DOI 10.26724/2079-8334-2024-1-87-197-201
UDC [591.4+612.084]:616.716.8

O.V. Dienha, L.B. Tsevukh, B.M. Mirchuk[1], M.F. Konovalov, Zh.O. Novikova, I.Y. Tarasenko, O.O. Yudina
Odesa National Medical University, Odesa,
[1]Danylo Halytsky Lviv National Medical University, Lviv

MORPHOLOGIC ASSESSMENT OF THE EFFECTIVENESS OF OSTEOTROPIC PREPARATIONS ON BONE TISSUE REGENERATION IN EXPERIMENTAL ANIMALS

e-mail: oksanadenga@gmail.com

The research was devoted to the study of the processes of regeneration of the alveolar process of the jaws of experimental animals under the influence of osteotropic drugs. The study was conducted on 50 Wistar rats of herd breeding aged 30 days at the beginning of the experiment. The duration of the experiment was 30 days. The animals were withdrawn from the experiment under thiopental anesthesia, and the jaws were isolated. Excised fragments of jaw bone tissue were fixed in neutral 10 % formalin, decalcified, and embedded in paraffin. The sections were stained with hematoxylin and eosin. The experimental study on animals made it possible to study the effectiveness of the phased application of osteotropic drugs on the processes of local bone resorption. These studies should be taken into account in the development of treatment and prevention complexes for children with osteogenesis disorders.

Key words: rats, experimental research, osteogenesis, complex of preparations, carcinogenic diet.

О.В. Дєньга, Л.Б. Цевух, Б.М. Мірчук, М.Ф. Коновалов, Ж.О. Новікова, І.Й. Тарасенко, О.О. Юдіна

МОРФОЛОГІЧНА ОЦІНКА ЕФЕКТИВНОСТІ ВПЛИВУ ОСТЕОТРОПНИХ ПРЕПАРАТІВ НА РЕГЕНЕРАЦІЮ КІСТКОВОЇ ТКАНИНИ ЕКСПЕРИМЕНТАЛЬНИХ ТВАРИН

Дослідження було присвячено вивченню процесів регенерації альвеолярного відростка щелеп піддослідних тварин під впливом остеотропних препаратів. Дослідження проведено на 50 щурах лінії Вістар стадного розведення віком 30 днів на початок експерименту. Тривалість експерименту становила 30 днів. Тварин виводили з експерименту під тіопенталовим наркозом, виділяли щелепи. Висічені фрагменти кісткової тканини щелепи фіксували в нейтральному 10 % формаліні, декальцинували, заливали в парафін. Зрізи забарвлювали гематоксиліном та еозином. Проведене експериментальне дослідження на тваринах дало змогу вивчити ефективність поетапного застосування остеотропних препаратів на процеси локальної резорбції кісткової тканини. Дані досліджень необхідно врахувати при розробці лікувально-профілактичних комплексів для дітей із порушенням остеогенезу.

Ключові слова: щури, експериментальні дослідження, остеогенез, комплекс препаратів, карієсогенний раціон.

The work is a fragment of the research project "Improvement of diagnostics, prevention and treatment of teeth hard tissues mineralization processes violations in children", state registration No. 0121U114421.

The problem of bone tissue regeneration in dentistry is one of the most pressing and challenging tasks of modern medicine. The development of new treatment methods based on the use of osteotropic drugs opens up new opportunities for the restoration of damaged bone structures, especially in pediatric dentistry. The importance of this issue is due to the growing need for effective methods of treating bone defects that can occur as a result of trauma, congenital anomalies, or diseases such as periodontitis [2, 6, 9, 13, 14].

Current research in the field of osteoregeneration focuses on the impact of various osteotropic drugs on bone repair processes. This includes analyzing morphological changes in bone tissue, studying collagen metabolism, and evaluating the effectiveness of various implants and materials used to stimulate bone growth [8, 11, 12]. Particular attention is paid to studies that help to understand the mechanisms of interaction between osteotropic drugs and bone tissue, which is key to the development of new therapeutic strategies.

Understanding the mechanisms of action of osteotropic preparations and their impact on bone regeneration will allow us to develop more effective methods of treatment and prevention of bone diseases in children, thereby improving their quality of life and health.

The purpose of the study was to research the processes of regeneration of the alveolar outgrowth of the jaws of experimental animals under the influence of osteotropic preparations.

Materials and methods. The studies were conducted on 50 Wistar rats of the herd breeding line with an average weight of 52±4 g at the age of 30 days at the beginning of the experiment.

Dental caries was reproduced in rats at 1 month of age by transferring the animals to a carcinogenic diet (CD) according to Stefan [5, 10], which they received for 30 days.

The animals were represented by the following groups of 10 rats each:

– Intact group;

– CD;

– CD+ Cinkteral (ZnSO4);
– CD+hydroxyapatite;
– CD+ Osteomag (D3, Ca, Mg, Cu, Zn) (360 mg/kg).

The intact group of rats received a normal vivarium diet.

The third group of rats, in addition to reproducing the caries model, received the drug "Cinkteral" (Teva Pharmaceutical Industries, Poland) at a dose of 25 mg/kg for 30 days.

The fourth group of rats in addition to CD received hydroxyapatite at a dose of 300 mg/kg for 30 days.

The fifth group of rats received Osteomag (Unipharm, United States of America) at a dose of 360 mg/kg for 30 days in addition to CD.

The duration of the experiment was 30 days. The animals were withdrawn from the experiment under thiopental anesthesia (40 mg/kg), and the jaws were dissected. Dissected fragments of jaw bone tissue were fixed in neutral 10 % formalin, decalcified, and embedded in paraffin. Sections were stained with hematoxylin and eosin [1].

Experimental studies were conducted at the Laboratory of Biochemistry and Vivarium of the SE "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine" (SE "ISMFS NAMS"). All experiments on rats were conducted according to standard operating procedures approved by SE "ISMFS NAMS", developed in accordance with the Guidelines of the Pharmacological Committee of the Ministry of Health of Ukraine and the International Regulations for the Use of Laboratory Animals [3, 7].

The results were processed by variational statistical methods of analysis using the Microsoft Office Excel 2016 software [4].

Results of the study and their discussion. One month after the beginning of the experiment, the animals on cariesogenic diet showed significant signs of bone destruction of alveolar processes. Microscopic examination revealed areas of bone tissue decalcification, where basophilic bone reaction was observed in the form of striations with indistinct edges. The number of osteocytes in these areas was significantly reduced (Fig. 1, a). The remaining osteocytes increased in size due to swelling and vacuolization of their cytoplasm. Bone marrow islets were found between the bone plates, although marrow was sometimes absent. Small islands of irregularly shaped unformed connective tissue were also found between the bone plates, slightly infiltrated with lymphoid elements.

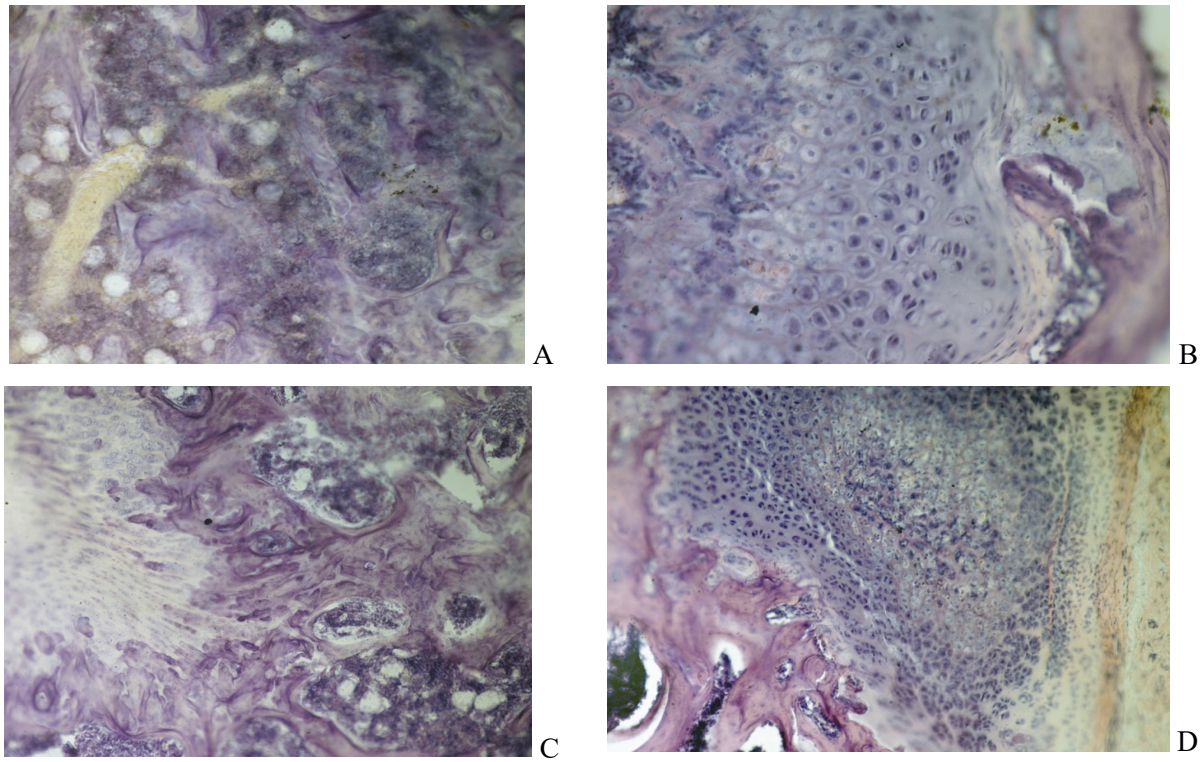


Fig. 1. Morphological changes in bone and cartilage tissues of rats on cariesogenic diet. A – destruction of bone tissue, accompanied by its basophilia, decrease in the number of osteocytes. Hematoxylin-eosin. X 280. B – formation of cartilage tissue in the place of damage to bone plates of alveolar processes of rats on cariesogenic diet. Hematoxylin-eosin. X 280. C – cartilage tissue formation at the site of damage to the bone plates of alveolar processes of rats on a cariesogenic diet. Hematoxylin-eosin. X 280. D – site of bone tissue damage in a rat on a cariesogenic diet. Hematoxylin-eosin. X 140.

By the same time, signs of regeneration were also seen, manifested by the formation of a relatively thick layer of cartilage tissue. This cartilage tissue consisted of large cells with round or oval nuclei containing a dense round nucleus and cytoplasm showing weak basophilia. The cells were tightly adherent to each other (Fig. 1, b). In the immediate vicinity of the cartilage tissue there was an already formed narrow plane of dense fibrous connective tissue containing fragments of destructively altered bone tissue. Inflammatory infiltration of this tissue was not observed. A small number of blood vessels of different caliber was detected near the bundles of fibrous tissue and in the tissue itself.

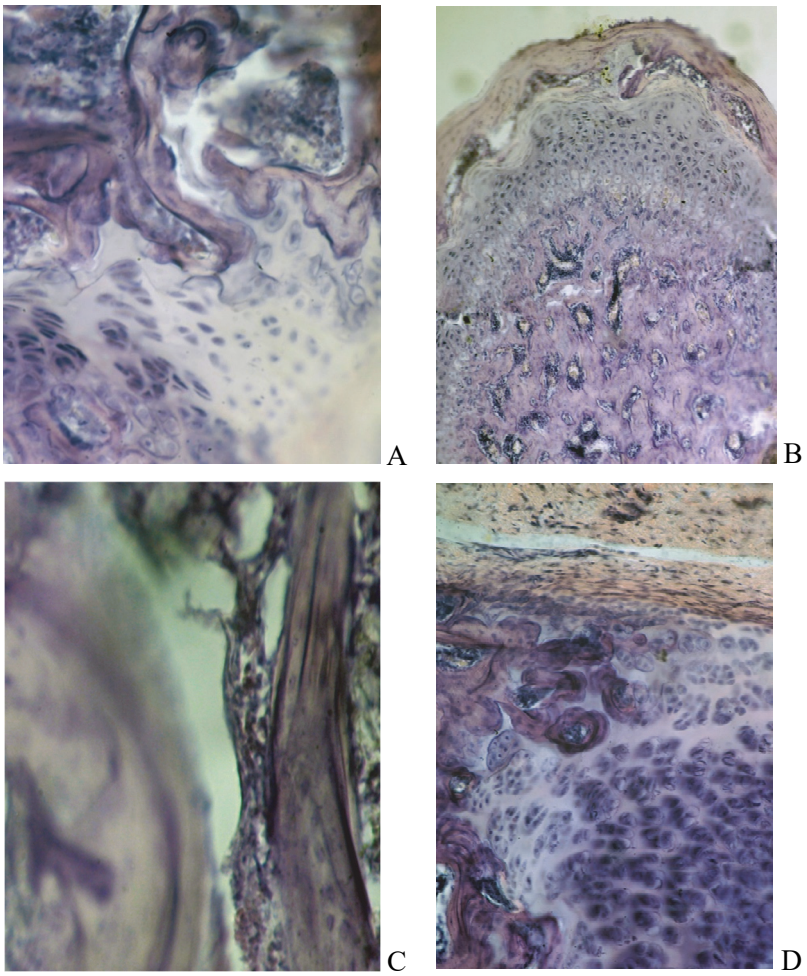


Fig. 2. The site of bone tissue damage in a rat after placing "Osteomag" in the well. A – a small island of cartilaginous tissue with ossification and bone formation phenomena. Hematoxylin-eosin. X 280. B – a narrow strip of cartilaginous tissue with ossification and bone formation phenomena. Intensive vascularization of the tissue located on the border of bone and cartilage tissue. High activity of osteoblasts osteoblasts located in the cortex. Hematoxylin-eosin. X 140. C – Formation of cartilage tissue at the site of damage to the bone plates of alveolar processes of rats on a keriessogenic diet. Hematoxylin-eosin. X 280. D – a small area of preserved cartilage tissue with pronounced phenomena of bone formation as a result of differentiation of chondrocytes. Hematoxylin-eosin. X 280.

The presence of cartilage tissue is accompanied by the activity of osteogenic cells located in the bone marrow. This activity is most pronounced near islets of cartilage tissue (Fig. 1, c).

Vacuolated degeneration phenomena with subsequent disintegration of cartilage sections located in the central areas are noted in some places (Fig. 1, d). Apparently, this is associated with insufficient blood supply to the central areas.

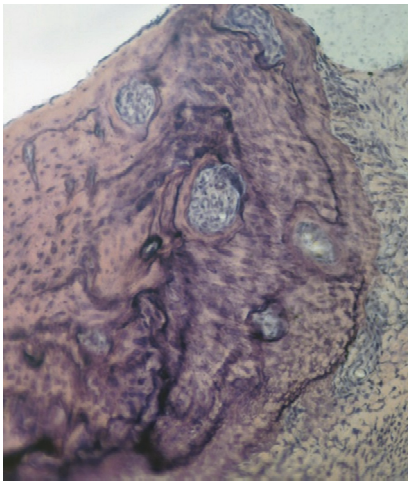
The application of "Cinkteral" results in a different regeneration pattern in the dental cavity. Microscopic examination confirms the presence of a narrower band of cartilage in the regeneration zone without signs of degeneration or necrosis. Characteristically, the cartilage tissue demonstrates good differentiation, consisting of columns of chondrocytes surrounded by a homogeneous basophilic substance. At the border with bone tissue, an even distribution of chondrocytes in two to three layers parallel to the bone tissue is observed, as well as their differentiation towards osteocytes. In this area, the formation of bone plates is noticed, most of which are already well differentiated, as demonstrated by the figures (Fig. 2, a, b). In some areas, the absence of cartilage is accompanied by complete bone regeneration. The cavity is filled with dense fibrous tissue without signs of inflammation, as seen in the figure (Fig. 2, c).

During this time interval, the osteogenic activity of bone marrow cells is preserved near the areas of the most intense destruction of bone tissue, promoting regeneration. The interalveolar spaces are filled with bone marrow, and there are no signs of inflammation in the surrounding tissues.

Microscopic examination of the areas of destruction of alveolar processes after "Osteomag" injection revealed no significant qualitative or quantitative differences in the regeneration processes compared to animals from the previous experimental group. One month after the beginning of the experiment, the process of bone tissue regeneration is not yet complete, but is at late stages of development, as confirmed by the detection of small areas of cartilaginous tissue near the bone plates (Fig. 2, d).

At the same time, the rest of the regeneration process was completed with the formation of bone plates of normal structure (Fig. 3, a, b). The interbulbar spaces are filled with bone marrow. Mature bone plates are joined by dense unformed well-vascularized connective tissue without signs of inflammation.

The morphological features of the regeneration processes in the animals that were injected with hydroxyapatite into the well are generally similar to those observed in the animals from the previous groups of experiments. However, it should be noted that in these animals a wider cartilage tissue lamina was detected. This may indicate a slower rate of regeneration compared to animals in the previous groups where a different compound was used for injection into the well. This difference indicates a possible influence of hydroxyapatite on the speed and character of bone tissue regeneration processes.


A

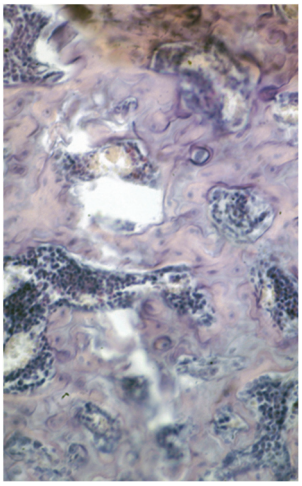

B

Fig.3. Introduction of "Osteomag" into the well. A – Mature bone tissue is identified, to which dense, unformed, well-vascularized connective tissue without signs of inflammation is adjacent. Hematoxylin-eosin. X 280. B – Mature bone tissue. The interbulbar spaces are filled with bone marrow. Hematoxylin-eosin. X 280.

One month after the beginning of the experiment, the animals receiving cariesogenic diet with hydroxyapatite added to the well showed a noticeable destruction of bone plates of alveolar processes. Areas of bone tissue decalcification and its basophilic changes were detected during this period. A state of edema and vacuolar degeneration of osteocytes was observed, similar to animals on a cariesogenic diet. The presence of significant areas of loose fibrous connective tissue permeated with lymphoid elements attracts special attention. By the indicated time, a prominent layer of cartilaginous tissue with a typical structure had formed between the bone tissue and the loose fibrous tissue, as shown in the figure (Fig. 4). The cytoplasm of cells in this area is characterized by weak basophilicity. A significant number of arterial and venous vessels of various diameters were found near the bundles of fibrous tissue and in the tissue itself. The activity of osteogenic cells in the bone marrow is preserved during the whole studied period of time.

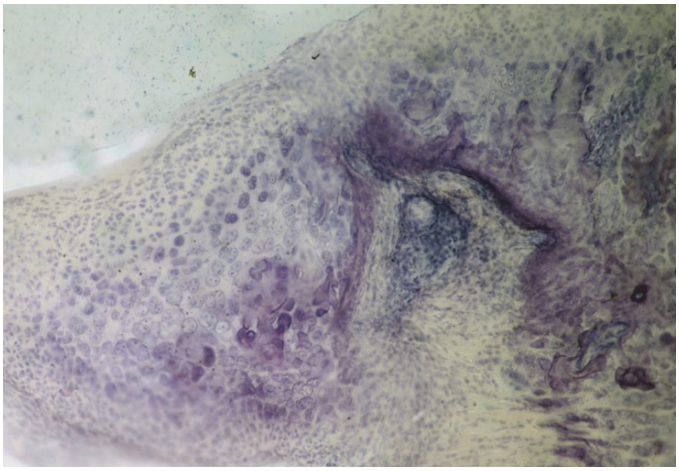


Fig. 4. Injection of hydroxyapatite into the well between the bone tissue and loose fibrous tissue reveals a rather thick layer of cartilage tissue. Hematoxylin-eosin. X 280.

It should be noted that the described character of changes is not determined throughout. In some places the regeneration process is completed and bone tissue of normal structure is formed.

The morphological studies demonstrate that the process of bone tissue regeneration of alveolar processes in rats on cariesogenic diet proceeds through an intermediate stage of cartilage tissue formation, which is consistent with the generally accepted ideas about the mechanisms of osteogenesis [6]. Our results show that the most effective in stimulating bone tissue regeneration were the preparations "Cinkteral" and "Osteomag", which confirms the data of previous studies on the importance of trace elements and minerals in the processes of bone regeneration [12]. Comparison with the results of other studies shows that the process of bone tissue regeneration can vary significantly depending on the drugs used and experimental conditions. For example, a study conducted by Gontar indicates that the use of polylactide and tricalcium phosphate in combination with mesenchymal stem cells can accelerate the process of bone tissue regeneration [9]. At the same time, our results show that the use of hydroxyapatite is less effective, which may be due to the slowing down of the processes of vascular network formation, as noted in the study by Zhang et al. [1]. It is especially important to note that in our study no significant differences in the degree of regeneration were observed when using "Cinkteral" and "Osteomag", indicating their similar efficacy. This is important for practical application in dentistry, especially in pediatric practice, where the choice of safe and effective treatment methods is of prime importance. Thus, the results of our study emphasize the importance of further research in the field of osteoregeneration and the development of new

osteotropic drugs to improve the dental health of children. This is particularly relevant for the development of comprehensive approaches in the treatment of diseases associated with osteogenesis disorders and may contribute to the improvement of patients' quality of life.

Conclusions

1. Morphological studies of the influence of various osteotropic preparations allow us to conclude that in the experiment on the model of dental caries it was proved that the most "qualitative" regeneration processes develop when such preparations as "Cinkteral" and "Osteomag" are introduced into the alveolar well after trauma.

2. One month after the beginning of the experiment, complete regeneration of bone plates is noted practically throughout the damaged bone tissue, and only in some places cartilage tissue areas in the form of narrow strips or small islands are preserved.

3. The conducted experimental study on animals allowed us to study the effectiveness of step-by-step application of the composition of osteotropic preparations on the processes of local bone tissue resorption. These studies should be taken into account when developing therapeutic and prophylactic complexes for children with osteogenesis disorders.

References

1. Horalskyy LP, Khomych VT, Kononskyy OI. Osnovy histolohichnoyi tekhniky i morfofunktsionalni metody doslidzhen u normi ta pry patolohiyi: navchalnyy posibnyk. Vyd. 3-ye, vypr. i dopov. Zhytomyr: Polissya, 2015;286. [in Ukrainian].

2. Yelinska AM, Starchenko II, Kostenko VO. Vplyv modulyatoriv redokschutlyvykh transkryptsiynykh chynnykiv na patomorfolohichni zminy parodonta shchuriv za umov systemnoyi zapalnoyi vidpovidi. Aktualni problemy suchasnoyi medytsyny: Visnyk Ukrayinskoyi medychnoyi stomatolohichnoyi akademiyi. 2019;3(63):127–132. doi: 10.31718/2077-1096.19.3.127 [in Ukrainian].

3. Nakaz Ukrayiny "Pro zatverdzhennya Poryadku provedennya naukovymy ustanovamy doslidiv, eksperymentiv na tvarynakh". Ministerstvo osvity i nauky Ukrayiny. 2012;249. [in Ukrainian].

4. Rohach IM, Keretsman AO, Sitkar AD. Pravylno vybranyy metod statystychnoho analizu – shlyakh do yakisnoyi interpretatsiyi danykh medychnykh doslidzhen. Naukovyy visnyk Uzhhorodskoho universytetu, seriya "Medytsyna". 2017;2(56):124–128. [in Ukrainian].

5. Shnayder SA, Levitskiy AP. Eksperymentalna stomatologiya. Pt.I. Eksperymentalni modeli stomatologichnykh zakhvoriuvan. Odesa: KP «Odeska miska drukarnya». 2017:167. [in Ukrainian].

6. Ashukina N, Vorontsov P, Maltseva V, Danyshchuk Z, Nikolchenko O, Samoylova K, Husak V. Morphology of the repair of critical size bone defects which filling allogeneic bone implants in combination with mesenchymal stem cells depending on the recipient age in the experiment. Orthopaedics traumatology and prosthetics. 2023;3-4:80–90. doi: 10.15674/0030-598720223-480-90.

7. European convention for the protection of vertebrale animals used for experimental and other scientific purposes. Strasburg.Council of Europe. 1986; 123:51.

8. Fu D, Qin K, Yang S, Lu J, Lian H, Zhao D. Proper mechanical stress promotes femoral head recovery from steroid-induced osteonecrosis in rats through the OPG/RANK/RANKL system. BMC Musculoskelet Disord. 2020;21(1):281. doi: 10.1186/s12891-020-03301-6.

9. Gontar N. Changes in markers of collagen metabolism in the blood serum of white rats during the filling of femur defects with implants based on polylactide and tricalcium phosphate with allogeneous mesenchymal stem cells. Reports of Morphology. 2023;29:12–19. doi: 10.31393/morphology-journal-2023-29(3)-02.

10. Ivanov VS, Tkachenko YeK, Dienha OV, Schnayder SA, Pyndus TO. Correction by the preparation of plant polyphenols of metabolic changes of tissues of rats oral cavity under conditions of intrauterine hypoxia and cariogenic diet. World of Medicine and Biology. 2021;3(77):214–219. doi: 10.26724/2079-8334-2021-3-77-214-219.

11. Li M, Bai Y, Li M, Zhou J. Performance evaluation of two antigen-extracted xenogeneic ostein and experimental study on repairing skull defects in rats. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi. 2021;35(10):1303–1310. doi: 10.7507/1002-1892.202103177.

12. Tarafder S, Bose S. Polycaprolactone-coated 3D printed tricalcium phosphate scaffolds for bone tissue engineering: in vitro alendronate release behavior and local delivery effect on in vivo osteogenesis. ACS Appl Mater Interfaces. 2014;6(13):9955–65. doi: 10.1021/am501048n.

13. Vishnevskaya AA, Schnayder SA, Reyzvikh OE, Babenya AA, Khrystova MT. Morphological assessment of the regeneration of the periodontal ligamentum in the treatment of generalized periodontitis with plasmogel from plately autoplasma. World of Medicine and Biology. 2020;4(74):174–179. doi: 10.26724/2079-8334-2020-4-74-174-179

14. Zhang D, Zong X, Guo X, Du H, Song G, Jin X. Influence of different sintering temperatures on mesoporous structure and ectopic osteogenesis of biphasic calcium phosphate ceramic granule materials. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi. 2021;35(1):95–103.doi: 10.7507/1002-1892.202007074.

Стаття надійшла 17.03.2023 р.