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# APPLICATION OF TISSUE-ENGINEERED STRUCTURE BASED ON AUTOLOGIC ECTOMESENCHYMAL STEM CELLS AND POROUS TITANIUM FOR SUBANTRAL MAXILLARY AUGMENTATION

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**Abstract:**

This paper deals with the use of porous titanium as a basis for tissue-engineering structure for subantral maxillary augmentation. Experimental study was conducted on rams. Experimental and histological studies revealed that porous titanium granules are biocompatible with the bone tissue, have the optimal parameters of the surface microrelief, which creates favorable conditions for adhesion, expansion and migration of osteoblasts, have insignificant kinetics of resorption, and are porous enough to ensure effective neovascularization of the de novo bone tissue. It was found that the porous titanium, used in combination with autologous ectomesenchymal stem cells, is an effective alternative to materials based on calcium phosphates and bone collagen for subantral augmentation of the upper jaw bone in case of dental implantation and reconstructive plastic surgery conducted in the maxillary sinus.

**Keywords:** tissue-engineering structure, porous titanium, subantral augmentation, dental implants.

## 1. Introduction

Secondary edentia accelerates atrophy of lateral maxilla. Loss of teeth leads to a decrease in bone tissue volume, making unfavorable prognosis for dental implant in the maxilla [1, 6, 3]. Subantral augmentation or a sinus lifting is one of the ways to improve the efficiency of the dental implantation results in significant atrophy of lateral maxilla [15,16,17]. By increasing the amount of bone tissue in sinus, the subantral augmentation allows the physician to use use longer implants, which creates optimal conditions for adequate resistance to occlusal forces. Sinus lifting is indicated in case when insufficient length of the alveolar process of the maxilla prevents placing the implant greater than 10 mm in length [2, 4, 23].

In most cases, to increase bone volume, the surgeons use a resorbable hydroxyapatite, demineralized freeze-dried autologous bone, and allogenic bone [7, 8, 22]. The latter is most preferable since it does not cause rejection and

provides optimal timing of implant osseointegration [5, 12]. However, the amount of donor bone in the oral cavity is limited; extraoral dissection of autograft involves additional surgical trauma and requires involvement of experts of related areas of medicine [9, 10, 20]. The disadvantages of allogeneic and synthetic osteoplastic materials include immune response, infection, low strength characteristics, long resorption and lack of primary stability of dental implants placed in the area of the conducted sinus lifting [11, 15, 21]. The literature and some authors, studies showed the possibility of using tissue-engineered structure based on ectomesenchymal autologous cells immobilized on porous titanium in the subantral augmentation [13, 14, 24]. At the present stage of development of dental implantology and otorhinolaryngology there is an urgent need to expand the indications for use of ectomesenchymal autologous cells, as well as porous titanium as a cell data carrying matrix. Studying the possibility of the use of this tissue-engineered structure for the purpose of restoring the integrity of the bottom of the maxillary sinus in case of its perforation and/or subsequent dental implantation is of particular interest.

**2. Objective of Research**

Improving the efficiency of the dental implantation under bone tissue atrophy in the lateral maxilla due to an experimental justification of the use of tissue-engineered structure based on ectomesenchymal autologous cells and porous titanium in subantral maxillary augmentation.

**3. Materials and Research Methods**

Experimental study was conducted on 12 yearling rams. Choosing a ram's maxilla as an experimental model is due to the following reasons: a large amount of available bone in the studied area, ease of maintenance and low level of animal aggression, a high regenerative potential of donor areas that allows not to kill the animal upon material sampling. The operation was conducted in compliance with aseptic regulations through the external access - the surgical site before surgery was cut out and treated with antiseptic solutions.

Before surgery, 2% xylazine hydrochloride solution and 2% ketamine hydrochloride solution were administered intramuscularly based on animal body weight (0.15 ml per 1 kg), then infiltration anesthesia was carried out in the intervention zone with 2% Lidocaini solution with adrenalin 1:100000, the skin and muscle fascia were dissected sectionally, ensuring thereby an optimal access to the required area for bone preparation. A fragment of cortical bone 2 cm in diameter was sawed out with a ball-shaped burr in the anterior wall of the maxillary sinus. The resulting autograft was carefully separated from the mucosa of the maxillary sinus, the mucous surface was covered with tissue-engineered structure consisting of PuraMatrix/3DM hydrogel with pre-cultivated ectomesenchymal autologous

cells immobilized on the porous titanium in the form of granules with a granular size of 0.7-1.0 mm and a porosity of 80%. This tissue-engineered structure is a synthetic biodegradable matrix gel based on oligopeptide fragments that forms nanowires and pre-cultured cells ectomesenchymal ram cells treated with 5-azacytidine. The finished tissue-engineered product was obtained by mechanical mixing of gel with precultivated mesenchymal cells and a porous titanium *in situ.*

The autograft was then fit in the same place, the wound was sutured and treated with antiseptics, X-ray examination was conducted. During the repeated surgical procedure after 1, 2 and 3 months the bone fragments were sawed out in the previously operated area together with the mucosa of the maxillary sinus. The isolated bone blocks were fixed in 10% neutral formalin, decalcified in Trilon B, and were normally histologically processed with potting in paraffin. Sections of 8-10 mm thick were stained with hematoxylin-eosin, according to Van Gieson, Bichat and Mallory methods.

**4. Results**

Visual assessment of interaction between the systems of macropreparations "titanium-periosteum-bone" and "bone-titanium-mucosa" at each period of observation was of particular interest. On day 30 of the experiment, it is clearly seen upon visual examination of the bone fragment of maxilla with implanted porous titanium that the titanium granules are overgrown with connective fibers.

Obviously, the surface roughness and high porosity (up to 80%) of the titanium granules facilitates active migration of fibroblasts with further colonization of individual areas thereof and formation of connective tissue within 4 weeks after surgery; in addition, the porosity creates conditions for effective vascularization. The granules at this stage of observation are soldered tightly enough into the periosteum and are peeled together with this tissue.

On day 60 of the experiment, the main part of the porous titanium introduced to the bone defect shows a characteristic tendency towards consolidation into a single unit. Upon probing, all the granules are well adjacent to each other and the introduction of the probe into the depth of the filled defect is extremely difficult. Upon visual assessment of macropreparations obtained on day 60 of the experiment, it was found that all titanium granules introduced to the bone defect are tightly connected with each other, with the periosteum and the sinus mucosa.

On day 90, the "titanium-bone" conglomerate adjoins tightly the walls of the defects with no visual boundary observed between them. Upon stripping of sinus mucosa we can easily see the connective tissue fibers, small and medium blood vessels growing through the pores of titanium granules both from the side of sinus mucosa and from

the side of bone defect walls. The spaces between the granules are filled with newly-formed semi-translucent bone substance. During histological examination 1 month after the start of the experiment a porous titanium took form of both the large conglomerates and small clumps, located next to each other. There were accumulations of monocytes, macrophages, fibroblasts and connective tissue observed in the location area of titanium granules. No signs of inflammation were observed around the porous titanium. At high magnification, there were signs of outgrowth and even peripheral organization of the material with thin connective tissue fibers around the porous titanium. Bone trabeculae became more mature and structured by this time of observation. The formation of collagen fiber bundles in parallel rows became symmetrical and unidirectional.

By day 60, multiple intra- and intercellular crystalline and beamed inclusion in intergranular deposits of connective tissue can be observed in close vicinity of the major volume of porous titanium, and on the periphery - different size cavities and residues of small granules of porous titanium between the osteons. There is a clearly visible proliferation of connective tissue and the formation of proliferating active fibroblasts in the intertrabecular space with active angiogenesis of the microvasculature in the studied specimens. There is also a typically stable number of macrophages and neutrophils, and as a consequence, the low level of resorption of newly formed bone tissue, which, in turn, is caused by the absence of cellular activity of collagenolytic enzymes (collagenase, cathepsin, metalloproteinase). After 3 months, the bone trabeculae acquired a more ordered structure, the formation of bundles of collagen fibers in parallel rows with the formation of the trabeculae as thin arcs anastomosing with each other was traced.

Close to osteones, there was a pronounced striation of collagen fibers, which is one of the signs of penetration of cellular elements and a newly formed bone substance into the porous titanium granules. Newly formed osteons were of different sizes, often large or irregular shape, with broad Haversian channels. The patterns of trabecular formation by active ingrowth of collagenous connective tissue and blood vessels in the damaged trabecular matrix were observed in areas where the bone base of the sinus was heavily damaged.

**5. Summary**

Thus, by month 3 of the observation, there are titanium granules sealed in bone material clearly visible in the compacting tissue; at the same time, a newly formed bone in these periods of observation shows a sufficient maturity and the nature of the bone regeneration has all signs of active angiogenesis and osteogenesis.

Scanning electron microscopy showed that already in 60 days after filling the defect, a peripheral proliferation and organization of the material with thin connective tissue fibers can be observed around the porous titanium (**Fig. 1**).

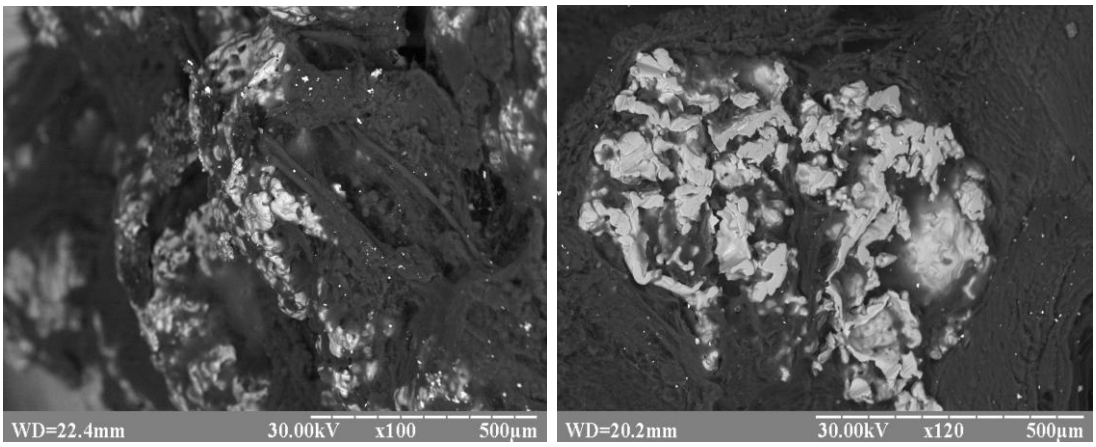


**Fig. (1). Scanning electron microscopy of the granules of non-resorbable porous titanium 60 days after filling the bone defect.**

## 6. Conclusion

The porous titanium granules included in the composition of the tissue-engineered structure together with precultivated ectomesenchymal autologous cells are biocompatible with the bone tissue, have the optimal parameters of the surface microrelief, which creates favorable conditions for adhesion, expansion and migration of osteoblasts, have insignificant kinetics of resorption, and are porous enough to ensure effective neovascularization of the de novo bone tissue. The porous titanium is an effective alternative to materials based on calcium phosphates and bone collagen as a carrying matrix for tissue-engineered structure for subantral augmentation of the upper jaw bone in case of dental implantation and reconstructive plastic surgery conducted in the maxillary sinus.

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## References

1. Budzinskii N.E. Determination of antimicrobial effect of Miramistin immobilized on the composite polisorbate on the microflora of root canals in acute and exacerbated chronic periodontitis and the ossification in animals / N.E. Budzinskii, S.V. Sirak, E.M. Maksimova, A.G. Sirak // Fundamental research. - 2013. - No. 7-3. - Pp. 518-522.
2. Grigoriants L.A. Indications and effectiveness of different surgical procedures in treating patients with odontogenic sinusitis caused by excretion of the filling material in the maxillary sinus./L.A. Grigoriants, S.V. Sirak, R.S. Zekeriaev, K.E. Arutiunian//Dentistry. - 2007. - Vol. 86. - No. 3. - Pp. 42-46.

3. Sletov A.A. Experimental determination of the regenerative potential of bone marrow cells/Sletov A.A., Pereverzev R.V., Ibragimov I.M., Kodzokov B.A., Sirak S.V.//Dentistry for all. - 2012. - No. 2. - Pp. 29-31.
4. Grigorian A.A. Development and clinical application of a new wound-healing agent for the treatment of diseases of the oral mucosa in children and adolescents / A.A. Grigorian, S.V. Sirak, A.G. Sirak, S.A. Khanova // Modern problems of science and education. - 2013. - No. 2. - P. 41.
5. Sletov A.A. Extraoral device for the anatomical positioning of fragments of jaw bones / A.A. Sletov, S.V. Sirak, A.B. Davydov, A.V. Arutiunov, R.A. Avanesian, A.G. Sirak, R.A. Mozheiko, I.A. Kopylova, T.T. Meboniia, Iu.I. Nikitina, I.E. Kazieva // Utility patent RUS 2541055 of 01.28.2014.
6. Sirak A.G. Morphofunctional changes in the dental pulp of experimental animals in the treatment of deep caries and acute focal pulpitis with the use of the developed drug compositions / A.G. Sirak, S.V. Sirak // Modern problems of science and education. - 2013. - No. 2. - P. 44.
7. Sirak S.V. The use of porous titanium for subantral bone augmentation in dental implantation (experimental research) /S.V. Sirak, A.A. Sletov, A.K. Martirosian, I.M. Ibragimov, M.G. Perikova // Medical Bulletin of the North Caucasus. 2013. - Vol. 8. No. 3. - Pp. 42-44.
8. Sirak S.V. Clinical and experimental use of osteoplastic materials in combination with electromagnetic radiation for accelerated regeneration of the maxillary bone defects/Sirak S.V., Kazieva I.E., Martirosian A.K.//Fundamental research. - 2013. - No. 5-2. - Pp. 389-393.
9. Sirak S.V. Dental incidence of child population of Stavropol region before and after the implementation of prevention programs / S.V. Sirak, I.A. Shapovalova, E.M. Maximova, S.N. Prigodin // Pediatric Dentistry and Prevention. - 2009. - Vol. 8, No. 1. - Pp. 64-66.
10. Sirak S.V. Dental morbidity of the adult population of the major climatic zones of Stavropol Territory / S.V. Sirak // Thesis for the degree of candidate of medical sciences / Stavropol State Medical Academy. Stavropol, 2003.
11. Sirak S.V. Experience in the use of local wound healing substances in the treatment of pemphigus vulgaris with localization in the oral mucosa and lips / Sirak S.V., Chebotarev V.V., Sirak A.G., Grigorian A.A.// Medical Bulletin of the North Caucasus. - 2013. - Vol. 8. - No. 1. - Pp. 59-62.

12. Sirak S.V. The effect of porous titanium on the osteogenic potential of bone marrow cells in vitro/Sirak S.V., Sletov A.A., Ibragimov I.M., Kodzokov B.A.// Medical Bulletin of the North Caucasus. - 2012. - Vol. 27. - No. 3. - Pp. 22-25.
13. Sirak S.V. Use of multicomponent adhesive ointment in combination with disease-modifying drug in adjuvant therapy of pemphigus/Sirak S.V., Kopylova I.A., Chebotarev V.V., Al-asfari F.M.S.//Paradonthology. - 2012. - Vol. 17. - No. 2. - Pp. 62-65.
14. Sirak S.V. diagnostics, treatment and prevention of maxillary sinusitis occurring after endodontic procedures/Sirak S.V., Sletov A.A., Loktionova M.V., Loktionov V.V., Sokolova E.V.//Paradonthology. - 2008. - No. 3. - Pp. 14-18.
15. Sirak S.V. A method of treating the radicular cyst of the jaw/Sirak S.V, Fedurchenko A.V., Sirak A.G., Mazharenko T.G.// Utility patent RUS 2326648 09.01.2007.
16. Sirak S.V. A method of dubantral bone augmentation for the installation of dental implants under atrophy of the alveolar process of the maxilla / S.V. Sirak, I.M. Ibragimov, B.A. Kodzokov, M.G. Perikova // Utility patent RUS 2469675 09.11.2011.
17. Radzievskaia N.G. Prevention and treatment of alveolitis after removal of third molar complex of the mandible / N.G. Radzievskaia, S.V. Sirak, E.V. Shchetinin, A.B. Hodzhaian, A.V. Arutiunov, R.A. Avanesian, G.G. Petrosian // Modern problems of science and education. - 2014. - No. 4. - P. 343.
18. Shchetinin E.V. The pathophysiological aspects of the regeneration of tooth extraction wells in the experiment / E.V. Shchetinin, S.V. Sirak, A.B. Hodzhaian, N.G. Radzievskaia, G.G. Petrosian // Medical Bulletin of the North Caucasus. - 2014. - Vol.9. - No. 3 (35). - Pp. 262-265.DOI: 10.14300/mnnc.2014.09073.
19. Grimm, W.D. Translational research: palatal-derived ecto-mesenchymal stem cells from human palate: a new hope for alveolar bone and cranio-facial bone reconstruction / W.D.Grimm, A.Dannan, B.Giesenhagen, I.Schau, G.Varga, M.A.Vukovic, S.V.Sirak // International Journal of Stem Cells. – 2014. - 7(1). – P.23-29.
20. Grimm, Dr.W.-D. Complex, three-dimensional reconstruction of critical size defects following delayed implant placement using stem cell-containing subepithelial connective tissue graft and allogenic human bone blocks for horizontal alveolar bone augmentation: a case report as proof of clinical study principles / Dr.W.-D. Grimm, M.Ploger, I.Schau, M.A.Vukovic, E.V.Shchetinin, A.B.Akkalaev, R.A.Avanesian, S.V.Sirak // Medical Bulletin of North Caucasus. - 2014. - T. 9. - № 2. - P. 125-127. DOI: 10.14300/mnnc.2014.09037.

21. Grimm W.D. Prefabricated 3d allogenic bone block in conjunction with stem cell-containing subepithelial connective tissue graft for horizontal alveolar bone augmentation: a case report as proof of clinical study principles/Grimm W.D., Plöger M., Schau I., Vukovic M.A., Shchetinin E., Akkalaev A.B., Arutunov A.V., Sirak S.V.//Medical Bulletin of North Caucasus. - 2014. - T.9. - №2(34). - C. 175-178.
22. Mikhalchenko, D.V. Influence of transcranial electrostimulation on the osseointegration of dental implant in the experiment/Mikhalchenko D.V., Poroshin A.V., Mikhalchenko V.F., Firsova I.V., Sirak S.V.//Research Journal of Pharmaceutical, Biological and Chemical Sciences. - 2014. - T.5. - № 5. C. - 705-711.
23. Sirak, S.V. Microbiocenosis of oral cavity in patients with dental implants and over-dentures/Sirak S.V., Avanesyan R.A., Akkalaev A.B., Demurova M.K., Dyagtyar E.A., Sirak A.G.//Research Journal of Pharmaceutical, Biological and Chemical Sciences. - 2014. - Vol.5. - No. 5. - Pp. 698-704.
24. Sirak, S.V. Clinical and morphological substantiation of treatment of odontogenic cysts of the maxilla/Sirak S.V., Arutyunov A.V., Shchetinin E.V., Sirak A.G., Akkalaev A.B., Mikhalchenko D.V.//Research Journal of Pharmaceutical, Biological and Chemical Sciences. - 2014. - Vol.5. - No. 5. - Pp. 682-690.

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