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# EXPERIMENTAL STUDY OF SKULL BONES RECONSTRUCTION WITH THE USE OF AUTOGRAFTS AND HYDROXYAPATITICAL GEL

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## Abstract

**Introduction.** To conduct reconstructive surgical interventions at pathological processes of skull base bone, defects of the temporal bone and *dura mater,* is especially complicated with the appearance of liquorrhea. This necessitates the improvement of operative technique and the use of new plastic materials. **Objective:** to study the features of meninges and skull bone defects plastic using autobone, fascia and HAP gel based on clinical observation, and macroscopic and microscopic experimental studies. **Materials and methods:** 36 mature nonlinear rats of both sexes up to 6 months old, weighing 190-250 g were divided into 2 groups. In the 1st (experimental) group there were 18 animals and autofragments of fascia and tibia with the addition of GAP gel were used for implantation; and in the 2nd group (comparison, n=18) the same plastic material without gel was used. The introduction of implant was carried out under general anesthesia in a standardized defect of skull frontal bones. The method of clinical observation, stereoscopic, common histological examination after staining with hematoxylin-eosin was applied. **Results:** The introduction of bone's and fascia autografts into skull defect and *dura mater* provides the structural function of the damage tightly closing, preventing or stopping of liquorrhea, besides fascia contributes to vascularization of tissues in the healing zone. Additional administration of HAP ge, provides pronounced adhesion to bone tissue, gaps filling in the graft bed, creates a

microenvironment for metabolism in the zone, shows a positive effect on osteogenesis, promotes calcification and bone reconstruction in the area of the defect. The results obtained also indicate the peculiarities of reparative processes in the bones of the skull, expressed in the dependence of bone repair and / or the development of fibrous tissue in the defect area and the density of adherence of the bone to the mother tissue, which is accompanied by improved bone restructuring under tight contact of the mother bone with the implanted material and the prevalence of fibrous tissue in case of their violation. This data confirm the effectiveness and promise HAPG use along with autofascia and autobone to close the defects of the meninges and skull bones and are important for improving reconstructive-restorative surgical interventions.

**Key words: reconstruction of skull bones defects, autofascia, autobone, hydroxyapatite gel (HAPG).**

To improve the methods and means for plastic surgery under the conditions of rapid development of reparative medicine and achievements in the development of new materials for implantation is today's urgent problem for various branches of medicine, including otolaryngology. In particular, the conduct of reconstructive surgical interventions in temporal bone defects, bones of the skull base, *dura mater,* accompanied by liquorrhea, necessitates the improvement of operation technique and the use of new plastic materials [1-5]. Among such materials, an amorphous nanostructured hydroxyapatite gel (HAPG), which has proven to be an active osteoplastic factor [6, 11], attracts attention.

Taking into account the above, **the objective** of this work was to study the peculiarities of skull bones reconstruction using autobone, fascia and HAPG.

The work included clinical observation, and experimental macroscopic and microscopic investigations.

**Material and methods.** The study was conducted on 36 sexually mature non-linear rats of both sexes up to 6 months old, weighing 190 - 250 g. The rules for ensuring thrifty and humane treatment of animals in accordance with the decisions of the CE Convention and the relevant Laws of Ukraine and ethics commission of SI "Prof. A.I. Kolomiychenko Institute for Otolaryngology NAMS of Ukraine ".

There were carried 2 groups of studies: in the 1st (experimental group, 18 animals), the auto-fragments of thigh muscle wide fascia and the tibia with the addition of HAPG were used for implantation, and in the 2nd (comparison group, 18 animals) the same plastic material without HAPG use.

Operations were performed under intra-abdominal anesthesia with sodium etamininal. The implant material was inserted into the frontal bones of the skull. A standardization technology for experimental bone damage was developed for research, which, after skin incision, included careful cutting of the bone with a dental boron and the formation of an oval defect with an average size of 2 x 5 mm. For simulated liquori, a defect of *dura mater* 0.2 mm in size was formed by its scarification with a needle.

During the operation, the defect of the cerebral sheath was sealed with fascia's fragment, and a rat frontal bone defect was closed with a prepared fragment of bone autograft. Animals of the experimental group in the bed of the graft additionally made HAPG, which filled the gaps between the auto- and maternal bones.

The gel used in the work is one of the variants of the Sintekost complex of ceramic composites I-PLANT Ltd. (Ukraine), State Registration Certificate No. 14719/2015 dated May 13, 2015. It has 10–20% concentration of inorganic matter without any foreign elements and contains only phosphate and calcium ions and water, the ratio of which corresponds to the stoichiometry of hydroxyapatite. Gel exhibits high hydrophilicity, tightly fills all cavities between the implant and the tissues of the skull, providing a seal of the created defect. After the operation was completed, the wound was sutured with 2-3 sutures and treated with an antiseptic. To prevent the development of inflammatory processes immediately after the operation, the antibiotic ceftriaxone was injected intramuscularly at the dose of 0.1 ml per 100 g of an animal's weight.

To assess the nature of the reparative processes course in the area of plastic surgery, animals were removed from the experiment by decapitation under ether anesthesia after 2 weeks, 1 - 1.5 and 2.5 - 3 months after surgery. In this case, for each period of the study 6 animals were taken. Samples of the material after removal were subject to conventional histological processing with decalcification, obtaining paraffin sections, staining with hematoxylin and eosin to assess the histological characteristics of tissues.

The studies were conducted with a high-end "Olympus SZX16" stereomicroscope and an "Olympus BX53" system microscope with a computer attachment. The samples were photographed with a digital camera "Olympus DP 72" and "Olympus DP 21".

**Results and discussion**

Clinical observations of the animals' state were made on the next day after surgery and in the subsequent terms of the study. They did not reveal changes in the animals' appearance, general behavior, or any movement disorders, etc.

In the wound zone, in the first days after the operation, a scab, slight edema and hyperemia were observed, which indicated the development of reactive processes. Skin healing by primary intention was noted already in 3-4 days after surgery, although soft tissue edema and hyperemia in the surgical area were still present, but were less pronounced in animals of the 1st group.

In 1-1.5 months after the operation, the restoration of the hair coat was detected and the pronounced tendency to decrease reactive edematous processes and hyperemia in the 1st group of the study was preserved.

In 2.5-3 months after the operation, the defect overgrown and absence of edema were observed in the group with autotransplants and HAPG, while in the comparison group the reactive thickening of the tissue structures remained.

When examining the operation area after removing the skin with stereoscopy in 2 weeks, on the edges of the oval defect of the maternal bone there were found *to fit snugly* to the edges of the bone defect and the presence of a light rim was noted. This may indicate a good adhesion of the introduced HAPG with the surrounding maternal bone tissue. An additional factor in this was the absence of a similar rim in the comparison group, where there was a pronounced reddening (hyperemia) in the contact areas on the border with the introduced bone, which may be associated with the development of reactive processes and vascular reactions.

A similar macroscopic picture was observed in 1-1.5 months after surgery, which also proves a pronounced affinity and a high level of adhesion of HAPG to the bone tissue, and which is evidenced by the literature data [6, 7, 9,10] (Fig. 1, 2).

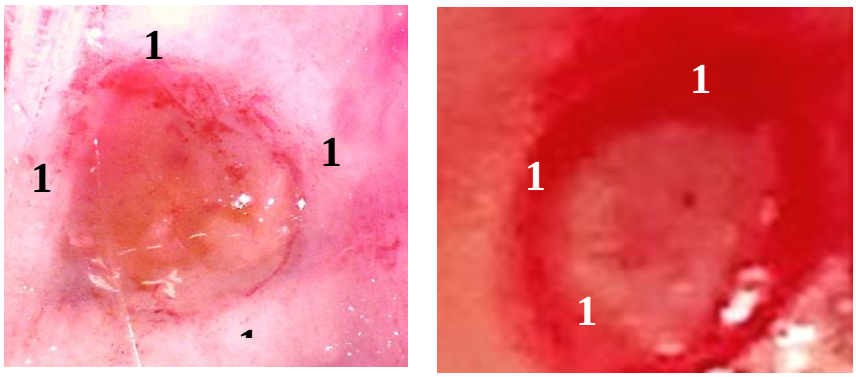


Fig.1. The bright rim in the bed of the defect (1) after the implantation of autobone, fascia and HAPG in 1 month after operation. Stereoscopy.

Fig.2. Redness (1) in the area of the defect and implantation of autobone and fascia in 1 month after operation. Stereoscopy.

In 3 months in the samples of the experimental group animals, almost complete closure of the defect was macroscopically noted, although the thickness of the newly formed bone tissue was uneven. At the same time, in the comparison group, thinning of the previously introduced autobone is revealed and areas of hyperemia are still detected, which may indicate a characteristic restructuring of the autograft during osteointegration, the manifestation of vascular reactions and the formation of fibrous tissue (Fig. 3, 4).

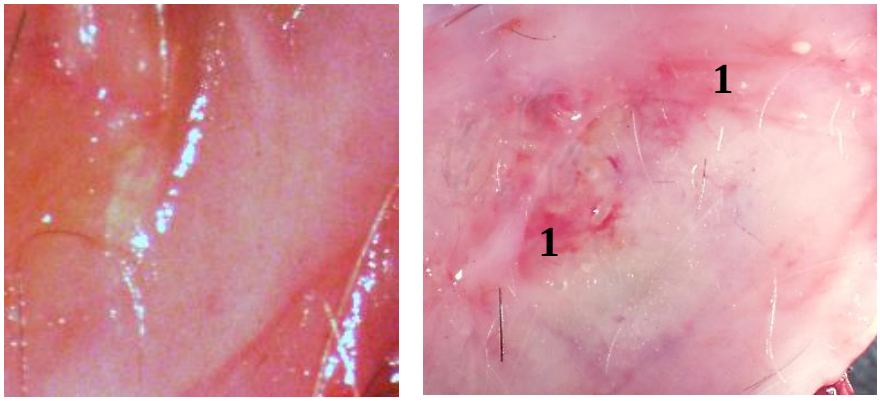


Fig. 3. Defect healing zone in 3 months after implantation of autobone, fascia and HAPG in animals of the experimental group. Stereomicroscopy.

Fig. 4. Areas of redness and hyperemia (1) in the area of the defect after the implantation of autobone and autofascia in 3 months after surgery in animals of the comparison group. Stereomicroscopy.

The results obtained indicate a more effective adhesion of autografts and closure of the defect in the frontal bones of the skull with osteogenesis appearance if autobone, fascia and HAPG were introduced during 3 month period of observation, while in the absence of GAPG hyperemia is more pronounced, which may indicate long preservation of vascular reactions and the development of fibrous tissue.

During the morphological study of microscopic preparations from the zone of skull defect in the first group of animals in 2 weeks after fascia's implantation, autologous bone and HAPG introduction in the area of fascia, the development of reactive changes with minor cell infiltration, fibroblast activation and formation of blood vessels (Fig. 5) was observed.

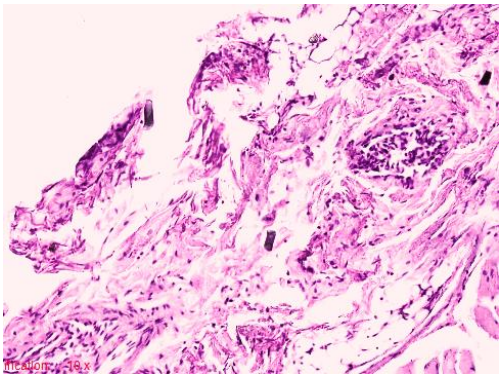

Fig. 5 Development of reactive processes with insignificant cellular infiltration, activation of fibroblasts and formation of blood vessels in the area of the introduction of fascia and autobone with HAPGl in the skull defect in 2 weeks after implantation. Microphoto. Stained with hematoxylin and eosin. 10, approx. ten.

The changes revealed are combined with activation and proliferation of *dura mater* cells, which is also reflection of the reparative processes characteristic for the healing of skull bones defects. This coincides with the data of literature [12, 13].

At this time, attention is drawn to the identification in the places of close proximity of the injected material and the mother bone in the area of the defect of active development of granulation tissue and vessel formation in the absence of any pathological manifestations. At the same time, single macrophages, active osteoblasts and osteoclasts are detected as well as the presence of fibroblasts and fibrous structures. The latter indicates the development of granulation tissue and the accumulation of osteoid substance (Fig. 6).

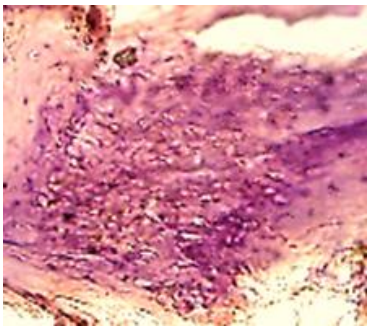

Fig.6. The development of granulation tissue and the accumulation of osteoid substance in the repair zone after the introduction of fascia and autobone with HAPG into the defect of the skull bones, 2 weeks after implantation. Microphoto. Stained with hematoxylin and eosin; a) About. 20 approx. 10.

A similar pattern in the graft bed with the introduction of HAPG may be due to its positive effect on osteogenesis, the creation of peculiar microenvironment for metabolism, the availability of calcium ions, which contributes to the calcification of newly formed structures and reconstruction of bone tissue in the area of the defect, as indicated by other researchers [6, 9, 10].

In addition, in the same period, especially in the places of close contact between the introduced and maternal bones, the development of healing processes with the formation of vascular and connective tissue bridges between auto and maternal bones in the areas of damage with the appearance of coarse fibrous callus (Fig. 7) take place.

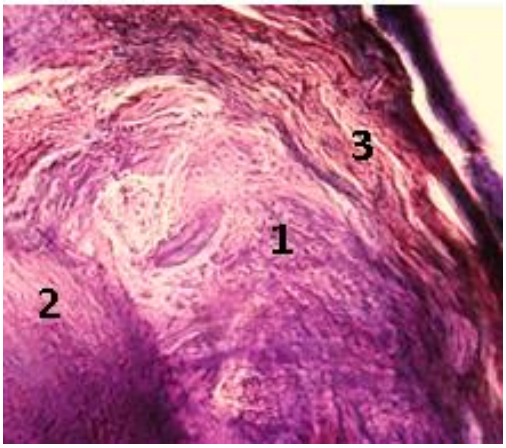


Fig. 7. The development of coarse-fibrous tissue (1) between the implanted (2) and the maternal bone of the skull (3). 2 weeks after the implantation of autografts and HAPG. Stained with hematoxylin and eosin. Microphoto. About. 10. ok.10

In the comparison group, at this period the development of reparative processes with the formation of vascular and connective tissue structures (bridges) between autologous and maternal bones in the areas of damage with the appearance of granulations, activation of fibroblasts and endothelial cells are observed. However, more pronounced resorptive processes of the injected bone take place as well. This is manifested by the identification of a large number of osteoclasts, the presence of extended gaps between the autograft and the maternal bone and areas with loose connective tissue, as well as more sparse placement of tissue structures characteristic of edema (Fig. 8).

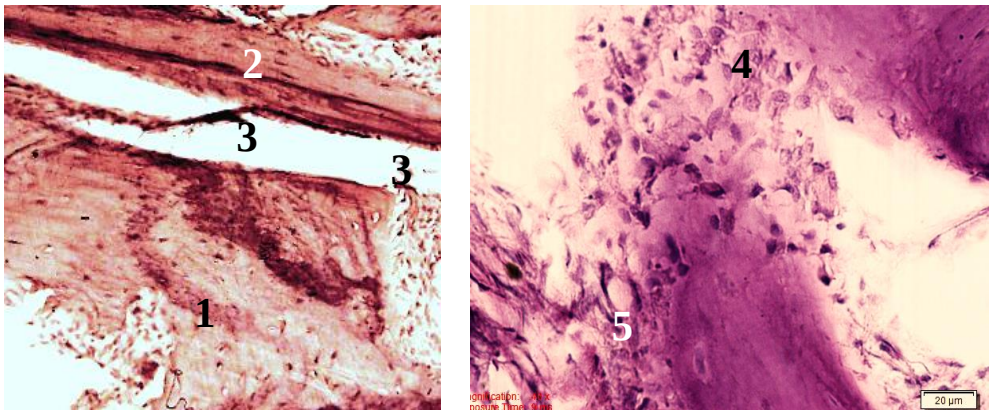


Fig. 8. a) Development of reparative processes between the areas of auto (1) and maternal (2) bones with the manifestation of resorption of the inserted autobone, the appearance of connective tissue (3) in the area of the defect; b) activation of osteoclasts and osteoblasts (4), endotheliocytes (5) of the vessels in the inserted fascia and autobone with the formation of a connective tissue ligament in 2 weeks after the operation without HAPG introduction. Microphoto. Stained with hematoxylin and eosin. a) About 10, approx. 10, b) O. 40, approx. ten.

In the study of histological preparations of the experimental group in the next 1 and 1.5 months after implantation, restructuring phenomena are detected, which correlates with the active manifestation of calcification in areas of newly formed bone tissue (Fig. 9).

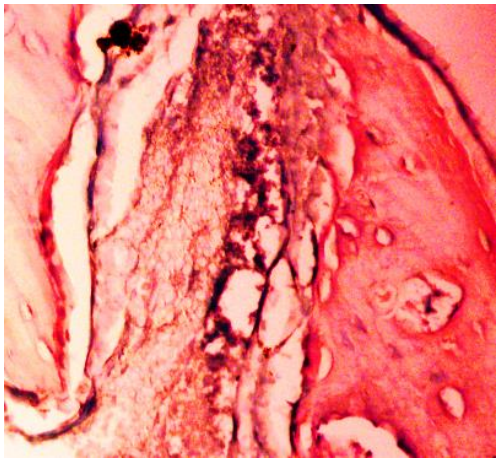

Fig. 9. The restructuring zone and the accumulation of osteoid substance after the introduction of fascia and autobone with HAPG into the defect of the skull bones in 1.5 months after implantation. Microphoto. Stained with hematoxylin and eosin. About 20, approx. 10.

The presented results indicate that the implanted HAPG exhibits adhesion and affinity for bone tissue, ensures the filling of gaps in the graft's bed and creates microenvironment for metabolism and assimilation of injected calcium in the area of the defect, further contributes to calcification processes and restoration of bone tissue. The phenomena of active calcium accumulation and improvement of bone tissue regeneration after the use of HAPG are also evidenced by other researchers [6, 9, 10].

Subsequently, in 3 months after surgery with HAPG, the bone defect is filled with newly formed bone tissue with activation of bone mash and remnants of individual sections of coarse fibrous tissue, preservation of osteogenesis signs, and the presence of areas of architectonically disturbed plate-shaped bone (Fig. 10).

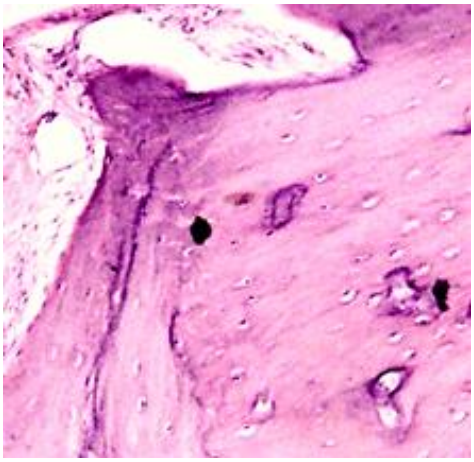

Fig.10. Violation of the architectonics of the newly formed bone tissue in the area of the defect 3 months after the implantation of autografts and HAP gel. Microphoto Stained with hematoxylin and eosin. A. 20 approx. ten.

At the same time, in the second group, destructive changes in the bone tissue and defect filling with fibrous structures still remained (Fig. 11).

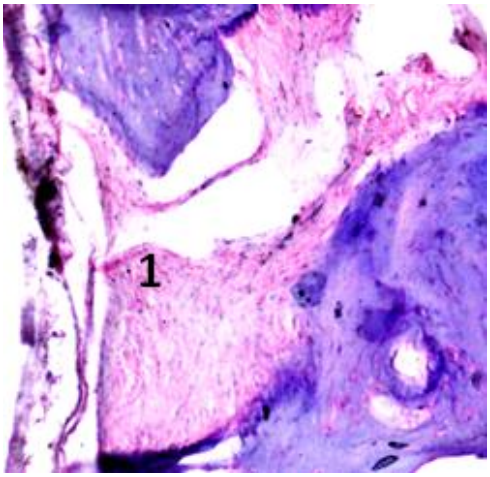


Fig.11. Filling the defect area with fibrous tissue in 3 months after the implantation of autografts. Microphoto. Stained with hematoxylin and eosin. A. 20 approx. ten.

It should be noted that when autobone is used, bone tissue recovery in the defect zone is found to depend on the adhesion density of the injected bone to the maternal bone, which is accompanied by a corresponding proliferation of fibrous connective tissue and is equally characteristic of using autotransplants with or without HAPG (Figure 12).

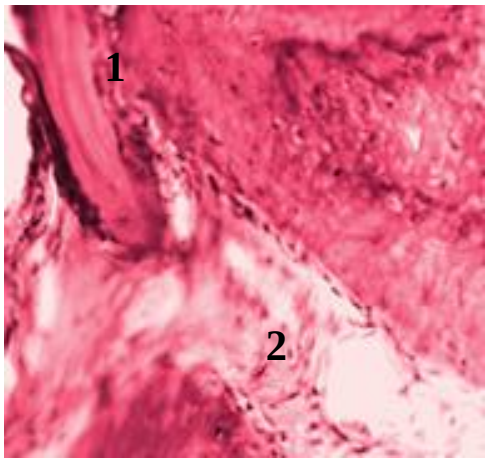


Fig. 12. Engraftment of autograft to the skull bone with their close fit (1) and the development of fibrous scar tissue in areas of increased distance by bone structures (2) in 3 months after the introduction of autografts and HAPG. Microphoto. Stained with hematoxylin and eosin. A. 20 approx. ten.

The results presented can be explained by the characteristic features of the reparative processes course in skull bones, which is due to the importance of ensuring the necessary contact of fragments; the diastasis should not exceed 1 mm. In the absence of such a contact, cell-fibrous, then fibrous tissue is formed, which persists for years without bone formation.

Thus, an experimental study of the plastic features at meninges and skull defect using autobone, fascia and HAPG suggests that the introduction of bone and fascia autografts into the defects mentioned provides a structural function of tightly closing damage, preventing or stopping liquorrhea and promotes vascularization of tissues in the area of healing. Additional administration of HAPG has a pronounced adhesion and affinity for bone tissue, provides gaps filling in the bed of the graft, creates a microenvironment for metabolism, has a positive effect on calcium aspiration and osteogenesis, promotes calcification of newly formed structures and reconstruction of bone tissue in the area of the defect.

The dependence of reparative processes in skull bone tissue in the defect area on the adhesion density of the injected bone to the maternal bone is significant, which results in successful bone resustation with a tight fit and development of fibrous tissue in the site where the autograft does not fit in the bed. This should be considered when performing surgeries and is important for reconstructive surgery results improvement.

**Conclusions:**

1. Experimental observations made, as well as macro- and microscopic studies indicate that the introduction of bone and fascia autografts into skull zone defect provides the structural function of damage tightly closing, preventing or stopping liquorrhea.

2. Fascia's introduction into the area of the defect also contributes to the vascularization of tissues and the processes of ostification.

3. HAPG's additional administration provides pronounced adhesion to bone tissue, does not cause adverse effects on surrounding tissues, fills gaps in the graft bed, creates a microenvironment for metabolism in the zone, positively affects calcium inhibition and osteogenesis, promotes calcification of newly formed structures and reconstruction bones in the area of the defect.

4. The results obtained also indicate the peculiarities of reparative processes in skull bones which is expressed in the dependence of bone repair and / or development of fibrous tissue in the defect area on the adhesion density of the injected bone to the mother tissue, which is accompanied by improved bone restructuring under conditions of tight contact of the mother bone with implanted material and the prevalence of fibrous tissue in case of their violation.

5. In general, the data obtained confirm the effectiveness and availability of HAPG use along with autofascia and autobone to close the defect of *dura mater* and skull bones and are important for improving reconstructive-restorative surgical interventions.

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