

A COMPARATIVE HISTOLOGICAL STUDY OF CALCIUM-SILICATE CEMENTS FOR DIRECT PULP CAPPING IN WISTAR RATS

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Abstract: Maintaining pulp vitality is essential for the long-term health of teeth. Direct pulp capping aims to stimulate reparative dentin formation and prevent pulp necrosis. This study aimed to evaluate the effects of novel calcium silicate-based materials on the pulpal inflammatory response and dentin bridge formation using a Wistar rat model.

A total of 18 Wistar rats (72 maxillary molars: 36 first and 36 second) were included and divided into two groups (A and B; n = 9). In Group A, Theracal LC was applied to the exposed pulp of the right maxillary molars, while MTA was applied to the left molars. In Group B, Calxyl was applied to the right molars, and Biodentine and MTA were applied to the first and second left molars, respectively. The Wilcoxon signed-rank test was used for statistical analysis.

After 30 days, the lowest pulpal inflammatory response was observed with Theracal LC, followed by Biodentine, which showed a comparable level of inflammation but with slightly more polymorphonuclear leukocytes. The highest inflammatory response was recorded with Calxyl. A statistically significant difference in inflammation levels was found between Theracal/Calxyl (p = 0.041), Theracal/MTA (p = 0.034), Biodentine/Calxyl (p = 0.044), and Biodentine/MTA (p = 0.039). All tested materials demonstrated a strong capacity to induce dentin bridge formation.

In conclusion, the new calcium silicate-based materials exhibited favorable biological properties, with Theracal LC and Biodentine showing the lowest inflammatory response. All materials promoted reparative dentinogenesis, confirming their potential for use in direct pulp capping procedures.

Keywords: pulp capping, Theracal LC, Biodentine.

1. INTRODUCTION

Preservation of pulp vitality is a key objective in modern endodontics, as vital pulp ensures the long-term functionality and health of the tooth [1]. Direct pulp capping is a minimally invasive therapeutic procedure designed to stimulate reparative dentinogenesis and prevent pulpal necrosis following exposure caused by caries or trauma [2–5]. The choice of an appropriate biocompatible material with

favorable bioactive properties is critical for the success of the therapy [3].

Calcium silicate-based materials have become the standard in vital pulp therapy in recent decades due to their high biocompatibility, capacity to stimulate dentin bridge formation, and antimicrobial properties [6–9]. Materials such as mineral trioxide aggregate (MTA), Biodentine, and newer materials such as Theracal LC are increasingly used in clinical practice [10–14]. In contrast, Calxyl, a

calcium hydroxide-based material, has a long history in direct pulp capping but demonstrates different biological responses compared with calcium silicate materials, particularly in terms of inflammatory reaction and the ability to stimulate reparative processes [15–17].

Animal studies, particularly in rats, provide valuable insights into the biological response of the pulp to different materials and enable a systematic evaluation of their biocompatibility and potential for dentin regeneration [18,19].

The aim of this study was to compare the inflammatory response and dentin bridge formation following direct pulp capping with different materials—calcium silicate-based materials and calcium hydroxide (Calxyl)—in Wistar rats, with particular emphasis on the newer materials Theracal LC and Biodentine, alongside MTA and Calxyl.

2. MATERIAL AND METHODS

The study was conducted in the vivarium of the Faculty of Natural Sciences and Mathematics, University of Banja Luka, after obtaining approval from the Ethics Committee of the University Clinical Center Banja Luka (No. 01-9-192.2/15), Bosnia and Herzegovina.

2.1. Tested Materials

The composition of all tested materials is shown in Table 1.

2.2. Design of the Study

The study included 18 laboratory rats of Wistar strain ie. 72 teeth (36 first upper molars and 36-second upper molars). The animals were 10-11 weeks old and weighed an average of 190-280 g. During the experiment, all rats were provided with free access to food and water, a 12-hour shift of light and darkness, air temperature 20-23°C, while the humidity was 60% ± 10%. Before the dental procedure, rats were anesthetized by induction of general anesthesia (Ketamine Hydrochloride Injection USP Rotexmedica-Germany at a dose of 50 mg/kg body weight). Immediately before the start of work, all teeth were mechanically cleaned of soft deposits with a soft brush and toothpaste and then disinfected with chlorhexidine digluconate (0.1% Chlorhexamed-Fluid, GlaxoSmithK, Buhl, Germany). Due to difficult access and reduced visibility of rat molars, a magnifying glass (4.5 ×, Zeiss, Oberkochen, Germany) was used.) Rats were divided into two experimental groups, A and B, with 9 rats in each group.

* In group A, Theracal LC was applied to the open pulp of the upper molars on the right side and MTA on the left side.

* In group B, Calxyl was applied to the open pulp of the upper molars on the right side, while Biodentine was applied to the first upper molar on the left side, and MTA to the second upper left molar.

Class I cavity preparations on the occlusal surface of non-carious first and second upper molars were done with a technical micromotor and a ster-

Table 1. Tested Materials - Composition and Manufacturers

| Material | Manufacturer | Composition |
|--------------------|-----------------------------|---|
| TheraCal LC | BISCO, USA | Resin-modified calcium silicate liner; Portland cement (Type III), radiopaque fillers (barium zirconate), calcium oxide, strontium glass, fumed silica, barium sulfate, hydrophilic resin monomers (HEMA, Bis-GMA, PEGDMA). |
| Biodentine | Septodont, France | Powder: Tricalcium silicate, dicalcium silicate, calcium carbonate, calcium oxide, iron oxide, zirconium oxide. Liquid: Calcium chloride solution (accelerator), hydrosoluble polymer (water-reducing agent). |
| MTA Angelus | Angelus, Brazil | Powder: Tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, bismuth oxide (radiopacifier). Liquid: Distilled water. |
| Calxyl | OCO Präparate GmbH, Germany | Aqueous suspension of calcium hydroxide; calcium hydroxide, distilled water, sometimes with barium sulfate (radiopacity). |

ile ISO 008 round diamond drill bit, with continuous water cooling, until the depth of the cavity was approximately half the size of the drill bit. After cavity preparation in one rat, a drill bit was replaced. Dental pulp was exposed with a sterile sharp endodontic explorer (DG16, Dental USA, Mc Henry, IL, USA) (*Figure 1*) pulpal blood was removed with sterile paper denominations, and the cavity was washed with saline to remove possible blood residues and dentin dust. Subsequently, DPP materials were applied to the tip of the endodontic explorer as described above. The cavities were restored with glass ionomer cement (GC Fuji VIII, GC Corporation, Tokyo, Japan). According to the study plan, the animals were sacrificed by intravenous injection of pentobarbital after 30 days of observation. After separating the upper jaws with a surgical scalpel and scissors (decapitation) (*Figure 2*), they were stored in 10% neutral buffered formalin, and the material was delivered to the Laboratory of the Department of Pathology of the Clinical Center in Banja Luka and prepared for pathohistological analysis.



Figure 1. Pulp cavity trepanation



Figure 2. Decapitation

2.3. Pathohistological Analysis

Material preparation began with decalcification of the jawbones in EDTA (ethylene-diamine-tetraacetic acid) for three weeks, after which the pulp sections were embedded in paraffin blocks. Sections were cut with a microtome at a thickness of 4 μm . Cross-sections were stained using the hematoxylin-eosin method.

Qualitative evaluation of the microscopic preparations was performed by a pathologist who was not affiliated with the laboratory where the slides were prepared, ensuring unbiased assessment. For this purpose, a light microscope (Celestron Labs CB2000CF Compound Binocular Microscope) was used. The following parameters were analyzed: absence of bacteria, presence of bacteria in one-third of the pulp, presence of bacteria in two-thirds of the pulp, and diffuse bacterial presence throughout the pulp.

The pulpal inflammatory response was evaluated using the following scale: absence of inflammation (none or few scattered inflammatory cells), presence of a small number of polymorphonuclear leukocytes, presence of a large number of polymorphonuclear leukocytes, and pulp necrosis. Formation of the dentin bridge at the end of the observation period was also assessed as a potential indicator of therapeutic success.

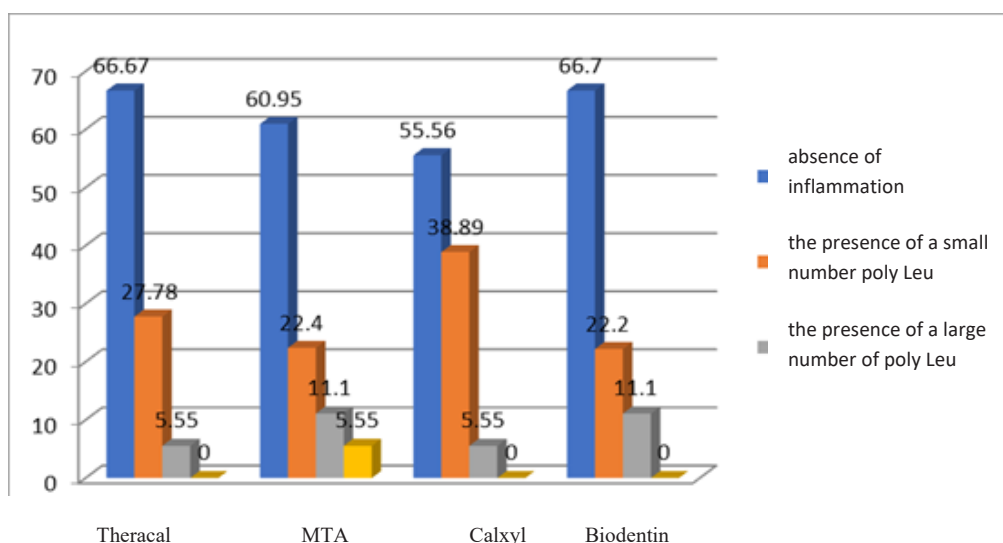
2.4. Statistical Data Analysis

A nonparametric Wilcoxon signed-rank test for paired samples was used to analyze the results of direct pulp capping in Wistar rats. The level of statistical significance was set at 5% ($p < 0.05$).

3. RESULTS

Analysis of the pulpal inflammatory response in teeth treated with Theracal LC showed absence of inflammation in 66.67% of cases, presence of a small number of polymorphonuclear leukocytes in 27.78% of cases, and presence of a large number of polymorphonuclear leukocytes in 5.55% of cases, with no necrosis observed (Graph 1).

After direct pulp capping (DPC) with Biodentine, inflammation was absent in 66.7% of cases, a small number of polymorphonuclear leukocytes was observed in 22.2% of cases, and a large number of



Graph 1. Inflammatory response of the dental pulp to the tested materials

polymorphonuclear leukocytes was noted in 11.1% of cases (Graph 1).

Following DPC with Calxyl, inflammation was absent in 55.56% of cases, a small number of polymorphonuclear leukocytes was present in 38.89% of cases, and a large number of polymorphonuclear leukocytes was observed in 5.55% of cases (Graph 1).

In teeth treated with MTA, inflammation was absent in 60.95% of cases, a small number of polymorphonuclear leukocytes was observed in 22.4% of cases, a large number of polymorphonuclear leukocytes in 11.1% of cases, and pulp necrosis was present in 5.55% of cases (Graph 1).

As shown in Graph 1, all four tested materials (Theracal LC, Biodentine, MTA, and Calxyl) exhibited the highest percentage of cases with no pulpal inflammatory response.

The lowest inflammatory response was observed with Theracal LC, followed by Biodentine, which displayed similar percentages of pulp inflammation as Theracal LC, although with a slightly higher occurrence of a large number of polymorphonuclear leukocytes.

MTA ranked next in terms of inflammatory response, showing a higher overall percentage of pulpal inflammation compared with Theracal LC and Biodentine, similar or higher values for the presence of a small number of polymorphonuclear leukocytes, a higher percentage of cases with a large number of polymorphonuclear leukocytes, and the only cases

with pulp necrosis. A statistically significant difference in pulpal inflammation was found between Theracal LC and MTA ($p = 0.034$) and between Biodentine and MTA ($p = 0.039$).

The highest inflammatory response was observed with Calxyl, which showed the highest percentage of cases with a small number of polymorphonuclear leukocytes. Statistically significant differences in pulpal inflammation were found between Theracal LC and Calxyl ($p = 0.041$) and between Biodentine and Calxyl ($p = 0.044$).

Table 2. Formation of a dentinal bridge after application of test materials

| | Theracal LC | Biodentine | MTA | Calxil |
|----------------|-------------|------------|-------|--------|
| Formed (%) | 72,22 | 61,11 | 55,56 | 55,56 |
| Not formed (%) | 27,78 | 38,89 | 44,44 | 44,44 |
| Sum | 100 | 100 | 100 | 100 |

No bacteria were detected in any of the samples. Analysis of dentin bridge formation after 30 days showed that all four tested materials promoted dentin bridge formation in a high percentage of cases (Table 2).

Theracal LC exhibited the highest frequency of dentin bridge formation (72.22%), whereas dentin bridge formation was absent in 27.78% of cases. Statistically significant differences were observed between Theracal LC and MTA ($p = 0.025$) as well

as between Theracal LC and Calxyl ($p = 0.025$) (Table 2).

Following Biodentine application, dentin bridge formation occurred in 61.11% of cases. The lowest dentin bridge formation was observed with MTA and Calxyl, each showing formation in 55.56% of cases. The difference in dentin bridge formation between Biodentine and MTA/Calxyl was also statistically significant ($p = 0.037$) (Table 2).

MTA and Calxyl demonstrated identical dentin bridge formation rates (55.56%) over the 30-day observation period.

4. DISCUSSION

Preservation of vital pulp is essential for long-term dental health, as it allows continued physiological function, structural strengthening, and maintenance of proprioception. Compared with endodontic treatment, successful direct pulp capping represents a more biologically acceptable, simpler, and more cost-effective approach, with a significantly higher rate of native tissue preservation. Advances in bioactive material development, particularly calcium silicate cements, have further improved the clinical success of this procedure.

After 30 days of treatment in rat teeth, all four tested materials—Theracal LC, Biodentine, MTA, and Calxyl—showed predominantly absent pulpal inflammatory response: Theracal LC 66.7%, Biodentine 66.7%, MTA 60.95%, and Calxyl 55.56%. Statistically significant differences were observed between Theracal LC/Calxyl ($p = 0.041$), Theracal LC/MTA ($p = 0.034$), Biodentine/Calxyl ($p = 0.044$), and Biodentine/MTA ($p = 0.039$).

The most pronounced inflammatory response after Calxyl application can be attributed to its solubility, weak sealing properties, tunnel defects in the dentin bridge, and greater biological variability. Similar findings have been reported in other histological and clinical studies [20–23].

Our results align with recent reviews and meta-analyses showing that calcium silicate cements (MTA, Biodentine, and resin-modified Theracal LC) generally induce favorable histological and clinical outcomes. Cushley et al. (2021) reported that MTA and Biodentine consistently achieved higher success rates than $\text{Ca}(\text{OH})_2$ in direct pulp capping [23]. Sabetti et al. (2021) highlighted that bioceramic materials,

particularly MTA and Biodentine, demonstrate very high success rates in vital pulp therapy, significantly outperforming calcium hydroxide [24]. Komora et al. (2024) concluded in a network meta-analysis that MTA ranks highest in success rate, with Biodentine closely following, while $\text{Ca}(\text{OH})_2$ materials show inferior long-term results [25]. Fasoulas et al. (2023) also confirmed better clinical and radiographic outcomes for MTA and Biodentine compared with $\text{Ca}(\text{OH})_2$ [26], and Pinto et al. (2024) reported success rates of 70–100% for calcium silicate direct pulp capping, whereas $\text{Ca}(\text{OH})_2$ maintained pulp vitality with greater variability [1].

Regarding Theracal LC, the evidence is more heterogeneous. Singh et al. (2024) reported a higher frequency of complete dentin bridge formation and lower pulpal inflammatory response compared with Biodentine in premolars scheduled for orthodontic extraction [27]. In contrast, Silva et al. (2023) observed that Theracal LC can elicit a favorable pulpal response, but results are less consistent than those of MTA and Biodentine. Komora et al. (2024) found that the success of Theracal LC was inferior to MTA and Biodentine in clinical follow-ups of 12–24 months [28,25].

Thirty days after DPC with Theracal LC, Biodentine, MTA, and Calxyl, no bacteria were detected in any of the 72 analyzed teeth or surrounding tissues. Standard microbiological analyses confirmed the complete absence of bacterial cells, indicating sterile working conditions, effective marginal sealing of the glass ionomer cement, and high procedural control [29,30].

At the end of the observation period, dentin bridge formation was observed for all materials: Theracal LC 72.2% > Biodentine 61.1% > MTA = Calxyl 55.6%, with a statistical advantage of Theracal LC over MTA and Calxyl ($p = 0.025$) and Biodentine over MTA/Calxyl ($p = 0.037$). The faster initial sealing and rapid alkalization of Theracal LC may explain earlier mineralization at 30 days. Clinical-histological studies with premolar extraction after six weeks also demonstrate a higher frequency and thickness of the dentin bridge with Theracal LC compared with Biodentine [27].

Theracal LC, a resin-based calcium silicate agent, has shown good efficacy and applicability in direct pulp capping of exposed posterior teeth, with predictable clinical and radiographic success [31]. In

contrast, an experimental histological study in rabbits (8 weeks) reported more favorable dentin bridge quality with Biodentine than with Theracal LC, while both materials had a similar inflammatory effect on the pulp [11]. These differences may be influenced by experiment duration, tissue type, animal model, hemostasis technique, material layer thickness, polymerization protocol, and histological scoring criteria.

One limitation of this study is the short observation period of 30 days, which captures only the early phase of repair; differences between materials may change with longer follow-up (8–12 weeks or more).

5. CONCLUSION

The new calcium silicate materials demonstrated favorable biological properties, with Theracal LC and Biodentine eliciting the lowest inflammatory responses. All materials promoted reparative dentin formation, confirming their potential for use in direct pulp capping therapy. Resin-modified calcium silicate cement (Theracal LC) may provide the advantage of early sealing; however, the literature reports heterogeneous histological outcomes, highlighting the need for longer follow-up and dentin bridge morphometry to draw definitive conclusions.

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КОМПАРАТИВНА ХИСТОЛОШКА СТУДИЈА КАЛЦИЈУМ-СИЛИКАТНИХ ЦЕМЕНАТА ЗА ДИРЕКТНО ПРЕКРИВАЊЕ ПУЛПЕ КОД ВИСТАР ПАЦОВА

Сажетак: Очување виталитета пулпе је од суштинског значаја за дугорочно здравље зуба. Директно прекривање пулпе има за циљ подстицање формирања репаративног дентина и спречавање некрозе пулпе. Циљ ове студије био је да се испита утицај нових материјала на бази калцијум силиката на инфламаторни одговор пулпе и формирање дентинског моста код пацова Вистар соја. У студију је укључено 18 пацова Вистар соја (укупно 72 максиларна молара: 36 првих и 36 других), подијељених у двије групе (А и Б; $n = 9$). У групи А, Theracal LC је примијењен на десне максиларне моларе, док је МТА коришћен на лијевим. У групи Б, Calxil је примијењен на десне моларе, а Biodentin и МТА на први и други леви молар. За статистичку анализу коришћен је Wilkoxsonov тест за зависне узорке.

Након 30 дана, најслабији инфламаторни одговор забиљежен је код материјала Theracal LC, а затим код Biodentina, који је имао сличне резултате, али са нешто већим присуством полиморфно-нуклеарних леукоцита. Најизраженији инфламаторни одговор показао је Calxyl. Статистички значајна разлика у инфламацији утврђена је између материјала Theracal/Calxyl ($p = 0,041$), Theracal/МТА ($p = 0,034$), као и између Biodentina/Calxyla ($p = 0,044$) и Biodentina/МТА ($p = 0,039$). Сви испитивани материјали су у великом проценту довели до формирања дентинског моста.

Закључно, нови калцијум силикати су показали повољне биолошке особине, при чему су Theracal LC и Biodentin изазвали најмање упалне реакције. Сви материјали су подстакли формирање репаративног дентина, што потврђује њихов потенцијал у терапији директног прекривања пулпе.

Кључне ријечи: Прекривање пулпе, Theracal, Biodentin, МТА.

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