

Effect of Tricalcium Silicate on Direct Pulp Capping: Experimental Study in Rats

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Abstract

Objectives Conduct a histological comparison of the pulp response to different materials, with a focus on the continuity and morphology of the mineralized barrier after direct pulp capping.

Materials and Methods One hundred and eight maxillary first molars of 54 Wistar rats were subject to direct pulp capping and divided into three groups according to the materials used: calcium hydroxide (CH), mineral trioxide aggregate (MTA), and Biodentine. All cavities were sealed, and the animals were euthanized at 7, 14, and 21 days. Descriptive histological evaluation of the inflammation and formation of the mineralized barrier was performed.

Statistical Analysis Statistical analyses were performed using the Kruskal–Wallis test, which was complemented by the Dunn test; differences with $p < 0.05$ were considered statistically significant.

Results The results showed that MTA and Biodentine elicited less intense inflammatory reactions than CH. With respect to the formation and quality of the dentin barrier formed, differences were observed at 21 days between the analyzed groups; the best results being obtained following treatment with MTA and Biodentine.

Conclusion MTA and Biodentine induced formation of a more continuous and uniform mineralized barrier with less intense pulp response than CH.

Keywords

- ▶ Direct pulp capping
- ▶ mineral trioxide aggregate
- ▶ Biodentine
- ▶ tricalcium silicate

Introduction

Direct pulp capping is a treatment for exposed vital pulp; it involves the placement of a dental material over the exposed area to facilitate both the formation of a protective barrier and the maintenance of the vital pulp.^{1,2} From a more precise clinical perspective, direct pulp capping is a clinical technique that lies between indirect pulp capping and pulpotomy.² The direct pulp capping procedure aims to maintain pulp vitality. This procedure utilizes calcium hydroxide (CH) which, in contact with the pulp tissue, promotes superficial necrosis with zones of necrosis and inflammatory response; these may be attributed to its pH (almost 13.0) and the formation

of a hard tissue barrier with an amorphous pattern.³ This material presents disadvantages such as porosity in the dentin bridge produced, poor adherence to the dentin, and failure to produce good long-term sealing.⁴

In 1993, Torabinejad developed a calcium silicate cement, known as mineral trioxide aggregate (MTA), which was indicated for root perforations and later for pulp caps.⁵ The chemical reaction of this material in direct contact with the pulp leads to the formation of calcium crystals, similar to those found in CH.² The superior nature of MTA in comparison with CH in terms of sealing capacity, dentin quality⁶ and biocompatibility⁷ supports the replacement of CH by this material in direct pulp capping procedures.⁸

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New tricalcium silicate-based cement Biodentine (Septodont, France) was released in 2011.⁹ These are composed of tricalcium silicate, dicalcium silicate, calcium carbonate, calcium oxide, and zirconium oxide (radiopacifier) as well as liquid containing water, calcium chloride (water-soluble polymer, responsible for accelerating the setting time of the material), and modified polycarboxylate (which imparts plasticity to the material).¹⁰ Initially, these cements were developed as substitutes for dentin; however, they presented favorable results in inducing the formation of mineralized tissue when in direct contact with the pulp.^{11,12} In addition to its biocompatibility,^{13,14} it also presented advantages such as viscosity and reduced setting time in comparison with MTA.¹⁵ However, a deeper understanding of the influence of the constituents of the pulp capping materials on their properties is necessary.¹⁶

Research on teeth from animal models has been widely used; according to Dammaschke,¹⁷ the Wistar rat molar, which is anatomically, biologically, and histologically similar to the human tooth, is considered a miniature molar and exhibits a superior repair speed compared with the human tooth. Owing to ease of handling and similar histological response in direct pulpal capping procedures, the Wistar rat molar represents a valid model for research.¹⁸

In view of the need to better understand the performance of various materials indicated for the protection of histologically exposed pulps, the aim of this study was to conduct a histological comparison of the pulp response to different materials, with a focus on the continuity and morphology of the mineralized barrier after direct pulp capping.

Materials and Methods

Sample Size

From the data obtained in a pilot test, the sample size was determined using the BioStat 5.3 program (Instituto Mauá, Amazonas, Brazil), considering an analysis power of 0.80, minimum difference between the means of 0.22, and standard deviation (SD) of the mean error 0.11, which guaranteed a minimum of six sample units per experimental group.

Animals

A total of 54 male rats weighing approximately 200 g,¹⁹ of the 56-day-old Wistar variety, were purchased from the Western State University of Paraná (UNIOESTE) Central Bioterium. They were adapted and kept in the Sectoral Bioterium of the UNIOESTE, Cascavel, Paraná, Brazil, in collective cages

of polyethylene (43 × 30 × 15 cm), in pairs, under controlled temperature conditions ranging between 22°C and 25°C, relative humidity of 55%, and photoperiod of 12 hour (light period from 7:00 am to 7:00 pm). The experimental procedures were in accordance with the Ethical Principles on Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA), and they were analyzed and approved by the Committee on Ethics in Animal Experimentation (CEEAA) of UNIOESTE, no. 14/18-CEUA.

Experimental Groups

The animals were randomly assigned to three treatment groups (CH, MTA, and Biodentine), with 18 animals per group. Each group was subdivided into three time periods of 7, 14, and 21 days, with six animals per group per period (►Table 1).

Pulp Capping

At 56 days, the animals were anesthetized with a combination of 1 mL of ketamine (injectable Dopalén, Ceva Saúde Animal, Paulínia, SP, Brazil) and 0.2 mL of xylazine (injectable Anasedan, Ceva Saúde Animal, Paulínia, SP, Brazil) and diluted in 3.8 mL of saline, intraperitoneally (0.1 mL of solution/50 g of animal body weight).¹⁷ After anesthesia, the animals were placed on an appropriate surgical table,²⁰ which allowed the opening of the oral cavity, facilitating access to the maxillary teeth (►Fig. 1).

On the occlusal side of the maxillary first molars (right and left), circular cavities were made using an electric motor (Driller, BLM 600 Plus, Carapicuíba, SP, Brazil) at 3000 rpm with an ISO 006 LN 28-mm drill bit (D 205 LN; Dentsply, Maillefer, Ballaigues, Switzerland), and pulp exposures verified with C+#15 files (Dentsply, Maillefer, Ballaigues, Switzerland).^{17,21}

Pulp hemorrhage was controlled, and the cavity was dried with sterile absorbent paper cones (►Fig. 2A). Capping was performed on pulp exposures (►Fig. 2B) for the following groups G1: CH (Calcium Hydroxide P.A., Biodynamic, Ibioporã, PR, Brazil) in powder form; G2: MTA (White MTA; Angelus, Londrina, PR, Brazil), handled according to the manufacturer's guidelines on glass plate; and G3: Biodentine (Septodont; Saint-Maur-des-Fosses, France), handled on a glass plate. The materials were applied with the aid of a metallic device, the MTA Applicator (MTA Applicator; Angelus, Londrina, PR, Brazil), and the cavities were sealed to avoid exposure to the resin-modified glass ionomer (Vitremere; 3M ESPE do Brasil Ltda., Sumaré, SP, Brazil) (►Fig. 2C) and photoactivated for 20 seconds with the Rádi-cal device (SDI-Innovative

Table 1 Groups of experimental animals with details of periods of euthanasia and material used in each group

Description of group	Animals	Euthanasia		
		7 days	14 days	21 days
CH	18	6	6	6
MTA	18	6	6	6
Biodentine	18	6	6	6

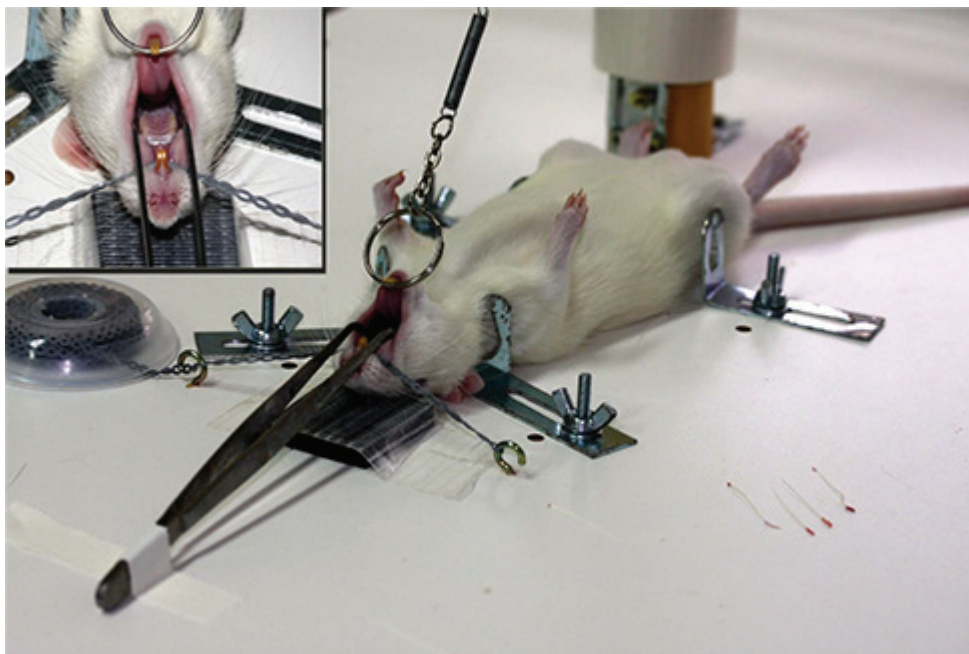


Fig. 1 Operating table for immobilization and maintenance of the oral cavity opening of the animal.

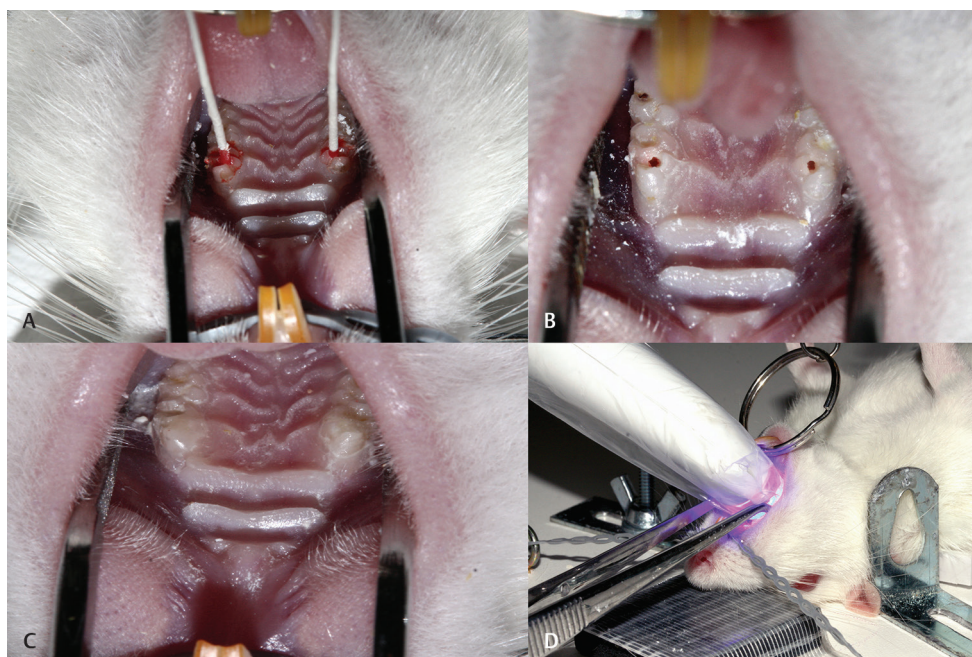


Fig. 2 (A) Hemorrhage control with paper cones; (B) rounded cavities; site of exposure; (C) sealing with Vitremer glass ionomer (3M-ESPE); (D) photopolymerization for 20 seconds.

Dental Products, Australia), with a power of 1200 mW/cm² (► **Fig. 2D**). At the end of the experimental period of 7, 14, and 21 days after pulp capping, the animals were euthanized by guillotine decapitation.

Histological Processing

The jaws were removed, dissected, identified, and fixed in 10% formalin solution for 48 hours, washed in running water, and stored in 70% alcohol. After this period, the samples were washed in running water for 1 hour and immersed in

a decalcifying acid solution (hydrogen chloride; UN 1789, Allkimia, Campinas, SP, Brazil) for 24 hours at room temperature and evaluated to verify the degree of decalcification achieved.

After decalcification, the samples were again washed in tap water for 1 hour for automatic histological processing, which lasted approximately 12 hours (Automatic Tissue Processor; Leica Microsystems® TP1020, Nussloch, Germany), with paraffin blocks (Purified Paraffin; Vetec Química Fina, Rio de Janeiro, RJ, Brazil). Cuts of 5-µm thickness were obtained using

a semiautomatic microtome (Hestion; ERM3000, Daintree Scientific, St. Helens, Australia), and histological sections were stained with Harris hematoxylin and eosin (HE).

Histological Analysis

The histological analyses were performed by a blinded calibrated observer, in which the on-site aspects of the pulp exposure were evaluated using an optical microscope with 40x- and 100x-objective lenses (Leica Microsystems ICC50 HD; Nussloch, Germany) and an image capture system (Las Ez–Leica, version 2.10, 2012). The following parameters were analyzed: inflammatory response, continuity and morphology of the dentine barrier formed. The results were classified according to the scores described in ▶Table 2. The results were evaluated by scores using the image capture system.²²

Statistical Analysis

Statistical analyses were performed using the BioStat 5.3 program (Instituto Mauá; Amazonas, Brazil), using the Kruskal–Wallis test and complemented by the Dunn test; differences with $p < 0.05$ were considered statistically significant.

Results

Pulp response presented severe hyperemia at 7 days (▶Fig. 3) in all experimental groups, irrespective of the material used. The analysis of the acute inflammatory infiltrate demonstrated varying extensions: the group treated with CH exhibited the greatest extension of the inflammatory processes, followed by the MTA and Biodentine groups, respectively. Necrotic remains and dentinal scrapings were present in all animals of the various groups.

At 14 days, 70% of the samples capped with CH showed necrosis and microabscess areas in the root canals, with single-cell chronic inflammatory infiltrate in the periapical region. The other samples exhibited initiation of amorphous formation of mineralized material in the middle of cellular tissue. Further, the pulps, capped with MTA and Biodentine,

presented amorphous formation of mineralized tissue at the site of exposure and in contact with the material used, both in evolution and continuity with dentin (▶Fig. 4). Analysis of the mineralized barrier revealed statistical differences in continuity, with those capped with CH differing from those of the MTA group (▶Table 3). Differences in terms of morphology were also observed: the morphology of those capped with CH was different from those capped with either the MTA and Biodentine. However, between CH and MTA, no statistically significant differences were found (▶Table 4).

At 21 days, higher amounts with discrete inflammatory infiltrate of pulp and formation of hard tissue were present in all cases; however, in the MTA and Biodentine groups, the barrier formed obliterated, on occasion, the entire root canal, with a formation aspect similar to that of pulpal calculus (atubular dentin) (▶Fig. 5). No statistically significant differences were found in the morphology of the mineralized barrier (▶Table 3). However, between CH and Biodentine, statistically significant differences were found for continuity of the mineralized barrier (▶Table 4).

Discussion

In this study, an experimental animal model (Wistar rat) was used, owing to the ease of experimental handling, coupled

Table 2 Scores used for histological analysis

Mineralized barrier	
Scores	Continuity
1	Complete
2	Partially present
3	Absent
Scores	Morphology
1	Tubular dentin
2	Irregular deposition of hard tissue or dentin
3	Only small layer of hard tissue deposition
4	Absence of hard tissue deposition

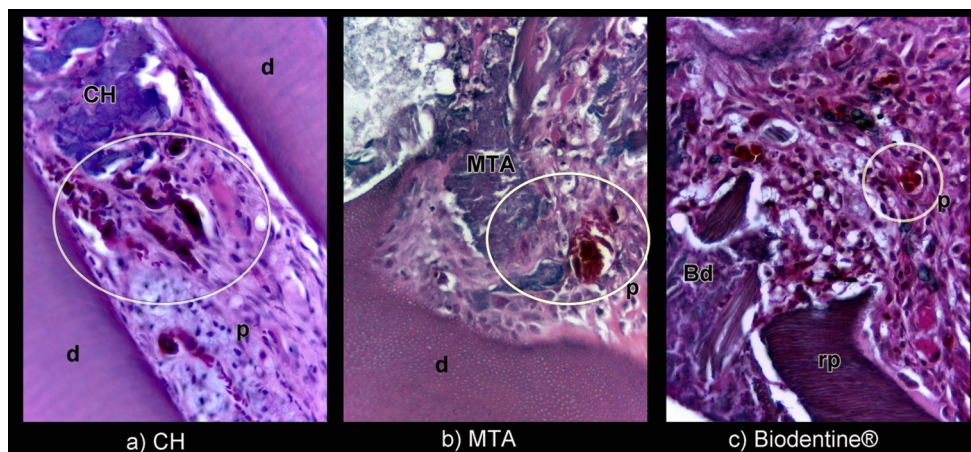


Fig. 3 Photomicrograph of the pulps of the euthanized animals at 7 days after pulp exposure in the various experimental groups, according to the material used: (a) CH; (b) MTA; and (c) Biodentine. Severe pulp hyperemia in all samples (circle) with higher intensity for CH, followed by MTA and Biodentine; p = pulp; d = dentin; rd = dentin scrapings; CH = calcium hydroxide; MTA = MTA; Bd = Biodentine. Hematoxylin and eosin staining. Magnification: 40x.

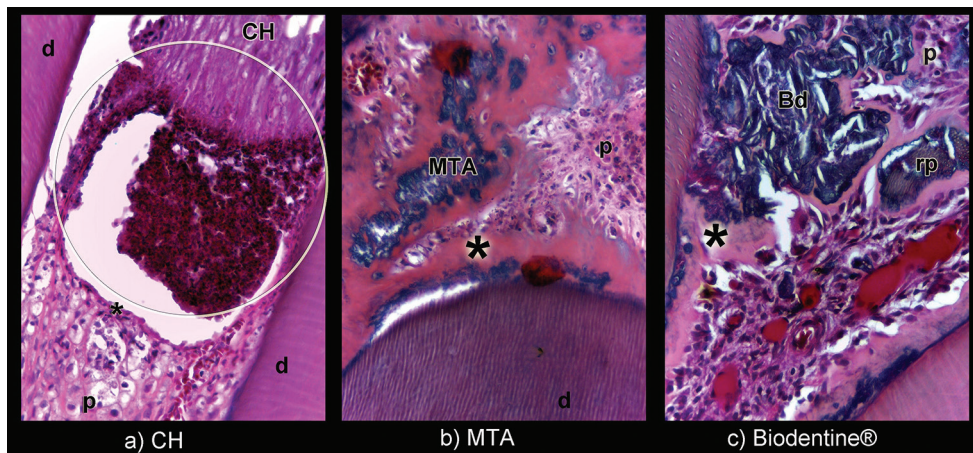


Fig. 4 Photomicrograph of the pulps of the euthanized animals at 14 days after pulp exposure in the various experimental groups, according to the material used (a) CH; (b) MTA; and (c) Bd. Analysis of the dentin barrier formed (a) CH = calcium hydroxide—the circled region highlights the transition zone of necrosis (top of the circle) and microabscess (bottom of the circle) induced by CH and the beginning of amorphous formation of mineralized tissue (*); (b) MTA = amorphous formation of mineralized tissue (*) and pulpar microabscess area (p); (c) Bd = Biodentine—amorphous formation of mineralized tissue (*); p = pulp; d = dentin; * = dentin bridge; rd = dentinal scrapings. Staining of hematoxylin and eosin. Magnification: 40x.

Table 3 Histological analysis of the tooth pulp for mineralized barrier–continuity in experimental animals according to time

Continuity	7 days	14 days	21 days
CH	3.00 (± 0.00) Aa	2.90 (± 0.32) Aa	2.67 (± 0.50) Aa
MTA	3.00 (± 0.00) Aa	2.00 (± 0.00) Ba	1.91 (± 0.94) Aba
Biodentine	3.00 (± 0.00) Aa	2.10 (0.48) ABa	1.70 (± 0.48) Bb

Abbreviations: CH, calcium hydroxide; MTA, mineral trioxide aggregate.

Values expressed as mean ± standard deviation; Kruskal–Wallis test, complemented by the Dunn test. Lower-case letters (line) and different capital letters (column) mean statistically significant differences = $p < 0.05$.

Table 4 Histological analysis of the tooth pulp for mineralized barrier–morphology in experimental animals according to time

Morphology	7 days	14 days	21 days
CH	4.00 (± 0.00) Aa	3.70 (± 0.48) Aa	3.22 (± 0.97) Aa
MTA	3.63 (± 0.52) Aa	2.50 (± 0.55) Ba	2.82 (± 0.98) Aa
Biodentine	3.90 (± 0.32) Aa	2.70 (± 0.67) Ba	2.20 (± 0.42) Ab

Abbreviations: CH, calcium hydroxide; MTA, mineral trioxide aggregate.

Values expressed as mean ± standard deviation; Kruskal–Wallis test, complemented by the Dunn test. Lower-case letters (line) and different capital letters (column) mean statistically significant differences = $p < 0.05$.

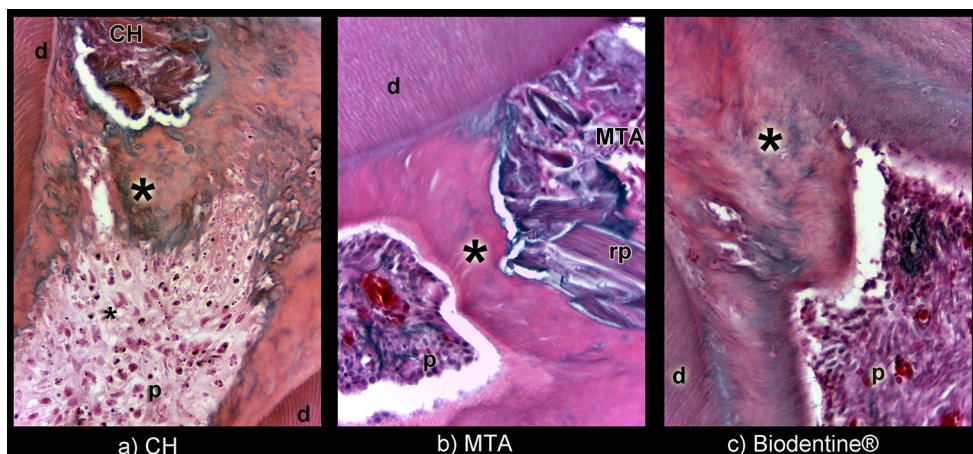


Fig. 5 Photomicrograph of the pulps of the euthanized animals at 21 days after pulp exposure in the various experimental groups. Analysis of the formed dentin barrier. (a) CH = calcium hydroxide—formation of the dentin barrier (*); (b) MTA = formation of the dentin barrier (*); (c) Bd = Biodentine—formation of the dentin barrier (*); p = pulp; d = dentin; * = dentin bridge; rd = dentinal scrapings. Staining of hematoxylin and eosin. Magnification: 40x.

with the fact that the pulp response and recovery after direct pulp capping of rodent teeth are histologically comparable with that in humans.^{17,19,21}

Dentin barrier formation occurs in response to various injuries, and this can be characterized as reactional dentin (primary odontoblasts) or reparative dentin, composed of neo-odontoblasts originating from dental pulp stem cells (DPSCs), cells with multiple differentiation potentials and capacity for tertiary dentinogenesis.²³⁻²⁵ For the mineralized barrier formation, the DPSCs migrate, proliferate, and differentiate into neo-odontoblasts, which then synthesize a matrix to form the tertiary dentin at the injury sites.²⁶

There are several types of stem cells of dental origin (DSCs), for example, DPSCs,²³ stem cells from human exfoliated deciduous tooth,²⁷ periodontal ligament stem cells,²⁸ alveolar bone derived mesenchymal stem cells,²⁹ dental follicle progenitor cells,³⁰ stem cells from the apical part of the dental papilla,³¹ teeth germ progenitor cells (Ikeda et al, 2008), and gingival mesenchymal stem cells.^{31,32} DPSCs are the first type of DSCs to be isolated; they exhibit pluripotency and a typical fibroblast-like morphology.²³

DPSCs were isolated and their differentiation potential was demonstrated in various media, such as dentinogenic, neurogenic, osteogenic, chondrogenic, adipogenic, and myogenic media.³³ In dentistry, DSCs can be used in the repair of damaged dentin, pulp regeneration and revascularization, osseointegration, and periodontal disorders.^{34,35}

In this study, we used the three materials as direct pulp capping protect materials: CH, MTA, and Biodentine. CH cement was one of the first materials used in the treatment of live pulps.³⁶ CH releases a large amount of relatively short time. The factors influencing the alkaline environment required for pulp healing, to promote immediate selection, the stimulation of hydroxyapatite, and the formation of tertiary dentin in the affected tissues were determined. In addition, the hydroxyls of this material neutralize the effects of osteoclasts, bond with hydrogen and form the H₂O molecule, and maintain the optimum pH for auxiliary pyrophosphate and remineralization activity.³⁷ However, CH may possibly wound the primary tooth pulp to permit internal resorption or dystrophic calcification.

MTA and Biodentine have a very similar chemical reaction: first, the solid-liquid interface is formed on the surface of the particles with immediate dissolution of ions, such as Ca⁺ that migrate to the solution forming CaOH₂; second, CaOH₂ is attracted by the hydroxyl groups, resulting in the formation of calcium silicate hydrate. This gelatinous layer has a negative character and can act as a nucleation site to form hydroxyapatite. In the third phase, the hydrated calcium silicate provides significant and continuous amounts of CH during the first hours of the setting reaction, leading to an increase in pH and the concentration of calcium ions in the adjacent areas. Compared with CH and MTA, Biodentine has more ions. It consists of tricalcium silicate, dicalcium silicate as a second core material, calcium carbonate, oxide as filler, iron oxide shade, and zirconium oxide as a radio-opacifier. The liquid, on the contrary, contains calcium chloride as a setting accelerator and a water-reducing agent.^{38,39}

In our study, histological analysis of samples from the 7-day period demonstrated the capacity of the pulp to respond to damage, which manifested during this period as inflammation of the pulp tissue. According to Goldberg,⁴⁰ cell repair begins after inflammation control, with replacement of the injured or necrotic region by undifferentiated cells. After transformation, these cells give rise to tissue similar to the previous undamaged tissue, with three successive phases of cell renewal: slight inflammation associated with cell recruitment, cell proliferation filling the lesion site, and cell differentiation in the pulp, creating neo-odontoblasts for the production of reparative dentin. These findings were similar to our results.

Our results indicate an outcome characterized by initial pulp inflammation and a zone of necrosis, similar to that described in other studies, thereby corroborating previous research.⁴¹⁻⁴³ Analysis of dentin bridge formation at 7 days did not reveal statistically significant differences between the three materials tested, similar to Dammaschke et al,¹⁸ with similar immunohistochemical analysis results for CH and MTA in the first week after direct pulp capping.

Although the use of CH in the pure form was recommended in a previous study,²⁵ this material promoted a more intense inflammatory reaction^{41,44} compared with MTA and Biodentine, which demonstrated similar results. Previous studies have shown that CH elicits the generation of zones of necrosis when in contact with pulp tissues, which may be attributed to its initial caustic effect.⁴⁵

Therefore, the intense inflammatory response elicited by CH, in comparison with that generated by MTA and Biodentine, may influence the formation of a mineralized barrier. This finding coincides with the difference revealed at 14 days by the analysis of the continuity of the mineralized barrier formation, where the CH presented an inferior result compared with MTA. All samples capped with MTA showed partial dentin bridge formation. In addition, morphological analysis at 14 days showed that CH presented statistically lower values than MTA and Biodentine, whose results were similar to each other.

The barriers formed using CH were porous, and therefore not ideal for a proper bacterial sealing, as described by Paranjpe et al.⁴⁶ The CH presented a porous barrier as from the second week, as also reported by Tran et al,⁴³ who concluded that CH used in direct pulp capping produces porous barriers at 14 days in rat pulps compared with Biodentine. In contrast, denser and more extensive formations were observed when MTA or Biodentine were used, in which the mineralization formed appeared as an atubular dentin, similar to pulp calcification and different from the reactional dentin.⁴⁷

In our study, morphological analysis of the mineralized barrier formed indicated superior irregular deposition of hard tissue for MTA and Biodentine when compared with CH, which presented small depositions of hard tissue at 14 days. According to Laurent et al,¹⁰ Biodentine exhibits a 12-minute setting time and increased sealing capacity. All materials promote alkalization of the medium, owing to the pH of around 12.5, which confers antibacterial power

to the materials. In routine clinical settings, the Biodentine material deserves special attention because it induces the formation of a mineralized barrier, similar to that induced by MTA, and a reduced setting time, thereby allowing definitive restorations to be made after direct pulp capping. This circumvents the need for additional clinical sessions, thereby eliminating the risk of new pulp exposure and secondary contamination, significantly reducing patient stress and cost of treatment.

At 21 days, morphological analysis of the mineralized barrier formed in the various groups revealed superior results for Biodentine relative to CH. It is known that the availability of calcium ions stimulates the formation of the calcium carbonate precipitate, which contributes to mineralization. It seems clear that the form and time for the release of calcium and hydroxyl ions interfere with the formed mineralized barrier. In CH, the release of the calcium ions occurs rapidly, which acts as an irritant to the tissues and generates a caustic surface effect.²⁹ Calcium silicate materials exhibit a slower and more gradual release, and disperse larger amounts of calcium ions compared with CH-based cements. Biodentine, however, exhibits greater calcium release and extended alkaline activity, as well as better mechanical properties than MTA.^{11,48}

Our results demonstrate the superiority of the new materials, MTA and Biodentine over CH, when used for direct capping of live pulps. The use of new materials based on calcium silicate presents a viable alternative, with favorable histological characteristics relative to CH, which was previously considered the gold standard for this treatment.

Conflict of Interest

None declared.

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