RESEARCH ARTICLE

HISTOPATHOLOGICAL PULP RESPONSE OF TEETH CAPPED WITH CALCIUM ALUMINATE CEMENT AND BIODENTINE: EXPERIMENTAL STUDY ON RODENTS

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ABSTRACT

This study aimed to evaluate the pulpal response after pulp capping using experimental nanostructured calcium aluminate cement (ALBO-CA) and tricalcium silicate cement Biodentine (Septodont, Saint Maur des Fosses, France). Class I cavities were prepared on 72 teeth (36 first upper molars and 36-second upper molars) in 18 Wistar strain rats. The dental pulp was capped in 36 teeth with experimental ALBO-CA (group A) and 36 teeth with Biodentine (group B). All cavities were restored with glass ionomer. After 28 days animals were sacrificed and the following were analyzed: the inflammatory response of the pulp, the presence of bacteria, and the formation of a dentinal bridge. After 28 days, bacteria were not present. Mann-Whitney U test did not show a statistically significant difference in the inflammatory response of the pulp between the groups ($U = 640.00$; $Z = 0.105$; $p = 0.916$). The chi-square test did not show a statistically significant difference in the formation of the dentinal bridge between the examined groups (Chi-square = 1,443; $p = 0,230$). ALBO-CA and Biodentine had similar effects on inflammation, pulp response, and formation of dental bridges in rats.

Keywords: ALBO-CA, pulp capping, rats, tricalcium silicate

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INTRODUCTION

Cement materials in dentistry have been developed to imitate the lost dentine tissue, to mimic biological features as much as possible, and to display bioactive characteristics (Ilić et al., 2019).This is not always simple because of dentine specificity, namely, its close contact with the pulp. In that sense, the local bioactivity of these cement materials is important to induce mineralization within the adjacent dentine (Ilić et al., 2019).

Calcium hydroxide (CH) became recognized as a valuable capping material. The initial effect of CH applied to the exposed pulp is the development of superficial necrosis. Namely, a chemical injury is provoked by hydroxyl ions leading to the formation of a zone of firm necrosis over the vital tissue. The necrosis causes slight irritation and stimulates the pulp to regenerate. Thereafter, the repair process occurs, including migration and proliferation of mesenchymal and endothelial pulp cells as well as collagen formation. The presence of Ca ions stimulates precipitation of $CaCO_3$ contributing to the mineralization initiation (Dammashke, 2008). Shortcomings of CH-based materials include poor mechanical characteristics, inability to preserve high pH values at the site of administration for a certain period, the possibility of primary tooth resorption, dissolution after one year and degradation during acid etching or tooth flexure, poor marginal seal with composite/amalgam restoration and weakening of the root during apexogenesis in a long-term therapy (Saad, 1988). Indeed, long-term use of CH capping may cause progressive calcification of the root canal space.

New generations of cementitious materials began to be produced to overcome the disadvantages of CH. A significant improvement in this field occurred in 1993 when Torabinejad introduced a novel material mineral trioxide aggregate (MTA) that is based on calcium silicate (CS) particles. The main advantage of CS in comparison to CH is its better mechanical and bioactive characteristics. MTA also has some drawbacks such as a long setting time, high cost, and potential for discoloration (Parirokh and Torabinejad, 2017; Jafari, 2017). Biodentine is tricalcium silicate cement. Contrary MTA(Mineral Trioxide Aggregate), it contains zirconium oxide as an X-ray contrast agent. The addition of 15% calcium carbonate to its composition improved the microstructure and facilitated the handling of cement. Biodentine has several advantages which include good sealing ability, adequate compressive strength, short setting time, biocompatibility, bioactivity, and biomineralization properties. Biodentine is mechanically stronger, less soluble, and gives a tighter seal. These features make Biodentine a suitable direct pulp-capping material (Deshmukh et al., 2018).

There is far less data in the scientific literature on materials based on calcium aluminates (Chang et al., 2014; Woodmansey et al., 2015; Janković et al., 2018; Walsh et al., 2018; Paraš et al., 2019; Čolović et al., 2019; Janković et al., 2020). The Endobinder calcium aluminate material has been successfully used for the repair of bony defects (Čolović et al., 2019). It confirmed biocompatibility during subcutaneous implantation in rats (Janković et al., 2018). According to Chang et al. (2014), given optimal pressure resistance, short setting time, and a high degree of biocompatibility, tricalcium aluminum cement may be a suitable material for vital pulp therapy. However, a small number of studies have considered this issue, and in doing so, calcium aluminate cements exhibited a beneficial effect on the reparative abilities of the pulp (Chang et al., 2014; Woodmansey et al., 2015; Walsh et al., 2018).

ALBO-CA is newly synthesized nanomaterial based on calcium aluminate. The particle size of nanomaterials is similar to the size of biological molecules and structures, which gives these materials a great advantage in tissue engineering and regenerative medicine. A nanostructured calcium aluminate cement ALBO-CA was synthesized by a special method, a combination of the hydrothermal salt gel method and the method of self-combustion waves. Nanoparticles improve particle activity and shorten the curing time to 10-15 minutes (Janković et al., 2018). It is expected that newly synthesized nanomaterials

based on calcium aluminates will have a beneficial effect on the reparative abilities of the pulp. This study aimed to evaluate the pulpal response after pulp capping using experimental nanostructured calcium aluminate ALBO-CA and tricalcium silicate Biodentine (Septodont, Saint Maur des Fosses, France).

MATERIAL AND METHODS

Approval of the ethics committee

The study was conducted in the vivarium of the Faculty of Natural Sciences and Mathematics in Banja Luka, after obtaining the consent of the Ethics Committee of the University Clinical Center in Banja Luka, number 01-9-192.2/15, Bosnia and Herzegovina.

Tested materials

Experimental nanostructured ALBO-CA based on calcium aluminate (CA) was compared with tricalcium silicate cement Biodentine (Septodont, Saint Maur des Fosses, France).

ALBO-CA ($CaO \bullet Al_2O_3 + CaCO_3 + Bi_2O_3$) was obtained by mixing $CaCO_3$, Bi_2O_3, and $BaSO_4$ with a calcium aluminate phase in a ratio of 2: 2: 1. The mixture was finally mixed with distilled water in a ratio of powder/water 2: 1, to achieve the consistency of cement paste.

BiodentineTR is composed of a highly purified C3S powder prepared synthetically from a mixture of powder constituents: SiO_2-16.9 %, CaO-62.9 %, and ZrO_2-5 %. C_2S and C_3S particles form 70 wt% of the above mixture's dehydrated powder. Biodentine does not contain $CaSO_4$, aluminate, or alumina ferrite. The liquid component is distilled water with the addition of $CaCl_2$ (Jafari and Jafari, 2017; Singh et al. 2014).

Resin-modified glass ionomer Fuji VIII (GC Corporation, Tokyo, Japan) was used for the restoration of all cavities, the composition of which is represented: powder: silicate glass and liquid: polyacrylic acid.

Design of the study

The study included 18 laboratory rats of the Wistar strain and 72 teeth, i.e. 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 of 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). The dose of anesthetic was determined according to the body weight of the rats, which was measured for each rat individually. 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, only the first and second upper molars of rats were included in the experiment. A magnifying glass (4.5 ×, Zeiss, Oberkochen, Germany) was used for the same reasons. Rats were divided into two experimental groups, A and B, with 9 rats in each group.

* In group A, ALBO-CA was applied to the opening pulp of the first and second upper molar on the right and left sides.

* In group B, the tricalcium silicate cement Biodentine (Septodont, Saint Maur des Fosses, France) was used in the same way as ALBO-CA.

Class I cavity preparations on the occlusal surface of non-carious first and second upper molars were done with a technical micromotor and a sterile 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), pulpal blood was removed with sterile cotton balls, and the cavity was washed with saline to remove possible blood residues and

dentin dust. After that, the tested materials (ALBO-CA and Biodentine) were applied to the pulp. 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 28 days of observation.

After separating the upper jaws with a surgical scalpel and scissors (decapitation), 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.

Pathohistological analysis

Preparation of the material was started by decalcification of the jawbones in EDTA (ethylene-diamino-tetracycline-acetate) for 3 hours, after which the pulp sections were molded into the paraffin blocks. Cutting was performed with a microtome (every 4μm). Cross-section staining was performed by the hematoxylin-eosin method. For these purposes, we used a light microscope (Celestron Labs CB2000CF Compound Binocular Microscope).

The following were analyzed: the absence of bacteria, the presence of bacteria in 1/3 of the tooth pulp, the presence of bacteria in 2/3 of the tooth pulp, and the diffuse presence of bacteria in the pulp.

Furthermore, the inflammatory response of the pulp was evaluated, using the modified criteria of Accorinte et al. (2008): the absence of inflammation, i.e. none or a few scattered inflammatory cells, the presence of a low number of polymorphonuclear leukocytes, the presence of a high number of polymorphonuclear leukocytes and pulp necrosis.

The formation of the dentinal bridge at the end of the observation period was also taken into account as one of the possible indicators of the success of the therapy. Dentine bridge was quantified using the modified criteria of Accorinte et al. (2008) as formed and not formed.

Statistical Data Analysis

The Man-Whitney U test and the Chi square test were used for statistical analysis of the obtained results of direct pulp capping in rats of the Wistar strain.

RESULTS

The results of the histological analysis are shown in Tables 1 and 2, and Figures 1 and 2.

Analysis of the inflammatory response of the pulp of the teeth of rats in group A, which ALBO-CA was applied to, indicated the absence of inflammation in 63.9% of cases, the presence of a low number of polymorphonuclear leukocytes in 250% of cases, the presence of a high number of polymorphonuclear leukocytes in 8.3% of cases, and pulp necrosis in 2.8% of cases (Table 1).

After direct pulp capping of rat teeth with Biodentine in group B, inflammation was completely absent in 61.1% of cases, in 30.6% of cases the presence of a low number of polymorphonuclear leukocytes was noted, while in 8.3% of cases, the presence of a high number of polymorphonuclear leukocytes was noted. Pulp necrosis was absent (Table 1).

It is clear from Table 1 that both examined materials, experimental ALBO-CA (group A) and tricalcium silicate cement Biodentine (group B), gave the largest percentage of the absence of the inflammatory response of the pulp. In group B in which Biodentine was applied to the pulp of rat teeth, a slightly higher presence of a small number of polymorphonuclear leukocytes was observed, but the Mann-Whitney U test did not show a statistically significant difference in the inflammatory response of the pulp between the groups ($U = 640.00$; $Z = 0.105$; $p = 0.916$). Bacteria were not present in any of the samples.

Analysis of the formation of a dentinal bridge on the teeth of rats after 28 days shows that both examined materials in a higher percentage led to the formation of the dentinal bridge (Table 2, Figure 1 and 2). ALBO-CA material had a higher frequency of dentinal bridge formation (66.7%), while dentinal bridge formation was absent in 33.3% of cases.

After the application of Biodentine (group B), dentinal bridge formation occurred in 52.8%, while the same was absent in 47.2% of cases. The Chi-square test did not show a statistically significant difference in the formation of the dentinal bridge between the examined groups (Chi-square = 1,443; p = 0,230).

Table 1 Inflammatory response of rat tooth pulp after application of ALBO-CA and Biodentine

			Inflammatory pulp response				Total
			Absence of inflammation	Low no. polymor-phonuclear Le	High no. polymor-phonuclear Le	Necrosis	
Group	A	N	23	9	3	1	36
		%	63.9%	25.0%	8.3%	2.8%	100.0%
	B	N	22	11	3	0	36
		%	61.1%	30.6%	8.3%	0.0%	100.0%
Total		N	45	20	6	1	72
		%	62.5%	27.8%	8.3%	1.4%	100.0%

Table 2 Dentinal bridge formation of rat tooth pulp after application of ALBO-CA and Biodentine

			Dentine bridge		Total
			Formed	Not formed	
Group	A	N	24	12	36
		%	66.7%	33.3%	100.0%
	B	N	19	17	36
		%	52.8%	47.2%	100.0%
Total		N	43	29	72
		%	59.7%	40.3%	100.0%

Figure 1 Longitudinal section of dental pulp section, odontoblast layer with fully formed dentinal bridge with dentinal tubules continuous with surrounding dentin after direct pulp capping ALBO-CA, HE 400x

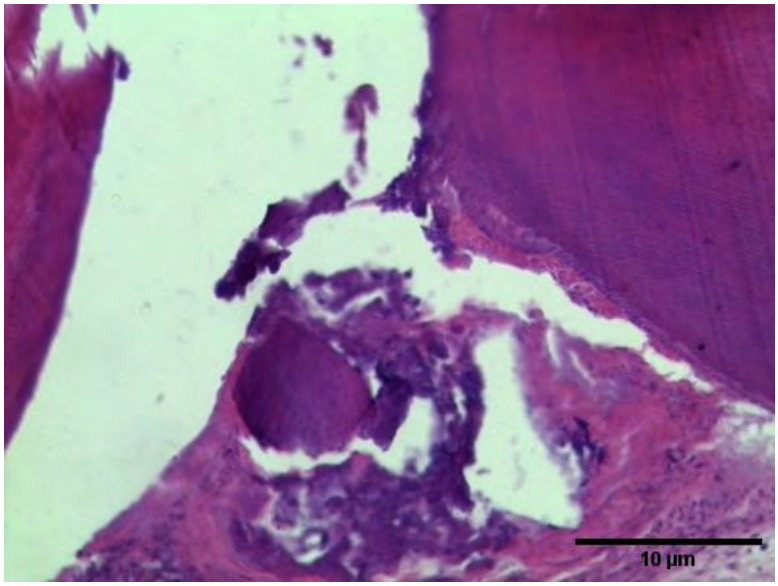

Figure 2 Section of the tooth, part of the pulp and dentin with a perforation of the pulp chamber of the tooth covering an incompletely formed dentin bridge (fibrin and calcifications of the dentin island) can be seen after directly covering the pulp with Biodentine HE 400x

DISCUSSION AND CONCLUSION

Pulp capping materials protect the vital pulp tissue threatened by caries or trauma. Most of the available pulp capping materials have numerous advantages and disadvantages (Da Rosa et al., 2018; El-Mal et al., 2019; Al-Sherbiny et al., 2020). Therefore, the studies on new pulp capping materials are still very current.

Recently, a new calcium aluminate-based material called ALBO-CA has been synthesized. According to our knowledge, there are only a few *in vivo* studies on the use of calcium aluminate cement in vital pulp therapy and pulpotomy procedures (Woodmansey et al., 2015; Walsh et al., 2018). Therefore, this study compared the efficacy of ALBO-CA and Biodentine as direct pulp capping materials. We selected Biodentine for comparison due to its so far excellent results as a pulp-capping agent.

Both animal and human teeth are usedto demonstrate the effects of pulp-capping materials onvital pulp tissue (Negm et al. 2017). Rats have significant advantages in terms of ethical and

economic reasons. The advantages of rats are their economic cost and good adaptation to life in laboratory conditions (Katica and Delibegović, 2019; Katica et al., 2020). In the last half a century, several studies have been published that have used rat molars to assess the response of pulp tissue to a direct pulp capping procedure or pulpotomy (Kramer et al., 2014; Kim et al., 2016).

According to the results of this study, both the examined materials, the newly synthesized ALBO-CA and Biodentine gave the absence of the inflammatory response of the pulp in most cases, indicating the biocompatibility of these materials. After the application of ALBO-CA, the inflammation of the pulp tissue was absent in a slightly higher number of cases, and a weaker presence of a small number of polymorphonuclear leukocytes was noted, but this difference was not statistically significant.

Good bond strength with the tooth also ensures lower micropermeability, which, in addition to working in aseptic conditions, resulted in complete absence of bacteria in all tested samples.

In the available literature, dealing with the problem of antimicrobial activity of calcium aluminate cement (Souza et al., 2013; Silva et al., 2014; Radović et al., 2019), calcium aluminates mainly exhibited a comparable antimicrobial effect with MTA, while in the study of Radović et al., the ALBO-CA material even exhibited a slightly greater antibacterial effect against E. coli compared to MTA. The antibacterial properties of Biodentine are attributed to its high pH, which is achieved by the action of hydroxyl ions on the surrounding tissue (Bhavana et al., 2015).

In this study, both examined materials after an observation period of 28 days, in a higher percentage led to the formation of a dentinal bridge. An interesting earlier study by Carmo et al. (2018), demonstrated the ability of several formulations of calcium aluminate cement (CACb) and Biodentine to form apatite crystals on their surface, after contact with PBS or deionized water for 14 days. Cement surfaces were analyzed using SEM, EDS-X, and FTIR.

The percentage of formed dentinal bridges was slightly higher after direct coating of the pulp with nanostructured calcium aluminate ALBO-CA, but without a statistically significant difference.

Many studies have evaluated the capacity of Biodentine in direct closure of exposed pulp in animals (Popović-Bajuć et al., 2014; Kim et al., 2016; El-Din et al., 2020; Souza et al., 2021) and humans (Jayanandan et. al., 2021). They reported good results for direct pulp capping with Biodentine.

In a study by Jayanandan et. al. (2021), performed was pulp capping with calcium hydroxide, mineral trioxide aggregate (MTA), Biodentine and EndoSequence root repair material (ERRM). 15 orthodontic patients requiring the extraction of four premolars (60 teeth in total) were included in the study. After closing the pulp, the teeth were extracted after 8 weeks. We analyzed the extracted teeth with cone beam computed tomography (CBCT) and histological sections to determine the quality of the dentinal bridge and pulp response. A dentine bridge was formed in 9/15 cases of teeth treated with MTA and ERRM (60%). In contrast, only 6/15 calcium hydroxide cases and 7/15 Biodentine cases showed complete bridging (40–46%). These differences were not statistically significant ($p > 0.05$), which is somewhat in line with our results because in this study Biodentine showed a lower quality of the dentine bridge in comparison to MTA and ERRM, although without a significant difference. There was also a slightly more pronounced inflammatory response of the pulp tissue after the application of Biodentine, as in our study, with the fact that here the MTA and ERRM groups differed significantly from the calcium hydroxide and Biodentine groups.

However, in the available literature, in most studies Biodentine showed either a similar effect to the materials with which it was compared in direct pulp coverage or, contrary to our knowledge, a more favorable therapeutic effect.

De Souza et al. (2021) evaluated the pulpal responses to mineral trioxide aggregate (MTA Angelus) and Biodentine, focusing on mineralized barrier

formation and inflammatory and degenerative events in 80 male Wistar rats. Lower first molars were mechanically exposed, covered with either MTA or Biodentine, and restored with silver amalgam. Teeth covered with gutta-percha and restored with silver amalgam served as a positive control, while untreated teeth served as a negative control. Pulp responses were evaluated after 14 or 21 days. Biodentinr and MTA showed satisfactory results, showing a milder inflammation response ($p<0.0001$) and a more pronounced formation of mineralized barriers ($p<0.0001$) compared to teeth covered with gutta-percha. Biodentine has shown favorable properties in vital pulp therapy, similar to MTA.

Biodentine was shown to be more successful after direct pulp capping of rabbit incisors compared to TheraCal LC in a study by Kayad et al. (2023). It had better results in terms of thickness and continuity of the dentin bridge, while both Biodentine and TheraCal LC had a similar inflammatory effect on the pulp.

Studies examining the dentinogenic potential of materials in which the basic component is calcium aluminate have shown good results. The direct pulp capping results for calcium aluminate obtained by this study can be compared with the results of the author Janković (2018) in which calcium aluminate cements showed a higher ability to form dentinal bridges compared to experimental calcium silicate cements and commercial MTA.

The findings of the authors Josipović (2020) and Radović (2019) also confirmed that the newly synthesized calcium aluminate-based material ALBO-CA was biocompatible, as well as having had the ability to induce calcified tissue after 30 days on sheep teeth after direct pulp coating. The authors attribute this finding to the ALBO-CA synthesis process, which results in higher reactivity of the material particles.

Several studies have considered the use of calcium aluminate cement in the pulpotomy procedure (Kramer et al., 2014; Woodmansey et al., 2015) and apicotomy procedures (Walsh et al., 2018). On that occasion, confirmed was the similar dentinogenic potential of calcium aluminate cement with MTA (Kramer et al., 2014; Walsh et al., 2018), similar effects on inflammation, pulp reaction, periodontal ligament, cement formation, and apical tissue healing in dogs (Walsh et al., 2018). The only exception is in the study by Woodmansey et al. (2015), where inflammation in samples with calcium aluminate cement Quick-Set was somewhat more pronounced compared to MTA, contrary to our findings, which the authors attributed to differences in the chemical composition of the material.

By the limitations of this study, ALBO-CA and Biodentine had similar effects on inflammation, pulp response, and formation of dental bridges in rats. That can be considered a reasonable therapeutic result. One limitation of this study was that pulp capping was performed on healthy, non-carious rat teeth. It is recommended to continue *in vivo* the research of nanostructured calcium aluminate cement on caries lesions on animal and human teeth.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Conception – OJ; Design – OJ, RA; Supervision - TA; Materials – OJ, IĐ; Data Collection and/or Processing – OJ, JK, JL, KV; Analysis and/or Interpretation – OJ, RA, JK, KV, IĐ; Literature Search – OJ, RA, TA, JL, KV; Writing Manuscript – OJ; Critical Review – TA, JL.

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PATOHISTOLOŠKI ODGOVOR ZUBNE PULPE NAKON PREKRIVANJA KALCIJ ALUMINATNIM CEMENTOM I BIODENTINOM: EKSPERIMENTALNO ISTRAŽIVANJE NA GLODARIMA

SAŽETAK

Cilj istraživanja jeste evaluacija odgovora zubne pulpe na prekrivanje eksperimentalnim nanostrukturnim kalcij aluminatnim cementom (ALBO-CA) i biodentinom, trikalcij silikatnim cementom (Septodont, Saint Maur des Fosses, Francuska). Na 72 zuba kod 18 Wistar štakora su pripremljeni kaviteti 1. klase (36 prvih gornjih molara i 36 drugih gornjih molara). Kod 36 zuba, zubna pulpa je prekrivena s eksperimentalnim ALBO-CA (grupa A), a kod 36 zuba sa biodentinom (grupa B). Svi kaviteti su ispunjeni sa staklenim jonomerima. Životinje su nakon 28 dana žrtvovane nakon čega su analizirani upalni odgovor zubne pulpe, prisustvo bakterija i stvaranje dentinskog mosta. Nakon 28 dana nije uočeno prisustvo bakterija. Mann-Whitney U test nije dokazao statistički signifikantnu razliku u upalnom odgovoru zubne pulpe između grupa ($U = 640.00$; $Z = 0.105$; $p = 0.916$). Chi-square test nije dokazao statistički signifikantnu razliku u formiranju dentinskog mosta između ispitivanih grupa (Chi-square = 1.443; p = 0,230). ALBO-CA i biodentine su imali slično djelovanje na upalu, odgovor zubne pulpe i formiranje dentinskih mostova kod štakora.

Ključne riječi: ALBO-CA, prekrivanje zubne pulpe, štakori, trikalcij silikat