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Dynamics of regeneration following transplantation of octacalcium phosphate into an experimental defect in the rabbit mandibular bone: electron microscopic and morphometric study

I. V. Chelpanova*

Danylo Halytsky Lviv National Medical University, Ukraine

Keywords: mandible, dentoalveolar apparatus, bone tissue, bone regeneration, osteoplastic materials, morphometric study, scanning electron microscopy, rabbits.

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***E-mail:** ilona.med75@gmail.com

The relevance of this research lies in the in-depth exploration of bone tissue remodeling dynamics following the creation of a defect and its filling with osteoplastic material. Despite the known results of osteoplastic materials application in clinical practice, complete and high-quality regeneration of the maxillofacial bone, its mechanisms and dynamics remain incompletely understood, requiring further clarification and detailed study.

Aim. The aim of this study is to determine the dynamics of histoarchitectural changes in the bone-ceramic regenerate after transplantation of octacalcium phosphate into an experimental defect in the rabbit mandible.

Materials and methods. Adult male rabbits aged 6–7 months and weighing 2.5–3.0 kg were used for the study. The control group consisted of animals with a bone defect that healed under a blood clot. The experimental group consisted of rabbits in which the bone defect was filled with an osteotropic material containing octacalcium phosphate. Post-traumatic bone tissue status within the defect area was monitored for 84 days. Ultrastructural changes were studied using transmission and scanning electron microscopy. To determine changes in the regenerate composition, three parameters were counted. The data was analyzed using the Student's t-test, and a difference at $p < 0.05$ was considered statistically significant.

Results. Studying the surface relief characteristics of the experimental bone defect in the lower jaw after implantation of the octacalcium phosphate material revealed numerous regenerative changes that occurred after the injury and correlated with the dynamics of changes in the relative area of bone tissue, osteoplastic material, and connective tissue in the regenerate. Morphometric analysis of the relative area of the regenerative components of the experimental defect established a phased nature of the dynamics of the studied changes. It was established that the osteocytic lacunar-canalicular system formed after implantation of the material acquired features of typical structure. Foci of incomplete osteogenesis were not visualized. Unlike control animals, in the zone of the outer bone plate after application of the octacalcium phosphate material, the osteons of the regenerate in their structure and geometry did not differ from the typical structure of the maternal bone.

Conclusions. It was established that in the experimental group of animals where the defect was filled with octacalcium phosphate material, a regular increase in the relative area of bone tissue in the regenerate was observed, which in terms of dynamics was similar to that in the control group, however, in terms of intensity of changes it significantly differed from the control, approaching the norm in terms of the studied indicators.

Ключові слова: нижня щелепа, зубощелепний апарат, кісткова тканина, регенерація кісткової тканини, остеопластичні матеріали, морфометричне дослідження, сканувальна електронна мікроскопія, кролики.

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Динаміка регенерації після трансплантації октакальційфосфату в експериментальний дефект нижньої щелепи кролика: електронномікроскопічне та морфометричне дослідження

І. В. Челпанова

Актуальним залишається вивчення динаміки перебудови кісткової тканини після нанесення дефекту та заповнення його порожнини остеопластичним матеріалом. Незважаючи на вже відомі результати застосування остеопластичних матеріалів у клінічній практиці, повна та якісна регенерація кісток щелепно-лицевої ділянки, її механізми та динаміка залишаються остаточно не з'ясованими, потребують уточнення і деталізації.

Мета роботи – визначити динаміку гістоархітектурних перебудов кістково-керамічного регенерату після трансплантації октакальційфосфату в експериментальний дефект нижньої щелепи кролика.

Матеріали і методи. Дослідження здійснили на статевозрілих кроликах-самцях віком 6–7 місяців, маса тіла – 2,5–3,0 кг. До контрольної групи увійшли тварини з дефектом кісткової тканини, що загоювався під кров'яним згустком. До експериментальної групи включили кроликів, у яких кістковий дефект заповнювали остеотропним матеріалом з октакальційфосфатом. Контроль посттравматичного стану кісткової тканини в ділянці дефекту здійснювали впродовж 84 діб. Ультраструктурні зміни вивчали з використанням трансмісійної та сканувальної електронної мікроскопії. Для визначення змін складу регенерату визначали три параметри. Дані проаналізували за допомогою t-критерію Стьюдента, різницю при $p < 0,05$ визначили як статистично значущу.

Результати. У результаті дослідження особливостей рельєфу поверхні експериментального кісткового дефекту нижньої щелепи після імплантації матеріалу з октакальційфосфатом виявили численні регенераційні зміни, що відбулися після нанесення травми та корелювали з динамікою змін відносного об'єму кісткової тканини, остеопластичного матеріалу та сполучної тканини в регенераті. Морфометричне вивчення відносного об'єму компонентів регенерату експериментального дефекту дало змогу зробити висновок про фазовий характер динаміки змін. Встановлено, що остеоцитарна лакуно-канальцева система, що формувалася після імплантації матеріалу, набувала ознак типової будови. Осередки незавершеного остеоґенезу не візуалізовані. На відміну від контрольних тварин, у зоні зовнішньої кісткової пластинки

після застосування матеріалу з октакальційфосфатом остеони регенерату за структурою та геометрією не відрізнялися від типової будови материнської кістки.

Висновки. В експериментальній групі тварин, у яких пластику дефекту здійснили, використавши матеріал з октакальційфосфатом, визначили закономірне збільшення відносного об'єму кісткової тканини в регенераті, що за напрямом динаміки схоже на зміни, зафіксовані в контрольній групі, але за інтенсивністю процесів значно відрізнялося та наближалося до норми.

One of the key challenges in modern dentistry and traumatology is the development of effective methods for bone tissue restoration [1]. Jaw defects arising from infections, trauma, tumors, or congenital anomalies are common. Although bone has an inherent ability to regenerate, large defects, pathological fractures, or infections can hinder the healing process. Literature review, published by M. P. Ferraz, indicates that surgical intervention using bone substitutes is necessary in such cases [2]. Bone grafting is a common method for treating bone defects. The choice of material for bone grafting depends on many factors such as availability, defect size, biomechanical properties, ease of handling, cost, ethical aspects, biological properties, and potential complications [3]. For large defects, autologous bone taken from the iliac crest is most often used. This material is considered the "gold standard" for bone grafting because it contains osteogenic cells that can create new bone, and its structure serves as a natural scaffold. However, this method has its drawbacks [3,4,5].

Alternative bone materials are becoming increasingly popular for treating small and medium-sized bone defects, as they help to reduce the risk of complications. However, for effective use, such materials must be able to integrate with the bone surface without the formation of fibrous tissue. To achieve optimal results, bone graft materials must meet certain criteria. They must stimulate osteogenesis without eliciting an immune response. In addition, the materials should promote rapid revascularization, stimulate osteoinduction and osteoconduction, which ultimately will lead to replacement of the graft with bone tissue that matches the quality and quantity of the host organism's tissues [3,5].

Materials of animal origin, known as xenogenic materials or xenografts, are also widely used. They have been investigated for over thirty years and are known for their osteoconductive properties, which are due to their inorganic structure, which is primarily composed of hydroxyapatite [6]. After removal of the organic components, a scaffold remains that mimics the structure of human bone and promotes bone regeneration. Xenogenic materials are favorably distinguished from other materials by their chemical structure, which is very close to the structure of human bone. In particular, the calcium to phosphate ratio in xenogenic materials (1.67) exactly matches this indicator in human bone, making them ideal for transplantation [7].

Such similarity, especially in materials from bovine bone, such as Bio-Oss®, significantly improves their ability to stimulate bone tissue regeneration. Octacalcium phosphate is among the most commonly used materials for filling bone defects. It rapidly degrades in the body, and its structure is easily replaced by natural bone tissue [8]. The advanced crystalline material octacalcium phosphate promotes factors that stimulate bone regeneration [9]. It has long been one of the most popular and frequently used xenogenic materials, especially in dentistry [10].

Aim

The aim of this study is to determine the dynamics of histomorphological changes in the bone-ceramic regenerate after transplantation of octacalcium phosphate into an experimental defect in the rabbit mandible.

Materials and methods

The study was conducted on 45 adult male rabbits aged 6–7 months, weighing 2.5–3.0 kg. The animals were divided into control and experimental groups (20 animals in each). An additional 5 intact animals were used to study the normal structure of bone tissue in the studied area of the lower jaw.

Under general anesthesia, by intraperitoneal injection of Thiopenate ("Brofarma", Ukraine), at a dose of 25 mg/kg of body weight, the animals in the control and experimental groups were subjected to a bone-destroying injury, a cavity 4 mm deep and 3 mm wide at the level of the toothless area of the mandibular part of the lower jaw using a dental burr.

The control group consisted of animals with a bone defect that healed under a blood clot. The experimental group consisted of rabbits in which the bone defect was filled with CompactBoneB osteotropic material (Dentegris, Germany) – a natural bovine bone substitute, the main crystalline material of which is native octacalcium phosphate (OCP-N). The study of the condition of bone tissue in the area of the inflicted defect was performed at 1, 7, 14, 21, 28, 35, 56 and 84 days after the injury was inflicted.

The dynamics of histomorphological remodeling of bone tissue in the area of the experimental defect in the lower jaw were studied using scanning electron microscopy, performed on a JEOL T220A scanning electron microscope at the Laboratory of Physical Research Methods in Geology, Ivan Franko Lviv National University. Samples for study were extracted by breaking from different areas at the interface between the regenerate and the maternal bone. For obtaining micrographs of fragments of the lower jaw, samples were fixed in a 2.0 % solution of glutaraldehyde and washed three times (10 min each) with distilled water. After washing, a sublimation drying procedure was carried out, with freezing of the wet samples and their subsequent placement in a vacuum chamber for sublimation of moisture for 1.5–2.0 hours until complete evaporation. Dried tissue samples were glued to cylindrical copper holders, after which their surface was metallized by thermal deposition of a thin layer of copper (up to 20 nm). Spraying was carried out in a VUP-5 sputterer. The surfaces of the samples were photographed on ×15–200 magnification, the accelerating voltage in all experiments was 20 kV [11].

For ultrastructural study, samples of the mandible bone were fixed at a temperature of +2 °C for 3–4 hours in

a 2.5 % glutaraldehyde solution in a 0.2 M phosphate buffer (pH = 7.4) followed by postfixation for 1 hour in a 1 % buffered (pH = 7.4) solution of osmium tetroxide ("SPI", USA), dehydration in alcohols of increasing concentration and propylene oxide, and production of epoxy blocks using an epon-araldite composition. Ultrathin sections placed on Mesh Regular Grid 200 ("SPI", USA) copper reference grids. Double contrast was performed according to the Reynolds method. The study was performed using transmission electron microscope PEM-100-01 ("SELMI", Ukraine) at an acceleration voltage of 75 kV and primary magnifications from 1500 to 25000 according to the standard scheme [12].

For the preparation of histological slides, fragments of the mandible bone with osteotropic materials were fixed in freshly prepared 10 % formalin solution, demineralized in a 10 % aqueous solution of nitric acid (HNO_3), which allowed for the maximum preservation of the regenerate structures. After this, the bone fragments were passed through ascending concentrations of alcohols followed by embedding in paraffin. Sections with a thickness of 5–7 μm were made from the obtained paraffin blocks using a microtome type MS-2 TU-64-1-1629-78. The obtained sections were placed on glass slides, deparaffinized and stained with hematoxylin and eosin according to a standard method [13], visualized using a UlabXSP-137TLED light microscope (China) and photographed with an XCAM-1080 P camera (China). To determine changes in the composition of the regenerate, the relative area of bone tissue, osteoplastic material, connective tissue in the regenerate, which were determined on demineralized histological sections of fragments of the lower jaw, stained with hematoxylin and eosin, were counted. The calculation of the relative area value was performed using the ImageJ 1.47v software package and the built-in Analyze Particles procedure for final segmentation and calculation of values (Analyze > Analyze Particles) according to the recommendations [14].

To determine the conformity of the obtained data to the normal distribution law, an analysis of the distribution histogram, asymmetry indicators and extinction coefficients and the Shapiro–Wilk test were used. The results of each group at different time points followed a normal distribution law, and are presented in the form of M ± m, where M – is the arithmetic mean, m – is the standard deviation of the mean. To determine the probable differences between the average values of the indicators at different times of the experiment, as well as to compare the data of the control group with the experimental group at the same observation times, the Student's t-test was used. The difference between the groups was considered significant at $p < 0.05$. To perform statistical calculations, create graphs and tables, the RStudio v1.2.5042 software was used, as well as Excel spreadsheets from the MS Office 2010 package using the licensed Statistica program (version 6.1, serial No. AGAR909E415822FA). The sequence of statistical procedures was performed in accordance with the recommendations [15].

All animals were housed in a vivarium and procedures for cleaning, inspection, marking and all other manipulations were carried out in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985), the "General Ethical Principles of Experiments on Animals" adopted by the First National Congress on Bioethics (Kyiv, 2001), the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Treatment" in accordance with the Directive of the Council of the European Union 2010/63/EU on compliance with the regulations, laws, and administrative provisions of the EU Member States on the protection of animals used for scientific purposes [16,17].

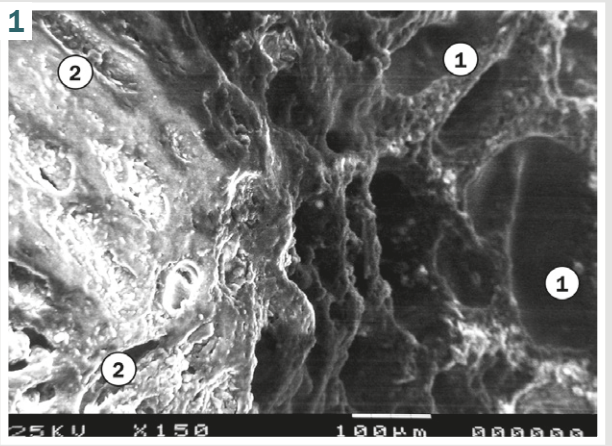
Results

The study of the topographic features of the bony surface in the area of the experimental defect of the lower jaw in the control group animals, which was carried out using scanning electron microscopy, revealed a number of characteristic changes that occurred during wound healing. In particular, 1 day after the formation of the experimental trepanation hole, numerous damaged microvessels were determined on the smooth surface of the compact cortical plate defect, containing conglomerates of blood cells in their lumen surrounded by small-mesh hemorrhages *(Fig. 1)*. Between the bony trabeculae and the damaged spicules of the cancellous bone of the alveolar process at the edges of the cavity, a moderate amount of small polymorphic fragments was observed along with perivascular accumulations of erythrocytes and a significant number of leukocytes. In the tissue of the maternal bone adjacent to the defect, there were expressed signs of perivascular and intracellular edema.

During the first 3 weeks of the experiment, numerous osteoid mounds were formed on the surface of the tissue of injured osteons and bone trabeculae, varying in size and shape. Perivascular edema, leukocyte infiltration, and other signs of inflammation in the injury zone were reduced. Fibroblast-like cells were located in a moderate amount diffusely in the space of small foci of intramembranous ossification in the peripheral areas of the regenerate and were found singly in deep areas of post-traumatic detritus that filled the experimental cavity.

Four and five weeks after the experimental injury, numerous polymorphic osteoid trabeculae were visualized on the inner surface of the defect, with predominantly radial orientation and dense anastomosing. In the deep areas of the experimental defect, the formation of islands of newly formed primary woven bone tissue surrounded by a dense network of primitive blood microvessels was observed. Osteoblasts identified by transmission electron microscopy were typically grouped in the spaces between osteogenic islands and hemocapillaries *(Fig. 2)*, indicating their active migration to deeply located foci of desmal osteogenesis, where a significant number of fibroblast-like cells were observed. In the peripheral newly formed trabeculae, the regenerate contained single fibroblasts with a significant predominance of osteoblasts and the appearance of single primary osteocytes.

The microrelief of the surface of the newly formed trabeculae had a hilly appearance and was characterized by an expressed variability *(Fig. 3)*. At the border of the bone regenerate with the lamellar bone tissue of the maternal bone, small foci of membrane osteogenesis were



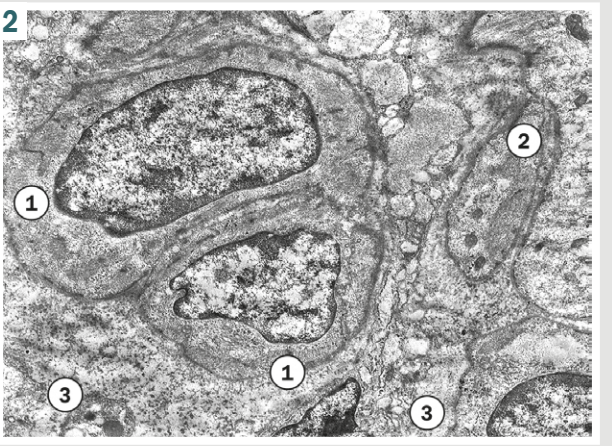
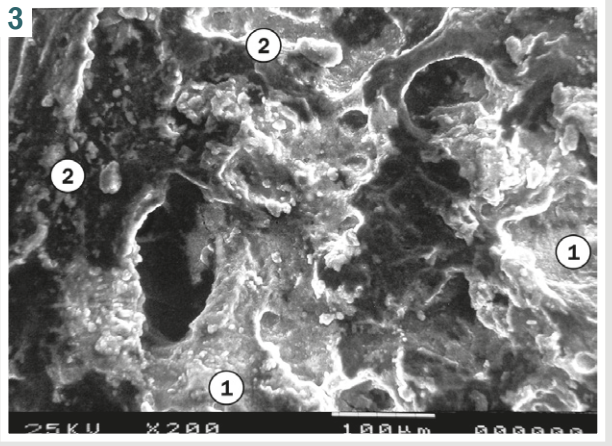


Fig. 1. Scanning electron micrograph of the bone defect area of the rabbit mandible in the control group 1 day after the injury. ×150. **1:** damaged surface of the outer bone plate with hemorrhages; **2:** deformed bone marrow spaces between the trabeculae of the cancellous bone of the interdental area of the alveolar part.

Fig. 2. Transmission electron micrograph of the bone defect area of the rabbit mandible in the control group 4 weeks after the injury. ×3000. **1:** osteoblast; **2:** poorly differentiated osteogenic cell; **3:** extracellular matrix.



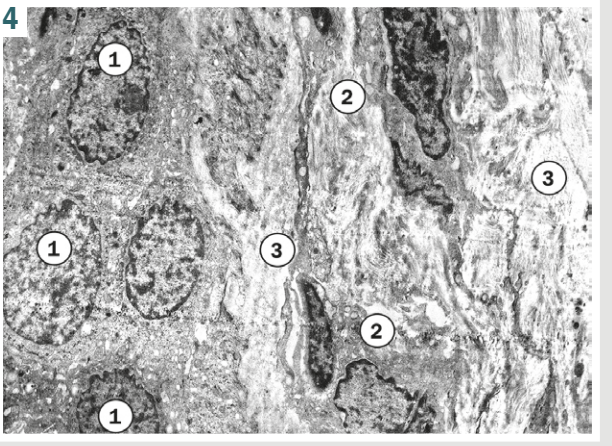
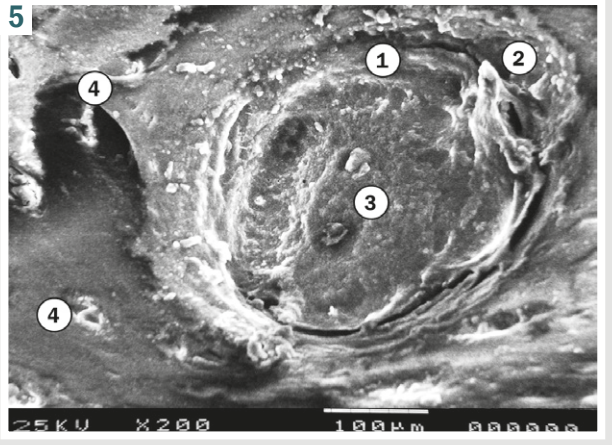


Fig. 3. Scanning electron micrograph of the bone defect area of the rabbit mandible in the control group 4 weeks after the injury. ×200. **1:** trabecula on the periphery of the regenerate; **2:** primary osteoblast on the surface of the trabecula in the focus of desmal osteogenesis.

Fig. 4. Transmission electron micrograph of the bone defect area of the rabbit mandible in the control group 4 weeks after the injury. ×3000. **1:** osteoclast nuclei; **2:** fibroblast and its processes; **3:** locus of desmal osteogenesis.



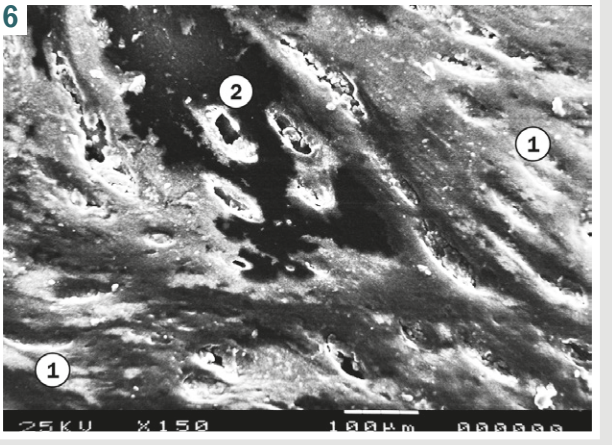


Fig. 5. Scanning electron micrograph of the bone defect area of the rabbit mandible in the control group 5 weeks after the injury. ×200. **1:** area of bioresorption on the surface of the newly formed bone trabecula; **2:** osteoclast; **3:** osteoblast; **4:** primitive hemocapillary in the osteoid of the regenerate.

Fig. 6. Scanning electron micrograph of the bone defect area of the rabbit mandible 1 day after the injury and filling of the defect with OCP-N material. ×150. **1:** injured surface of the outer bone plate with damaged microvessels; **2:** focus of perivascular edema between the deformed plates of the interdental area of the alveolar part.

observed with a significant numerical predominance of osteoblasts over osteocytes.

Osteoclasts were found in greater numbers than in the first 3 weeks of observation, were mostly compact oval in shape with sizes up to 70 μm. Under transmission electron microscopy, osteoclasts contained several nuclei with signs of moderate activity and were in direct contact with foci of desmal osteogenesis *(Fig. 4)*. Some of the activated osteoclasts were visualized on the surface of bone trabeculae, which indicated the beginning of bioresorption processes in the regenerate *(Fig. 5)*.

Eight weeks into the experiment, numerous polymorphic trabeculae on the periphery of the defect were joined with islands of osteogenesis in the deep areas of the regenerate, forming a continuous cancellous structure. Significant variability in the microrelief of the surface of bone trabeculae indicated active processes of osteogenesis of cancellous bone to fill the space of the experimental defect. Along with the presence of a dense network of hemocapillaries, osteoblasts didn't form groups and were located singly. Unlike the previous term of the study, a significant number of osteoclasts were found, indicating intensive remodeling of the extracellular matrix of the tissue in the regeneration zone. Even more significant resorptive activity of osteoclasts accompanied the compaction of the cortical plate of the bone regenerate. At the border of the regenerate with the lamellar bone tissue of the maternal bone, numerous foci of remodeling of primary woven bone tissue with the formation of primitive osteons were observed.

Twelve weeks after the experimental injury, the surface relief of most trabeculae of the cancellous bone of the alveolar process was noticeably smoothed compared to the previous study period. The number of osteogenic cells and osteoclasts in the regenerate, including at the border of the experimental defect with the maternal bone, was lower than at the 8th week of observation, but significantly exceeded the zones of bone that were not injured. Along with signs of active formation of the osteocyte lacunar-canalicular system, the microarchitecture of the osteons of the regenerate differed significantly from the typical structure, but completely restored the space of the experimental defect. Foci of incomplete osteogenesis were not observed.

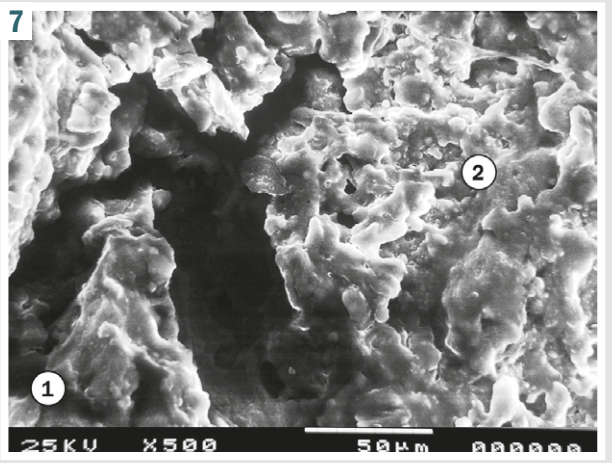
The study of the surface relief features of the experimental bone defect of the lower jaw after implantation of OCP-N material in animals revealed numerous regenerative reconstructions that occurred after the injury. In particular, 1 day after the experimental intervention, damaged blood microvessels with blood clots in the lumen were observed on the smooth surface of the trepanation cavity in the compact cortical plate. Small hemorrhages were visualized around the vessels. Between the fractured trabeculae and spicules of cancellous bone tissue at the edges of the defect of the maternal bone, a moderate number of small fragments of different shapes were determined along with perivascular accumulations of blood cells. Signs of perivascular and intracellular edema in the tissue structures located near the experimental defect were expressed *(Fig. 6)*. Significant accumulation of exudate was also observed between the fragments of the implanted OCP-N material, especially at the implant edges.

During the first 3 weeks of the experiment, intensive formation of numerous osteoid hillocks and outgrowths occurred on the surface of the tissue of damaged osteons and bone trabeculae of the maternal bone, varying in size, shape, and almost completely covering the surface of the trabeculae. Perivascular edema, leukocyte infiltration, and other signs of inflammation in the defect zone were reduced during the first two weeks after intervention and were not detected after 3 weeks of the experiment. In the composition of numerous small foci of desmal osteogenesis, fibroblast-like cells were located diffusely and, unlike the control group, were found in large numbers. In the peripheral areas of the regenerate, newly formed trabeculae between fragments of the implanted OCP-N material merged with the hilly trabeculae of the maternal cancellous bone. In the deep areas of the regenerate, a moderate number of blood microvessels were determined surrounded by active fibroblasts and dense spicule-like connective tissue outgrowths.

Four and five weeks after the implantation of OCP-N material, polymorphic osteoid trabeculae with predominantly radial orientation and dense anastomosing were observed on the periphery of the regenerate. Individual small fragments of osteotropic material were rarely found. In the deep zones of the experimental defect, significant areas of primary woven bone tissue were found, in the thickness of which a dense network of primitive blood microvessels and loosely located remnants of resorbed OCP-N material were located. A large number of osteoblasts formed numerous clusters between osteogenic islands and hemocapillaries. In a few small areas of the deep zone of the regenerate around the particles of the implant, foci of desmal osteogenesis with a significant number of fibroblast-like cells were observed. In the peripheral newly formed trabeculae between individual small remnants of the material, osteoblasts outnumbered, but a moderate number of primary osteocytes significantly exceeded the content of these cells in the study of bone samples of the control group. An expressed heteromorphic bumpiness was visualized on the surface of most newly formed trabeculae *(Fig. 7)*.

A significant number of osteoclasts were found at the border of the bone-ceramic regenerate with the lamellar bone tissue of the cortical plate of the maternal bone. Unlike the previous observation period, foci of membrane osteogenesis in the superficial localization were not determined. A boundary of tight adhesion of primary woven bone tissue of newly formed trabeculae to the osteon tissue system of the maternal bone was determined. Some of the activated osteoclasts with sizes of 90–120 μm were visualized on the surface of bone trabeculae, as well as near the particles of the implanted material, indicating active bioresorption processes in the regenerate (*Fig. 8*). The total number of osteoclasts significantly exceeded their content as in comparison with the previous term of the experiment, as well as in animals of the control group.

After 8 weeks of the experiment, the polymorphic trabeculae in the bone-ceramic regenerate varied significantly in their histoarchitecture. In particular, in the depth of the applied bone defect, on the periphery of the regeneration zone, lamellar bone tissue with a small content of secondary osteoblasts and osteoclasts



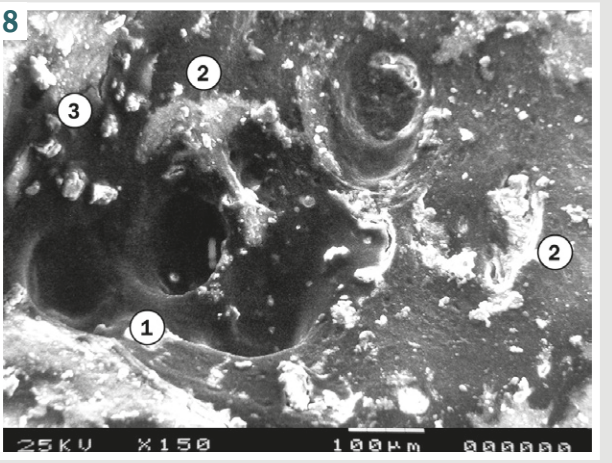


Fig. 7. Scanning electron micrograph of the bone defect area of the rabbit mandible 4 weeks after the injury and filling of the defect with OCP-N material. ×500. **1:** trabeculae from the inner surface of the shaft; **2:** trabeculae on the periphery of the bone regenerate.

Fig. 8. Scanning electron micrograph of the bone defect area of the rabbit mandible 5 weeks after the injury and filling of the defect with OCP-N material. ×150. **1:** area of bioresorption on the surface of the newly formed bone trabecula; **2:** activated osteoclast; **3:** group of osteoblasts.

predominated in the trabeculae, moreover, the boundary between the newly formed trabeculae and the maternal cancellous bone was not visualized. Inside the regenerate, a heteromorphic cancellous structure was observed, which consisted mainly of primitive bone plates and, to a great extent, of woven bone with an admixture of partially resorbed fragments of the implanted material. Connective tissue areas around the microvessels were rarely encountered and had the appearance of thin layers enriched in randomly grouped collagen fibers. Among the osteogenic cells, diffusely located osteoblasts prevailed in a moderate amount. Unlike observations in the control group, a limited number of osteoclasts were found only in areas of primary woven bone tissue of a small part of bone trabeculae, indicating remodeling of these areas. At the border of the regenerate with the outer bone plate of the maternal bone, newly formed primitive osteons were determined with signs of a moderately developed lacunar-canalicular architecture.

Twelve weeks after the experimental injury and implantation of OCP-N material, the trabeculae of the cancellous bone of the alveolar process consisted mainly of lamellar tissue. In some deep areas of the regenerate, foci of primary woven bone tissue were found, which contained a moderate number of activated osteoclasts, but without remnants of the implanted material. In the peripheral areas of the regenerate, near the maternal cancellous bone that was not injured, the microrelief of the surface of most trabeculae was relatively smooth, without signs of osteoid formation. The number of osteogenic cells and osteoclasts in the regenerate of this localization was lower than at the eighth week of the experiment and approached the characteristics of the bone of the alveolar process that was not injured. Overall, the osteocyte lacunar-canalicular system that formed after the implantation of the material acquired features of a typical structure. Foci of incomplete osteogenesis weren't visualized. Unlike the group of control animals, in the zone of the outer bone plate after the use of OCP-N material, the osteons of the regenerate didn't differ in their structure and geometry from the typical structure of the maternal bone.

Morphometric study of the dynamics of changes in the relative area of bone tissue in the regenerate of the experimental defect in the control group showed an active increase in the parameter from the first to the fifth week after the defect was applied *(Table 1).* In particular, the relative content of bone tissue in the regenerate after 2 weeks of the experiment was 2.75 times ($p < 0.05$) higher than the value of the 1st week; after 3 weeks, the parameter increased by 60.4 % ($p < 0.05$) relative to the previous period; after 4 and 5 weeks – by 68.0 % ($p < 0.05$) and 32.0 % ($p < 0.05$), respectively. In the period from the 5th to the 8th week, the increase in the relative area of bone tissue in the regenerate formed under the blood clot was 24.5 % ($p < 0.05$). Subsequently, until the 12th week of the experiment, changes in the indicator were not statistically significant compared to the value of the 8th week, stabilizing at the level 70.3 ± 4.8 %.

In the experimental group of animals where the defect was filled using OCP-N material, a consistent increase in the relative area of bone tissue in the regenerate was observed. While the dynamic pattern was similar to that in the control group, the intensity of changes was significantly different. One week after the injury, the parameter value was 64.6 % ($p < 0.05$) higher than the indicator of the first day, not significantly different from the control level. On the contrary, after 2 weeks of implantation of OCP-N material, a sharp increase in the parameter significantly exceeded the degree of its increase in the control group – by 38.3 % ($p < 0.05$); after 3 weeks – by 57.9 % ($p < 0.05$); after 4 weeks – by 25.5 % ($p < 0.05$); after 5 weeks – by 18.1 % ($p < 0.05$). In the period from the 5th to the 8th week, the studied parameter continued to increase moderately and stabilized at a level that was statistically significantly different from the indicators of the control group. After 12 weeks of the experiment, the relative area of bone tissue in the regenerate that formed after implantation of OCP-N was 76.8 ± 6.1 %.

Table 1. Dynamics of changes in the relative area of bone tissue, osteoplastic material and connective tissue in the regenerate (%), M ± m

Exposure	Bone tissue		Material	Connective tissue	
	Control	OCP-N	OCP-N	Control	OCP-N
1 day	3.7 ± 0.6	4.8 ± 0.7	80.7 ± 7.2	6.9 ± 0.4	7.3 ± 0.6
7 days	5.6 ± 0.8#	7.9 ± 0.9#	65.3 ± 5.4#	14.7 ± 1.1#	16.3 ± 1.4*.#
14 days	15.4 ± 1.2#	21.3 ± 2.4*.#	34.9 ± 3.8#	30.5 ± 2.4#	44.8 ± 3.8*.#
21 days	24.7 ± 1.8#	39.0 ± 3.4*.#	22.5 ± 2.4#	56.1 ± 3.5#	42.9 ± 3.6*
28 days	41.5 ± 3.4#	52.1 ± 3.8*.#	16.1 ± 2.3#	41.8 ± 3.1#	35.0 ± 2.7*.#
35 days	54.8 ± 3.7#	64.7 ± 4.0*.#	10.5 ± 1.4#	34.2 ± 2.3#	26.3 ± 2.1*.#
56 days	68.2 ± 4.5#	74.9 ± 5.7#	5.8 ± 0.7#	20.7 ± 1.7#	18.4 ± 1.6#
84 days	70.3 ± 4.8	76.8 ± 6.1	1.8 ± 0.7#	24.5 ± 2.0	16.1 ± 1.5*

*: the difference is statistically significant when compared to the control group; #: the difference is statistically significant when compared to the previous time point of the experiment.

Morphometric study of the relative area of osteoplastic material in the regenerate at the stages of augmentation of the experimental defect after implantation of OCP-N material revealed a general dynamic of bioresorption, which consisted of a sequential alternation of periods of uneven intensity of resorption. In particular, during the first week after implantation, the relative content of the material decreased by 19.1 % ($p < 0.05$) relative to the value after 1 day. During the second week, the decrease in the parameter was the most intense among all the studied periods – by 46.6 % ($p < 0.05$) compared to the indicator of the first week. After 3, 4, and 5 weeks of the experiment, the parameter value decreased by 35.5 % ($p < 0.05$), 28.4 % ($p < 0.05$), and 34.8 % ($p < 0.05$), respectively, compared to the previous period. At the final terms of the experiment, the relative area of OCP-N material fragments in the regenerate reached minimal values.

Morphometric study of the relative area of connective tissue in the regenerate of the control group experimental defect allowed us to establish the phase nature of the dynamics of changes in the parameter. In the first three weeks of the experiment, an active increase in the content of the connective tissue component in the regenerate was observed. In particular, one week after the injury, the parameter increased by 2.1 times ($p < 0.05$) relative to the value after 1 day; after 2 weeks of the experiment – by 107.5 % ($p < 0.05$) compared to the value of the 1st week; after 3 weeks, the parameter increased by 83.9 % ($p < 0.05$) relative to the level of the previous period and reached the highest value 56.1 ± 3.5 %. In the further dynamics of changes in the content of connective tissue, a gradual decrease in the indicator was observed: after 4 weeks – by 25.5 % ($p < 0.05$) relative to the peak value of the 3rd week; after 5 and 8 weeks – by 18.2 % ($p < 0.05$) and 39.5 % ($p < 0.05$), respectively, compared to the previous term of the study. In the period from the 8th to the 12th week, the relative area of connective tissue in the regenerate that formed under the blood clot didn't change significantly and stabilized at the level 24.5 ± 2.0 %.

In the experimental group of animals where the defect was filled using OCP-N material, a characteristic phase dynamic of changes in the relative area of connective tissue in the regenerate was observed, which had significant differences from the changes in the control group. One week after the injury, the parameter value was 2.2 times ($p < 0.05$) higher than the indicator of the first day, not significantly different from the control level. After 2 weeks of implantation of OCP-N material, a sharp increase in the parameter compared to the value of the first week (2.7 times; $p < 0.05$) led to its exceeding the control level by 46.9 % ($p < 0.05$), reaching the highest value for the entire observation period – 44.8 ± 3.8 %. After three weeks, the connective tissue content in the bone-ceramic regenerate did not differ statistically significantly from the previous time point after implantation of OCF-N, however, it was 23.5 % ($p < 0.05$) lower than the control group. Starting from day 3, an intensive reduction of the connective tissue component of the regenerate was observed in the experimental group, the intensity of which exceeded the control dynamics. Specifically, four weeks after implantation of the OCF-N material, the studied parameter was 16.3 % lower than the control ($p < 0.05$), and five weeks later, it was 23.1 % lower ($p < 0.05$). Unlike the control group, after applying the osteoplastic material, further reduction of the connective tissue content of the regenerate was observed after 8 and 12 weeks of the study; at the end of the observation, the indicator of the experimental group was 34.3 % lower ($p < 0.05$) than the control level.

Discussion

Bone grafting is widely used in dentistry. This procedure is relevant in a variety of clinical scenarios. In particular, in periodontal surgery, tooth implantation, sinus lifting, socket preservation, and many other procedures [18,19,20,21,22,23].

According to many researchers, xenogenic materials play a crucial role in promoting bone regeneration, offering a promising alternative to autogenous and allogenic bone grafts [18,19]. These materials have a number of advantages, especially compared to the more invasive surgical procedures required for taking autografts.

Other advantages include biocompatibility, osteoconductivity, ease of handling, and the possibility of performing minimally invasive procedures[20,21]. Sohn H.-S. & Oh J.-K. emphasize that, unlike autogenous grafts, they don't require additional surgery to take the required bone, which reduces the risk of infection, scarring, and other complications, as well as shortens the operation time [22].

Another significant advantage of xenogenic bone materials is their wide availability – they can be mass-produced, which meets the growing demand for bone reconstruction. Zhao R. et al. state that this demand has stimulated extensive research on xenomaterials in experimental and clinical practice [23]. As a result, various

types of xenogenic materials have been developed and widely used, including those based on calcium phosphate, which have osteoconductive properties. Literature review published by Gupta H. et al. and other authors indicate that these materials promote bone tissue regeneration, allowing it to penetrate and grow into the scaffold, making them ideal for the treatment of small and medium-sized bone defects, sinus lifting, and bone augmentation for dental implants [24,25,26,27].

Calcium phosphate materials are often used due to their compositional similarity to natural bone. Excellent biocompatibility makes it a popular choice for bone grafting. Different authors believe that calcium phosphate-based materials can be molded into granular or paste-like forms, which provide improved moldability and reduce application time during surgical procedures [20,25,28,29]. However, these materials face limitations when applied to large defects due to the lack of osteoinductive properties – the ability to actively induce the formation of new bone [30].

As a result of the conducted study and the examination of the surface relief characteristics of the experimental bone defect in the lower jaw after implantation of octacalcium phosphate material, we identified numerous regenerative changes that occurred after the injury and correlated with the dynamics of changes in the relative area of bone tissue, osteoplastic material, and connective tissue in the regenerate. Morphometric analysis of the relative area of the regenerate components of the experimental defect established a phased nature of the dynamics of the studied changes. Specifically, it was found that the osteocytic lacunar-canalicular system, which formed after the implantation of the material, acquired features of typical structure. Foci of incomplete osteogenesis were not visualized. Unlike control animals, in the zone of the outer bone plate after application of octacalcium phosphate material, the osteons of the regenerate in their structure and geometry did not differ from the typical structure of the maternal bone.

Therefore, our results are consistent with the literature and confirm that the OCP-N is effective when used for bone tissue regeneration, as the bone component of the regenerate significantly outweighs the connective tissue component, which ensures the restoration not only of the lost area but also of the qualitative characteristics of the jaw bone. Taking into account the mechanical load on the jaws and the crucial importance of bone tissue quality in dental prosthetics, particularly implantation, we consider the use of the OCP-N to be relevant and justified in clinical dental practice. In wartime conditions, the timeframes for bone defect regeneration using OCP-N are also of particular importance, significantly outpacing the control group and, as evidenced by a comparison of the obtained results with literature data, the healing times for bone defects filled with auto- and allografts [2].

Conclusions

1. In the experimental group of animals where the defect was repaired using OCP-N material, a regular increase in the relative area of bone tissue in the regenerate was observed, which in terms of dynamics was similar to that in the control group, however, in terms of intensity of changes it significantly differed from the control, approaching the norm in terms of the studied indicators.

2. In deep zones of the defect after 4 and 5 weeks of the experiment, significant areas of woven bone, newly formed microvascular networks, and decompacted remnants of resorbed OCP-N material were detected in the regenerate, which indicates effective bone tissue regeneration in the defect zone at this time point compared to the control.

3. The presence of activated osteoclasts on the surface of bone trabeculae and near implanted material particles after 4 weeks of the experiment indicated active bioresorption processes in the regenerate.

4. Unlike the control group, in the zone of the outer bone plate after application of OCP-N material, the osteons of the regenerate did not differ in structure and geometry from the typical structure of the maternal bone.

5. Morphometrically, it was determined that using OCP-N material resulted in an increase in the relative area of bone tissue in the regenerate, which in terms of intensity of changes significantly exceeded the control, and the overall dynamics of bioresorption of the osteoplastic material in the regenerate showed its highest intensity during the second week.

6. In the dynamics of changes in the relative area of connective tissue, 2 weeks after implantation of OCP-N material, a sharp increase in the parameter compared to the value of the first week led to its exceeding the control level by 46.9 % ($p < 0.05$), reaching the highest value for the entire observation period. Starting from the 3rd week in the experimental group, intense reduction of the connective tissue component of the regenerate was observed, the severity of which exceeded the control dynamics.

Prospects for further researches. Further studies are planned to investigate the ultrastructural features of regeneration of experimental bone tissue defects under conditions of using different osteoplastic materials, which will allow for the development of more effective innovative solutions that will improve bone regeneration outcomes in the maxillofacial region.

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Information about the author:
Chelpanova I. V., MD, PhD Associate Professor, Head of the Department of Histology, Cytology and Embryology, Danylo Halytsky Lviv National Medical University, Ukraine.
ORCID ID: 0000-0001-5215-814X

Відомості про автора:
Челпанова І. В., канд. мед. наук, доцент, зав. каф. гістології, цитології та ембріології, Львівський національний медичний університет імені Данила Галицького, Україна.

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