Adaptation of Endosequence Root Repair Material on Biological Samples

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Abstract

Introduction: The introduction of mineral trioxide aggregate (MTA) and bioceramic sealers increased the success rate of endodontic surgery and perforation repair. The aim of this study was to evaluate the marginal adaptation at different times of endosequence root repair material (ERMM) in order to evaluate its dimensional stability using variable pressure-scanning electron microscope (VP-SEM). **Material And Methods:** Forty-eight teeth were selected shaped up to a master apical size of 25. Then a 3 mm cut perpendicular to the long axis and a retrograde cavity preparation were performed. In order to obtain 2 mm thick sample a second cut was done and, in this disk, ERMM was inserted. The samples were stored at 37°. The samples were divided into four time-depending groups observed with VP-SEM at time 0 (Group 1) and after 2 (Group 2), 7 (Group 3) and 30 days (Group 4) after ERMM setting. Statistical analysis with one way-ANOVA test was performed (95%). **Results:** None of the four groups analyzed showed a complete marginal adaptation between dentin and ERMM. Instead, in all groups ERMM exhibited a completely preserved marginal adaptation to the dentin wall in all time-dependent groups. The mean (\pm SD) gap value was for time 0, 3.91 (\pm 2.55) μ m after 2 days, 4.32 (\pm 2.69), after 7 days 4.49 (\pm 2.53), and after 30 days 4.81 (\pm 2.85) μ m. No statistically significant difference was found between the four groups. **Conclusions:** The results of the present study demonstrate the dimensional stability over time of ERMM.

Keywords: Apicoectomy, dental marginal adaptation, electron scanning microscopy, endodontics, endosequence root repair material

INTRODUCTION

Root canal treatment is minimally invasive, it has a high success rate over 90%,^[1,2] and a positive cost-benefit ratio.^[3] However, failures can unfortunately occur, this can be due to several factors, such as the anatomy of the root canals, the presence of complex and highly organized pathogenic microbial communities technological limitations in dental instruments and/or in obturation techniques, and the presence of leakage in coronal restorations that allows bacterial penetration inside the root canal systems.^[4,5]

For the above-mentioned factors, it is clearly inevitable that some root canal therapies will not be able to heal. A careful and well-done initial root canal treatment will minimize the risk of failure.^[6] Surely after an initial failure, it is possible to retry the therapy with a conservative approach. Several studies, with a 4-year follow-up reported the significantly higher success rate of surgical endodontic treatment (78%)

with respect to the success rate of nonsurgical retreatment with the same follow-up period (71%).^[7]

On the basis of clinical studies,^[8] the apical leakage has been identified as the main cause of failure of surgical endodontic therapies. Several studies have shown that to avoid leakage formation, other than adopting a good filling technique, the filling material must not undergo dimensional changes over time, but must maintain its stability.^[9]

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In the last 15 years, mineral trioxide aggregate (MTA) became the gold standard among the materials used for the root filling after surgical endodontic treatment. MTA can improve healing thanks to its sealing properties, dimensional stability, and its bio-stimulating tissue response.^[9,10] On the other hand, the white MTA has some disadvantages such as the setting time. In fact the initial set is 40 ± 2.9 minutes while the final set is of 140 ± 2.6 minutes.^[9]

Bioceramic materials have the same useful properties and, moreover, a shorter setting time and a uniform consistency during placement which improves handling. EndoSequence root repair material (ERRM) (Brasseler USA, Savannah, GA) is a bioceramic material delivered in two different kinds of configuration: premixed moldable putty (ESP) and preloaded syringeable paste (ESS) with delivery tips for the intracanal delivery of the material.

According to the manufacturer, both configurations of ERMM have a working time of 30+ minutes. The setting reaction, due to its highly alkaline pH, allows the material to have antibacterial properties. Indeed, previous studies have already demonstrated the antibacterial efficacy *in vivo*.^[10,11] Different ERMM characteristics were evaluated in several studies, hardness after setting,^[12] material setting time,^[13] marginal adaptation, and sealing ability.^[14,15]

Until now, the dimensional stability of the ERRM has not yet been studied. To achieve our goal, we used the scanning electron microscopy (SEM), a fundamental tool in dental research. Although conventional field emission SEM (FE-SEM) operating conditions (high vacuum and high voltage) give very informative images with a high resolution of biocompatible prosthetic materials,^[16] there is an innovative type of SEM microscope, the variable pressure (VP-SEM) that works at variable pressure and humidity, conditions that avoid MTA and bioceramic filling materials (like ERRM) to be damaged under the electron beam. Moreover, the use of this kind of microscope does not require any procedure of dehydration or sputter-coating, methods that alter natural surface features.^[17]

The aim of the present study is to measure the marginal adaptation at different times (ERRM just inserted, time 0, after 27, and 30 days after ERRM setting) in order to evaluate the dimensional stability of ERRM using VP-SEM.

METHODS AND MATERIALS

Sample preparation

A total of 82 freshly extracted teeth were selected and evaluated with an optical microscope (Zeiss, Axioscop 40) at 20x magnification and 34 teeth were excluded due to cracks or defects. All the remaining 48 samples were stored in sodium chloride (NaOCl) 0.9% (OGNA, Monza, Italy), for maximum 20 days at 4°C until used. Experimental procedures were performed from the same operator and with the same conditions. The methodology used was already validated in previously published study.^[14] The root canal system of each

tooth was instrumented and flared up to a master apical size of 25 using K-files (Dentsply, Maillefer, Ballaigues, Switzerland), followed by pro-taper universal rotary Ni-Ti instruments (Dentsply, Maillefer, Ballaigues, Switzerland) and mounted on a low-speed endodontic handpiece 16:1 (Kavo Kerr corporation orange, USA). The root canals were irrigated among each instrumentation with 1 ml of 5.25% NaOCl (OGNA, Monza, Italy), and the smear layer was removed with a final rinse using 17% Ethylenediaminetetraacetic acid (EDTA) (OGNA, Monza, Italy). Absorbent paper points were used to dry the canals. Teeth were cut with a diamond fissure bur mounted on a high-speed handpiece under water spray irrigation. To create samples with standardized length, the teeth were marked on the root surface with a demographic pencil and were sectioned horizontally with an angle of 90° to the major axis of the root 3 mm away from the apex and sectioning a piece of root of 2 mm thick. The root canals were instrumented with special narrow periapical ultrasonic tips for cavity preparation, then were filled with ERRM according with the manufacturer's instructions (Brasseler USA dental, Savannah USA). The ERRM was placed into the canal using a proper syringe, the overflow was removed, and the sample's surface was polished using special burs mounted on laboratory low-speed handpiece.

Specimens were divided in four time-dependent groups: group 1, marginal adaptations were evaluated immediately after ERRM application; group 2: marginal adaptations were evaluated after 2 days; group 3: marginal adaptations were evaluated after 7 days; group 4: marginal adaptations were evaluated after 30 days.

Vp-SEM observation

The samples were mounted on aluminum stubs with adhesive carbon tape and observed with the variable pressure SEM Hitachi SU-3500 (Hitachi Japan), at 30 Pa and 6 kV operating conditions. This particularly innovative microscope is equipped with: secondary electrons detector (SE), ultra variable-pressure detector (UVD); back scattered electrons detector (BSE). This instrument enables teeth observation at variable pressure, humidity, and low voltage.^[18] In Figure 1 image output from the UVD and BSE image compo mode is shown. Images were captured at several magnifications between 50X and 1000X.

Each sample was observed at four different times to detect any change in the marginal adaptation between ERRM and dentin. Measurements were carried out on photos at 1000X magnification. On each photo eight reference points were selected in which the measurements were performed [Figure 1]. The points were always aligned in the same way in order to ensure measurement standardization. The image-processing program Image J (command: */line measure/*, after image calibration at the proper magnification) was used to obtain gap measures. The data were analyzed by Med Calc© (2019 MedCalc Software bvb) statistical program. The mean

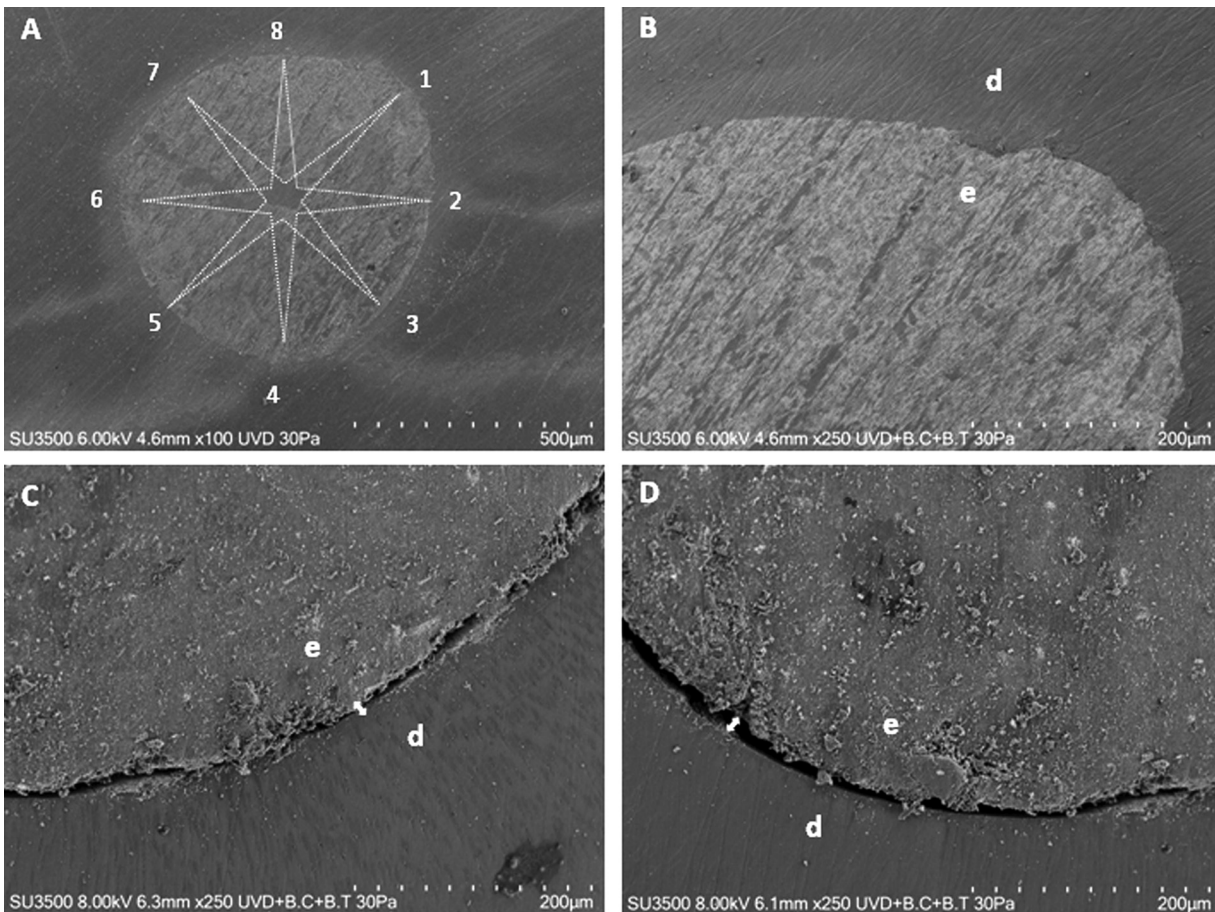


Figure 1: A) Apex filled with ERRM, numbers represent selected sites of gap measurement; B) SEM micrograph shows no gap between ERRM (E) and dentin (d); C) Image shows the surface of a representative quarter of group 1 samples. Small arrow indicates a mean gap value of 1; D) Image shows the surface of a representative quarter of group 4 samples. Arrow indicates one of the widest gap value.

gap and standard deviation for the marginal adaptation data were performed using one-way analysis test. Significance was set at the 95% confidence level.

RESULTS

None of the four groups analyzed showed a complete marginal adaptation between dentin and ERRM [Table 1, Figure 1] instead in all groups, both gap-free and gap-filled regions were observed. ERRM exhibited a completely preserved marginal adaptation to the dentin wall in all time-dependent groups [Table 1, Figure 1].

The average mean \pm standard deviation, the minimum, and the maximum values of the applied apical marginal adaptation to dentin in different time-dependent groups are shown in Table 1. The maximum mean average gap was found at group 4: $4.81 \pm 2.85 \mu\text{m}$, followed by group 3: $4.49 \pm 2.53 \mu\text{m}$, and group 2: 4.32 ± 2.69 , while the minimum mean was found at group 1: $3.91 \pm 2.55 \mu\text{m}$ (Table 1). Statistical analysis of One Way-ANOVA test showed no significant difference in mean average gap at dentin—ERRM interface, comparing group 1 with group 2, group 3, and group 4 ($P=0.756$, $P > 0.05$).

DISCUSSION

The introduction of MTA in the endodontic market was thought to supply the lack of endodontic materials. An ideal endodontic root repair material should provide some important characteristics such as the capability of sealing the pathways between endodontic anatomy and periodontal tissue, the biocompatibility, the insolubility to oral fluids, and the dimensional stability.^[19] MTA provides these properties, although some studies have shown it has also some of disadvantages such as: short working time, a prolonged setting time, difficult handling properties, and the absence of a solvent to remove the material.^[13,20] In order to supply these problems with MTA, new materials have been introduced on the market: bioceramic-based materials. The bioceramic-based materials include a group of components made by the combination of different molecules, calcium silicate, and calcium phosphate. Thanks to their advantageous physical and biological properties these materials are applicable for biomedical and dental use, having a special focus on endodontic practice.^[21] The composition of ERRM is calcium silicates, calcium phosphate, and zirconium oxide mixed with a filler and thickening agents, as listed by the

Table 1: The mean (SD) of gap value (μm) after ERRM application between the apical plug and dentine wall of the root canal system in different times (0 day, 2 days, 7 days and 30 days)

	0 day	2 days	7 days	30 days	P-value
Mean (SD) of gap value (μm)	3.91(\pm 2.5)	4.32(\pm 2.6)	4.49(\pm 2.53)	4.81(\pm 2.85)	
Min./Max. (μm)	0.00/9.56	0.00/10.83	0.00/10.91	0.00/12.7	

ns, not statistically significant.

manufacturer. ERRM has been object of many in vitro and in vivo studies that have shown the biocompatibility, the absence of toxicity, and shrinkage. Moreover, other studies have investigated the chemical stability within the biological environment.^[22-24] Other than the chemical and physical properties, also the antibacterial activity of the ERRM has been studied.^[25] Previously published studies considered also the possibility that bioceramic-based materials could create hydroxyapatite when in a humid environment and form a bond between dentin and the filling material.^[21] The reported setting time of ERRM has been studied and ranges between 4 hours and 48 hours.^[13] One of the most important characteristics is the marginal adaptation of the material. Although the quality of marginal adaptation is not directly related with long-lasting in vivo success, it has been demonstrated that it is a proper way to evaluate the ability to seal and the leakage resistance of the material.^[26] Quantitative analysis of the marginal adaptation at different times (0, 2, 7, and 30 days after ERRM use), in order to evaluate the dimensional stability of ERRM, was the aim of the present study.

In this study, the VP-SEM was used for its capability of working at variable pressure and humidity, avoiding MTA and bioceramic filling materials (like ERRM) damage under the electron beam. These operating parameters ensure absence of artifacts due to unideal observation conditions. Therefore, we can say that the measures we report correspond to the real condition of the materials. The presence of BSE detector allows to better distinguish different material phases, to avoid incorrect measurements, clarifying the boundaries between materials. BSE detector counts the number of backscattered electrons (BSE); this number is proportional to the mean atomic number of the sample. */Bright/* in a BSE image correlates with greater average atomic number in the sample and */dark/* correlates with lower average atomic number; this allows to distinguish different phases in the sample.^[18] BSE image compo mode in low vacuum means that the image results from the overlapping of images generated by secondary electrons and BSE. BSE image compo mode in low vacuum contains information about sample composition, but less topography. These working conditions are essential for non-destructive observations on biological, non-conductive, and vulnerable specimens as teeth. This study, despite the limitation of this kind of model, demonstrates the mid-term dimensional stability of the endosequence root repair. The marginal adaptation in the long term is a direct consequence of material stability,

because a really low-shrinking percentage avoids new gaps formation between the dentin walls and the materials. On the basis of the marginal adaptation time-depending measurements reported, we can state that ERRM has good stability over time. Indeed, the gap size of the material after setting time is coherent with a previously published study^[27] and does not seem to increase in the mid-term. These results can be explained by the physical and mechanical properties of the calcium silicate-based materials, their smaller particle size shows a favorable flow characteristics.^[28]

CONCLUSION

The present study seems to demonstrate that ERRM keeps dimensional stability over time. These results may enhance the clinical use of this material for both retrograde filling in endodontic surgery and perforation repair, to seal communication between endodontic space and periodontal tissues. Moreover, these results were obtained using VP-SEM, therefore these findings may lead to the conclusion that for this kind of study the metallization of the sample does not influence the long-term result. However, in order to confirm these results, further studies seem necessary to better evaluate the longstanding dimensional stability of the material in vivo and the influence of the sample treatment on the results.

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Conflicts of interest

There are no conflicts of interest.

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