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Research article

APPLICATION OF CALCIUM ALUMINATE-BASED MATERIALS FOR DIRECT PULP CAPPING – *IN VIVO STUDY*

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The aim of this paper is the histopathological assessment of the effects of a newly synthesized nanomaterial based on calcium aluminate *ALBO-CA* on the pulp of rat teeth. In 18 Wistar rats, 54 Class I cavities and exposed pulp of maxillary molars were treated with the tested materials: two experimental materials *ALBO-CA* (18 teeth), *ALBO-CS* (18 teeth), and *MTA* control (18 teeth), and cavities were restored with glass ionomer. The histopathological analysis included the following parameters: presence of pulp inflammation, degree of dentin bridge formation, and presence of bacteria in the pulp. Complete absence of pulp inflammation was noted in 12 (66.67 %) teeth with *ALBO-CA*, 10 (55.56 %) teeth with *ALBO-CS,* and 11 (60.95 %) teeth with *MTA* cement. A statistically significant difference in the results of pulp inflammatory response was found only when comparing the presence of a small number of polymorphonuclear leukocytes between *ALBO-CS* and *MTA* cement (Kruskal Wallis H test p=7.8255). A fully formed dentine bridge was recorded only after the application of *ALBO-CA* with a statistically significant difference compared to *ALBO-CS* and *MTA* (F test p=0.519, S-test p=0.656, Man-Whitney test p=2.802, Chi-square test p=4.747). Thirty days after the direct pulp capping with *ALBO-CA*, *ALBO-CS,* and *MTA* cements, bacteria were absent in rat teeth and surrounding tissue. Newly synthesized calcium aluminate *ALBO-CA* showed good reparative abilities and possible use in direct pulp capping therapy.

Keywords: calcium aluminates, calcium silicates, direct pulp capping, nanomaterials.

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INTRODUCTION

A long history of clinical practice attests to treatment of exposed pulp through the therapeutic procedure of direct pulp capping. The treatment of vital pulp has been performed for over 200 years [1]. Direct pulp capping (DPC) is a procedure in which the exposed pulp chamber is covered, with the primary goal of preserving its vitality and stimulating the pulp's defensive response and reparative dentin formation [2].

For a long time, calcium hydroxide held the status of the most commonly used material for direct pulp capping. In an effort to overcome the known shortcomings of this material-such as the occurrence of dystrophic calcifications, the potential for creating a discontinuous dentin bridge with so-called "tunnel defects," marginal leakage, and inconsistent material properties-many researchers of the modern era have begun to intensively explore the application of other materials for DPC, primarily calcium silicate cements [3].

The effectiveness of calcium silicate cements as DPC materials has been demonstrated through experimental and clinical studies, particularly for Mineral Trioxide Aggregate (MTA) [4,5]. However, it is now known that this material also has some negative properties: short working time, long setting time, and a granular consistency of the mixed material, which complicates clinical manipulation and everyday use [5]. Improving these characteristics while maintaining the biocompatibility and biofunctionality of MTA has been a primary goal in recent research on dental materials [4,5].

Calcium aluminate cements have demonstrated good physicochemical properties [6-8], low toxicity, and adequate marginal sealing [9,10]. However, differing findings have also been reported. Some studies noted a greater solubility and water absorption, as well as significantly higher marginal microleakage for calcium aluminates compared to MTA [11,12]. Attempts to use these materials for direct restorative fillings have ended unsuccessfully [13,14]. As endodontic materials, they have been examined in multiple *in vitro* and *in vivo* studies. During these studies, they demonstrated good antimicrobial activity [15] and biocompatibility, showing a comparable or even better tissue response than MTA in subcutaneous implantation in rats [16,17]. A similar effect of calcium aluminates and White Mineral Trioxide Aggregate (WMTA) on inflammation, the formation of newly mineralized apical tissue, and the thickness of the periodontal ligament was observed after filling the root canals of sheep teeth, and their ability to promote full bone recovery after bone defect repair was confirmed [18,19].

The first attempt to use calcium aluminates in pulp treatment was in pulpotomy procedures on rat teeth. In this study confirmed a similar dentinogenic potential to WMTA [20]. To the best of our knowledge, only one study to date has examined the application of calcium aluminate cements for direct pulp capping, underscoring the significance of this research [20]. In a recent study, calcium aluminate cement demonstrated a comparable effect to Biodentine on the inflammatory response of the pulp and dentin bridge formation in rats [21]. The additional value beyond the research

mentioned above, lies in the fact that nanomaterials were examined in this study. The benefit of nanomaterials is their great potential for application in regenerative medicine and tissue engineering, given the size of their particles (<100 nm) [17]. Furthermore, the specific synthesis methods, including hydrothermal synthesis via sol-gel methods and self-propagating wave methods, ensure high particle activity, short setting times, and faster hydration [17].

The aim of this study was to investigate the effect of nanostructured calcium aluminate cement on the exposed pulp of rat teeth. And to compare its effectiveness with two calcium silicate based cements.

MATERIALS AND METHODS

The study was conducted with the approval of the Ethics Committee of the University Clinical Center Banja Luka, number 01-9-192.2/15, Bosnia and Herzegovina. The experiment took place in the vivarium of the Faculty of Natural Sciences and Mathematics at the University of Banja Luka and in the laboratory of the Institute of Pathology at the Clinical Center Banja Luka.

Tested Materials

The experimental nanostructured calcium aluminate-based biomaterial (*ALBO-CA*) whose nanostructure was previously confirmed by XRD analysis (Philips PW 1050) [8], was compared with calcium silicate cement (*ALBO-CS*), while white MTA (*MTA Angelus*®, Tulsa OK, USA) was used as the positive control. The calcium aluminate system ($CaO \bullet Al_2O_3 + CaCO_3 + Bi_2O_3$) i.e. a mixture named *ALBO-CA*, obtained by mixing $CaCO_3$, Bi_2O_3, and $BaSO_4$ with a calcium aluminate phase in a 2:2:1 ratio. To prepare the calcium aluminate endodontic mixture, the individual components, calcium aluminate ($CaO \bullet Al_2O_3$, CA) and calcite ($CaCO_3$), were first synthesized. The mixture was then combined with distilled water in a powder-to-water ratio of 2:1 to achieve the consistency of a cement paste. The second material tested was the calcium silicate *ALBO-CS*, which contains a mixture of dicalcium and tricalcium silicates (60 %) with the addition of 20 % calcium carbonate ($CaCO_3$) and 20 % $BaSO_4$ (Merck, Germany). Both materials were synthesized using the method developed by Jokanović et al., employing a new technology, a combination of the hydrothermal sol-gel method and self-propagating wave synthesis [17].

Study Design

The research procedure was approved by the Local Ethics Committee and conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH Publication No. 8023, revised 1978). All interventions were performed under anesthesia, with minimal pain and distress to the animals. The study involved 18 laboratory Wistar rats, corresponding to 54 teeth (36

first upper molars and 18 second upper molars). The animals were 10–11 weeks old, with an average weight of 265–280 g. During the experiment, the rats were housed in plexiglass cages, one animal per cage, with free access to food and water, a 12-hour light/dark cycle, room temperature of 20–23 °C, and air humidity of 60 % ± 10 %. Before the dental procedure, the rats were anesthetized with general anesthesia (Ketamine Hydrochloride Injection USP Rotexmedica-Germany at a dose of 50 mg/kg body weight). Immediately before the procedure, all teeth were mechanically cleaned of soft deposits with a soft brush and paste, and then disinfected with chlorhexidine digluconate (0.1 % Chlorhexamed-Fluid, GlaxoSmithK, Buhl, Germany). Due to the limited access and visibility of the rat molars, a magnifying glass (4.5×, Zeiss, Oberkochen, Germany) was used. The rats were divided into two experimental groups, with 9 rats in each group:

- In the first group, the open pulp of the first and second upper molars on the right side received calcium aluminate (*ALBO-CA*), while *MTA* was applied to the first upper molar on the left side.
- In the second group, the open pulp of the first and second upper molars on the right side received calcium silicate (*ALBO-CS*), while *MTA* was applied to the first upper molar on the left side.

Class I cavity preparation on the occlusal surface of non-carious first and second upper molars was performed using a technical micromotor and sterile round diamond burs (ISO 008, RENDELL + ZWILLING, Quezon City, Philippines) with continuous water cooling until cavity depth reached approximately half the bur's size. After preparing the cavity in one rat, the bur was replaced. The dental pulp was exposed using a sterile sharp endodontic explorer (DG16, Dental USA, McHenry, IL, USA). Pulpal blood was removed with sterile paper points, and the cavity was rinsed with saline to remove any remaining blood and dentin debris. The tested materials for DPC—*ALBO-CA* (18 teeth), *ALBO-CS* (18 teeth), and *MTA* control (18 teeth)—were applied using an endodontic explorer as previously described. The cavities were definitively restored with glass ionomer cement (GC Fuji VIII, GC Corporation, Tokyo, Japan). The observation period lasted 30 days. Throughout this time, the animals' health status (behavior, changes in skin and fur, food and water consumption, urination, and defecation) was monitored daily. After the observation period, the animals were sacrificed with an intravenous injection of pentobarbital (Pentobarbital sodium salt 100 mg/ml, Sigma-Aldrich Chemie GmbH, Steinheim, Germany). After separating the upper jaws using a surgical scalpel and scissors (decapitation), they were stored in 10 % neutral buffered formalin and delivered to the Laboratory at the Institute of Pathology at the Clinical Center in Banja Luka for preparation for histopathological analysis.

Histopathological Analysis

The preparation of the samples began with the decalcification of the jaw bones in EDTA for 3 weeks, followed by embedding the pulp sections in paraffin blocks.

The sections were cut using a microtome (4 μm per section). The tissue sections were stained using the hematoxylin-eosin method. Qualitative quantification of the microscopic slides was performed by a pathologist, who was not affiliated with the laboratory where the samples were prepared for microscopy, to avoid any subjective influence on the results. The pathologist used a light microscope (Olympus BX-51 microscope, Japan) for this purpose.

The inflammatory response of the pulp was evaluated using a modified criterion by Accorinte et al. (2008): no inflammation (no or a few scattered inflammatory cells), the presence of a small number of polymorphonuclear leukocytes, the presence of a large number of polymorphonuclear leukocytes, and pulp necrosis [22]. Furthermore, the presence of bacteria was analyzed as follows: absence of bacteria, bacteria in 1/3 of the pulp, bacteria in 2/3 of the pulp, and diffuse presence of bacteria throughout the pulp. Dentin bridge formation at the end of the observation period was also considered as one of the possible indicators of treatment success. The dentin bridge was quantified using the modified criteria from Accorinte et al. (2008) as completely formed, incompletely formed, or absent [22].

Statistical Analysis

Parametric (Student's t-test) and non-parametric tests (Fisher's test, Mann-Whitney test, Chi-square test, Kruskal-Wallis H test) were used for statistical analysis of the rat DPC results, depending on data distribution. All p-values <0.05 were considered significant. Data were analyzed using SPSS 20.0 statistical software (IBM Corp, Armonk, NY, USA).

RESULTS

Inflammatory Response of the Pulp

The inflammatory responses of the pulp after a 30-day treatment of rat teeth with dental cements: *ALBO-CA* (calcium aluminate cement), *ALBO-CS* (calcium silicate cement), and *MTA* (mineral trioxide aggregate) were categorized into four categories: necrosis (NK), presence of a large number of polymorphonuclear leukocytes (LNPoliLeu), presence of a small number of polymorphonuclear leukocytes (SNPoliLeu), and absence of inflammation (AI). The number of teeth under each category of inflammatory response is presented numerically (N) and as a percentage in Table 1.

After 30 days of treatment of rat teeth with dental cements, pulp necrosis was recorded in only one tooth (1.85 %) following treatment with *MTA*. No necrosis was observed after treatment with *ALBO-CA* or *ALBO-CS* cements. The presence of a LNPoliLeu was observed in one tooth treated with *ALBO-CA* and one tooth treated with *ALBO-CS*, while *MTA* treatment showed the presence of a LNPoliLeu in two teeth

(11.11 %). A SNPoliLeu was observed in five teeth (27.78 %) after 30 days of treatment with *ALBO-CA*, and in seven teeth (38.89 %) after treatment with *ALBO-CS* cement. In the biocompatible *MTA* cement group, a SNPoliLeu was detected in four teeth (22.44 %). Complete AI at the end of the observation period was confirmed in 10 teeth (55.56 %) treated with *ALBO-CS*, 11 teeth (60.95 %) treated with *MTA*, and 12 teeth (66.67 %) treated with *ALBO-CA* cement (Table 1, Figures 1 and 2).

Table 1. Inflammatory responses of the pulp after a 30-day treatment of rat teeth with dental cements: *ALBO-CA, ALBO-CS*, and *MTA*

			Inflammatory pulp response				
			NK	**LNPoLeu**	**SNPoILeu**	**AI**	**total**
material	**ALBO-CA**	**N**	**0**	**1**	**5**	**12**	**18**
		%	0.00 %	5.55 %	27.78 %	66.67 %	100.0 %
	ALBO-CS	N	0	1	7	10	18
		%	0.00 %	5.55 %	38.89 %	55.56 %	100.0 %
	MTA	N	1	2	4	11	18
		%	5.54 %	11.11 %	22.44 %	60.95 %	100.0 %
total		N	1	4	17	33	54
		%	1.85 %	5.56 %	31.48 %	61.11 %	100.0 %

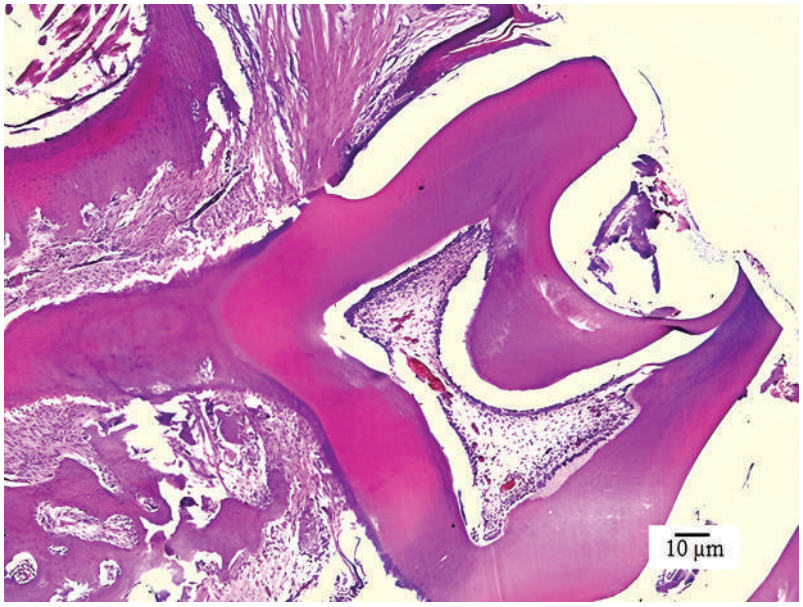

Figure 1. Longitudinal section of a tooth and pulp-absence of inflammation, no inflammatory cells after direct pulp capping with *ALBO-CA*, HE x 200.

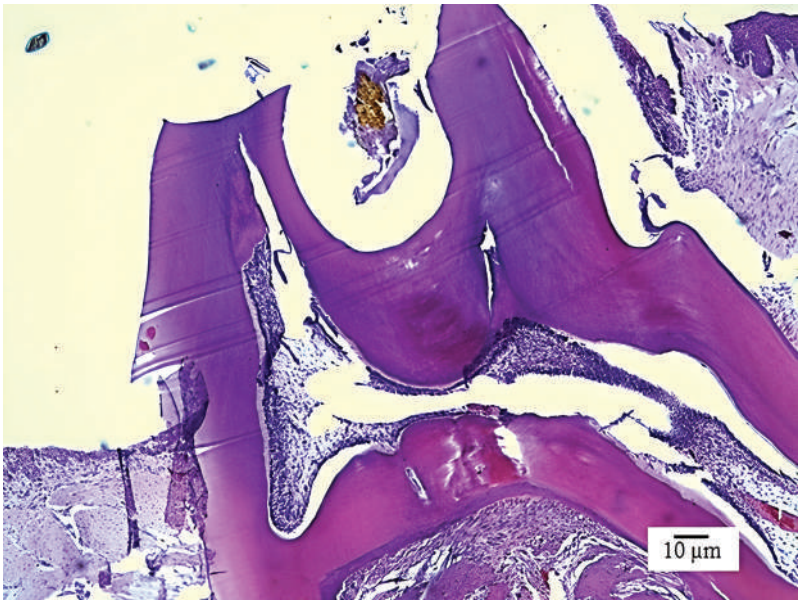

Figure 2. Longitudinal section of a tooth and pulp – absence of inflammation, no inflammatory cells after direct pulp capping with *ALBO-CS*, HE x 200.

The Kruskal-Wallis H test showed a statistically significant difference in the inflammatory response of the pulp only when comparing the presence of a SNPoliLeu between *ALBO-CS* and *MTA* cements (Table 2, Figure 3).

Table 2. Overview of the level of statistical significance of differences in inflammatory responses of the pulp after a 30-day treatment of rat teeth with dental cements: *ALBO-CA*, *ALBO-CS*, and *MTA*.

materials	NK	LNPoLeu	SNPoILeu	AI
ALBO-CA / ALBO-CS	0.0000	0.0000	4.9465	4.8122
ALBO-CA / MTA	1.3225	2.4483	2.4483	3.7415
ALBO-CS / MTA	1.3225	2.4483	7.8255**	3.7415

**significant differences

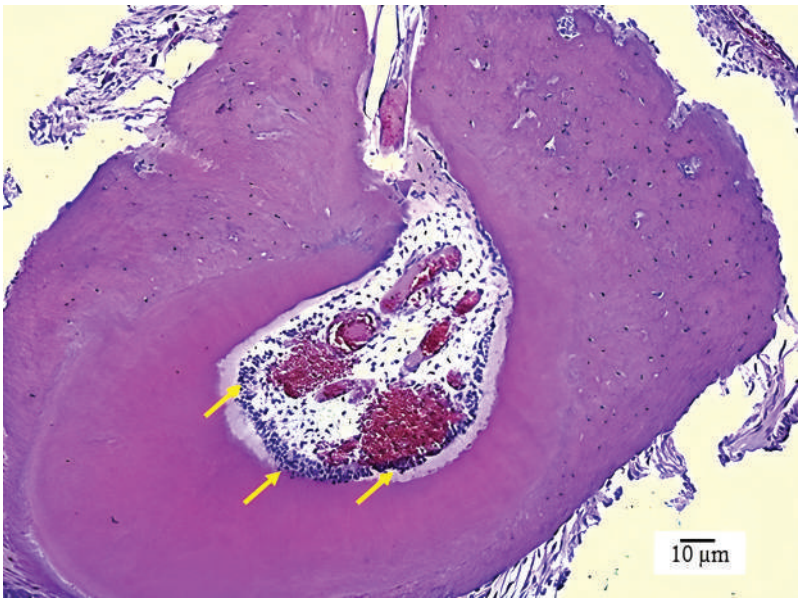

Figure 3. Cross-section of a tooth and pulp-a few scattered inflammatory cells after direct pulp capping with *MTA*, HE x 200.

Presence of Bacteria

Thirty days after direct pulp capping of rat teeth with biocompatible cements *ALBO-CA*, *ALBO-CS*, and *MTA*, no bacteria were present in the teeth or surrounding tissues.

Dentin Bridge Formation

The degree of dentin bridge formation after 30 days of treatment with *ALBO-CA, ALBO-CS*, and *MTA* was categorized into three groups: fully formed dentin bridge (Table 3), partially formed dentin bridge (Table 4), and absence of dentin bridge formation (Table 5).

Table 3. Fully formed dentin bridge after pulp capping with *ALBO-CA, ALBO-CS* and *MTA* materials (n = number of rat teeth)

fully formed dentine bridge (n= number of rats teeth)	**n=9**	**n=0**	**n=0**
significance of differences:	**ALBO-CA / ALBO-CS**	**ALBO-CA / MTA**	**ALBO-CS / MTA**
Fisher's test (p > 0.05)	p=0.519**	p=0.519**	p=0.000
Student's test (p > 0.05)	p=0.656**	p=0.656**	p= 0.000
Man-Whitney (p > 0.05)	p=2.802**	p=2.802**	p= 0.000
Chi-square test (p > 0.05)	p=4.747**	p=4.747**	p= 0.000

** significant differences

Table 4. Partially formed dentin bridge after pulp capping with *ALBO-CA*, *ALBO-CS* and *MTA* materials (n = number of rat teeth)

partially formed dentin bridge (n= number of rats teeth)	n=6	n=11	n=10
significance of differences:	**ALBO-CA / ALBO-CS**	**ALBO-CA / MTA**	**ALBO-CS / MTA**
Fisher's test (p > 0.05)	p=0.609**	p=0.033	p=0.012
Student's test (p > 0.05)	p=0.718**	p=0.048	p=0.018
Man-Whitney (p > 0.05)	p=3.044**	p=1.905**	p=0.032
Chi-square test (p > 0.05)	p=3.125**	p=2.086**	p=0.029
Man-Whitney (p > 0.05)	p=1.916**	p=3.188**	p=0.029
Chi-square test (p > 0.05)	p=2.133**	p=4.095**	p=0.028

**significant differences

Table 5. Absence of dentin bridge formation after pulp capping with *ALBO-CA*, *ALBO-CS* and *MTA* materials (n = number of rat teeth)

absence of dentine bridge formation (n= number of rats teeth)	n=3	n=7	n=8
significance of differences:	**ALBO-CA / ALBO-CS**	**ALBO-CA / MTA**	**ALBO-CS / MTA**
Fisher's test (p > 0.05)	p=0.035	p=0.656**	p=0.011
Student's test (p > 0.05)	p=0.047	p=0.805**	p=0.016
Man-Whitney (p > 0.05)	p=1.916**	p=3.188**	p=0.029
Chi-square test (p > 0.05)	p=2.133**	p=4.095**	p=0.028

** significant differences

A fully formed dentin bridge was recorded only after the application of *ALBO-CA*, showing a statistically significant difference when compared to *ALBO-CS* and *MTA* (F test p=0.519, S-test p=0.656, Mann-Whitney test p=2.802, Chi-square test p=4.747) (Table 3). After 30 days of *ALBO-CA* application, a dentin bridge was not formed in 3 teeth (16.67 %), 7 teeth (38.89 %) with *ALBO-CS*, and 8 teeth (44.45 %) with *MTA*. A significantly lower number of unformed dentin bridges was observed after *ALBO-CA* application compared to *ALBO-CS* (Mann-Whitney p=1.916, Chi-square test p=3.125) and *ALBO-CA* compared to *MTA* (F test p=0.656, S-test p=0.805, Mann-

Whitney test p=3.188, Chi-square test p=4.095).The highest dentinogenic potential was observed with *ALBO-CA*, with dentin bridges formed in 15 teeth (83.33 %), followed by *ALBO-CS* with 11 teeth (61.11 %), and *MTA* with 10 teeth (55.55 %) (Figures 4-6).

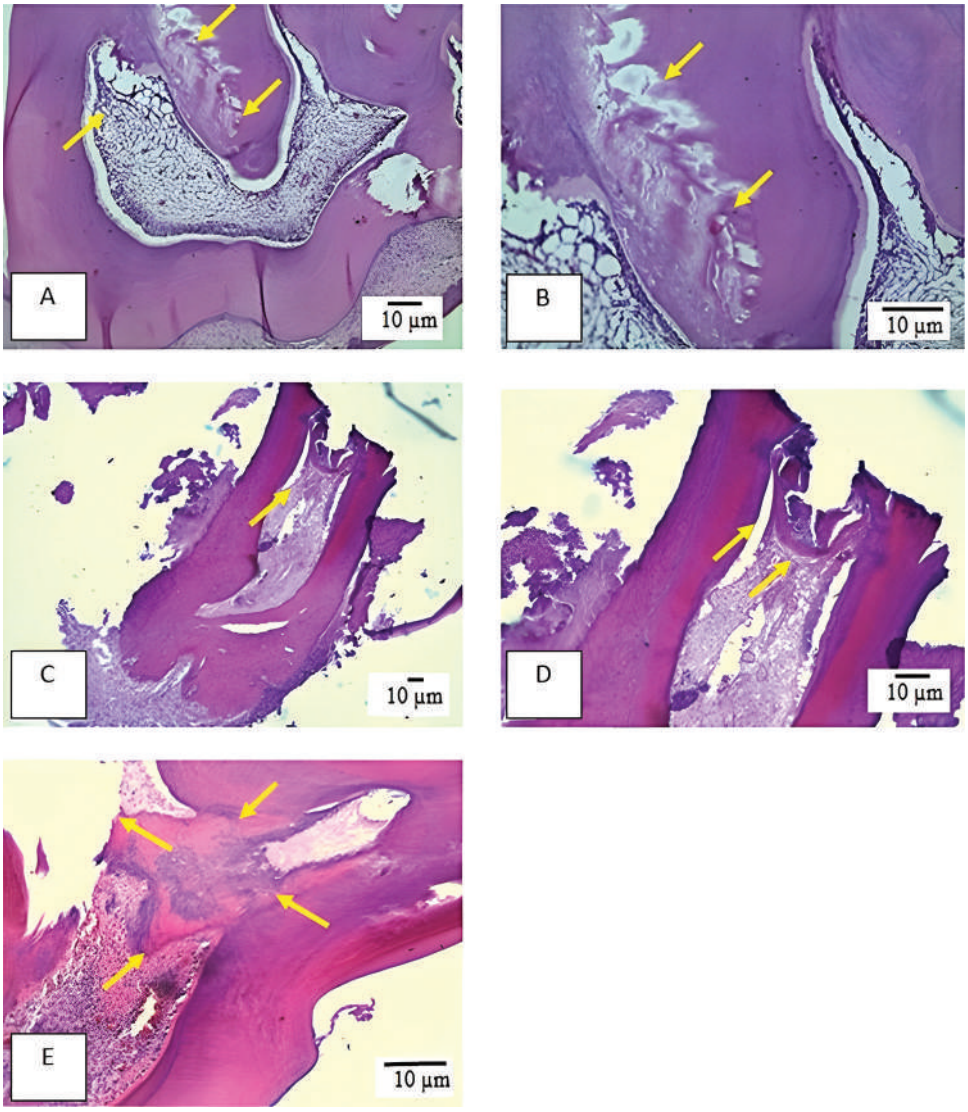

Figure 4. Different stages of dentin bridge formation after DPC with *ALBO-CA*:
A and **B** Cross-section of a tooth with a fibrin matrix at the perforation site and early pulp disintegration, showing the early stage of dentin bridge formation after direct pulp capping with *ALBO-CA,* a) HE x200, b) HE x400. **C** and **D** Longitudinal section of a tooth showing dentin islands in the process of closing the cavity perforation after direct pulp capping with *ALBO-CA*, a) HE x100, b) HE x200. **E** Longitudinal section of a tooth, part of the pulp and a layer of odontoblasts with a fully formed dentin bridge, featuring dentin tubules continuous with the surrounding dentin after direct pulp capping with *ALBO-CA,* HE x400.

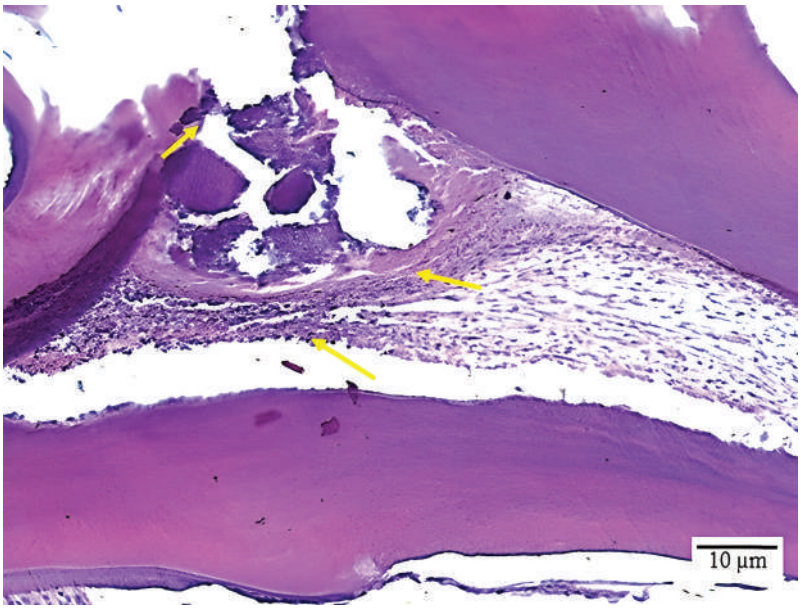

Figure 5. Cross-section of a tooth, showing part of the pulp and dentin with a perforated pulp chamber covered by a partially formed dentin bridge (fibrin and calcified dentin islands) after direct pulp capping with *ALBO-CS,* HE x200.

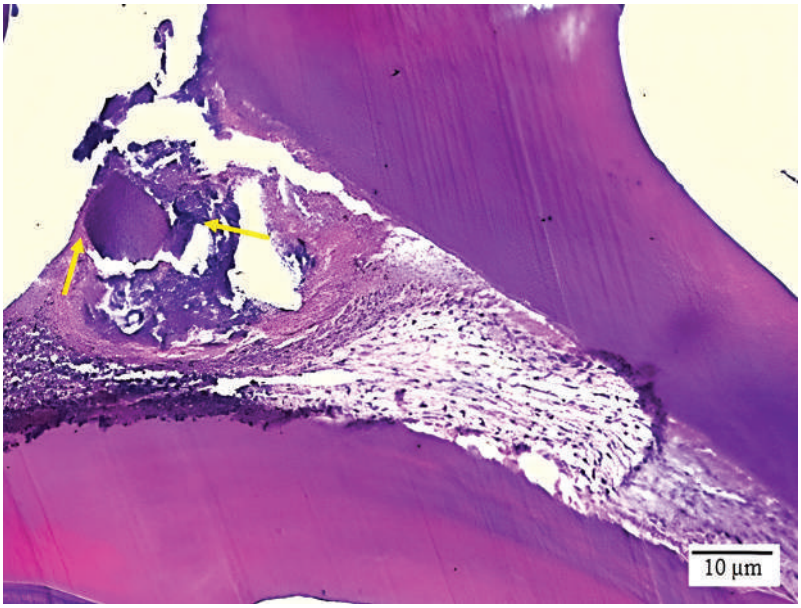

Figure 6. Cross-section of a tooth, showing part of the pulp and dentin with a perforated pulp chamber covered by a partially formed dentin bridge (fibrin and calcified dentin islands) after direct pulp capping with *MTA*, HE x200.

DISCUSSION

The imperative of biocompatible materials in the process of DPC and tooth treatment is to facilitate and promote the natural healing process of pulp damaged in any way. In addition to pulp healing, a crucial prerequisite for a successful therapy is good cavity sealing after tooth cleaning. The most important criterion for assessing the success of the therapy is the absence or degree of inflammatory response of the tooth tissue to the material used for DPC. After a 30-day treatment of rat teeth in our experiment, the three tested dental cements, *ALBO-CA*, *ALBO-CS*, and *MTA*, demonstrated excellent biocompatibility with the treated tissue and a minimal inflammatory response of pulp. *ALBO-CA* and *ALBO-CS* showed no cytotoxic effects on the growth of dental pulp cells, and moreover, they increased cell viability, similar to their predecessor *MTA* [9]. This finding supports the fact that no tissue necrosis was present in teeth treated with *ALBO-CA* and *ALBO-CS* cements, while a very small percentage of teeth showed necrosis after *MTA* treatment. Furthermore, the presence of a LNPoliLeu was observed in one tooth (5.55 %) after DPC with *ALBO-CA* and *ALBO-CS*, and in two teeth after DPC with *MTA*. This result supports the fact that all three cements are biocompatible and enable the pulp healing process. Calcium aluminates, calcium silicates, and *MTA* significantly stimulate the mineralization of the extracellular matrix, which includes the deposition of calcium phosphate crystals in the fibrous extracellular matrix [23].

A statistically significant difference based on the Kruskal-Wallis H test was found only in the greater presence of a SNPoliLeu with *ALBO-CS* compared to *MTA* cement. Although the number of teeth with a SNPoliLeu was higher with *ALBO-CS* compared to *MTA* and *ALBO-CA* biocompatible materials, *ALBO-CS* represents a promising alternative to *MTA* in pulp-dentin complex regeneration, dentin bridge formation, and surrounding tissue regeneration [24]. The parameter of the presence of a LNPoliLeu was slightly higher with *MTA* compared to *ALBO-CA* and *ALBO-CS* cements, though it generally showed very low values for all three cements. This parameter indicated the absence of major inflammatory processes in tooth tissue after their 30-day treatment. Calcium aluminate-based biocompatible materials demonstrate a high degree of maintaining a sterile environment for optimal tissue regeneration [17], as observed with both *ALBO-CS* and *MTA* in this study. The absence of inflammation in the teeth after 30-day treatment was most evident with *ALBO-CA* cement, where two-thirds of the treated teeth exhibited no inflammatory processes. This was followed by *MTA* at 60.95 %, and finally, *ALBO-CS*, with the lowest absence of inflammation in approximately 55.56 % of teeth. Generally speaking, this is a very high percentage of inflammation-free tooth tissues for all three materials after direct pulp capping in rats. Proper sealing with DPC materials prevents the entry of potential bacteria from the oral cavity and the formation of inflammatory processes in the pulp [21,25].

Thirty days after DPC of the experimental rats' teeth with biocompatible materials *ALBO-CA*, *ALBO-CS*, and *MTA*, no bacteria were detected in any of the analyzed

teeth (n=54) or in their surrounding tissue. Standard microbiological analyses confirmed the complete absence of any bacterial cells. The presence of a SNPoliLeu in the tissues is a result of tissue adaptation to the biocompatible material, not a response to bacterial infection.

This finding of complete absence of bacterial cells in the tooth tissue was also noted after direct pulp capping with *ALBO-CA* and Biodentine 28 days after application in rat teeth [21]. *ALBO-CA* has a good ability to maintain a sterile environment in the contact zone with the pulp. The shorter setting time and associated microstructure of *ALBO-CA* compared to *MTA* [15,17] are cited as reasons for this. In the contact zone with the pulp, *ALBO-CA* creates compounds with lower calcium content, which are therefore stronger and less prone to bacterial colonization [21]. On the other hand the advantage of the *ALBO-CS* material is that, in contact with moisture, *ALBO-CS* exhibits less unwanted expansion than *MTA* [24], and thus a lower likelihood of bacterial colonization. Indirectly the capacity of DPC materials to seal is influenced by the axial forces during tooth function [26]. *ALBO-CA* and *ALBO-CS* resist these forces for a longer period compared to *MTA*. Similarly, *ALBO-CA* and *ALBO-CS* are biocompatible materials with stronger bond strength between the cement and the tooth wall than *MTA*. The greater bond strength is due to the smaller particle size of *ALBO-CA* and *ALBO-CS* compared to *MTA*, which contributes to enhanced impregnation and the creation of stronger layers in the dentinal tubules [8,26]. Bond strength can be justified by the strength of material interactions with dentin, which primarily depends on the availability of ions for bonding the structural units of the biocompatible material [26,27]. Good bond strength with the tooth results in lower microleakage, and combined with aseptic working conditions, this supports the fact that the biocompatible materials used in our experiment adhered well to tooth tissue and prevented the creation of a bacterial-friendly environment. Previous research confirms that calcium aluminate cements have antimicrobial effects comparable to, or even superior to, *MTA,* particularly against bacteria like *E. coli* [6,15,28].

Biocompatible materials *ALBO-CA* and *ALBO-CS* facilitate the migration of stem cells to the site of the future dentin bridge and their differentiation [21,24]. These materials also influence the maturation, differentiation and mineralization of developing odontoblasts [21,24]. This fact is supported by the results of this study, which show a higher percentage of dentin bridge formation after 30 days of treatment with dental cements: *ALBO-CA*, *ALBO-CS*, and *MTA*. The results of this study unequivocally indicate that the tested cements *ALBO-CA*, *ALBO-CS*, and *MTA* generally lead to initiation of dentin bridge formation, with *ALBO-CA* resulting in a complete formation within 30 days, while the other materials required a longer period. The best biocompatibility in forming dentin bridges in treated animals was demonstrated by *ALBO-CA*. *ALBO-CS* showed intermediate results, performing slightly better than *MTA*, although the difference was not statistically significant, meaning the process of osteogenesis and dentin bridge formation for these cements took longer than for *ALBO-CA*. Similar results in the ability to form dentin bridges between *ALBO-CS* and

MTA are understandable and expected, as both materials contain high percentages of dicalcium and tricalcium silicate in their composition.

The significance of a stable chemical structure and the insolubility of biocompatible cement in bodily fluids is very important. This material stability is crucial because time is needed for dentin bridge formation. The solubility of *ALBO-CA* material is higher than *MTA* in the first few hours and in the initial period after application, while later the solubility of *ALBO-CA* material becomes lower than *MTA* [9,11,15,17]. Besides stability the process of dentin-pulp complex repair and regeneration is significant for forming a new dentin bridge. The growth and maturation of dental pulp cells during odontogenesis are facilitated by the composition of the cement used for DPC. Great attention must always be given to the free calcium released from biocompatible materials, which is incorporated into odontogenic cells during dentin bridge formation [29]. In this study, the better formation of the dentin bridge with *ALBO-CA* and *ALBO-CS* materials was partly due to calcium binding in odontogenic cells.

The results of DPC for *ALBO-CA*, with the exception of one study, cannot be compared with those of other researchers, as these are new materials still in preclinical and *in vivo* animal experiments. The only publication addressing DPC was the study by Janković and colleagues, where *ALBO-CA* was compared with tricalcium silicate cement Biodentine in the therapeutic procedure of pulp capping in 72 teeth (36 first upper molars and 36 second upper molars) of 18 Wistar rats. *ALBO-CA* and Biodentine had similar effects on inflammation, pulp response, and dentin bridge formation in rats. After 28 days, no bacteria were present, and there was no significant difference between materials in terms of inflammatory response and dentin bridge formation.

Calcium aluminates have generally shown good results when used in pulpotomy procedures. Kramer and colleagues confirmed the similar dentinogenic potential of calcium aluminate cement (Quick-Set) and calcium silicate cements (ProRoot MTA and MTA Plus) in a study on Sprague-Dawley rats. After 30 and 60 days, dentin bridge formation was observed with all three cements, and all teeth maintained vitality [20].

In a study on dogs the effect of Quick-Set and white ProRoot MTA materials was examined after pulpotomy. The results showed no statistically significant difference in reparative dentin formation and dentinogenesis quality between the two materials after 70 days. Inflammation was somewhat more pronounced in samples with Quick-Set compared to MTA, contrary to our findings. The authors attributed this to differences in the chemical composition of the materials [30].

Walsh et al. compared the healing of pulp and periapical tissues in dogs after exposure to NeoMTA Plus and Quick-Set2 in pulpotomy and apicoectomy procedures. After 90 days, Quick-Set2 and NeoMTA Plus showed similar effects on inflammation, pulp response, periodontal ligament, cement formation, and apical tissue healing in dogs. The only significant difference was in the quality of dentin bridge formation after

pulpotomies, with NeoMTA Plus showing a slightly superior dentin bridge quality compared to Quick-Set2, which contrasts with the results obtained in this study [31].

The biocompatibility results for *ALBO-CS* obtained in this study correspond to the findings of Popović Bajić et al. [23]. In their study, histological analysis of tooth pulp in Vietnamese pigs after 28 days of DPC showed favorable therapeutic effects of *ALBO-CS* and *ALBO-CSHA* compared to *MTA*. All teeth showed dentin bridges (experimental and control groups) and mild pulp inflammation. No necrosis or presence of Gram-positive bacteria in the pulp was observed in any case. The authors attributed this finding to the special sol-gel synthesis method, which favored the bioactivity of the tested *ALBO-CS* [24].

An *in vivo* study by Opačić Galić et al. on a rabbit model also confirmed favorable biological response of pulp tissue to the newly synthesized nanostructured biomaterial *ALBO-CS*, noting mild to moderate inflammatory reactions only in close proximity to the implanted material, with complete absence of bacteria [32].

One limitation of this study was that pulp capping was performed on healthy, noncarious rat teeth. One of the limitations is also the duration of the treatment period for the animals; real results will only be available over a longer period of time when end users wear the nanostructured ALBO-CA.

CONCLUSION

Within limitations of this study the nanostructured calcium aluminate-based biomaterial *ALBO-CA* demonstrated good reparative capabilities and achieved the status of a potential material for use in vital pulp treatment. Today, optimism is growing regarding the use of biological cements *ALBO-CA* and *ALBO-CS* in pulp treatment through the stimulation and generation of biological tissue. It is certainly recommended that the obtained results be verified in future experimental and clinical studies.

Authors' contributions

OJ designed the study, collected the samples, interpreted the data, and wrote the manuscript. SP performed and interpreted the statistical analyses and contributed in the writing of the manuscript. LJTL performed the laboratory and histopathological analyses. TA, RA, KV, VP interpreted the data and contributed in the writing of the manuscript. IĐ, SM, MB participated in the practical part of the experiment on rats. VJ synthesized the material used in the study. All authors have read and approved the manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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PRIMENA MATERIJALA NA BAZI KALCIJUM ALUMINATA ZA DIREKTNO PREKRIVANJE PULPE - *IN VIVO* STUDIJA

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Cilj ovog rada je patohistološka procena efekta novosintetisanog nanomaterijala na bazi kalcijum aluminata *ALBO-CA* na pulpu zuba pacova. Kod 18 pacova Wistar soja na 54 kaviteta I klase i eksponiranu pulpu maksilarnih molara aplikovani su ispitivani materijali: dva eksperimentalna materijala *ALBO-CA* (18 zuba), *ALBO-CS* (18 zuba) i *MTA* kontrola (18 zuba), a kaviteti restaurisani glasjonomercementom. Patohistološka analiza je uključivala parametre: prisustvo upale pulpe, stepen formiranja dentinskog mosta i prisustvo bakterija u pulpi. Potpuno odsustvo upale pulpe je konstatovano kod 12 (66,67 %) zuba sa *ALBO-CA,* 10 (55,56 %) zuba sa *ALBO-CS* i 11 (60,95 %) zuba sa MTA cementom. Statistički značajna razlika u rezultatima inflamatornog odgovora pulpe utvrđena je samo pri poređenju prisustva malog broja polimorfonuklearnih leukocita između *ALBO-CS* i MTA cementa (Kruskal Wallis H test p=7,8255). Potpuno formiran dentinski most je zabeležen samo nakon primene *ALBO-CA* uz statistički značajnu razliku u poređenju sa *ALBO-CS* i *MTA* (F test p=0,519, S-test p=0,656,

Man-Whitney test p=2,802, Chi-square test p=4,747). Trideset dana nakon direktnog prekrivanja pulpe *ALBO-CA, ALBO-CS* i MTA cementima bakterije nisu bile prisutne u zubima pacova i okolnom tkivu. Novosintetisani kalcijum-aluminat *ALBO-CA* je pokazao dobre reparatorne sposobnosti i mogućnost korišćenja u terapiji direktnog prekrivanja pulpe.