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Reprint

Impact of silver nanoparticle concentration in alginate microcapsules on their effects on morphological changes in periodontitis

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Abstract: Objective: to evaluate the effects of alginate microcapsules on morphological changes in periodontitis depending on silver nanoparticle concentration in them.
Materials and Methods. The study was conducted on 30 rats distributed among three groups of 10 animals: the comparison group comprising animals with an experimental model of periodontitis and two experimental groups including animals with 5-week periodontitis, which were subjected to applications of the gel containing microcapsules with high (0.25 M, Group 1) and low (0.1 M, Group 2) concentrations of silver on the surface of their gums. To assess morphological changes, the mandible was sampled for subsequent examination.
Results. In animals with periodontitis, changes in the supporting structure of the tooth took place, including defibration and perivascular edema in periodontal ligament of the tooth, and bone resorption via its replacement with connective tissue. The application of a gel containing microcapsules with silver nanoparticles led to a partial reduction of such disorders: particularly, it reduced bone resorption and its replacement with connective tissue. Destructive changes in periodontal tissues caused by gel with a high content of silver nanoparticles (0.25 M) were less pronounced than those caused by capsules with a low content of silver (0.1 M).
Conclusion. Gels containing microcapsules with silver nanoparticles contributed to the reduction of destructive changes in the supporting structure of the tooth in periodontitis. The severity of the effects of the gel increased with an increase in the content of silver nanoparticles in alginate microcapsules.

Keywords: periodontitis, nanoparticles, bone destruction.

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Introduction

Periodontal disease is a common oral infection affecting the tissues surrounding and supporting teeth. The initial signs of these diseases are pain, bleeding and hyperemia of the gums; this condition is characterized as gingivitis. Subsequently, if left untreated, it progresses to periodontitis, which leads to structural changes of a more destructive nature, such as a loss of periodontal ligament and supporting bone. In 2009, The European Seminar on Periodontal Health and Cardiovascular Disease reported that the prevalence of periodontal disease ranged from 20% to 50% worldwide [1]. According to the World Health Organization (2016), periodontitis was the 11th most common disease in the world [2]. Periodontitis is one of the main causes of tooth loss, leading to aesthetic and functional disorders that negatively affect the nutrition and general health of patients. Due to the fact that this disease progresses rapidly, has high prevalence and is difficult to treat, issues related to the search and development of new effective treatment methods remain currently relevant.

Silver nanoparticles have recently gained great popularity in the field of biomedicine. There are many published studies demonstrating the antibacterial effect of silver, which allows using it for treating certain ailments, including periodontal diseases [3–5].

Promising technologies, such as nanostructuring and encapsulation of various substances, create new opportunities for improving therapeutic schemes for the treatment of periodontal diseases. Previous studies confirmed that the gel containing microcapsules with silver nanoparticles was imposing a positive effect on the microcirculatory bed condition in periodontal tissues of rats with experimental periodontitis [6]. However, other published sources suggested that silver nanoparticles could irritate the mucous membrane [7, 8], triggering an adverse side effect, the progression of which may cause the appearance of erosive formations on the mucous membrane. In the future, such formations may lead to the destruction of tissues, which necessitates minimizing the risks of their occurrence. In this regard, the optimization of silver nanoparticle concentration in microcapsules represents an option of solving this problem, which substantiated the scope of our research.

Objective – to evaluate the effects of alginate microcapsules on morphological changes in periodontitis, depending on the concentration of silver nanoparticles in them.

Materials and Methods

Our research was conducted on 30 white male rats randomly distributed among three groups of 10 animals in each. The comparison group included animals with experimentally modeled periodontitis; while treatment groups comprised animals with 5-week periodontitis, which (after removal of the ligature) were subjected to applications of the gel containing microcapsules with high (0.25 M, Group 1) and low (0.1 M, Group 2) concentrations of silver on the surface of their gums. The animals were kept under standard vivarium conditions with natural light, and free access to water and food. All experimental work was carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, the requirements of the national guidelines for the maintenance and care of laboratory animals, and were approved by the Ethics Committee at V.I. Razumovsky Saratov State Medical University (protocol No. 1 of 9 July 2021). Animals were anesthetized 10 min before the manipulation via intramuscular administration of Telazol (Zoetis Inc., Spain) at a rate of 0.1 mL/kg and Xylanit (Nita-Pharm LLC, Russia) at a dose of 1 mg/kg of animal weight.

The modeling of periodontitis in rats was carried out by the ligature method modified by A. Ionel et al. [9]. Under anesthesia, a polyfilament nonabsorbable suture was sewn into the gum tissue in the area of the central incisors of the lower jaw: this particular area is the most accessible option for applications and diagnostic manipulations. The ligature was removed on day 14 after suturing.

Animals in experimental groups were treated with alginate silver microcapsules three times: on days 14, 16 and 18 of the experiment. Microcapsules were prepared according to the earlier specified protocol [6]. Hollow silver alginate microcapsules were obtained by adsorption of 1 mL of sodium alginate at a concentration of 5 mg/mL on porous particles of calcium carbonate [10], followed by gelling of sodium alginate by silver ions with the addition of 1 mL of silver nitrate at concentrations of 0.1 M or 0.25 M. Complete reduction of silver nanoparticles and simultaneous dissolution of the porous core of calcium carbonate was carried out via adding 1 mL of ascorbic acid at a concentration of 0.1 M to the suspension. As a result, two types of specimens were prepared: hollow alginate silver microcapsules with different concentrations of silver, 0.1 M and 0.25 M.

Animals were taken out of the experiment by an overdose of anesthetic agents during the 5th week after the ligation. The mandible was sampled. The specimens were fixed in 10% formalin for subsequent histological examination. The collected tissues were demineralized with an electrolyte decalcifying solution (ErgoProduction, Russia) over the 24-hr period. After that, a section containing tissues of the supporting structure of the central incisors was cut out for making preparations, and it was used to prepare horizontal (transverse with respect to the axis of the incisors) sections. The tissues were subjected to alcohol treatment, after which they were embedded in paraffin. Cross-sections 5–7 μm thick were made using a semi-automated RMD 3000 microtome (MtPoint, Russia – Australia) and stained with Mayer's solution of hematoxylin (Biovitrum LLC, Russia) and eosin (Biovitrum LLC, Russia). Bio-Clear (BioOptica, Italy) was used for making sections transparent, while Bio-Monht (BioOptica, Italy) was used to place preparations under coverslips.

Microscopy of the obtained preparations was performed using a transmitted light microvisor of the μVizo-103 series (LOMO PHOTONIKA LLC, Russia). Microscopy of sagittal sections allowed evaluating the severity of inflammatory and destructive changes in the supporting structure of the tooth, including an assessment of morphological changes in the periodontal ligament of the tooth, the condition of its vessels, and manifestations of bone resorption. When assessing changes in the periodontal ligament of the tooth, the direction and course of its fibers were also taken into account. In addition, a qualitative assessment of the aggregate state of blood in the vessels of the periodontal ligament of the tooth was performed.

Results

Morphological analysis horizontal section preparations in comparison group animals showed that in the 5th week after the ligature application, there was a defibration in the periodontal ligament, a disorder of the orientation of fibers due to interstitial edema, and the presence of pronounced perivascular edema (*Figure A*). Along with this, in some vessels, we observed some signs of a disorder of the blood aggregate state: blood sludge separation. The predominant population of cells in the periodontal ligament was represented by fibroblasts (*Figure B*).

`In the bone tissue, bone rarefaction was observed of the lacunar resorption type with the ingrowth of connective tissue into the lacunae (*Figure B*).

In animals of the comparison group, on the 5th week after ligation, we revealed blood flow disorders in the form of blood sludging and perivascular edema, as well as bone resorption with proliferation of connective tissue, indicating a pronounced destruction of periodontal complex tissues.

In animals treated with a gel with low-concentration (0.1 M) silver microcapsules applied to the gingiva during the 5th week, we noted defibration in the periodontal ligament and a disorder in the orientation of fibers in some places (*Figures C, D*).

In the alveolar bone, rarefaction of bone tissue was observed mainly in the area of perivascular spaces (*Figure D*). At the same time, in animals of the treatment group I, both resorptive processes and bone tissue replacement with connective tissue were less pronounced vs. the comparison group.

For example, in animals that were subjected to a gel with a low content (0.1 M) of silver, applied to the surface of their gums, we revealed disorders of periodontal ligament integrity and destructive processes in the bone. The severity of bone tissue destruction and its replacement with connective tissue in animals of this group was less pronounced than in the comparison group.

Microscopy of cross-sectional preparations in animals of the treatment group II exhibited an ordered uniform orientation of periodontal ligament fibers, and interstitial edema was not detected. As in other groups, in animals of this group, perivascular edema persisted in the periodontal ligament (*Figure D*).

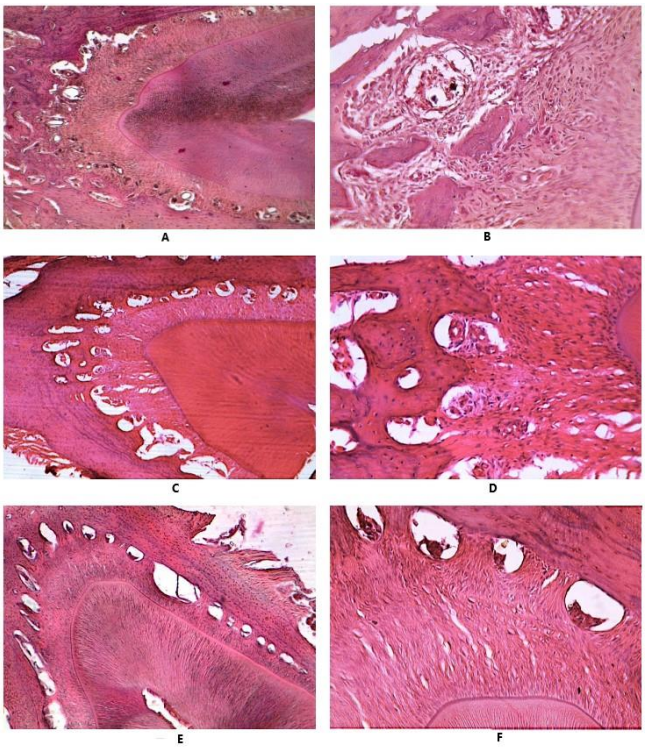

Figure. Morphological changes in rats with experimental periodontitis against the background of using gels with high (0.25 M) or low (0.1 M) silver concentrations:

A, B – periodontal ligament of an animal with experimental periodontitis (A, magnification ×20; B, magnification ×5); C, D – periodontal ligament of an animal with experimental periodontitis after application of a gel containing microcapsules with a low concentration of silver (0.1 M) (C, magnification ×20; D, magnification ×5); E, F – periodontal ligament of an animal with experimental periodontitis after application of a gel containing microcapsules with a high concentration of silver (0.25 M) (D, magnification ×20; E, magnification ×5)

After applying a gel containing high concentration of silver microcapsules (0.25 M), in rats with periodontitis in the alveolar bone, we observed weakly expressed signs of resorptive processes in the bone tissue of the alveolar process (*Figures E, F*).

Therefore, when a gel with a high silver content (0.25 M) was applied to the gum surface, destructive processes in the bone were not pronounced, which confirmed its higher efficacy vs. the gel containing capsules with a low concentration of silver nanoparticles.

Discussion. The results of our study demonstrated the development of degenerative inflammatory changes manifested by the presence of interstitial edema, along with destruction of bone tissue in the lower jaw in animals with experimental periodontitis, which was consistent with the published data [11]. However, in the course of our research, we established that these changes persisted for three weeks after removal of the ligature, which I implied their consistency. Therefore, the modification of periodontitis modeling method employed in our study can be used to identify the efficacy and safety of medicamentous therapy of developing disorders, since it reproduces the chronic course of periodontitis.

Applications of gels containing silver microcapsules with silver nanoparticles (0.1 M and 0.25 M) to the gingival mucosa in rats with inflamed gums prevented the development of destructive processes in the tissues of the supporting structure of the tooth. This effect may be indirectly due to the antibacterial action of silver nanoparticles, which is supported by [reviously published data [12–15].

In the course of this study, we noted that the gel containing microcapsules with silver (0.25 M) in experimental periodontitis does not have a significant effect on the morphological manifestations of microcirculatory disorders during the 5th week. The results of histological examination supported the data, obtained earlier in the study, on the effect of a gel containing high silver content microcapsules on the functional state of the gum microvasculature, and suggested the presence of a weak irritant response and the absence of a pronounced normalization of perfusion in the course of applications onto the inflamed periodontium [6]. Meanwhile, in animals of Group II, a leveling of the bone tissue destruction in the alveolar process was observed, which may imply the manifestation of the osteogenic effect of silver nanoparticles described by other authors [16].

As a result of morphological analysis in animals with experimental periodontitis, which were subjected to the application of a gel with a high concentration of silver in capsules, an effective decrease in the severity of destructive processes in the bone tissue was detected, as compared to rats with the gums, to which a gel with a low concentration (0.1 M) of silver was applied. Perhaps this was caused by the manifestation of antibacterial and osteogenic effects, which were much more pronounced in the high silver content (0.25 M) gel in microcapsules.

Conclusion

During the experiment, we detected no signs of a negative effect of applied gels of both concentrations, low (0.1 M) and high (0.25 M), on the affected gum tissue in animals, which confirmed the safety of their use. Gels containing alginate microcapsules with silver nanoparticles contributed to the reduction of destructive changes in the supporting structure of the tooth in periodontitis. This finding substantiates the prospects for their clinical approbation in patients with inflammatory periodontal diseases. The severity of the effects of the gel increased with an increase in the content of silver nanoparticles in alginate microcapsules.

Conflict of interest. The project was carried out within the framework of the Public Procurement to V.I. Razumovsky Saratov State Medical University of the Russian Federation Ministry of Healthcare, Development and Pathogenetic Substantiation of Using the System of Prolonged Release of Antibacterial and Anti-Inflammatory Agents for Correcting Microcirculatory Disorders in Experimental Periodontitis (Registration # 121032500024-2).

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