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Evaluation of biocompatibility of bioceramic sealers with periapical tissues: an experimental study in vivo

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Abstract

INTRODUCTION. In the view of significant changes in the pharmacotherapy of dental diseases, bioceramic sealers, which have recently been used for root canal obturation and differ significantly in composition from the widely used epoxy sealers, are of scientific and practical interest.

AIM. To develop a domestic calcium-silicate bioceramic material for endodontic treatment and to evaluate the biocompatibility of periapical tooth tissues in a comparative aspect with various sealers under experimental conditions on laboratory animals.

MATERIALS AND METHODS. Laboratory studies were conducted on 12 male chinchilla rabbits weighing 3.5 ± 0.1 kg. After the material was removed into the periodontal tissues of the animals on the 7th and 30th days of the experiment, fragments of the jaws with an experimental incisor were obtained, which were studied histologically. The study determined the biocompatibility of modern sealers: epoxy AH Plus (Dentsply Sirona, USA) and bioceramic sealers: TotalFill BC Sealer (FKG, Switzerland) and a new developed bioceramic composition Bioceramin (VladMiVa).

RESULTS. The biocompatibility of sealers had a statistically significant difference between the study groups during the 7-day observation period ($p \leqslant 0.01$). In the epoxy sealer group, reactive changes in the periapical tissues of the teeth were present throughout the observation period. At the same time, in the bioceramic sealer groups, proliferation of loose highly cellular fibrous tissue with rare small mononuclear inflammatory infiltrates was detected during the 7-day observation period, and reactive changes with periosteal osteogenesis in 30 days after the start of the experiment.

CONCLUSIONS. According to the results of the study, the calcium-silicate material of foreign manufacture and the newly developed bioceramic calcium-silicate sealer of domestic manufacture showed high biocompatibility with periodontal tissues of laboratory animals' teeth ($p<0.05$). At the same time, toxicity of the epoxy sealer on periodontal tissues was revealed in 7- and 30-day observation periods.

Keywords: bioceramic sealer, calcium silicate-based cement, import substitution, russian bioceramic material, root canal obturation, biocompatibility

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Оценка биосовместимости биокерамических силеров с периапикальными тканями зуба: экспериментальное исследование in vivo

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Резюме

ВВЕДЕНИЕ. Ввиду существенных изменений в фармакотерапии стоматологических заболеваний, научно-практический интерес представляют биокерамические силеры, которые с недавнего времени применяются при обтурации корневых каналов и значительно отличаются по составу от широко используемых эпоксидных силеров.

ЦЕЛЬ ИССЛЕДОВАНИЯ. Разработать отечественный кальций-силикатный биокерамический материал для эндодонтического лечения и оценить биосовместимость периапикальных тканей зуба в сравнительном аспекте с различными силерами в условиях эксперимента на лабораторных животных.

МАТЕРИАЛЫ И МЕТОДЫ. Лабораторные исследования проводились на 12 кроликах-самцах породы шиншилла, массой 3,5±0,1 кг. После выведения материала в ткани периодонта животных на 7 и 30 сутки эксперимента были получены фрагменты челюстей с опытным резцом, которые были изучены гистологически. В исследовании была определена биосовместимость современных силеров: эпоксидных AH Plus (Dentsply Sirona, США) и биокерамических: TotalFill BC Sealer (FKG, Switzerland) и новой разработанной биокерамической композиции отечественного производства Биокерамин (ВладМиВа).
РЕЗУЛЬТАТЫ. Биосовместимость силеров имела статистически значимую разницу между группами исследования в период 7 дней наблюдения ($p \leqslant 0,01$). В группе эпоксидного силера присутствовали реактивные изменения периапикальных тканей зубов на всем протяжении наблюдений. При этом, в группах биокерамических силеров было выявлено разрастание рыхлой высококлеточной фиброзной ткани с редкими мелкими мононуклеарными воспалительными инфильтратами в период 7 дней наблюдений, и реактивные изменения с периостальным остеогенезом через 30 дней после начала эксперимента.
ВЫВОДЫ. По результатам исследования кальций-силикатный материал зарубежного производства и новый разработанный биокерамический кальций-силикатный силер отечественного производства показали высокую биосовместимость с периодонтальными тканями зубов лабораторных животных ($p < 0,05$). При этом, выявлена токсичность эпоксидного силера на ткани периодонта в 7- и 30-дневный периоды наблюдения.

Ключевые слова: биокерамический силер, кальций-силикатный цемент, импортозамещение, российский биокерамический материал, обтурация корневых каналов, биосовместимость

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INTRODUCTION

The success of endodontic treatment is determined by the quality implementation of the endodontic treatment triad: shaping–irrigation–obturation of the root canal system [1]. A key and indispensable stage of this treatment is the hermetic filling of the root canal with a material that possesses the necessary biological (bactericidal activity, biocompatibility), physical (resistance to dissolution in tissue fluids, adhesion to canal walls), and practical properties (radiopacity, ease of insertion and removal) [2]. Biocompatibility is a critical property of the sealer used, as the filling material is in direct contact with the periapical tissues, including through the physiological apex of the tooth root [3]. Biocompatibility refers to the ability of a material to prevent an acute local immune response in the periodontal tissues, which is achieved by the sealer's molecular composition being similar to that of the surrounding periodontal tissues [3; 4].

At the same time, irritation of the surrounding periodontal tissues can impair periapical healing and disrupt bone regeneration when the material is extruded beyond the root apex through the apical foramen during obturation or the sealing of root perforations [5].

Currently, various types of materials are used in endodontics for root canal filling. Due to significant changes in the pharmacotherapy of dental diseases, bioceramic sealers–recently introduced for root canal obturation and markedly different in composition from widely used epoxy resin-based sealers–are of considerable scientific and clinical interest [6]. According to international literature, several in vitro studies have shown that bioceramic sealers do not exhibit cytotoxic effects on the tooth-periodontium complex [7].

Earlier studies have noted that three-dimensional obturation of the root canal system with bioceramic sealers prevents microleakage and reinfection, while also creating favorable conditions for the regeneration of periodontal tissues [8; 9].

However, data on new bioceramic materials – particularly those of domestic origin – remain limited, which highlights the relevance of ongoing research. The development of a domestic composition of a biocompatible and bioactive sealer for root canal obturation using bioceramic technologies may represent a significant scientific breakthrough in Russian dental science. To identify and confirm the effectiveness of the developed bioceramic sealer formulations, a series of experimental and clinical-laboratory studies (both in vitro and in vivo) must be conducted – one of which may be the present research project.

AIM

The aim of the present study is to develop a domestically produced calcium silicate-based bioceramic material for endodontic treatment and to evaluate the biocompatibility of periapical tooth tissues in a comparative aspect with various sealers under experimental conditions in an animal model.

MATERIALS AND METHODS

The design of the in vivo laboratory study, conducted as part of the development of a new bioceramic material for root canal obturation, consisted of two stages:

– the experimental stage, involving the evaluation of the materials' biocompatibility with periodontal tissues in rabbits under laboratory conditions;

– and the pathomorphological stage, involving histological examination of bone fragments with the treated incisor.

Experimental Stage

Animal experiments were carried out at the NIMSI laboratory in accordance with Directive 2010/63/EU of the European Parliament and the Council of the European Union. The laboratory study was conducted on 12 male Chinchilla rabbits weighing 3.5 ± 0.1 kg, in two stages.

In the first stage, experimental endodontic treatment was performed on the mandibular incisors of the animals (Fig. 1). The procedures were carried out under general anesthesia using Zoletil 100, with premedication using Meditin at a dose of 0.2 ml/kg. Anesthesia reversal was achieved with Antisedan.

The in vivo experiment consisted of opening the pulp chamber, forming endodontic access, creating a lateral root wall perforation, and subsequently obturating with the test sealer, ensuring partial extrusion beyond the apex to promote tight contact with periapical tissues. One of the tested materials was introduced into the canal according to the division into three experimental groups. Radiographic verification of the canal filling was performed to confirm proper material placement and apical extrusion.

In the second stage, the sealer was injected subcutaneously into the thigh area to further assess the material's biocompatibility with soft tissues. Following general anesthesia (as described above), the fur was shaved from the injection site using an electric clipper. The area was disinfected with 0.05% chlorhexidine solution, and the injection site was marked with brilliant green dye. Each material was injected in equal volume (0.4 ml). The animals were randomly assigned to one of three groups, according to the sealer used for root canal obturation.

Pathomorphological Stage

The evaluation of the condition of the periapical tissues was conducted on days 7 and 30 of the experiment. In the first phase of the morphological assessment, a 2×3 cm section of mandibular bone containing the treated incisor and surrounding gingival soft tissues was isolated from each animal. As a negative control, biological samples were collected from the contralateral side of the jaw or the thigh.

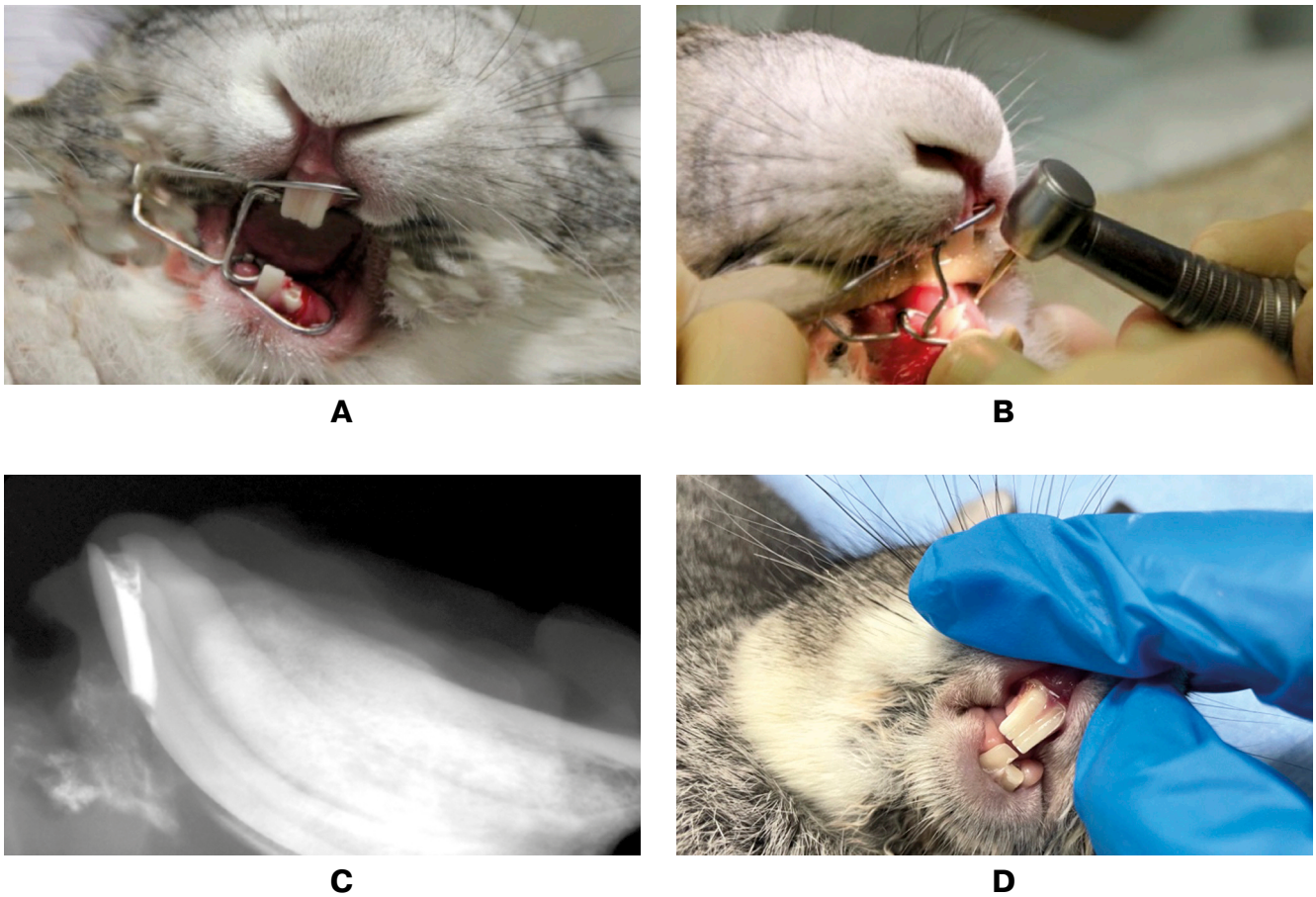


Fig. 1. Stages of modeling endodontic treatment of incisors in laboratory animals:
A – resection of the lower jaw incisor; *B* – creation of access to the root canal;
C – radiographic control of the injection of the test material into the periapical tissues;
D – application of a filling made of light-curing composite material

Рис. 1. Этапы моделирования эндодонтического лечения резцов у лабораторных животных:
А – резекция резца нижней челюсти; *Б* – создание доступа к корневому каналу зуба;
В – рентгенологический контроль выведения исследуемого материала в периапикальные ткани зуба;
Г – наложение пломбы из светоотверждаемого композитного материала

In the preparatory stage, the samples with the tooth were fixed in formalin for 24 hours, followed by decalcification using SoftyDec solution (BioVitrum) over 7 days. In the second phase, histological evaluation was performed on the thigh area where the material was subcutaneously injected. All samples were embedded in paraffin using standard protocols, and histological sections 7–8 μm thick were prepared. These sections were then stained with hematoxylin and eosin.

Tissue samples were assessed using the following scoring systems:

1. Inflammatory response: 0 – none; 1 – mild; 2 – moderate; 3 – severe.

2. Macrophage infiltration: 0 – fewer than 10 cells; 1 – 10 to <30 cells; 2 – ⩾30 cells.

3. Fibrous capsule thickness: 0 – absent; 1 – thin capsule; 2 – thick capsule.

4. Vascular changes: 0 – none; 1 – mild; 2 – moderate; 3 – severe.

Statistical Analysis

Data were analyzed using the Mann–Whitney U test to compare values between the two observation periods within each group, and the Friedman test with Bonferroni correction to compare values between the experimental groups at a single time point. Differences were considered statistically significant at $p < 0.05$.

RESULTS

The results of the histological analysis demonstrated that wound healing showed statistically significant differences between the experimental groups and observation periods ($p < 0.05$). At the same time, the material samples remained at the injection site throughout the observation period. In Group 1, surgical wounds healed with signs of material rejection, whereas in Groups 2 and 3, no signs of infection or rejection were observed. Table 1 presents the most relevant scoring results for all evaluated variables in both observation periods, based on the assessment of periapical tooth tissues, along with the outcomes of the statistical analysis.

Histological Analysis of Periapical Tissues in Animals

Macroscopic examinations conducted after 7 days of observation revealed reactive changes in the adjacent alveolar bone in Group 1. The bony trabeculae were lined with a layer of active, weakly polymorphic osteoblasts. In the intertrabecular spaces and periosteal region, there was proliferation of loose, highly cellular fibrous tissue with sparse small mononuclear inflammatory infiltrates. A pronounced inflammatory response was observed, with accumulation of segmented neutrophils, mononuclear inflammatory cells, and macrophages, as well as moderate to severe vascular occlusion (Fig. 2, *A*). After 30 days, a mild chronic inflammatory reaction was detected, accompanied by a well-developed fibrous capsule.

In Group 2, a small focus of foreign material was surrounded by mononuclear inflammatory infiltrate and immature loose fibrous tissue. Reactive changes were present in the adjacent alveolar bone (Fig. 3, *B*). On day 7, a mild inflammatory reaction and moderate vascular changes were observed. A thin, poorly defined capsule was present, and macrophage infiltration was assessed as moderate to severe, with active phagocytic features noted in the cells. After 30 days, the chronic inflammatory response was absent or mild, and a thin connective tissue capsule was present. Macrophage infiltration decreased from severe to moderate, and the vascular network appeared normal in most samples.

In Group 3, reactive changes with periosteal osteogenesis were present in the adjacent alveolar bone (Fig. 4, *C*). On day 7, a fibrous capsule with immature collagen connective tissue fibers and a mild acute inflammatory reaction–mainly involving neutrophils and macrophages–was observed. Vascular changes ranged from none to mild. After 30 days, the inflammatory response had resolved, and a mature, thin, dense connective tissue capsule with signs of periosteal osteogenesis was noted. Vascular changes and macrophage infiltration were minimal or absent.

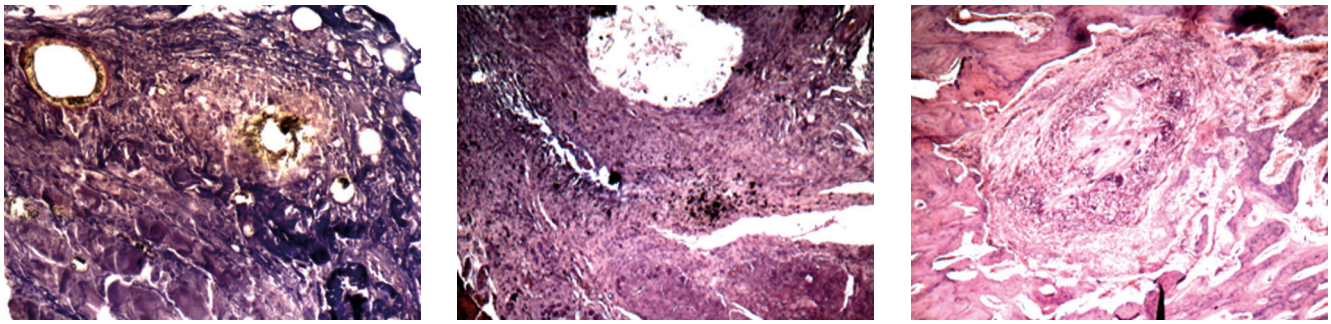
Table 1. Results of the biocompatibility assessment of sealers at days 7 and 30 of observation

Таблица 1. Результаты оценки биосовместимости силеров с периапикальными тканями зуба на 7 и 30 дни наблюдений

	Inflammatory reaction (0/1/2/3)			Macrophage infiltration (0/1/2)			Thickness of the fibrous capsule (0/1/2)			Vascular changes (0/1/2/3)		
	7 day	30 day	P	7 day	30 day	P	7 day	30 day	P	7 day	30 day	P
AH Plus	0/0/0/100	0/25/50/0	0.005*	0/25/75	75/25/0	0.025*	25/50/25	0/75/25	0.018*	0/15/45/40	15/50/25/10	0.018*
TotalFill BC Sealer	0/50/50/0	75/25/0/0	0.045*	10/15.8/74.2	50/50/0	0.184	0/50/50	0/100/0	0.025*	50/50/0/0	50/50/0/0	0.342
Biokeramin	0/25/75/0	100/0/0/0	0.002*	0/50/50	75/25/0	0.053	0/85/15	0/100/0	0.145	50/25/25/0	65/35/0/0	0.125
P-value	0.025*	0.010*		0.045*	0.132		0.255	0.123		0.035*	0.005*	

Note: Values are presented as relative frequencies (percentages) for each parameter. Asterisks (*) indicate statistically significant differences.

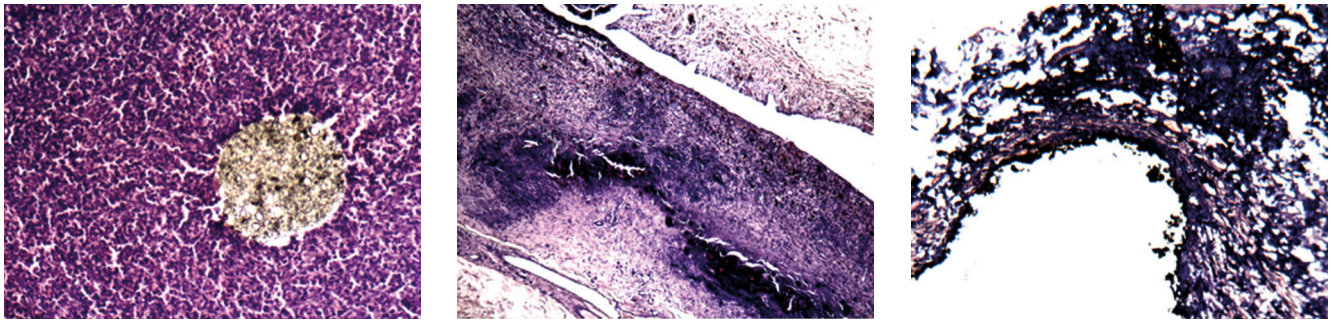
Примечание: Значения представлены как относительные частоты (проценты) каждого показателя. * Статистически значимые различия.



A B C

Fig. 2. Histologic specimen of the fragment of lower jaw bone of a laboratory animal: *A* – with epoxy sealer; *B* – with bioceramic sealer TotalFill BC Sealer; *C* – with the developed composition of calcium silicate sealer;

Рис. 2. Гистологический препарат фрагмента кости нижней челюсти лабораторного животного: *A* – с эпоксидным силером; *B* – с биокерамическим силером TotalFill BC Sealer; *C* – с разработанной композицией кальцийсиликатного силера



A B C

Fig. 3. Histologic specimen of a soft tissue fragment of the laboratory animal upper leg: *A* – with epoxy sealer; *B* – with bioceramic sealer TotalFill BC Sealer; *C* – with the developed composition of calcium silicate sealer

Рис. 3. Гистологический препарат фрагмента мягких тканей бедра лабораторного животного: *A* – с эпоксидным силером; *B* – с биокерамическим силером TotalFill BC Sealer; *C* – с разработанной композицией кальцийсиликатного силера

A B

Fig. 4. Clinical photo of the subcutaneous injection of epoxy (*A*) and bioceramic (*B*) sealers area into the laboratory animal's upper leg 7 days after injection

Рис. 4. Клиническая фотография области введения эпоксидного (*A*) и биокерамического (*B*) силеров подкожно в бедро лабораторного животного через 7 дней после введения

Histological Analysis of Soft Tissues in the Animal Thigh

Histological examination of the soft tissues in Group 1 revealed an abscess formation consisting of dense accumulations of destructive eosinophils, neutrophils, and macrophages (Fig. 3, *A*).

In Group 2, a pseudocystic cavity without internal contents was detected in the thigh region. It was surrounded by highly cellular, well-vascularized immature fibrous tissue with multiple foci of mineralization (Fig. 3, *B*).

The domestically produced calcium silicate-based sealer demonstrated the least pronounced infiltration by immune cells (Fig. 3, *C*).

Figure 4 show clinical photographs of the soft tissue reaction in the animal thigh to the injection of epoxy and bioceramic sealers. In the case of the epoxy sealer, ulceration was observed at the material injection site, while no signs of inflammation were noted at the injection site of the newly developed bioceramic sealer.

DISCUSSION

It was established that the tested calcium silicate-based sealers – both domestic and foreign – exhibited no toxic effects on periapical tooth tissues. The obtained results confirm the biocompatibility of the newly developed domestically produced calcium silicate-based sealer, which opens the prospect for its clinical application in root canal obturation for the treatment of pulp and periodontal tissue diseases, provided that further scientific and clinical studies yield positive results [10–12].

The results also demonstrated that the epoxy resin-based sealer AH Plus induces significant reactive changes in both periodontal and thigh soft tissues, which is considered a disadvantage of this class of materials. Moreover, unlike calcium silicate-based sealers, epoxy sealers do not contribute to the stimulation of reparative processes [13; 14].

The developed composition of the single-component bioceramic sealer for root canal filling is characterized by high early strength, controlled setting time, excellent biocompatibility, and osteoinductive activity. These properties enhance both the convenience and quality of treatment for patients with diseases of the pulp and periapical tissues. The sealer is designed to be used in conjunction with gutta-percha cones containing bioceramic nanoparticles, thereby enabling the formation of a hermetic, monolithic root filling through the use of advanced bioceramic technologies.

CONCLUSION

A 100% biocompatibility of both domestic and foreign bioceramic sealers was established during the 7- and 30-day observation periods. In contrast, the epoxy-based sealer exhibited toxic effects in all cases, accompanied by signs of necrosis in the surrounding tissues.

The conducted studies demonstrated the high biocompatibility of the newly developed sealer composition with the periapical tissues of teeth, as well as its pronounced osteogenic effect. The theoretical and practical feasibility of using the new domestically produced calcium silicate-based sealer in the treatment of pulp and periapical tissue diseases has been confirmed. This material is currently undergoing certification under the name *Biokeramin* and has no analogues in Russia.

Summarizing the findings of the laboratory study, it should be emphasized that, within the context of import substitution, the domestic material *Biokeramin*, protected by patent, is demonstrably competitive in addressing the challenges of regenerative dentistry. The Russian-developed material showed high quality on par with well-known next-generation foreign bioceramic sealers.

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