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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Biocompatibility of nanostructured biomaterials based on calcium aluminate

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SUMMARY

Introduction/Objective The aim of this paper was to verify the biocompatibility of the newly synthesized nanostructured material based on calcium aluminate after implantation into the subcutaneous tissue of rats.

Methods The study included 18 rats aged 10–11 weeks, divided into two experimental groups (n = 9). In all animals, incision took place on the back and two pockets of 15 mm in depth were made, in which sterile polyethylene tubes with test materials [calcium aluminate cement (*ALBO*-CA), calcium silicate cement with the addition of hydroxyapatite (*ALBO*-CSHA), and mineral trioxide aggregate (MTA) for the control group) were implanted. Six rats of each group were sacrificed in three observational periods (seven, 15, 30 days). Pathohistological analysis included inflammation, bleeding, fibrous capsule, and tissue integrity around the implanted material.

Results After seven days of treatment, *ALBO*-CA and *ALBO*-CSHA showed better tissue response compared to MTA, with a statistically significant difference in inflammation intensity (p = 0.2781). The difference in vascular congestion and thickness of the fibrous capsule after implantation of *ALBO*-CA material compared to MTA was also statistically significant (p = 0.5567). At the end of the 30-day evaluation period, an identical inflammatory response of connective tissue at the site of implanting *ALBO*-CA, *ALBO*-CSHA, and MTA (score of 0.7) was recorded. The formation of thick or moderately thick fibrous capsule was found to be the thickest in *ALBO*-CA (grade 3.7). There were no statistically significant differences between the parameters analyzed after 30 days.

Conclusion Newly synthesized *ALBO*-CA showed a satisfactory tissue response and confirmed biocompatibility after implantation in subcutaneous tissue of rats.

Keywords: nanomaterials; calcium aluminates; calcium silicates; tissue reaction

INTRODUCTION

In recent years, tendencies of scientific research focused on the discovery of new biomaterials that would overcome the shortcomings of the existing ones through changes in composition and improved characteristics. In contact with tissue, biomaterials must not exhibit potential cytotoxic, genotoxic, mutagenic, or allergic reactions [1].

Biocompatibility tests are carried out *in vitro* and *in vivo* conditions and include testing of materials in laboratory conditions on cell tissues or organ cultures, animal experiments, and clinical tests on humans. The efficiency of animal tests is more important than *in vitro* research. These tests are carried out by using subcutaneous implantation, allergy tests, and tests of acute and chronic systemic toxicity of dental materials [2].

Calcium aluminate cements (CAC) were developed at the beginning of the 20th century as chemically durable materials from Portland cement. Their hydraulic calcium phase contains 30–50% of aluminum, while in Portland cement, aluminum is less than 5% [3, 4, 5].

More thorough studies of the potential biomedical application of calcium aluminate cements have been carried out a decade ago.

Attempts of calcium aluminates use in dentistry for direct restorative fillings on lateral teeth (DoxaDent; Doxa AB, Uppsala, Sweden) (Ceramir C&B, Doxa AB), during a three-year clinical monitoring did not indicate clinical success, due to inferior mechanical properties and handling difficulties [6, 7].

Calcium aluminate in addition to X-ray contrast agent, mixed with water or 0.9% saline solution, resulted in an endodontic cement of similar indications as commercially available tricalcium silicate cements (EndoBinder, Binderweare, Sao Carlos, SP, Brazil). EndoBinder was developed with the intention of preserving the positive properties and clinical application

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of mineral trioxide aggregate (MTA), but without its negative properties. According to the results of several recent studies, EndoBinder showed appropriate biological and physico-chemical characteristics, good antimicrobial activity against *Enterococus fecalis*, *Staphylococcus aureus*, and *Candide albicans*, and after the implantation into subcutaneous tissue of rats caused a minor inflammatory reaction compared to MTA or an inflammatory reaction similar to MTA [8–11]. During the repair of bone defects, EndoBinder showed similar behavior as MTA, and stimulated complete bone recovery after three months [12].

Quick-set (Primus Consulting, Bradenton, FL, USA), calcium aluminate cement, confirmed the dentinogenic potential as White Mineral Trioxide Aggregate (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) in *in vitro* conditions on the MDPC-23 odontoblast cell line and *in vivo* conditions in pulpotomy [13, 14].

Attempts of combining calcium aluminate cements (CAC) with collagen, zirconium, zinc oxide, tricalcium phosphate, and hydroxyapatite have shown great potential for application in odontology and orthopedics. Compared with commercial products for implementation in dentistry (MTA, glass ionomer cement Vidrion; SSWhite Duflex, São Cristovão, RJ, Brasil) and orthopedics (polymethyl methacrylate), CAC mixtures showed greater alkaline phosphatase activity, higher cellular viability of osteoblast cells, and formation apatite on the surfaces of all CAC samples of homogeneous layer [15].

The combination of tricalcium aluminates ($Ca_3Al_2O_6$) with tricalcium silicates (Ca_3SiO_5) resulted in better physico-chemical properties (accelerated the hydration process, reduced the setting time and improved the compressive strength), higher bioactivity and biocompatibility (stimulating effect on L929 cell growth) compared to pure tricalcium silicates (Ca_3SiO_5) [16].

Despite the fact that in the last decade the emphasis has been placed on research of calcium aluminate cements, wider use of these materials in dental practice requires additional testing of a recent date.

Research on new calcium aluminates with similar physical and biological properties as well as MTA, but without its negative characteristics are still current.

Simultaneously, there is a growing influence of nanotechnology and nanomaterials in medicine.

The objective of this study was to investigate the biocompatibility of the newly synthesized nanostructured material based on calcium aluminate after implantation into the subcutaneous tissue of rats.

METHODS

The research was conducted after the approval of the Ethics Committee of the University Clinical Center in Banja Luka, No. 01-9-192.2/15, Bosnia and Herzegovina, and an experiment was realized at the Vivarium of the Faculty of Natural Sciences and Mathematics, University of Banja Luka, and in the Laboratory of the Institute of Pathology, Banja Luka Clinical Center.

Tested materials

The calcium aluminate-based nanomaterial system (*ALBO*-CA) was compared to the hydroxyapatite and calcium silicate-based material (*ALBO*-CSHA). White Mineral Trioxide Aggregate (MTA Angelus® Tulsa OK, USA) was used as a positive control.

The calcium aluminate system ($CaO \bullet Al_2O_3 + CaCO_3 + Bi_2O_3$) is a mixture called *ALBO*-MCCA, obtained by mixing $CaCO_3$ and Bi_2O_3, and $BaSO_4$ with a calcium aluminate phase in the ratio of 2:2:1. In order to obtain calcium aluminate endodontic mixtures, it was first necessary to synthesize particular components of the mixture: calcium aluminate ($CaO \bullet Al_2O_3$, CA) and calcite ($CaCO_3$). The mixture was finally mixed with distilled water in the 2:1 ratio of powder to water, according to the recommended protocol.

Another test material was calcium silicate with the addition of hydroxyapatite. Both of the materials were synthesized according to the method of Professor Jokanović and his associate using new technology, a combination of hydrothermal sol-gel method and the method of self-combusting waves [17].

Design of the study

The animal model comprised rats of the Wistar strain (18 rats, aged 10–11 weeks, with the average weight of 190–280 g). During the experiment, the rats had free access to food and water, 12-hour shifts of light and dark, air temperature ranged 20–23°C, and humidity was 60% ± 10%. The rats were divided into two experimental groups (n = 9). Six rats (three from each group) were sacrificed after seven, 15 and 30 days. Subcutaneous implantation of polyethylene tubes (length 10 mm, inner diameter 1 mm) was conducted, up to half filled with tested materials (*ALBO*-CA in group I, *ALBO*-CSHA in group II) and half empty. The empty half of the tube was the negative control. Two tubes were placed on the back of each rat, on the right side of the tube with the tested material, and on the left side of the spinal column of the tube with the MTA (positive control). The tubes were oriented so that the material was always turned toward the head, and the empty part of the tube to the tail.

Before the surgical procedure, the rats were put under general anesthesia (ketamine, 90 mg / kg of body weight, Ketamine Hydrochloride Injection USP, Rotexmedica GmbH Arzneimittelwerk, Trittau, Germany, in combination with xylazine, 5 mg / kg of body weight, 2% Xylazine, Cp Pharma, Bergdorf, Germany).

Preparation of the operational field was carried out, and then a blunt dissection, right and left of the spinal column, two pockets about 15 mm deep were formed. Sterile polyethylene tubes, previously filled with freshly mixed test materials, were placed in the pockets in this way. After that, the wound was sewed. The individual knotted seam was applied.

After the operation, the animals were placed in one cage in the controlled environment, with controlled diet and daily professional care.

Animal health control was carried out three times a day. Six animals were sacrificed in each observation period (seven, 15, 30 days). For this purpose, intravenous injection of pentobarbital (pentobarbital sodium salt, 100 mg/ml, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was used.

Prepared subcutaneous tissue, together with tubes, was immersed in 10% formalin and delivered to laboratories for histological analysis.

For histological analysis, four samples were taken from each animal. After that, tissue clip fixation together with polyethylene tubes was performed in 10% buffered formalin, followed by paraffin molding and paraffinic dyeing by hematoxylin and eosin (4 μm thickness slices). The analysis of the preparation was done on a light microscope (Olympus BX-51, Olympus Corporation, Tokio, Japan) by an experienced pathologist, who did not participate in the sampling of the material.

Histological analysis of the prepared samples was performed qualitatively and semi-quantitatively, and the inflammatory reaction, vascular congestion, fibrous capsule, and the preservation of the integrity of the connective tissue were considered.

Statistical analysis

Dunn's post-hoc test was applied for the significance of differences between pairs with a significance level of = 0.05 for statistical analysis of the obtained subcutaneous implantation results. Statistical analysis was done in the Minitab 16 software package (Minitab INC, State College, PA, USA).

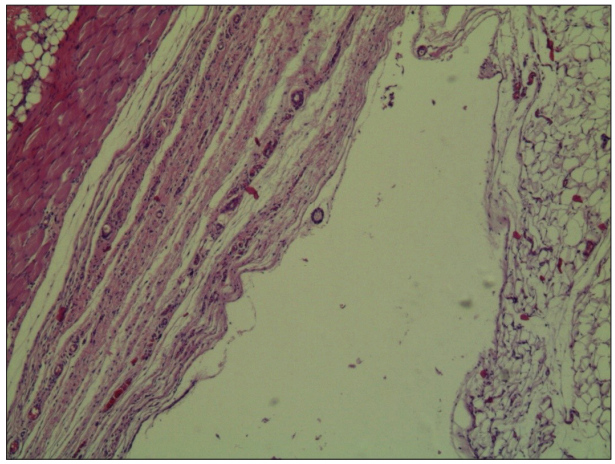

Figure 1. Calcium aluminate cement (ALBO-CA) after the experimental period of seven days; there is a defect (from the tube) with a very thin capsule and moderate vascular congestion (H&E, × 100)

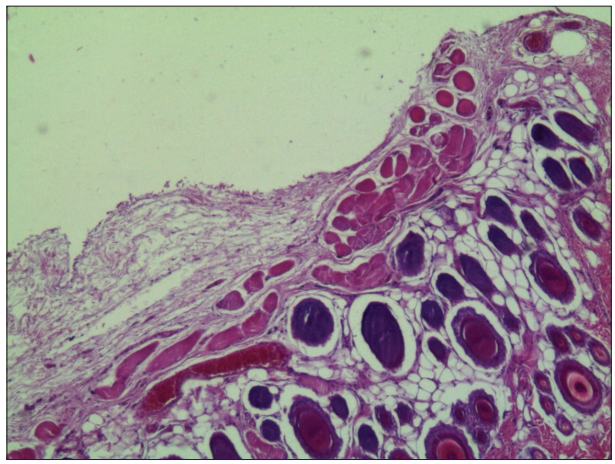

Figure 2. Calcium silicate cement with the addition of hydroxyapatite (ALBO-CSHA) after the experimental period of seven days; the fibrous capsule is not present (H&E, × 200)

RESULTS

Results of the histological analysis are shown in Table 1 and Figures 1–7.

After a seven-day histopathological analysis, a moderate inflammatory reaction was recorded (grade 3) with calcium aluminate *ALBO*-CA and calcium silicate hydroxyapatite *ALBO*-CSHA, while inflammatory reaction with MTA was moderate (grade 3) in three cases, and an intensive inflammatory reaction (grade 4) was noted in three cases, with a statistically significant difference (p = 0.2781). Moderate inflammation was traced by moderate vascular congestion with *ALBO*-CA (grade 3) and *ALBO*-CSHA material. After MTA implantation, equally moderate distribution (grade 3) and pronounced vascular congestion were observed (grade 4). In all tested

Table 1. Analyzes of inflammation, bleeding, fibrous capsules and integrity of binders of tested materials by observation periodicity

Parameters		7 days	15 days	30 days
Inflammatory reaction	ALBO-CA	3 ± 1.5	2 ± 1	0.666 ± 0.5
	ALBO-CSHA	3 ± 1.5	2 ± 1	0.666 ± 0.5
	MTA	3 ± 1.414	2 ± 1	1 ± 0.5
Vascular congestion	ALBO-CA	3 ± 1.2777	2 ± 0.8888	0.6666 ± 0.5211
	ALBO-CSHA	3.333 ± 0.5773	2.6666 ± 0.5773	0.6666 ± 0.57735
	MTA	3.6667 ± 0.5773	2.6666 ± 0.5773	1 ± 0.0
Fibrous capsule	ALBO-CA	1 ± 0.2778	2.3333 ± 1.156	4 ± 1.8888
	ALBO-CSHA	0.6666 ± 0.5774	2.3333 ± 0.5775	3.3333 ± 0.57735
	MTA	0.3333 ± 0.57735	2 ± 0.0	3.3333 ± 0.57735
Preserving the integrity of the connective tissue	ALBO-CA	3 ± 0.0	2 ± 0.0	1 ± 0.4444
	ALBO-CSHA	3 ± 0.0	2 ± 0.0	0.6666 ± 0.57735
	MTA	3 ± 0.0	2 ± 0.0	1 ± 0.0

ALBO-CA – calcium aluminate cement; ALBO-CSHA – calcium silicate cement with the addition of hydroxyapatite; MTA – mineral trioxide aggregate

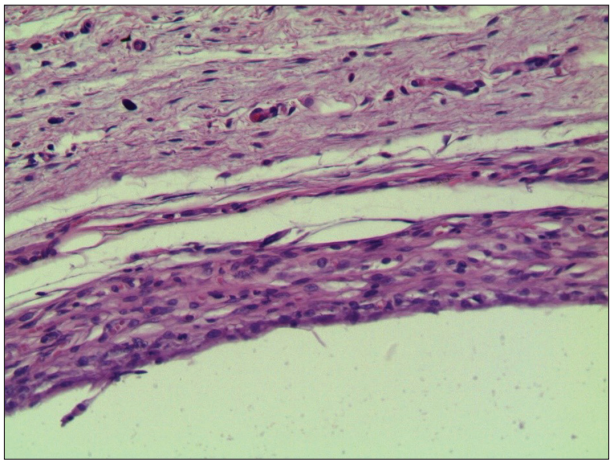

Figure 3. Calcium aluminate cement (ALBO-CA) after the experimental period of 15 days; there is a moderately thick capsule and mild vascular congestion and mild inflammation (H&E, × 400)

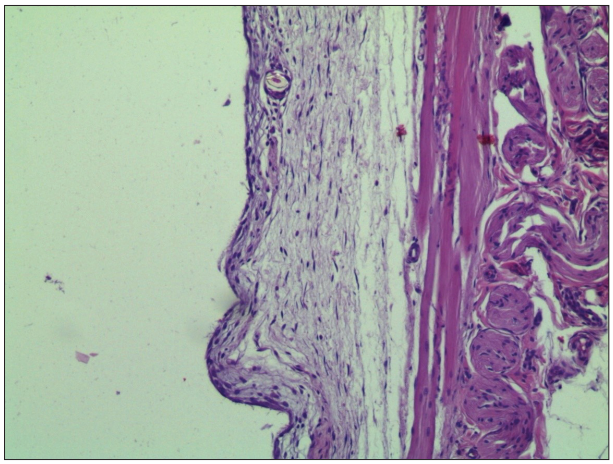

Figure 4. Calcium silicate cement with the addition of hydroxyapatite (ALBO-CSHA) after the experimental period of 15 days; there is a thin capsule and a mild inflammation reaction; lymphocytes and rare plasmocytes are present, which speaks in favor of a chronic inflammatory reaction (H&E, × 200)

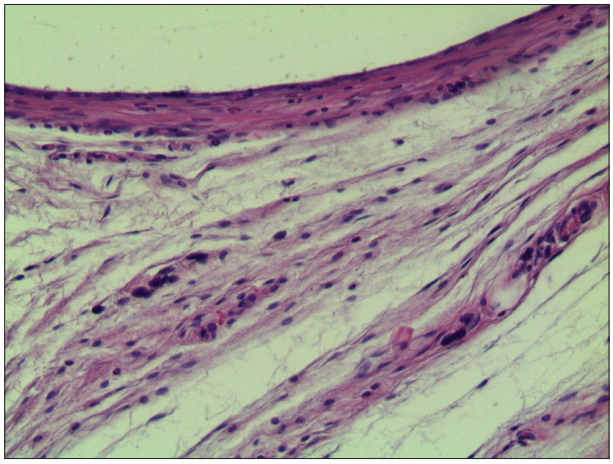

Figure 5. Calcium aluminate cement (ALBO-CA) after the experimental period of 30 days; the microphotography reveals a thick capsule and mild vascular congestion and mild inflammation (H&E, × 400)

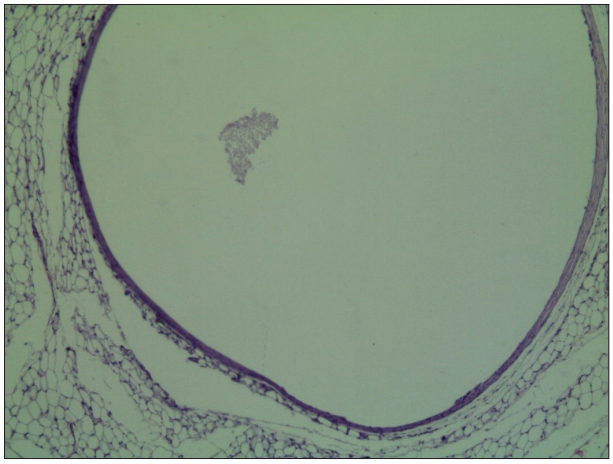

Figure 6. Calcium silicate cement with the addition of hydroxyapatite (ALBO-CSHA) after the experimental period of 30 days; implantation in the subcutaneous fat tissue is encircled by a thick capsule without inflammatory response and congestion (H&E, × 200)

materials, a moderate distortion of the connective tissue structure was registered, while the fibrous capsule after the implantation of *ALBO*-CA material was minimal (grade 1), i.e. minimal (grade 1) or absent (grade 0) after the implantation of *ALBO*-CSHA and MTA (Figures 1 and 2). The difference in vascular congestion and the thickness of the fibrous capsule after implantation of *ALBO*-CA material relative to MTA was statistically significant ($p = 0.5567$).

In the control preparations, seven days after subcutaneous implantation, the presence of moderate intensity inflammatory reaction and one case of pronounced inflammatory reaction was noted in the control of the *ALBO*-CA material and the MTA control. Blood vessels showed signs of moderate vascular congestion, which was more expressed in the control group of MTA. In all control preparations, there was a moderate disturbance of connective tissue structure, except in one case of the MTA control group with a slightly disrupted connective tissue structure (grade 2). The fibrous capsule was absent

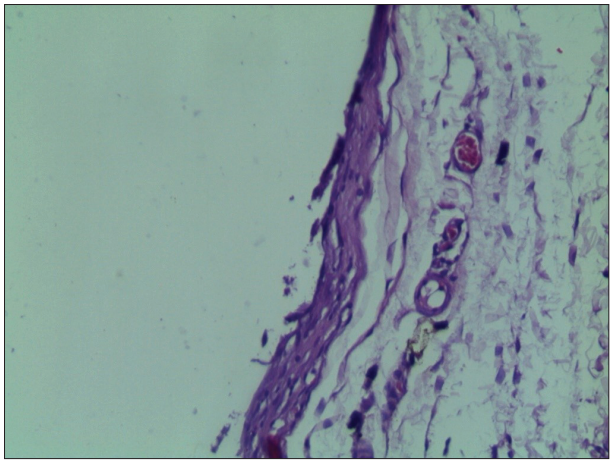

Figure 7. Negative control (empty tube) after the experimental period of 30 days; the microphotography reveals a thick capsule and a mild vascular congestion and mild inflammation (H&E, × 400)

in all control preparations of *ALBO*-CA, and in most of the preparations of the MTA control group, except in one case. Material control of *ALBO*-CSHA recorded a gentle thin capsule in two cases (grade 1), and complete absence of the capsule in one sample (grade 0).

A pathohistological analysis after 15 days at the site of implantation of all examined materials showed a slight inflammatory reaction thin or moderately thick fibrous capsule (Figures 3 and 4), and a mild disturbance of the connective tissue structure (grade 2). Vascular congestion is characterized as mild (grade 2) with the *ALBO*-CA material, or as moderate or mild to *ALBO*-CSHA and MTA, with a statistically significant difference ($p = 0.2974$).

In control preparations after 15 days, the weakest inflammatory response was recorded in the *ALBO*-CSHA control (grade 2), while control preparations for *ALBO*-CA were rated with mark 3 (moderate inflammatory response), and for MTA the score was 2.3 (mild to moderate inflammatory reaction). In all control preparations, a slight disturbance of the structure of the loose connective tissue (grade 2) was observed, followed by the formation of a thin fibrous capsule (grade 2). Vascular congestion was equally expressed in control group of *ALBO*-CSHA and MTA (grade 2.3), while in the *ALBO*-CA control, the score was rated 2.6.

At the end of the evaluation period of 30 days, an identical inflammatory response of loose connective tissue at the site of implantation *ALBO*-CA, *ALBO*-CSHA, and MTA (score of 0.7) was recorded. The formation of thick or moderately thick fibrous capsules (Figures 5 and 6) was established, which was the thickest with *ALBO*-CA (grade 3.7). There were no statistically significant differences between the analyzed parameters.

In control preparations after 30 days, a weaker inflammatory response, lower vascular congestion, and better integrity of the binders were observed in control samples of *ALBO*-CSHA and MTA (grade 0.7), compared with *ALBO*-CA control (grade 1). A moderately thick or thick fibrous capsule was formed (Figure 7), thicker in the control of the MTA material (grade 3.2), while the *ALBO*-CA and *ALBO*-CSHA controls were rated 3.

DISCUSSION

Animal testing is a common method of checking new materials before their clinical examination.

There are almost no species and subspecies in the animal world that have not been used for the purposes of scientific research. However, when it comes to dental research, preference is given to rats. Although there are now hundreds of pureblooded and about fifty crossed rat strains, the strains most commonly used in dental experiments are from Wistar and Sprague–Dawley strains. The advantage in dental research for these experimental animals is primarily the similarity between their and human molars. In addition, rats cannot vomit and are therefore frequently used as a starting model for numerous toxicity and carcinogenicity tests of dental materials. Of course, the advantage is their reasonable price and the possibility of simple securing. Like mice, rats have good reproductive skills and are well-grown in laboratory conditions [18].

Subcutaneous implants in rats are often used to evaluate the biological compatibility of various dental materials. The widespread use of this method comes from the fact that the implantation of the material into the subcutaneous tissue of the animal is accessible and simple, but also reliable in order to determine tissue irritation and the interaction between the tissue and the material itself [4, 11, 17]. Local reactions to the implant can be quantitatively and qualitatively evaluated using various methods: histological analysis, scanning electron microscopy, transmission electro-microscopy, histochemical analysis. However, the most common method for studying tissue compatibility of implanted material is histological analysis, and thickness of the fibrous capsule around the implant that is under the skin, which has been used as an indicator of the biocompatibility of the material for decades [19].

Although a wide range of different types of tubes are in use today – dentine [20], silicone [21], Teflon [3], polyethylene [22], polypropylene [23] – our choice were polyethylene tubes, considering their inert nature and wide application. They do not cause a tissue reaction and in controlled and effective way expose tested material to the living tissue. They are easy to apply and sterilize [11, 17, 22].

Tissue around the tested materials showed the highest level of inflammation in the first seven days, with moderate damage to the structure of the connective tissue. The connective tissue around the MTA showed signs of the most severe inflammatory reaction evaluated as moderate and pronounced inflammation. This is in line with the findings of some other researchers (Lotfi et al. [24], Camilleri and Pitt Ford [25]) according to which a slightly more expressed initial inflammatory response to tissue implantation by MTA is explained by high pH of this material, long-term follow-up of heat-trapping, as well as the stimulation of inflammatory cytokines.

In accordance with our results are also the results of the study by Opačić-Galić et al. [22], where seven days after the subcutaneous implantation of the nanostructured CS-HA, CS and the control MTA, in rats of the Wistar strain, the strongest inflammatory response was given by MTA (3.30 ± 0.48), while for CS and CS-HA it was graded with 3.00 ± 0.71.

The results of this research showed the correlation between the strength of the inflammatory response and the thickness of the fibrous capsule. With time lag, a decrease in intensity of inflammation was noticed, with an increase in the thickness of the fibrous capsule in all tested materials. Experimental calcium aluminate cement (*ALBO*-CA) at the end of the observation period, after 30 days, caused an identical tissue reaction such as calcium silicate hydroxyapatite (*ALBO*-CSHA) and commercial mineral trioxide aggregate (MTA), with the best organized fibrous capsule around the material, which is probably due to the chemical nature and method of synthesis of this cement. The bioactivity of one material is also related to the method of its synthesis. The calcium aluminate

nanostructured biomaterial, which was tested in this study, was produced by nanotechnology, by combining two methods: hydrothermal sol-gel method and the self-combusting wave methods. According to Chen et al. [26], the materials obtained by sol-gel processes are more bioactive than those obtained by other synthetic methods.

Garcia Lda et al. [4] obtained biocompatibility results that fully correspond to the results of this study in the absence of inflammation and a significant increase in the thickness of fibrous capsule after 30 days of subcutaneous implantation of calcium aluminate cement (EndoBinder), calcium silicate cement (mineral trioxide aggregates) and calcium hydroxide in rats. Despite the fact that EndoBinder releases a lower amount of calcium ions, it caused a tissue reaction similar to that induced by MTA and calcium hydroxide in all observation periods. The authors explain this by the methodology applied in this *in vivo* study. Garcia Lda et al. [4] concluded that, from the biological point of view, EndoBinder became a promising option in endodontic therapy, but with the recommendation that additional research into the biological and physical-chemical properties of this new cement could be carried out before its application in the endodontic therapy of human populations.

Aminozarbian et al. [27] confirmed that the inflammatory response of the tissue to the implantation of CAAC calcium aluminate cement and the mixture of volostonite and calcium aluminate cement of WOLCA was, after 30 days, in rats comparable to MTA, which is in accordance with our findings, while CAAC Plus did not confirm biocompatibility as it induced a more intense inflammatory response than MTA. The authors explain this by the composition of CAAC Plus cement, with adding 5% Na-HMP dispersant in order to improve its properties, which on the other hand negatively affected biocompatibility [27].

Aguilar et al. [11] confirmed a better tissue reaction after calcium aluminate cement EndoBinder was compared to GMTA after 42 days of subcutaneous implantation of these materials in rat tissue. This is explained by the process of EndoBinder's synthesis, low Ca content, which results in the release of a smaller amount of calcium ions, making it less irritant and cytotoxic to the tissues, without compromising its antimicrobial properties [11].

The reaction of the tissue to the empty tubes (negative control) in this experiment is similar to the findings of other researchers [19, 28]. After seventh day, the most pronounced inflammatory response was confirmed, and this initial inflammatory reaction to the empty tubules was explained by the reaction to the surgical implantation procedure [19, 28].

Results of the biocompatibility of *ALBO*-CSHA obtained in this study correspond to the findings of Petrović et al. [17] and Saghiri et al. [29]. In their studies, at the end of the observation, there was no significant difference in the tissue inflammatory response to CS-HA and MTA, i.e. BioAggregate, nanostructured tricalcium silicate cement with the addition of calcium phosphate, and MTA after subcutaneous implantation in rats. The authors explain this finding by the fact that calcium-containing good biological properties owe a common ability to released calcium ions, and a similar reaction is expected in a situation where the new calcium silicate system comes into contact with tissue fluids. In the study by Petrović et al. [17], the application of a special synthesis method further favored the bioactivity of the examined CS-HA.

Contrary to this, Batur et al. [30] have come up with results that are inconsistent with the results of this study, as they confirmed statistically significantly better tissue response and a weaker inflammatory response of DiaRoot BioAggregate compared to MTA. A possible reason for a better BioAggregate result may be the fact that their research was done *in vivo* conditions on laboratory animals (Sprague–Dawley rats), as opposed to previous *in vitro* studies, in which no statistically significant difference was found between MTA and BioAggregat [30].

CONCLUSION

The subcutaneous tissue of rats showed good tolerance of calcium aluminate nanostructured biomaterials and comparable to nanostructured calcium silicate *ALBO*-CSHA and commercial calcium silicate cement (MTA). The biocompatibility of this nanomaterial should be verified in other experimental studies before clinical screening on human population.

NOTE

This paper is part of a research thesis entitled „Biocompatibility nanostructured biomaterials based on calcium aluminate" by Ognjenka Janković.

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Биокомпатибилност наноструктурних биоматеријала на бази калцијум-алумината

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САЖЕТАК

Увод/Циљ Циљ овог рада је био да се провери биокомпатибилност новосинтетисаног наноструктурног материјала на бази калцијум-алумината после имплантације у поткожно ткиво пацова.

Методе У истраживање је укључено 18 пацова старости 10–11 седмица, који су подељени у две експерименталне групе ($n = 9$). Код свих животиња је урађена инцизија на леђима и формирана су два џепа дубине 15 *mm* у која су аплициране стерилне полиетиленске тубице са тестним материјалима (*ALBO-CA, ALBO-CSHA* и контрола *MTA*). По шест пацова сваке групе је жртвовано у три опсервациона периода (7, 15 и 30 дана). Патохистолошки су анализирани инфламација, крварење, фиброзна капсула и интегритет ткива око имплантираног материјала.

Резултати После седам дана *ALBO-CA* и *ALBO-CSHA* су показали бољи ткивни одговор у односу на *MTA* са статистички значајном разликом у интензитету инфламације ($p = 0,2781$). Разлика у васкуларној конгестији и дебљини фиброзне капсуле после имплантације материјала *ALBO-CA* у односу на *MTA* је такође била статистички значајна ($p = 0,5567$). На крају евалуационог периода од 30 дана забележен је идентичан инфламаторни одговор растреситог везивног ткива на месту имплантације *ALBO-CA, ALBO-CSHA* и *MTA* (оцена 0,7). Констатовано је формирање дебеле или умерено дебеле фиброзне капсуле, која је била најдебља код *ALBO-CA* (оцена 3,7). Статистички значајне разлике између анализираних параметара после 30 дана није било.

Закључак Новосинтетисани *ALBO-CA* је показао задовољавајућу ткивну реакцију и потврдио биокомпатибилност после имплантације у поткожно ткиво пацова.

Кључне речи: наноматеријали; калцијум-алуминати; калцијум-силикати, ткивна реакција