

Influence of the Vehicle on the Tissue Reaction and Biomineralization of Fast Endodontic Cement

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ABSTRACT

Objective: To investigate the tissue response and the biomineralization ability of CER prepared with epoxy resin or water compared to Mineral Trioxide Aggregate (MTA). **Material and Methods:** Polyethylene tubes containing materials or empty tubes for control were inserted into the subcutaneous tissues of 30 rats. After 7, 15, 30, 60, and 90 days, the rats were killed and the tubes were removed for analysis using hematoxylin-eosin staining, von Kossa staining, and under polarized light. Inflammation was graded through a score system; the thickness of the fibrous capsule was classified as thin or thick; the biomineralization ability was recorded as present or absent. The results were statistically analyzed using the Kruskal-Wallis test ($p < 0.05$). **Results:** Histologic analysis performed after 7 and 15 days for CER prepared with epoxy resin or water and for MTA showed moderate inflammation and a thick fibrous capsule ($p > 0.05$). After 30, 60, and 90 days, mild inflammation, and a thin fibrous capsule were observed in all groups ($p > 0.05$). **Conclusion:** All materials had structures positive for von Kossa and birefringent to polarized light. CER epoxy resin showed biocompatibility and biomineralization similar to CER water and MTA.

Keywords: Endodontics; Biocompatible Materials; Biomineralization; Root Canal Therapy.

Introduction

Endodontic cements need to be biocompatible once they will be in contact with vital tissues [1,2]. Mineral Trioxide Aggregate (MTA) is a repair cement that shows biocompatibility and stimulates deposition of mineralized tissue [2-7]. MTA is indicated for several situations, such as root perforation, pulpotomy, apexification/apexogenesis and root-end fillings [5-7]. However, MTA has several drawbacks, such as the handling difficulty, granular consistency and slow setting time [8].

CER is an experimental endodontic cement, whose name comes from *Cimento Endodôntico Rápido*, or fast endodontic cement in English [9]. CER has the same indication as MTA, and it is composed of a Portland cement hydrated, water, barium sulfate, and an emulsifier [9]. The cement has biological properties similar to MTA, a setting time lower than MTA, thermal expansion similar to dentin, releasing calcium and hydroxyl ions, favoring the process of tissue repair [4,5,9].

Epoxy resin is present in the composition of some endodontic sealers, e.g., AH Plus, considered a gold standard cement for root canals [4,10-12]. The cements based on epoxy resin have exhibited adhesion to the dentin, significant penetration into the dentin tubules, excellent apical sealing capacity, biocompatibility and maintenance of cell viability [5,13-15]. The preparation with resin can improve the physical-chemical properties of CER, and consequently, it could be an indication for as a root canal filling due to improved fluidity, but some biological hazard may occur.

In a previous study, Garcia et al. [16] investigated the cytotoxic effects of the CER on fibroblast-like MDPL-20 cells and found that at the 24-hour period, the CER cement presented the highest cytotoxic effects on the fibroblast-like cells when compared to conventional white MTA; however, at the 7-day period, the cytotoxicity of CER decreased and reached similar parameters to that of the white MTA. Since this was an in vitro study, new studies are needed to analyze these new MTA-based cement formulations. Thus, this study aimed was to evaluate the epoxy resin and water as vehicles in the biocompatibility and biomineralization ability of CER using the rat subcutaneous tissue implantation method. MTA was used as a gold standard for biocompatibility comparison. The null hypothesis is that the tested materials show no differences in inflammatory reaction and biomineralization capability.

Material and Methods

Ethical Clearance

The study was approved by the Research Ethics Committee (2015-00451).

Experimental Procedures

Thirty male rats (*Wistar*) weighing 250-280 g and 3 to 4 months of age were used in this study. The sample size was estimated based on data from a previous study [5] but using a power sample of 90%, which is corroborated by previous studies [3,5,17] and considering an alpha error of 0.05 to recognize a significant difference, a minimum number of six rats/group was considered necessary. The rats were housed in environments with temperatures between 22°C and 24°C with a controlled light cycle (12 hours light and 12 hours dark). Throughout the experimental period, the animals were fed with solid food and water "ad libitum".

One hundred and twenty polyethylene tubes (Abbott Laboratórios do Brasil Ltda., São Paulo, SP, Brazil) with 1.0 mm internal diameter, 1.6 mm external diameter and 10.0 mm length were filled with the tested materials [3,5,17]. The Angelus MTA (Angelus Indústria de Produtos Odontológicos, Londrina, PR, Brazil) and experimental cement CER (School of Engineering, Department of Physics and Chemistry, Ilha

Solteira, SP, Brazil) were prepared according to the manufacturer's recommendations (1 dose of MTA powder/1 drop of liquid; CER epoxy resin and CER water: 0.25 g of CER powder and 0.30 g of liquid) and inserted into the tubes. Thirty polyethylene tubes remained empty and were used as a control.

The animals received antiseptis with 5% iodine solution and were shaved under xylazine (10 mg/kg - Anasedan, Agribrands do Brazil Ltda., Paulínia, SP, Brazil) and ketamine (25 mg/kg, Dopalen, Sespo Ind. & Com. Ltda., Jacaréí, SP, Brazil) anesthesia. The shaved backs received a 2-cm incision in a head to tail orientation with a number 15 Bard-Parker blade (Becton Dickinson, Franklin Lakes, NJ, USA). The skin was reflected, creating 2 pockets in 1 side of the incision, one in the cranial portion and another in the caudal portion 6 cm away from each other, and another pocket on the opposite side of the incision. The tubes were implanted into the spaces created with blunt dissection, and the skin was closed with a 4/0 silk suture [3,5,17].

After 7, 15, 30, 60 and 90 days from the implantation time, the animals were killed with an overdose of anesthetic solution and the tubes and surrounding tissues were removed and fixed in 7% buffered formalin at pH 7.0. The tubes were bisected transversely and both halves were cut again longitudinally with the use of a sharp blade to allow the surfaces to be readily kept in contact with the processing solutions. The specimens were processed for glycol methacrylate (GMA) embedding, serially sectioned into 3-mm cuts, and stained with hematoxylin-eosin [3,5]. The 10-mm cuts were stained according to the von Kossa technique or remained without staining to be observed under polarized light.

Histological sections were analysed under light-field illumination using optical microscope microscopy (400, DM 4000 B; Leica, Wetzlar, Germany) by a calibrated investigator and who was unaware of the experimental groups being analysed. The von Kossa technique was used to observe mineralized structures in the tissue, which were dark stained [5,17]. The polarized light technique enables observing birefringent structures related to calcium carbonate crystals originated from the combination of calcium ions from the material with carbonic gas from the tissue [5,17].

Reactions in the tissue in contact with the material on the tube opening were scored according to previous studies [5,14,15] at 0 (none or few inflammatory cells and no reaction), 1 (less than 25 cells and mild reaction), 2 (between 25-125 cells and moderate reaction) and 3 (125 and more cells and severe reaction). Fibrous capsules were considered to be thin when thickness was < 150 µm and thick at > 150 µm. Necrosis and calcification were recorded as present or absent. An average value for each material was obtained from 10 separate areas. An increase of 400X was used for the analysis of inflammation and 100X for the analysis of biomineralization [3,5,17].

Data Analysis

Results were statistically analyzed using Kruskal-Wallis tests. The p-value was considered significant at 5%.

Results

Histological Findings

After 7 and 15 days, all the four groups: Control, MTA-Angelus, CER water and CER epoxy resin presented a moderate inflammatory infiltrate of lymphocytes and macrophages with the presence of a thick fibrous capsule (Figure 1: A1, A2 – B1, B2 – C1, C2 – D1, D2, respectively). There was no statistically significant difference ($p > 0.05$) among the scores of the different groups (median score = 2) (Table 1). During the experimental periods of analysis (30, 60 and 90 days), the inflammation significantly decreased, showing a mild infiltrate of lymphocytes and macrophages were predominant with a thin fibrous capsule for all the four

groups (Figure 1: A3-A5, B3-B5, C3-C5, D3-D5). There was no statistically significant difference ($p>0.05$) among the scores of the different groups (median score = 1) (Table 1).

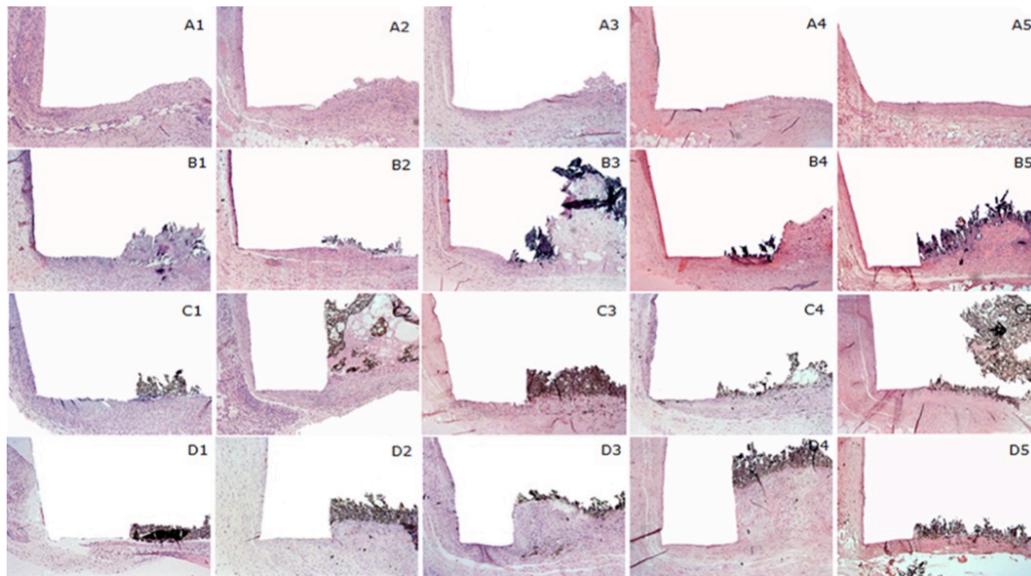


Figure 1. Representative images of the subcutaneous tissue reactions: Inflammatory response to the Control Group (empty tubes) at 7 (A1), 15 (A2) with intense inflammatory infiltrate and thick fibrous capsule; 30 (A3), 60 (A4) and 90 (A5) days, with mild inflammatory infiltrate and thin fibrous capsule. MTA at 7 (B1), 15 (B2) with moderate inflammatory infiltrate and presence of thick fibrous capsule; 30 (B3), 60 (B4) and 90 (B5) days with mild inflammatory infiltrate and thin fibrous capsule; CER water 7 (C1), 15 (C2) with intense inflammatory infiltrate and thick fibrous capsule; 30 (C3), 60 (C4) and 90 (C5) days, with mild inflammatory infiltrate and thin fibrous capsule; CER epoxy resin at 7 (D1), 15 (D2) with intense inflammatory infiltrate and thick fibrous capsule; 30 (D3), 60 (D4) and 90 (D5) days, with mild inflammatory infiltrate and thin fibrous capsule. Staining with hematoxylin-eosin (x100) shows fibrous capsule formation with infiltration of macrophages and lymphocytes.

Table 1. Inflammatory score, thickness of fibrous capsule and biomineralization ability - von Kossa (VK) and Polarized Light (PL).

Time	Material	Scores Inflammation				Median	Capsule	Biomineralization		N
		0	1	2	3			VK (%)	PL (%)	
7 Days	Control ^A	0	1	5	0	2	Thick	0	0	6
	MTA ^A	0	0	5	1	2	Thick	100	100	
	CER Water ^A	0	0	5	1	2	Thick	100	100	
	CER Epoxy Resin ^A	0	1	5	0	2	Thick	100	100	
15 Days	Control ^A	0	1	4	1	2	Thick	0	0	6
	MTA ^A	0	0	6	0	2	Thick	100	100	
	CER Water ^A	0	0	6	0	2	Thick	100	100	
	CER Epoxy Resin ^A	0	2	4	0	2	Thick	100	100	
30 Days	Control ^A	0	4	2	0	1	Thin	0	0	6
	MTA ^A	0	4	2	0	1	Thin	100	100	
	CER Water ^A	0	5	1	0	1	Thin	100	100	
	CER Epoxy Resin ^A	0	5	1	0	1	Thin	100	100	
60 Days	Control ^A	0	6	0	0	1	Thin	0	0	6
	MTA ^A	0	6	0	0	1	Thin	100	100	
	CER Water ^A	0	5	1	0	1	Thin	100	100	
	CER Epoxy Resin ^A	0	6	0	0	1	Thin	100	100	
90 Days	Control ^A	0	6	0	0	1	Thin	0	0	6
	MTA ^A	0	6	0	0	1	Thin	100	100	
	CER Water ^A	0	5	1	0	1	Thin	100	100	
	CER Epoxy Resin ^A	0	6	0	0	1	Thin	100	100	

*Same letters indicate no statistical difference among the groups ($p>0.05$).

The control groups were not positive for von Kossa and birefringent granulations polarized light were absent in any period (Figure 2: A1-A5 and Figure 3: A1-A5, respectively). Granulations birefringent to polarized light and von Kossa positive were observed near the tube opening for the three tested materials MTA-Angelus, CER water and CER epoxy resin (Figure 2 and 3: B1-B5, C1-C5 and D1-D5, respectively), proving the mineralizing capacity of the materials tested in the study.

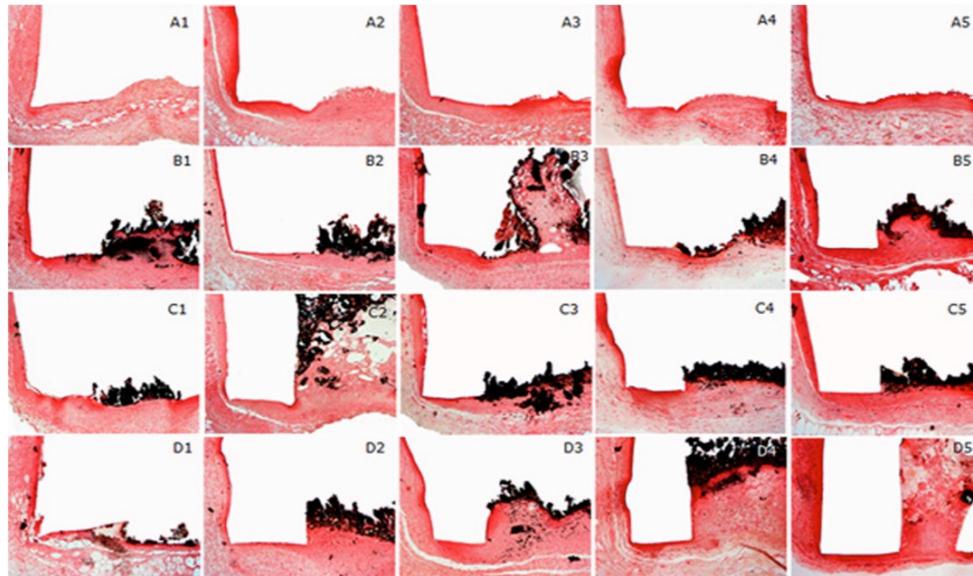


Figure 2. Biom mineralization in response to the Control Groups (empty tubes) at 7 (A1), 15 (A2), 30 (A3), 60 (A4) and 90 (A5) days; MTA at 7 (B1), 15 (B2), 30 (B3), 60 (B4) and 90 (B5) days, CER water at 7 (C1), 15 (C2), 30 (C3), 60 (C4) and 90 (C5) days and CER epoxy resin at 7 (D1), 15 (D2), 30 (D3), 60 (D4) and 90 (D5) days. Black areas represent mineralization from the von Kossa method. Both materials induced mineralization, which was not observed for control group (x100).

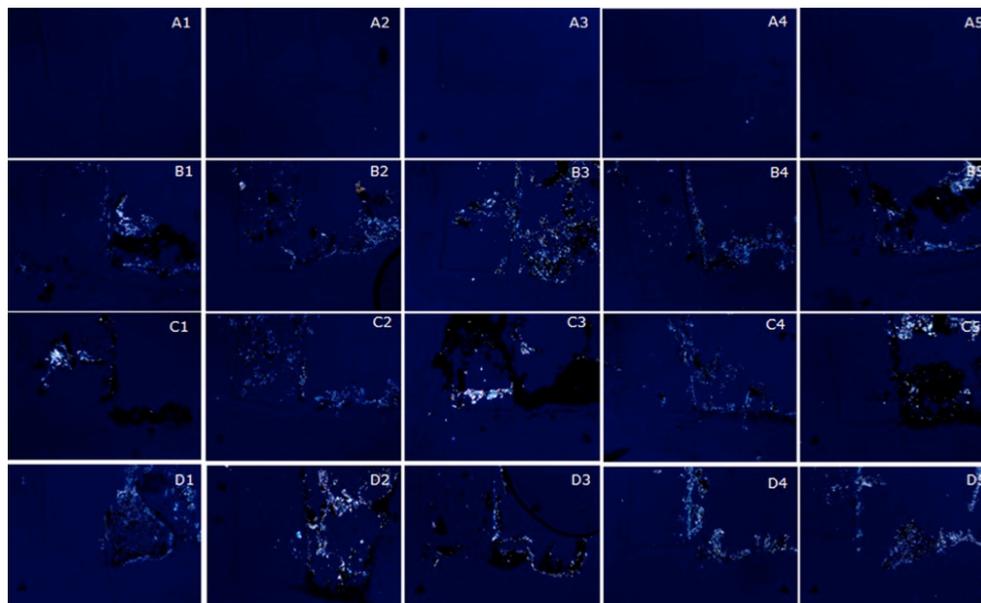


Figure 3. Biom mineralization in response to the Control Groups (empty tubes) at 7 (A1), 15 (A2), 30 (A3), 60 (A4) and 90 (A5) days; MTA at 7 (B1), 15 (B2), 30 (B3), 60 (B4) and 90 (B5) days, CER water at 7 (C1), 15 (C2), 30 (C3), 60 (C4) and 90 (C5) days and CER epoxy resin at 7 (D1), 15 (D2), 30 (D3), 60 (D4) and 90 (D5) days. Birefringent structures in the tissue represent calcite crystals, which were observed for both materials, but not for control group (x100).

Discussion

Endodontic cement must have high biocompatibility, adequate physicochemical properties, induce the deposition of biomineralized structures and have antimicrobial activity [4,5,13,18,19]. These properties should be evaluated before their clinical use to minimize adverse effects in patients. This study evaluated the biocompatibility and biomineralization ability of the endodontic CER manipulated with epoxy resin or water. These vehicles were used to improve the physical characteristics of CER and to enable their indication as root canal sealer, but without compromising the good biological properties already shown [5].

In this study, polyethylene tubes filled with material or unfilled (control) were used in subcutaneous rat. The specimens were included in glycol methacrylate. This strategy was chosen because this method of inclusion provides thinner cuts that provide an appropriate cellular definition, which allows for a better definition of the inflammatory process [15]. The empty tubes caused a more exacerbated inflammatory reaction after 7 and 15 days, but the inflammatory reaction decreased with time, which is in agreement with previous studies [5,14,15,20].

CER consists of Portland cement gel, barium sulfate and an emulsifier (proprietary) whose function is to improve handling properties. Portland cement was widely studied as a substitute for MTA because of its lower cost, similar chemical composition and, in particular, its MTA biocompatibility similarity [21-23]. Previous studies have shown that CER presented a greater ability to release calcium ions than MTA when in contact with an aqueous environment, and this characteristic could accelerate tissue repair in endodontic therapy [9,21]. Gomes-Filho et al. [5] showed that the tissue response and the biomineralization capacity of the CER were very similar to the results found with the MTA-Angelus. However, manipulation of the CER with other vehicles has not been studied.

The inflammation and thickness of the fibrous capsule are important criteria for evaluating endodontic cements' biocompatibility [14]. CER epoxy resin and water induced a moderate inflammatory response, and a thick fibrous capsule was found on days 7 and 15, and in the more advanced times of analysis, the inflammatory process and the fibrous capsule decreased. The presence of an organized fibrous capsule limits inflammation and prevents the inflammatory reaction from extending to regions distant from the area in contact with the cement [24]. The response of CER manipulated with epoxy resin in the subcutaneous tissue was similar to that observed with the CER water and MTA. In this study, the similarity is probably due to the presence of Portland cement in its composition. The literature shows that MTA and Portland behave similarly due to their composition [20-23].

The components di-tricalcium silicate and tricalcium aluminate are the main components of Portland cement and MTA [24]. When these components come into contact with tissue, a hydration reaction will occur, resulting in calcium hydroxide formation that dissociates into calcium and hydroxyl ions [20]. Calcium ions react with carbon dioxide present in tissues, giving rise to calcite crystals, calcium carbonate and a reduced inflammatory process [20]. The precipitation of calcium carbonate serves as the nucleus for a calcification estimation of deposition of mineralized tissue and can be detected by von Kossa and birefringent polarized light structures [17,20,25]. In this study, positive areas of von Kossa and birefringent polarized light structures were detected in all periods of analysis in CER epoxy resin and CER water, showing that CER associated with other vehicles can also stimulate the formation of mineralized tissue. Also, deposition of mineralized tissue into subcutaneous tissue is a sign of cements' osteoinductivity [25,26].

This is the first study that evaluates the biological properties of CER-manipulated epoxy resin, demonstrating its biocompatibility; however, as in any animal study, the results should be interpreted with

caution and not be extrapolated directly to clinical practice. Thus, more research is necessary to evaluate the physical and chemical properties of the CER manipulated with epoxy resin.

Conclusion

CER manipulated with epoxy resin is biocompatible and induces biomineralization similar to CER water and MTA.

Authors' Contributions

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All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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