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Pathomorphological features of the alveolar bone when applying osteoconductive materials on the background of generalized periodontitis

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Abstract

Modern methods of surgical preparation for orthopedic treatment of patients with periodontal diseases need improvement. The problems of optimization of reparative osteogenesis, prevention of atrophy of the alveolar process which affects the quality of osseointegration, are considered separately. The aim of the study is to establish preimplantation morphological changes of the alveolar bone after surgery to increase the effectiveness of surgical preparation for orthopedic treatment of patients with periodontal disease by developing scientifically sound diagnostic, treatment and rehabilitation measures. To find out the histological compliance of the alveolar bone with clinical and radiological parameters and to determine the general characteristics of the restored bone tissue, histological examination of the alveolar process biopsy was performed during dental implantation in 80 patients: in 20 cases – from areas where osteoplastic mixtures were not applied; in 60 cases – where osteoplastic mixtures were applied. The proposed surgical approaches to tooth extraction applying osteotropic material based on β-TCF with fibrin gel enriched with growth factors, the use of osteoplastic material based on β-TCF and myelocollagen transplant in patients with generalized periodontitis are quite effective in the prevention of postextractive and postoperative atrophy of alveolar processes, which is statistically proven during the evaluation of clinical, radiological, anthropometric, densitometric and morphological results of the study. The applied methods allowed to achieve a significant reduction in the loss of height, width and optical density of the alveolar process long time after treatment compared with the loss of these parameters in patients treated by traditional methods.

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Introduction.

The problem of replacement of dentition defects has been a topical issue of modern theoretical and practical dentistry for many decades. The use of traditional methods of orthopedic treatment is not always effective, especially in people of working age, who prefer fixed orthopedic structures [1, 2]. At the same time, the combination of dentition defects with periodontal disease, especially in young people, complicates the choice of orthopedic treatment, which together with other etiological factors worsens the pathology and reduces the term of use of orthopedic structures [3, 4, 1]. In patients with generalized periodontitis (GP) on the background of metabolic osteopathies, the biochemical characteristics and compensatory reactions of bone tissue deteriorate, which causes accelerated bone tissue loss around implants and alveolar processes [5, 6, 7, 8]. Such changes in the bone tissue of the jaws of patients with GP require a more balanced approach to the method and technique of treatment and additional measures to prevent the loss of dental implants during treatment stages [9, 10, 11, 12]. The methods of surgical preparation of patients with periodontal diseases for orthopedic treatment remain insufficiently studied. The problems of optimization of reparative osteogenesis, prevention of atrophy of the alveolar process, which affects the quality of osseointegration, are considered separately [13, 14, 15, 16, 17, 18].

Objectives.

To establish preimplantation morphological changes of the alveolar bone after surgery to improve the effectiveness of surgical preparation for orthopedic treatment of patients with periodontal disease by developing scientifically sound diagnostic, treatment and rehabilitation measures.

Material and methods.

759 people were examined: 49 somatically and dentistically healthy persons and 710 patients with dentition defects aged 21 to 70 years; 494 (69.58%) patients were diagnosed with GP of various stages of development. To achieve the end result of surgical interventions in the area of the alveolar process, which would satisfy the subsequent dental implantation (DI) 262 patients were divided into groups, depending on the method of extraction and filling of post-extraction defect: Group 1 – 80 patients who underwent conventional extraction technique; postextraction bone defect was filled with a blood clot; Group 2 – 69 patients whose postextraction bone defect was filled with bone-plastic material based on β-TCF; Group 3 – 67 patients whose postextraction bone defect was filled with bone-plastic material based on β-TCF and fibrin gel of patient's autoblood enriched with growth factors; Group 4 – 46 patients with GP, who underwent tooth extraction according to our proposed technique applying osteoplastic material based on β-TCF and bone marrow. After a 6-month healing period, all patients had dental implants placed. Ninety cylindrical biopsies of the bone tissue were obtained from areas where bone augmentation was performed. For this purpose, a trepan with an inner diameter of 2.5 mm and an outer diameter of 3.5 mm was applied. Bone biopsy specimens contained both osteotropic material used for alveolar augmentation of extracted teeth and for the sinus floor lift, and preserved own alveolar bone. However, the preserved bone of the alveolar process was not included in the histological examination. Bone tissue samples, obtained by biopsy, were processed according to generally accepted histological methods with staining of preparations with hematoxylin-eosin. Morphological studies were performed according to the principles of Parfitt et al. [19]. The samples were semi-automatically measured using an Olympus microscope (Tokyo, Japan) associated with computer software using a soft display analysis system (Münster, Germany). The total surface area of each sample, which consisted only of reconstituted bone tissue, as well as the surface consisting of osteotropic material, was measured in mm². The amount of bone tissue and osteotropic material was analyzed in their percentage to the total amount of test material. Student's t-value test was used to determine the statistical error of the results. The errors were $p<0.05$ and were considered insignificant.

Research results and their discussion.

Histological examination of biopsies of patients of Group 1 revealed that the defect was filled with connective tissue with trabecula ossea in it. In the morphometric study, the ratio of bone and connective tissue is 0.97 (connective tissue – 50.74%, bone tissue – 49.26%). The connective tissue is represented by a dense fibrous tissue of the unformed type with a predominance of connective tissue fibers. The fibers are mostly bundles; some of them are multidirectional, often wavy. The fibers are loose; there are connective tissue cells between the collagen fibers: fibroblasts, macrophages, plasma cells, lymphocytes. According to the morphometric study, the cell density is 1.03 cells per 1000 μm^2 of connective tissue. Blood vessels are mostly thin-walled and small in caliber; some of them contain erythrocytes in the lumen. Vessels are located unevenly, accounting for 3.57%

of the connective tissue area. Trabecula ossea of 116.44 ± 18.34 µm thick are noted in the thickness of the connective tissue. Trabeculae are mostly represented by groups of bone plates with osteocytes in the lacunae, covered with a layer of osteogenic cells – osteoblasts, which have an irregular cubic shape, slightly basophilic cytoplasm and a single nucleus. Some trabeculae consist of bone plates; between them in the lacunae there are osteogenic cells resembling osteoblasts (the nucleus of such cells is rounded or slightly elongated). Individual bone plates have primitive structure – osteoid structure with non-calcified bone matrix and with the above-described osteogenic cells in the thickness. Histological examination of biopsies of patients of Group 2 revealed that the postoperative defect was filled with bone tissue and connective tissue. The share of spongy bone tissue is 68.68%, which is 1.39 times more than in patients of Group 1. The share of connective tissue is 31.32% (ratio – bone tissue: connective tissue is 2.19%). The connective tissue is dense, fibrous, of unformed type. It consists mostly of bundles of collagen fibers, individual groups of which are intertwined at different angles. The share of connective tissue cells is 0.96 cells per 1000 µm^2. Vessels of various calibers, mostly small and medium in diameter, some filled with erythrocytes, are visualized among the fibers.

The proportion of vessels is 4.75% of the connective tissue area. The wall of some vessels is thickened, contains single smooth myocytes. Bone trabeculae of different length and thickness (average thickness is 123.52±38.64 µm) are placed at different angles and in different planes. The uniformity of their location in different areas of the studied biopsies (both in the peripheral and in the central part) is noticed. Trabeculae are constructed of bone plates, between which are mature osteogenic cellular elements – the cell nuclei are elongated, localized in the lacunae, the size of the latter is small. Bone trabeculae are in close contact with connective tissue fibers, as well as with single granules of β-TCF. β -TKF particles are colorless. If they fall out of the section, their location is easily recognizable due to the characteristic shape and size of the space they occupied or due to the remnants of β-TKF granules located on the border with the surrounding tissues. Localization of β-TKF granules is manifested in the form of different cavities in histological specimens. Bone-plastic materials occupy not only the space of the lost bone tissue, but also integrate with the surrounding bone, acting as a framework for the penetration of maternal vessels and the migration of determined osteogenic progenitor cells of the recipient (Fig. 1).

Figure 1. Patient M., 32 years old. Medical card of an inpatient No.1989. Biopsy of the alveolar bone of the patient (Group 2) after extraction of tooth 13. Staining: hematoxylin-eosin. Enlarged: × 100. 1 – bone trabeculae of different length and thickness, 2 – connective tissue, 3 – vessels, 4 – areas of β-TCF granules, 5 – focus of lymphocyte-macrophage infiltration.

It should be noted, the reactive cellular infiltration on the periphery of the formed bone trabeculae and around the remnants of granules of bone-plastic material is mostly absent.Histological examination of biopsies of patients of Group 3 shows that the postoperative defect (like in the previous group) is filled with lamellar bone and connective tissue. The share of spongy osseous tissue differs slightly from the share of patients of Group 2 and is 73.75%. Connective tissue is 26.25% (ratio of bone tissue: connective tissue is 2.81), represented, as in the previous group, by bundles of collagen fibers with a small number of connective tissue cells (0.85 cells per 1000 µm^2). The connective tissue fibers are mostly compact, with a small number of small-sized vessels with thin walls (4.69% of the connective tissue area); the vessels in the lumen contain erythrocytes. The spongy bone tissue that fills the postoperative defect is represented by bone trabeculae which are in close contact with the connective tissue. Despite the pronounced quantitative difference with patients of Group 2, there is a qualitative differentiation, which is characterized by a significant increase in the thickness of the bone trabeculae (132.14 µm). On the outside of the trabeculae, a layer of osteoblasts with cubic nuclei is clearly visualized (Fig. 2).

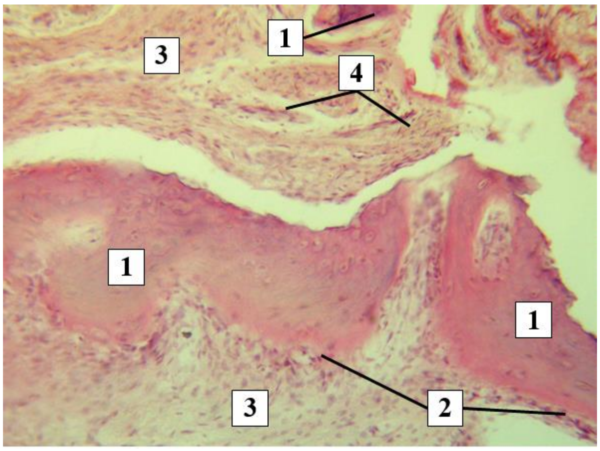

Figure 2. Patient S., 32 years old. Medical card of an inpatient No.2467. Biopsy of the alveolar bone of the patient (Group 3) after extraction of tooth 36. Staining: hematoxylin-eosin. Enlarged: × 100. 1 – bone trabeculae, 2 – layer of osteoblasts, 3 – connective tissue, 4 – blood vessels.

Trabeculae are made of bone plates, which are placed at angles to each other, between them in the lacunae there are mature osteocytes with processes that anastomose with each other. In the part of bone trabeculae on the periphery between the bone plates in the expanded lacunae the nucleus of osteogenic cells are oval, the number of osteoid cells is increased, the osseomucoid is basophilic, and the contours of the trabeculae are uneven. These changes are a manifestation of osteogenic regeneration and characterize the further increase in the proportion of spongy bone tissue in the area of the removed tooth. Histological examination of biopsies of patients of Group 4 revealed that the postoperative defect was filled with bone tissue and connective tissue. The share of spongy osseous tissue is significantly higher than in the previous groups – 80.20% (Fig. 3).

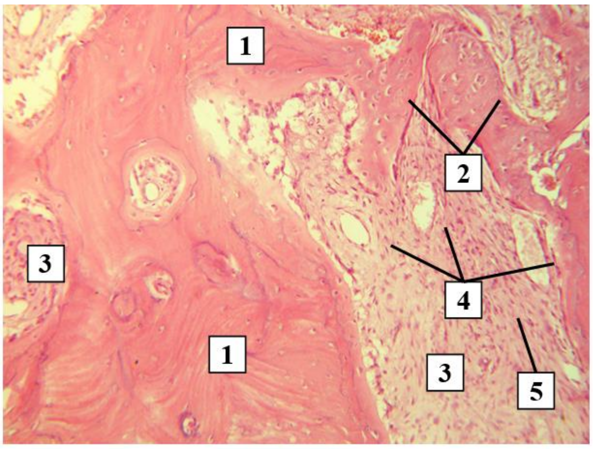

Figure 3. Patient A., 42 years old. Medical card of an inpatient No.1708. Biopsy of the alveolar bone of the patient (Group 3) after extraction of tooth 43. Staining: hematoxylin-eosin. Enlarged: × 100. 1 – bone trabeculae with calcified osteomucoid, 2 – osteoid trabeculae, 3 – connective tissue, 4 – blood vessels, 5 – connective tissue cells.

The ratio of bone tissue to connective tissue is 4.05, which indicates a pronounced and significant predominance of the formed lamellar bone over connective tissue. Bone trabeculae of different length and thickness (average thickness is 137.69±24.5 µm); a layer of osteoblasts is visualized from the outside. On the periphery of the studied biopsies, the trabeculae are thicker and are made of bone plates with osteocytes in the lacunae. The nuclei of osteogenic cells are elongated, the lacunae are small, and the osteogenic matrix is calcified. Closer to the central part of the studied biopsies, there are thinner trabeculae ossea, most of which contain osteoids and osteogenic cells with oval and rounded nuclei, which indicates their reparative potential (Fig. 4). Bone plates are in close contact with the connective tissue matrix of the postoperative are.

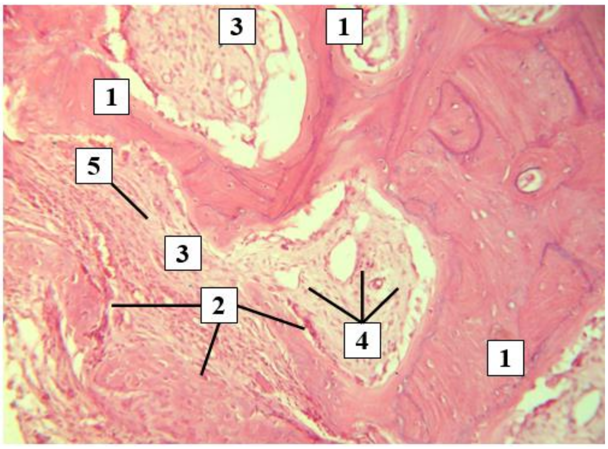

Figure 4. Patient A., 42 years old. Medical card of an inpatient No1708. Biopsy of the alveolar bone of the patient (Group 4) after extraction of tooth 43. Staining: hematoxylin-eosin. Enlarged: × 100. 1 – bone trabeculae with calcified osteomucoid, 2 – osteoid trabeculae, 3 – connective tissue, 4 –blood vessels, 5 – connective tissue cells.

Connective tissue is formed by connective tissue fibers with an increased number of vessels in it, compared with all previous research groups. The share of vessels is 5.25%; vessels are mostly small, thin-walled, filled with erythrocytes. Revascularization is one of the main indicators of graft integration. Growing into a graft, the vessels of the bone bed transport mesenchymal cells and initiate graft integration. Thus, blood supply and angiogenesis of cavitary defects of the jaw bones are important indicators of bone graft integration and restoration of the authentic structure of bone tissue at the defect site. In the thickness of connective tissue there is a moderate number of fibroblasts, macrophages, plasma cells, lymphocytes – 1.30 cells per 1000 μm^2, which indicates both further "maturation" of connective tissue and osteoregeneration, as these mesenchymal cells have osteoinductive potential. The results of histological studies showed significant advantages of applying synthetic osteoconductive material based on β-TCF with bone marrow in patients with GP in creating an adequate volume of bone tissue that is completely resorbed and replaced by newly formed bone tissue. According to the results of histomorphometric evaluation of biopsy samples, it is proved that when applying β-TCF-based material with bone marrow in patients with GP, the bone fraction reaches 80.20% after 12 months, compared with a mixture of β-TCF-based material with fibrin gel rich in growth factors – (73.75%) and independent use of material based on β-TCF – (68.68%). However, the results of our histological examination differ slightly from the results of morphological studies of M.M. Ilkiv and V.I. Hereliuk, 2010 [20], D.V. Korliakov and N.H. Korotkykh, 2004, who claim about rapid and 100% morphological reproduction of bone tissue with the use of osteoplastic materials and autocrine components after removal of teeth and jaw cysts. Al-Tarifi Fadi Mahmoud, 2011 and co-authors [21], claim that although the newly formed cellular bone in all patients does not meet 100% of the norm, it is close to it. Thus, our proposed operational approaches to tooth extraction applying osteotropic material based on β-TCF with fibrin gel enriched with growth factors, the use of osteoplastic material based on β-TCF and myelocollagen transplant in patients with GP are quite effective in prevention of postextractive and postoperative atrophy of alveolar processes, which is statistically proven in the evaluation of clinical, radiological, anthropometric, densitometric and morphological results of the study. Our methods have achieved a significant reduction in the loss of height, width and optical density of the alveolar process long time after treatment, compared with the loss of these parameters in patients who underwent traditional treatment.

Conclusions.

1. Osteoconductive material based on β-TCF, especially in combination with the patient's bone marrow, has high osteoconductive properties, high biocompatibility without a foreign reaction of cells to the osteotropic material, which promotes good regeneration of bone tissue and the achievement of stable osteointegration of the inserted implant in patients.
2. Systematization of bone defects after tooth root removal allows to determine the basic principles of treatment in order to achieve reliable osseointegration of the implant and ensure a highly aesthetic result.
3. Osteotropic material based on β-TCF with fibrin gel rich in growth factors has high osteoconductive properties, high biocompatibility, which contributes to good bone tissue regeneration and sustainable osteointegration of the implant in patients.
4. For the prevention of atrophy of the bone tissue of the jaws in patients with GP, the use of synthetic osteoconductive material based on β-TCF and myelocollagen graft in patients with GP has significant advantages in creating an adequate volume of bone tissue and its preservation.

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