

# COMPARISON OF THREE CALCIUM SILICATE CEMENTS USED AS RETROGRADE FILLING MATERIALS. AN IN VITRO STUDY

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## ABSTRACT

**Objective.** The purpose of the study was to evaluate the penetration into the dentinal tubules of the root canal of three different Calcium Silicate Cements (CSCs): ProRoot MTA White (Dentsply Tulsa Dental, USA), Biodentine (Septodont, France) and Endosequence RRM (Brassler, USA) used as retrograde filling materials. Also, their clinical properties as manipulation, setting time and apical seal capacity were compared.

**Material and Methods.** Twenty-one single-rooted human freshly extracted teeth were included. After endodontic treatment, an apicectomy of 3 mm height was performed in order to mimic the surgical procedure, followed by a 3 mm deep retrograde ultrasonic cavity preparation and filling. The teeth were divided into three groups (n=7), according to the retrofilling material used. A horizontal slice of 2 mm thickness (measured from the apex) was sectioned for each root and observed under a dental operating microscope at 1.6x. The diffusion of the retrograde filling material into the dentinal tubules was evaluated as shape and measured as length in millimeters on recorded photographs.

**Results.** Biodentine showed the highest penetration values (mean=1.574mm), but it was more difficult to be manipulated. ProRoot MTA showed good penetration, but it was also technical sensitive. Endosequence was the best regarding handling characteristics and showed large concentric diffusion areas surrounding almost the entire root canal perimeter, with lower penetration values (mean=1.56mm) comparative to Biodentine, but better than MTA (mean=1.252mm).

**Conclusions.** Calcium Silicate Cements offer a good apical seal in retrograde surgery and present the advantage of diffusing into the dentinal tubules of the root canal walls.

**Keywords:** Calcium Silicate Cements, apical surgery, retrograde filling, dentin penetration capacity.

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## INTRODUCTION

Apical surgery is often the last resort to surgically maintain a tooth with a periapical lesion that cannot be managed with conventional endodontic treatment/retreatment and the main goal of it is to prevent bacterial leakage from the root canal system into the periradicular tissues by placing a tight and persistent root-end filling which will allow periapical healing with good long-term prognosis (1).

A variety of materials have been propagated in the past years for the root-end filling but the clinical use of Calcium Silicate Cements (CSCs) has already a long history. In the 1990's Mineral Trioxide Aggregate (MTA) was developed with the purpose to serve as a root-end-filling material and to seal lateral root perforations. The sealing ability of most of the CSCs is extremely well documented in in vitro and in vivo on animals studies, and basically CSCs applied in root-end cavities demonstrated an efficient and durable seal (2). In addition, CSCs were more biocompatible than all the other materials showing cementogenic and healing ability (3). Their principal limitations are long setting time, low radiopacity and difficult handling, so new CSCs-based materials containing additional components

have been introduced since and have received a considerable attention from laboratory researchers and clinicians for their innovative properties (2). In order to overcome these limitations, a new bioceramic material was formulated using MTA-based cement technology, Biodentine (Septodont, Saint-Maur-des-Fossés, France). It was introduced in 2010 and has proved to be a second major break-through (4). Biodentine claims improvements of some of the properties of CSCs such as physical qualities and handling, including its other wide range of applications like endodontic repair and pulp capping in restorative dentistry (5). This biologically active material aids its penetration through opened dentinal tubules to crystallize interlocking with dentin and provide mechanical properties (4). EndoSequence (Brasseler, Savannah, GA, USA) is also a CS based material that has the same indications as MTA and Biodentine, but it has different components. This cement is a combination of CS and Calcium Phosphate, which is also known as Bioceramic. It has certain advantages like biocompatibility, non toxicity, dimensional stability and most importantly in endodontic applications, being bio-inert (6). The material is composed of nanoparticles, which makes the penetration into the dentine easier, favoring the mechanical seal and the moisture inside the dentinal tubules makes it easier for the material to enter

the tubules and eases the final setting. Another property is the high alkaline pH, which generates an antimicrobial environment and also it stimulates the hydroxyapatite development. This material has excellent radiopaque qualities, which helps the practitioner detecting the material (7,8).

Therefore, the purpose of this in vitro study was to evaluate the penetration of these three different root repair materials into the dentinal tubules of the root canal and to evaluate their sealing capacity as retrograde filling materials. During the study, the processing, manipulation and setting time of each material was also assessed, in order to help the clinicians choose the most appropriate material in correlation with the diagnosis, outcome expectations, endodontic and surgical techniques.

## **MATERIAL AND METHODS**

A number of twenty-one freshly extracted single-rooted human teeth were included in this study. The selected teeth were not previously endodontically treated, had an intact coronal structure free of caries and complete root apical development. After samples selection, the crown sectioning of each tooth at 2 mm above the cemento-enamel junction (CEJ) followed, and an orthograde endodontic treatment was performed. Root canals were shaped using the ProTaper Next system (Dentsply Maillefer, Ballaigues, Switzerland) connected to the handpiece of an Endo-Motor X-Smart Plus (Dentsply Sirona, Maillefer, Ballaigues, Switzerland) in association with irrigants. Teeth were obturated with the Continuous Wave of Condensation technique (CWC) using AH Plus sealer (Dentsply De Trey, Konstanz, Germany) and dedicated gutta-percha points for ProTaper Next (Dentsply). After 24 hours, an apicectomy of 3 mm height was performed with a high-speed diamond bur perpendicular to the long axis of the tooth, in order to mimic the surgical clinical procedure. A 3 mm deep retrograde cavity preparation with ProUltra Surgical Endo Tip (Dentsply Sirona, Ballaigues, Switzerland) mounted on an ultrasonic device (Satelec/Acteon, Merignac, France) followed.

The samples were then randomly divided into three groups (n=7) according to the material used as retrograde filling: Group 1 - ProRoot MTA White (Dentsply Tulsa Dental, Johnson City, TN, USA); Group 2 - Biodentine (Septodont, Saint-Maur-des-Fossés, France); Group 3 - Endosequence RRM consistency putty (Brassler, Savannah, GA, USA). Teeth were isolated with two layers of transparent nail varnish and all samples were immersed in Methylene blue dye 1% solution (TIS Farmaceutic SA, Bucharest, Romania) for 48 hours at room temperature. After 48 hours, teeth were thoroughly washed under running water and stored for another 21 days in a humid environment to simulate periapical conditions. From each root-end a horizontal slice of 2 mm thickness (measured

from the apex) was sectioned using a diamond disk. Each section was observed and photographed with a Cannon EOS 60D camera (Cannon, USA) connected to a dental operating microscope (Alltion, Wuzhou, China) with a focal distance of 250mm and an eyepiece power of 12.5x, at a magnification of 1.6x. The white penetration areas that have been observed at the microscopical examination around the condensed root-end material in each sample were considered as the diffusion areas of the retrograde filling material into the surrounding dentinal tubules, as they were not immediately present after the root-end obturation, neither after 48 hours. This diffusion was measured as length in millimeters on photographs starting from the most far ending point of the penetration zone to the contour of the root canal wall in each observed area; the numbers, sizes and shapes of the diffusion areas were also analyzed (Fig.1a-f). The measured lengths on photographs in millimeters were divided to a constant factor represented by the total magnification of the microscope and resolution of the Cannon camera. These lengths in mm were recorded in a table and compared between groups (Table 1).

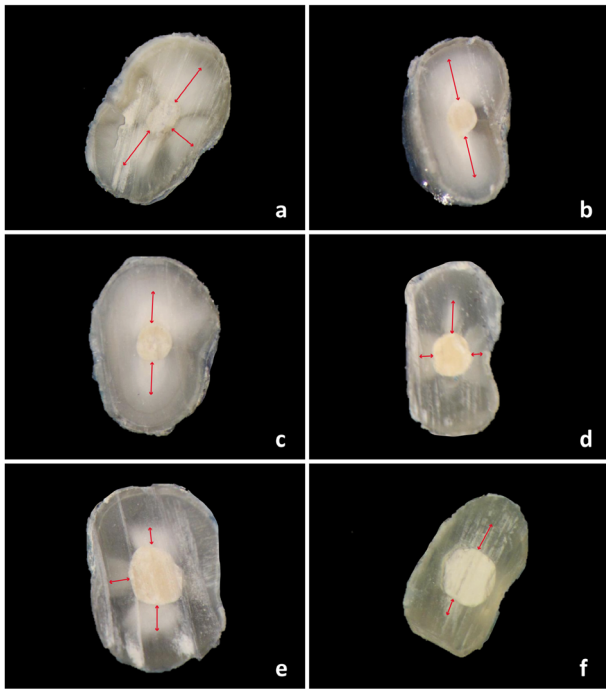
For each slice, areas of remaining sealer and gutta-percha into the retro-cavity were counted, the penetration of the dye solution into the retrograde filling material was analyzed, and the number of voids in the apical plugs or the presence of root fractures were recorded.

## **RESULTS**

### **Dentin penetration capacity**

In all the analyzed samples, concentric areas of diffuse penetration of the material into the dentinal tubules were observed, surrounding almost the entire contour of the root canal preparation, showing the good diffusion capacity of this material. Biodentine (Fig.1a,b) penetrated more into the dentinal tubules as length with highest values, varying between 0.72 and 3.64 mm and an average of 1.574 mm. Although it showed a higher number of penetration zones around the root contour comparative to the total root cross-sectional surface than MTA, the diffusion of the material was more located to a certain surface of the root perimeter and not concentric, surrounding the cement, as for Endosequence. ProRoot MTA (Fig.1e,f) had the lowest penetration values into the dentinal tubules comparative to Biodentine and Endosequence, with a maximum of 1.68 and a mean value of 1.252 mm. The diffusion of the material into the dentinal tubules was observed only in some areas surrounding the root canal contour, numerous in some of the samples, but reduced in others, covering as total a smaller cross-sectional area comparative to Biodentine and Endosequence.

Comparisons between the measurements in all samples are recorded in Figure 2.



**Figure 1.**

Samples of dentin penetration capacity highlighted by arrows: a, b) Biodentine penetrates best the dentinal tubules as length; c, d) Endosequence is showing deep, concentric and wide penetration areas into the dentin; e, f) ProRoot MTA presented lower penetration values into the dentinal tubules

Sample number	1	2	3	4	5	6	7	Mean value (mm)
<b>GROUP 1</b>	2.4	0.98	1.32	2.16	1.36	1.48	0.75	<b>1.252</b>
<b>MTA</b>	0.72	1.68	1.48	1.28	0.78	1.28	0.96	
	1.6	1.2		1.36	0.56		1.04	
	1.04			0.72	1.28		1.04	
				0.8				
<b>GROUP 2</b>	1.76	0.48	2.53	1.36	0.72	2.4	1.2	<b>1.574</b>
<b>BIODENTINE</b>	0.88	1.36	1.44	1.44	1.6	0.72	1.04	
	2.16		1.92			1.68	1.76	
			3.36			0.88	1.68	
			2.32					
			1.52					
<b>GROUP 3</b>	1.92	2.84	1.6	1.44	0.72	0.72	2.06	<b>1.560</b>
<b>ENDOSEQUENCE</b>	1.92	2.53	1.44	1.76	1.6	0.49	1.84	
	1.96		0.68	1.92			0.8	
	1.6			1.36				

**Table 1.**

Values (mm) of the materials penetration length into the dentinal tubules measured for each sample and observed area

### Sealing capacity

Regarding the sealing capacity of the three materials, in Group 1 (ProRoot MTA) in some of the samples (No 1, 2 and 4) the interface between gutta-percha and MTA was still visible, meaning that the US

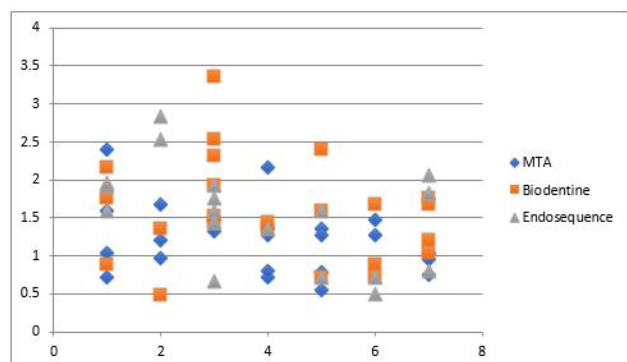
tip used in retro-preparation did not remove the entire root canal filling material prior to the placement of MTA. Also, in these samples, voids were present, either located in the mass of MTA (Sample no 1), showing lacks in the MTA-condensation process, or linear, in contact with the canal walls (Sample no 2). All these observations are strictly dependent by the operators technical skills during removal or condensation of the materials. In two of the analyzed samples, a dye penetration area of approximately 0.1 and 0.2 mm diameter was noticed, representing micro-leakage (Samples no 4 and 5). Sealer remnants at the interface with MTA were observed in only one sample.

In Group 2 (Biodentine) apical micro-cracks, appearing like horizontal fracture lines were observed in three of the samples. These could be induced by the rotary preparation of the root canals, by the retro-preparation with US tips or by the sectioning procedure of the slice samples, but were not present at the selection of the involved teeth. Point-like marginal voids were observed in only one sample, whereas three plug voids were present on two others. Also, gutta-percha remnants were observed in only one sample.

In Group 3 (Endosequence) in several samples (n=5) remnants of gutta-percha were observed at the interface with the CS-material. Marginal and plug voids were horizontally laid out and observed only in two samples, where marginal microleakage was also recorded.

### Handling properties

The material with the easiest manipulating properties proved to be for far EndoSequence (Brasseler, USA). This bioceramic material comes already in a pre-mixed form by the producer and ready for usage in the consistency putty, making utilization easier for the clinician. Also, the short setting time is a characteristic that makes this bioceramic material very friendly for clinical use.



**Figure 2.**

Graphic representation of the diffusion distances (mm) for Endosequence, ProRoot MTA and Biodentine in all samples

The tips for application, which are available in the packaging, make the placement of this cement even easier, especially during periapical microsurgery, where the field is moistured with blood or periapical fluids.

Room temperature was not influencing the setting time of the material, because due to the syringe presentation, the material needs no mixing procedure done in exterior by the clinician.

Biodentine was on the second place as manipulation properties after EndoSequence, because it is a two-phased material presented as capsules that contain a powder, which needs to be mixed with the liquid in a triturator. This different way of manipulation takes more time, is more temperature sensitive and if waited too long, the material gets wasted due to hardening. Biodentine was still easier manipulated and used than ProRoot MTA. ProRoot MTA is presented separately as a powder and a liquid (distilled water) in a pipette, which means the practitioner has to mix these two components in a proper proportion in order to achieve an optimal consistency of the cement to be used. This is technically sensitive and depends on the operator skills. MTA is also a very temperature sensitive material and hardens very fast in a warm environment, by losing its hydrating water. A dentist with less experience could waste a lot of material due to these problems.

## **DISCUSSIONS**

The presence of setting accelerator in Biodentine results in faster setting compared to ProRoot MTA, thereby improving its handling properties and strength (9). Long setting time of CSCs cements like ProRoot MTA leads to an increased risk of partial material loss and alteration of the interface, alter material solubility and can cause displacement of the cement (10, 11), especially when used in apical surgery as a retro-filling material, although hydroxyapatite crystals form over MTA when it comes in contact with tissue synthetic fluid. This can act as a nidus for the formation of calcified structures after the use of this material in endodontic treatments (12). On the other hand, contamination of MTA with blood affects the morphology of the set material and reduces the release of calcium ions (13-15). Therefore, fast setting materials like Endosequence are the ones preferred by the clinicians, because in a very short time after filling, the material is hard and will not be influenced by tissular fluids or blood. Although, the advantages of ProRoot MTA cannot be denied and are represented by its high biocompatibility due to its hydrophilic properties, its very low solubility and its excellent sealing capacity and low marginal leakage (12). Its sealing capacity is closely connected to the ability of the cement to penetrate the dentin and the results of this study regarding these two parameters are similar for each of the three studied materials.

A very recent *in vitro* study found, using scanning electron microscopy, that significant and better sealing ability and marginal adaptation was demonstrated by EndoSequence when compared to ProRoot MTA sealer (16). EndoSequence contains nanosphere particles that allow for the material to enter dentinal tubules, be moistened by dentine liquid, and create a mechanical bond upon setting (17), thereby improving the sealing capacity of the material. Also, EndoSequence seems to be a suitable material as a retro-filling material in apical surgery because has strengths and biological properties comparable with MTA and is easy to

EndoSequence seems to be a suitable material as a retro-filling material in apical surgery because has strengths and biological properties comparable with MTA and is easy to handle and apply, and thus can be considered an alternative to MTA [18].

Numerous recent studies showed that Biodentine, when used as a root-end filling material, showed significantly better sealing ability in comparison to MTA [18-20]. Biodentine is also a source of hydroxyapatite, but is more soluble than MTA in a wet environment, like the periapical region [18-20].

## **CONCLUSIONS**

Endosequence proved to be the best material regarding its handling properties and setting time. Presenting the easiest clinical manipulation, it can be used with no effort not only as a retrograde filling material but also in root and perforation repair more easily than ProRoot MTA and Biodentine.

Also, Endosequence proved to have the best penetration capacity because of the concentric areas of diffusion into the dentinal tubules observed surrounding almost the entire contour of the root canal wall. Although the measurements showed longer penetrations zones for Biodentine, Endosequence was considered better, taking into consideration the total penetrated area compared to the roots cross-sectional total surface.

Biodentine had the highest values in length measurements, but on the other hand it was more difficult to be manipulated. Also, the total surface covered by the diffusion of Biodentine comparative to Endosequence into the dentinal tubules was lower, but larger than in the MTA group. MTA showed the lowest penetrations scores as a mean, but with numerous diffusion areas in some of the samples, and it was the most difficult to be used clinically, being the most temperature-sensitive compared to the other two materials.

All three calcium silicate cements showed a good apical sealing capacity and are recommended as selective materials in root-end obturation during microendodontic surgery.

For the results to be more conclusive, further studies are necessary including a larger number of teeth and a more precise method to quantify the penetrated area in comparison with the total one. Also, a histological analysis has to be performed in order to establish the type and depth of the diffused material into the dentinal tubules, the assessment based only by visual measurements made on the photographs being subjective.



## REFERENCES

1. von Arx T. Apical surgery: A review of current techniques and outcome. *Saudi Dent J.* 2011; 23(1):9-15.
2. Prati C, Gandolfi MG. Calcium silicate bioactive cements: Biological perspectives and clinical applications. *Dent Mater.* 2015;31(4):351-70.
3. Katsamakis S, Slot DE, Van der Sluis LW, Van der Weijden F. Histological response of the periodontium to MTA: a systematic review. *J Clin Periodontol.* 2013;40:334-344.
4. Kaur M, Singh H, Dhillon JS, Batra M, Saini M. MTA versus Biodentine: Review of Literature with a Comparative Analysis. *J Clin Diagn Res.* 2017;11(8):ZG01-ZG05.
5. Septodont Corporate. Available online: <https://www.septodontcorp.com/files/pdf/Case-Studies-Collection-14.pdf> (accessed on 02.05.2020).
6. Raghavendra SS, Jadhav GR, Gathani KM, Kotadia P. Bioceramics in endodontics - a review. *J Istanbul Univ Fac Dent.* 2017;51(3 Suppl 1):128-37.
7. Damas BA, Wheeler MA, Bringas JS, Hoen MM. Cytotoxicity comparison of mineral trioxide aggregates and EndoSequence bioceramic root repair materials. *J Endod.* 2011;37(3):372-5.
8. Hirschman WR, Wheeler MA, Bringas JS, Hoen MM. Cytotoxicity comparison of three current direct pulp-capping agents with a new bioceramic root repair putty. *J Endod.* 2012; 38(3):385-8.
9. Grech L, Mallia B, Camilleri J. Investigation of the physical properties of tricalcium silicate cement-based root-end filling materials. *Dent Mater.* 2013;29(2):e20-8.
10. Gandolfi MG, Spagnuolo G, SiPoboni F, Procino A, Riviuccio V, Pelliccioni GA, Prati C, Rengo S. Calcium silicate/calcium phosphate biphasic cements for vital pulp therapy: chemicalphysical properties and human pulp cells response. *Clin Oral Investig.* 2015;19(8): 2075-89.
11. Silva GF, Guerreiro JM, Sasso-Cerri E, Tanomaru-Filho M, Cerri PS. Histological and histomorphometrical evaluation of furcation perforations filled with MTA, CPM and ZOE. *Int Endod J.* 2011;44(2):100-10.
12. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review--Part III: Clinical applications, drawbacks, and mechanism of action. *J Endod.* 2010; 36(3):400-13.
13. Duarte MAH, Marciano MA, Vivan RR, Tanomaru Filho M, Tanomaru JMG, Camilleri J. Tricalcium silicate-based cements: properties
17. Damas BA, Wheeler MA, Bringas JS, Hoen MM. Cytotoxicity comparison of mineral trioxide aggregates and EndoSequence Bioceramic Root Repair Materials. *J Endod.* 2011;37(3):372-5.
14. Nekoofar MH, Stone DF, Dummer PM. The effect of blood contamination on the compressive strength and surface microstructure of mineral trioxide aggregate. *Int Endod J.* 2010;43(9):782-91.
15. Nekoofar MH, Davies TE, Stone D, Basturk FB, Dummer PM. Microstructure and chemical analysis of blood-contaminated mineral trioxide aggregate. *Int Endod J.* 2011;44(11): 1011-8.
16. Patri G, Agrawal P, Anushree N, Arora S, Kunjappu JJ, Shamsuddin SV. A Scanning Electron Microscope Analysis of Sealing Potential and Marginal Adaptation of Different Root Canal Sealers to Dentin: An In Vitro study. *J Contemp Dent Pract.* 2020;21(1):73-77.
17. Damas BA, Wheeler MA, Bringas JS, Hoen MM. Cytotoxicity comparison of mineral trioxide aggregates and EndoSequence Bioceramic Root Repair Materials. *J Endod.* 2011;37(3):372-5.
18. Zafar K, Jamal S, Ghafoor R. Bio-active cements-Mineral Trioxide Aggregate based calcium silicate materials: a narrative review. *J Pak Med Assoc.* 2020;70(3):497-504.
19. Agrafioti A, Tzimpoulas N, Chatzitheodoridis E, Kontakiotis EG. Comparative evaluation of sealing ability and microstructure of MTA and Biodentine after exposure to different environments. *Clin Oral Investig.* 2016;20:535-40.
20. Soundappan S, Sundaramurthy JL, Raghu S, Natanasabapathy V. Biodentine versus Mineral Trioxide Aggregate versus Intermediate Restorative Material for Retrograde Root End Filling: An Invitro Study. *J Dent.* 2014;11:143-9.