

ISSN 1814-6023 (Print)

ISSN 2524-2350 (Online)

UDC 616.314-007.21:616.314-77-089.843]-085.837.3

<https://doi.org/10.29235/1814-6023-2020-17-1-20-27>

Received 18.12.2019

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## **MORPHOLOGICAL CHANGES IN THE BONE TISSUE AROUND DENTAL IMPLANTS AFTER LOW-FREQUENCY LOW-INTENSITY ULTRASOUND APPLICATIONS**

**Abstract.** The article presents the results of a histological study of morphological changes in bone tissue around established dental implants after exposure to low-intensity, low-frequency ultrasound during dental implantation.

Histological studies of tibia bone blocks were conducted in three groups of laboratory animals around installed dental implants, which were subjected to different modes of ultrasonic exposure.

In the course of studies, it was found that the processes of osseointegration of dental implants in animals of all groups occurred without staging. In the early stages, granulation tissue was formed, which was subsequently replaced by reticulofibrotic bone tissue, and then by more mature lamellar tissue. The timing and degree of bone maturation, as well as the indicators of osseointegration in groups using low-intensity pulsed ultrasound and without it, were significantly different.

It was shown that the ultrasound effect on peri-implant tissues induces osteoreparative processes, stimulating neoangiogenesis in granulation and newly formed bone tissue. It has been established that ultrasonic exposure of implants, and then peri-implant tissues during dental implantation, promotes the formation of bone tissue, the histostucture of which is similar to the histostucture of the maternal bone at earlier stages.

**Keywords:** bone regeneration, osteoblasts, low-intensity ultrasound, dental implants, histology

**For citation:** Rubnikovich S. P., Khomich I. S., Denisova Yu. L. Morphological changes in the bone tissue around dental implants after low-frequency low-intensity ultrasound applications. *Vesti Natsyyanal'nai akademii navuk Belarusi. Seriya meditsinskikh navuk* = *Proceedings of the National Academy of Sciences of Belarus. Medical series*, 2020, vol. 17, no. 1, pp. 20–27. <https://doi.org/10.29235/1814-6023-2020-17-1-20-27>

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## **МОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ КОСТНОЙ ТКАНИ ВОКРУГ ДЕНТАЛЬНЫХ ИМПЛАНТАТОВ ПОСЛЕ ВОЗДЕЙСТВИЯ НИЗКОЧАСТОТНЫМ УЛЬТРАЗВУКОМ НИЗКОЙ ИНТЕНСИВНОСТИ**

**Аннотация.** В статье представлены результаты гистологического исследования морфологических изменений в костной ткани вокруг установленных дентальных имплантатов после воздействия низкоинтенсивным низкочастотным ультразвуком при дентальной имплантации.

Проведены гистологические исследования блоков костной ткани вокруг установленных дентальных имплантатов, которые подвергались разным режимам ультразвукового воздействия большеберцовой кости, у лабораторных животных трех групп.

В ходе исследований установлено, что процессы остеointеграции дентальных имплантатов у животных всех групп происходили без нарушения стадийности. На ранних сроках формировалась грануляционная ткань, которая впоследствии замещалась ретикулофиброзной костной тканью, а затем и более зрелой пластинчатой. Сроки и степень созревания костной ткани, а также показатели остеointеграции в группах с использованием низкоинтенсивного импульсного ультразвука и без его применения значительно отличались.

Показано, что ультразвуковое воздействие на периимплантные ткани индуцирует процессы остеорепаляции, стимулируя неоангиогенез в грануляционной и новообразованной костной ткани. Установлено, что ультразвуковое воздействие на имплантаты, а затем и на периимплантные ткани при дентальной имплантации способствует формированию костной ткани, гистоструктура которой схожа с гистоструктурой материнской кости в более ранние сроки.

**Ключевые слова:** регенерация кости, остеобласты, ультразвук низкой интенсивности, дентальные имплантаты, гистология

**Для цитирования:** Рубникович, С. П. Морфологические изменения костной ткани вокруг дентальных имплантатов после воздействия низкочастотным ультразвуком низкой интенсивности / С. П. Рубникович, И. С. Хомич, Ю. Л. Денисова // Вест. Нац. акад. наук Беларусі. Сер. мед. навук. – 2020. – Т. 17, № 1. – С. 20–27. <https://doi.org/10.29235/1814-6023-2020-17-1-20-27>

**Introduction.** Dental implants can significantly improve the results of recovering chewing efficiency in patients with dentition defects, improve the fixation of removable dentures, or replace them with non-removable prostheses [1–4].

Since the beginning of the 1990s, studies of the potential therapeutic effects of ultrasound on the bones of the maxillofacial region were being performed, and most of these studies have reported positive results. Researchers have shown that the application of ultrasound increases the synthesis of angiogenesis-related cytokines such as interleukin 8, fibroblast growth factor, and vascular endothelial growth factor. Studies have confirmed the ability of ultrasound therapy to improve healing of mandibular fractures and osteoradionecrosis, enhance the proliferation of fibroblasts and osteoblasts. With its unique ability to accelerate bone repair, low intensity pulsed ultrasound may be a promising new method to improve the quality of osseointegration of dental implants [5–16].

Osseointegration is defined as direct bone formation on the implant surface and is a functional ankylosis. This concept has been described by prof. P. I. Branemark and in 1977 he formulated the phenomenon of osseointegration: “Direct structural and functional connection between the highly differentiated living bone and the bearing surface of the supporting implant, revealed at the level of a light microscope”. P. I. Branemark formulated the necessary conditions for the success of implantation and strong osseointegration: sterility, surface cleanliness, non-invasiveness, geometric congruence of the bed and structure, etc. He also proposed the use of implants from two parts – intraosseous and supra-gingival (abutment) [1].

Osseointegration belongs to the category of direct or primary healing. It can be compared with the direct healing of fractures, in which the ends of the fragments fuse together without intermediate fibrous or fibrocartilaginous tissue. There is, however, a fundamental difference: osseointegration does not integrate bone with bone, but bone with the implant surface. From the point of view of tissue engineering, the implant surface acts as a scaffold (frame) in this unique phenomenon. Modern dental and orthopedic implants have been developed based on this concept and are called osseointegrated. Osseointegration includes several cellular and extracellular biological processes that occur at the border of the bone – implant, and contribute to the formation of bone on the surface of the implant. Activation of osteogenic processes occurs due to growth and differentiation factors secreted by activated blood cells on the implant surface [2].

Immediately after implantation, the bone matrix contacts the extracellular fluid, and non-collagen proteins and growth factors are released and activate bone regeneration. Bone marrow cells by chemotaxis from the endocortical space and the bone endostium migrate to the lesion site. They multiply and differentiate into osteoblasts, creating a layer of a non-collagen matrix on the surface of the implant, which regulates cell adhesion and mineral binding [3].

The deposition of a new calcified matrix on the surface of the implant is formed in the period from 4 to 6 weeks after implantation and is a coarse fiber. It is often regarded as a primitive type of bone tissue and is characterized by random (like felt) orientation of collagen fibrils, numerous, irregularly shaped osteocytes, and relatively low mineral density. The coarse-grained bone fills the initial space at the implant-bone border. Organized in a three-dimensional network, it determines a sufficiently high resistance of the implant to early loading. Its physical architecture is a biological framework for cell attachment and bone formation, which provides secondary (biological) stability of the implant [4].

Starting from the second month, the coarse fibrous bone is gradually remodeled and replaced by the plate bone, which can achieve a high degree of mineralization. Three months after implantation, a mixed structure of bone tissue and plate matrix is found around the implant [5].

The last stage of osseointegration consists of bone tissue remodeling, begins around the third month and helps to adapt the bone structure to stress and mechanical stress. The bone renewal around the implant is characterized by the presence of bone marrow spaces containing osteoclasts, osteoblasts, mesenchymal cells, lymphatic and blood vessels near the surface of the implant. This process improves bone quality and functional adaptation by replacing an existing, necrotic bone and/or initially formed coarse fibrous bone with a mature, viable plate bone, or by changing the size and orientation of the trabeculae. Bone remodeling continues throughout life, preventing the accumulation of microdamage and bone fatigue and ensuring the long-term functioning of the implant [6].

The desire to minimize the percentage of complications after dental implantation, minimize the risk of rejection of implants and achieve the most complete osseointegration encourages researchers to constantly search for new and effective methods of surface treatment of implants, surgical techniques, physical methods of influencing the postoperative area and their combinations [7].

Physiotherapeutic procedures, it would seem, require a lot of time and the involvement of a doctor or nurse for the correct procedure, but the positive effect of this “additional” treatment is very significant. For example, the results of a number of studies indicate that pulsed ultrasound waves can accelerate the healing of fractures of the tubular bones and lower jaw. Low-intensity pulsed ultrasound has a direct effect on cellular physiology, increasing the incorporation of calcium ions in cartilage and bone cell cultures and stimulating the expression of numerous genes involved in the healing process. In addition to modulating gene expression, ultrasound can stimulate angiogenesis and increase blood flow around the fracture. In addition, acoustic pressure waves facilitate fluid flow, which increases the delivery of nutrients and waste disposal (the acoustic flow phenomenon), thereby stimulating the proliferation and differentiation of fibroblasts, chondroblasts, and osteoblasts [8, 13, 16].

Low-intensity pulsed ultrasound was used to stimulate the healing of fractures and osteotomies in orthopedics. In dentistry, we studied the effects of low-frequency ultrasound on periodontal tissues and bone regeneration in the postoperative period and obtained confirmed positive results from exposure to the organs of the maxillofacial region. Studies by foreign scientists have demonstrated the promise of using low-intensity ultrasound in the induction of bone formation around titanium blanks installed by experimental animals [9, 14, 17].

Although the exact mechanism of interaction of low-frequency ultrasound with living tissue and stimulation of bone healing remains unclear, studies in this direction have shown that low-intensity pulsed ultrasound can stimulate bone tissue regeneration [8, 15, 18].

Based on the information available on this topic, we conducted an experimental-clinical study aimed at studying the positive effects of ultrasound on implants and peri-implant tissues to optimize osseointegration processes during dental implantation.

The aim of the study is to perform histological evaluation of effectiveness of the use of ultrasound for bone restoration and formation in dental implant placement.

**Materials and research methods.** To study the nature of morphological changes in peri-implant bone tissue under the influence of contact exposure to the implant and peri-implant tissues with low-frequency ultrasound, experimental studies were carried out on 77 rabbits of the Chinchilla breed of both sexes, kept in stationary conditions on a high-grade standard diet according to the established norms in accordance with the rules for working with experimental animals. The animals were divided into three groups: the first – a comparison group (27 rabbits), the second and third – experimental groups (25 rabbits each).

In the first group (comparison group) were laboratory animals after the installation of dental implants by the traditional method recommended by the manufacturer (without the use of low-intensity pulsed ultrasound). The second group consisted of laboratory animals to which dental implants were contact-sounded with low-intensity pulsed ultrasound during their installation. In the third group, laboratory animals placed dental implants in the tibia, voiced them with low-intensity pulsed ultrasound and the subsequent contact exposure of low-intensity pulsed ultrasound to the peri-implant region. After the observation period, the animals were withdrawn from the experiment in compliance with the principles of bioethics (in accordance with GLP standards). Pathomorphological evaluation of biopsy samples of the studied groups was performed 1, 2, 4, 8 weeks after implantation.

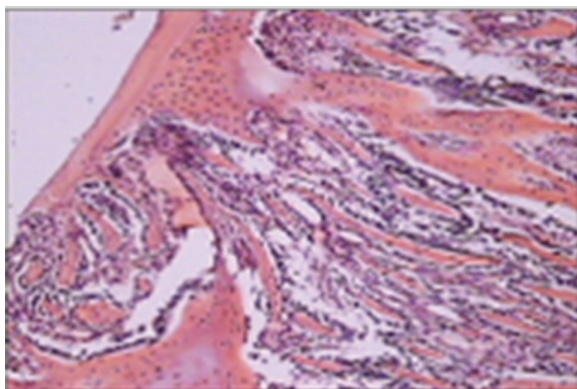


Fig. 1. Morphological features of peri-implant tissues in experimental animals of the second group at 1 week (H & E stain,  $\times 200$ )

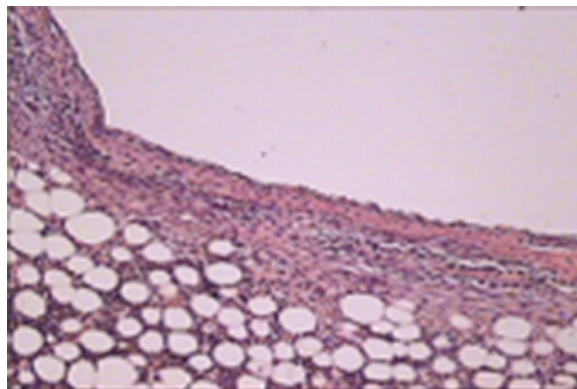


Fig. 2. Morphological features of peri-implant tissues in experimental animals of the third group at 1 week (H & E stain,  $\times 200$ )

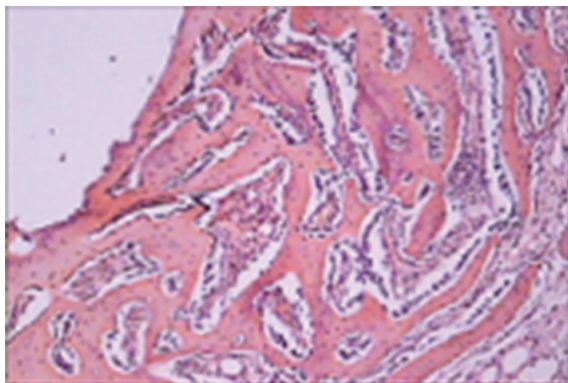


Fig. 3. Morphological features of peri-implant tissues in experimental animals of the first group at 2 weeks (H & E stain,  $\times 200$ )

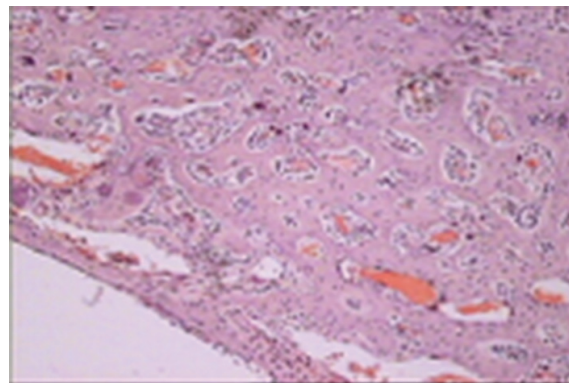


Fig. 4. Morphological features of peri-implant tissues in experimental animals of the second group at 2 weeks (H & E stain,  $\times 200$ )

Rabbit tibiae bone blocks containing dental implants were fixed in 10% neutral formalin for 48 hours. Decalcification was performed using EDTA with obligatory control of the process completeness with calcium oxalate. The implants were removed from bone after decalcification. Then washed in running water for 24 hours, dehydrated in alcohols of increasing concentration (70, 80, 96, absolute alcohol). The material carried through the alcohol-chloroform, chloroform, chloroform-paraffin and embedded in paraffin. The paraffin blocks were sectioned to 4–5 microns thick, which were stained with hematoxylin and eosin. The study of micropreparations and microphotographs was carried out at a magnification of 100 and 400 using Axio Imager (Zeiss) microscopes and using a DMLS microscope with software (Leica, Germany).

**Results and its discussion.** In 1 week after implantation in the first group of animals at the perimeter of the implant cavity areas of compact maternal bone, necrotic structureless masses and zones of bone matrix resorption could be defined. Small areas of granulation and reticular fibrous tissue were identified. Inter-trabecular spaces were filled with yellow bone marrow and small areas of red bone marrow with foci of necrosis. Necrotic death of single parent bone osteocytes, inflammatory infiltration in the periosteum and low blood vessels density was observed.

In the second group of animals the gap between the implant and regenerate was filled with connective tissue. Granulation tissue was more mature than in the control group, and was dominated by a fibrous component and blood capillaries (Fig. 1).

In the maternal bone resorption areas were filled with reticular fibrous tissue with a high density of osteogenic cells. Osteoblasts were single-row compact, maternal bone matrix had normal structure, osteocytes lie freely in bone lacunae, the number and shape of the Haversian canals were not changed.

In the third group of animals a connective tissue capsule formation, comprising of areas of reticular fibrous and granulation tissue, was observed throughout the implant cavity perimeter. Granulation tissue was replaced by reticular fibrous at considerable length of the perimeter of implant cavity (Fig. 2).

Granulation tissue was characterized by the presence of different diameter blood capillaries containing endothelial layer. Trabecular cavities were filled with bone marrow with high density of cells of fibroblastic differone. There were no necrotic areas.

Bone trabeculae were oriented at different angles and parallel to the surface of the implant. Inter-trabecular spaces were filled with reticular fibrous tissue with high osteoblastic density on the trabeculae surface. Not in all areas of newly formed bone osteogenic cells were observed, and coarse fibrous tissue dominated.

In the second group 2 weeks after implantation connective tissue capsule around implants formed mostly reticular fibrous newly formed bone and small areas of mature granulation tissue. The newly formed bone trabeculae were oriented for the most part parallel to the surface of the implant (Fig. 4).

In 2 weeks after implantation in the first group of animals peri-implant space was mostly occupied by a newly formed bone tissue, also small areas of granulation and dense fibrous tissue was determined (Fig. 3).

On the trabeculae surface single osteocytes and compactly situated osteoblasts were determined. In the inter-trabecular space areas of reticular fibrous tissue and red bone marrow with blood vessels was visualized.

In the third animal group, perimeter of the implant cavity is determined by newly formed bone tissue consisting of osteoid trabeculae forming marrow spaces (Fig. 5).

Inter-trabecular spaces are filled with reticular fibrous tissue and red bone marrow. Well distinguished are active osteoblasts on the trabeculae surface and osteocytes in the bone matrix. This marks the beginning of lamellar bone formation.

In 1 month after implantation, the animals of the first group were characterized by the presence of peri-implant reticular fibrous tissue, woven bone tissue, but with remaining small foci of granulation tissue, quite immature, multicellular and with low vascularity (Fig. 6).

Inter-trabecular spaces were filled with reticular fibrous tissue, areas of maternal bone trabeculae resorption and osteoclasts were visualized.

In the second group of rabbits at 1 month a significant thickening of the periosteum on the border with the implant is observed. Also an increasing proliferation of inner osteogenic layer of the periosteum and the formation of trabecular bone can be seen (Fig. 7).

In the newly formed bone the abundance of osteocytes in bone matrix and osteoblasts compactly arranged on the surface of bone trabeculae is observed.

In the third group of animals at 1 month was determined by newly formed predominately lamellar bone tissue throughout the implant cavity. Bone trabeculae oriented mainly parallel to the surface of the implant. A clear border connections are not traced to the parent bone. The Haversian cavities are filled with red bone marrow with functioning blood vessels.

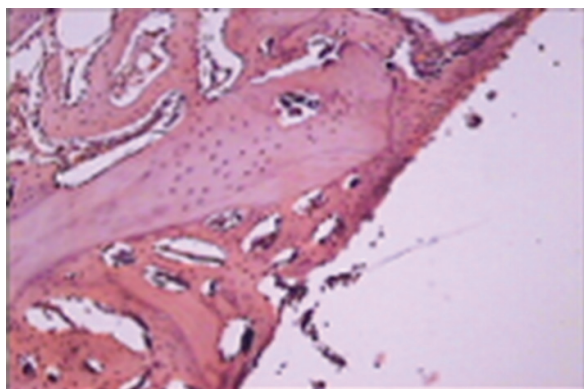


Fig. 5. Morphological features of peri-implant tissues in experimental animals of the third group after 2 weeks (H & E stain,  $\times 200$ )

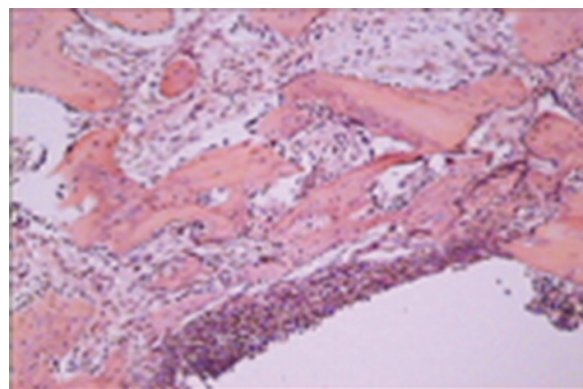


Fig. 6. Morphological features of peri-implant tissues in experimental animals of the first group at 1 month (H & E stain,  $\times 200$ )

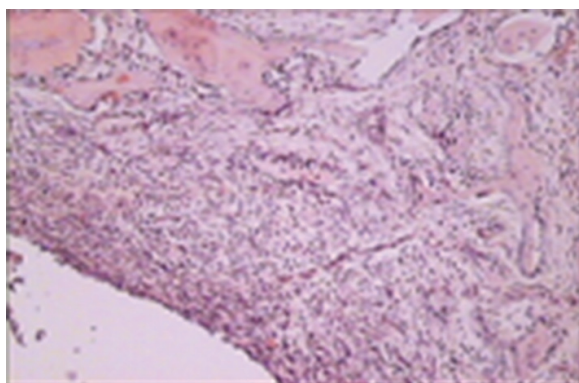


Fig. 7. Morphological features of peri-implant tissues in experimental animals of the second group at 1 month (H & E stain, ×200)

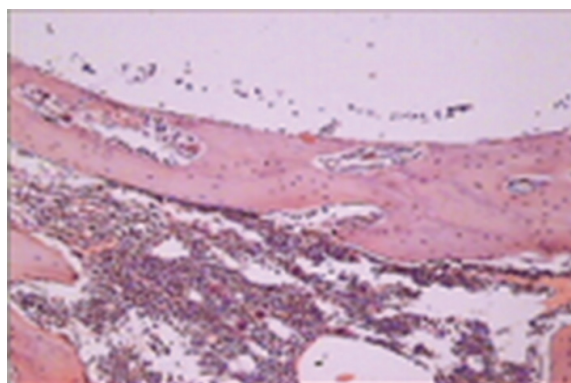


Fig. 8. Morphological signs of peri-implant tissues in experimental animals the first group after 2 months (H & E stain, ×200)

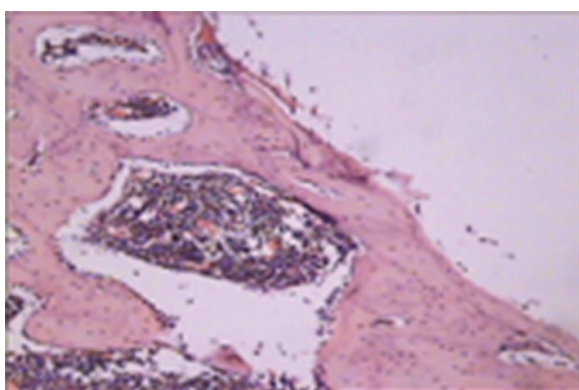


Fig. 9. Morphological signs of peri-implant tissues in experimental animals of the second group at 2 months (H & E stain, ×200)

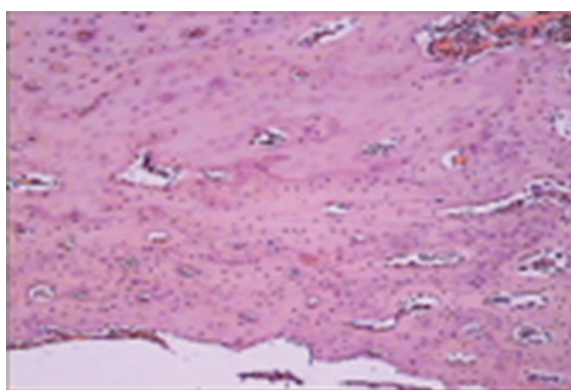


Fig. 10. Morphological signs of peri-implant tissues in experimental animals of the third group at 2 months (H & E stain, ×200)

After 2 months, in the second and third groups of animals compact bone with woven trabeculae and lamellar bone areas was observed. Newly formed osteons and areas of mature lamellar bone were identified. In peri-implant region vascular network with erythrocytes in the lumen of the newly formed blood vessels could be seen. Areas of reticular fibrous tissue were not present. In the first animal group peri-implant bone area was visually less than in the second and third groups (Fig. 8).

Woven bone areas with low density of osteogenic cells and areas of reticular fibrous tissue filling inter-trabecular spaces were observed. In the second group areas of lamellar bones prevailed over the newly formed woven bone (Fig. 9).

In the inter-trabecular spaces red bone marrow with numerous blood capillaries is visualized. The newly formed osteons and border with matrix bone can be seen. The animals of the third group for the most part had formed lamellar bone of normal histological structure around the perimeter of the implant cavity (Fig. 10).

There is a large number of osteocytes on the bone surface and red bone marrow in the inter-trabecular spaces. There is no border observed between newly-formed and matrix bone.

**Conclusion.** The processes of osseointegration of installed dental implants in all groups of animals take place without disturbing the staging. In the early stages granulation tissue is formed, and subsequently is being replaced by reticular fibrous, woven and more mature lamellar bone tissue in later stages. However, the timing and degree of maturation of bone tissue, as well as indicators of osseointegration in groups with low-intensity pulsed ultrasound and without differ significantly. So, in the first animal group bone maturation delay, presence of necrotic zones, immature granulation tissue,

matrix bone defects in the early stages, and the predominance of coarse-fibered bone tissue at a later date osteosynthesis is observed. Not indicated complete integration regenerate bone with the implant. Ultrasound application to implants in a second group of animals induces osteoreparation processes, stimulating angiogenesis in the granulation tissue and new bone formation. Dental implantation with subsequent ultrasound treatment on implants and the peri-implant tissues in the third group promotes the formation of bone tissue similar in histostructure to the parent bone in earlier stages. There is more complete and stronger integration of the newly formed bone with the implant surface than in the first group of animals.

**Conflict of interests.** The authors declare no conflict of interests.

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