HISTOMORPHOLOGICAL EVALUATION OF MAXILLARY AUGMENTATION AREAS AND SCHNEIDER'S MEMBRANE PLASTICALLY CLOSED BY VARIOUS METHODS

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ABSTRACT

Aim of the study. The aim of this study was to investigate, under experimental conditions and by means of histomorphological examination, the effect of platelet-rich fibrin on reparative osteogenesis in areas of maxillary bone augmentation and the effect of autologous fibrin glue on regeneration of the injured Schneiderian membrane. **Materials and methods.** The experimental studies were performed on sexually mature outbred rabbits (21 animals), in which open sinus lift procedures were carried out under intravenous anesthesia, with deliberate perforation of the Schneiderian membrane during the intervention. Depending on the methods used for plastic closure of the created defect, the rabbits were divided into two experimental groups: the main group, consisting of 12 animals, and the control group, consisting of 9 animals. In both groups, the perforated area was closed with an “Ossix Plus” collagen membrane, and maxillary bone augmentation was performed using the osteoplastic material “Cerabone Botiss”. However, in the main group of animals, autologous fibrin glue was preliminarily applied to the collagen membrane, and the osteoplastic material was mixed with platelet-rich fibrin. **Results.** In the control group of animals, 10 days after surgery, histological examination revealed morphological manifestations of an acute inflammatory reaction in the areas of Schneiderian membrane perforation; after 30 days, traces of hemorrhages or wall-adherent hematomas were observed in the lumen of the maxillary sinuses; after 90 days, while separate areas of the bone defect were replaced by mature fibrous connective tissue. In rabbits of the main group, on postoperative day 10, moderate inflammatory infiltration of the Schneiderian membrane mucosa was found in the biopsy specimens; after 30 days, morphological signs of reduction of the inflammatory process in the perforated area and complete restoration of its anatomical integrity were observed; after 90 days, osteoid had formed in the augmentation areas of the anterior wall of the maxillary sinus, and in separate zones it was already being replaced by mature bone tissue. **Conclusions.** Autologous fibrin glue ensures tight sealing of the Schneiderian membrane perforation area, stable hemostasis, and an anti-edematous effect, and accelerates its regeneration. Under the influence of platelet-rich fibrin (PRF), in the maxillary augmentation graft of rabbits, 3 months after osteoplastic procedures, osteoid formation is completed and mature bone tissue is formed more actively, which is confirmed histomorphologically.

Key words: autologous fibrin glue, platelet-rich fibrin, collagen membrane, Schneiderian membrane plastic closure, histomorphological study.

INTRODUCTION

During open sinus lift procedures, perforation of the mucous membrane of the maxillary sinus floor, the Schneiderian membrane (SM), may occur in 20–37% of dental patients [1, 2], which negatively affects integration of the bone graft and dental implant [3, 4]. Restoration of SM integrity and sealing of the perforation area are of decisive importance for the success of surgical intervention. Today, many methods for closing SM perforations are available [5-8]. When large perforations (≥10 mm) occur, they must be surgically closed with resorbable collagen membranes in order to prevent the bone graft from entering the lumen of the maxillary sinus and to avoid the development of an inflammatory process within it [3]. However, when collagen membranes are used, complete sealing of the perforation area is not always achieved, and defects of the maxillary sinus mucosa closed with this biomaterial are often replaced by fibrous tissue without restoration of the ciliated epithelium [6]. Collagen membranes used to close large SM perforations may become

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displaced under the pressure of grafting materials used for augmentation of the alveolar process of the maxilla [7]. To improve the effectiveness of treatment of SM perforations, a number of authors have proposed the use of bioadhesives, particularly fibrin glue [8]. It is simple to use clinically. Fibrin glue ensures complete sealing of the wound surface and has no adverse effects compared with synthetic adhesives. Autologous fibrin adhesives have several advantages, as they are biocompatible and safe. Autologous fibrin glue also has a better effect on the healing of palatal soft-tissue wounds compared with platelet-rich fibrin (PRF) [9]. However, for stimulation of reparative osteogenesis of the jaw bones, it is more effective to use platelet-rich plasma (PRP) or PRF mixed with xenografts [10-12]. However, in the available professional literature, we found no data concerning morphological evaluation of the effectiveness of platelet-rich fibrin for maxillary augmentation in the setting of plastic closure of a perforated SM with autologous fibrin glue.

AIM OF THE STUDY

To investigate, under experimental conditions and by means of histomorphological examination, the effect of platelet-rich fibrin on reparative osteogenesis in areas of maxillary bone augmentation and the effect of autologous fibrin glue on regeneration of the injured Schneiderian membrane.

MATERIALS AND METHODS

The experimental studies were carried out in the vivarium of the State Non-Profit Company Danylo Halytsky Lviv National Medical University and at the Department of Histology of the State Non-Profit Company Danylo Halytsky Lviv National Medical University. During animal manipulations and withdrawal of animals from the experiment, moral and ethical standards were fully observed in accordance with the principles of the Declaration of Helsinki, the Council of Europe Convention on Human Rights and Biomedicine, ICH GCP, and the current regulatory legal acts of Ukraine. Studies in rabbits were conducted in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of 18.03.1986, Council of Europe Directive 2010/63/EU, and the Law of Ukraine "On Protection of Animals from Cruelty." The study was approved by the Commission on Ethics of Scientific Research, Experimental Developments, and Scientific Works of the State Non-Profit Company Danylo Halytsky Lviv National Medical University on March 18, 2024, protocol No. 3. Sexually mature outbred rabbits (21 animals) were divided into two experimental groups: the main group, consisting of 12 animals, and the control group, consisting of 9 animals. In all animals, after shaving and antiseptic treatment of the skin in the frontal region of the maxilla, surgical interventions were performed under intravenous anesthesia with a sodium thiopental solution (25 mg/mL): a 15-mm vertical incision was made along the midline of the dorsum of the nose, the periosteum was detached with a raspatory, and the anterior wall of the maxillary bone was exposed. Using a round bur, a bone defect measuring 10 mm² was created above the maxillary sinus. After lifting, or elevation, of the Schneiderian membrane, a perforation 0.5 mm in diameter was made in it. Depending on the methods used for plastic closure of the created defect of the maxillary sinus mucosa and the materials used for bone augmentation, the rabbits were divided into two experimental groups. In the first, control group, the perforation area of the maxillary sinus mucosa was closed with an "Ossix Plus" collagen membrane (Dentsply Sirona, USA), and maxillary bone augmentation was performed with the osteoplastic material "Cerabone Botiss" (bovine bone), which had been preliminarily moistened with physiological saline. In the main group of animals, plastic closure of the Schneiderian membrane was also performed

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with an "Ossix Plus" collagen membrane, onto which autologous fibrin glue had been preliminarily applied. This glue was prepared according to the method of Alston S.M. et al. [13]: 7 mL of venous blood was taken from the animal's tail vein into sterile tubes with a 3.8% sodium citrate solution and centrifuged for 4 min at 3500 rpm; as a result, the blood was separated into two fractions: an erythrocyte-leukocyte clot and platelet-rich plasma containing fibrinogen and blood coagulation factors in physiological concentrations. The obtained platelet-rich autologous plasma was mixed with a component containing a 10% calcium chloride solution and thrombin, after which a bioadhesive was formed. Bone augmentation of the maxilla in rabbits of the main group was performed with the osteoplastic material "Cerabone Botiss," which was mixed with platelet-rich fibrin, obtained according to the method of D.M. Dohan Ehrenfest [14]: 7 mL of venous blood, taken from each animal of the main experimental group by venipuncture of the tail vessel, was placed into a glass tube without anticoagulant and immediately centrifuged (3000 rpm, 10 min). After centrifugation, two layers were formed in the tubes. The upper layer was a platelet-rich fibrin clot, and the basal layer consisted of erythrocytes. PRF was removed with sterile forceps, and erythrocytes at the bottom of the fibrin clot were removed.

Three rabbits from the control group and four rabbits from the main group were withdrawn from the experiment on postoperative days 10, 30, and 90. Euthanasia was performed using chloroform by inhalational overdose. Decapitation was then performed, followed by complete dissection of the skin and amputation of the auricles. The maxillae, nasal bones, choanae, and nasal septum were excised as a single block using a Gigli saw. The obtained fragment was fixed in a 10% buffered formalin solution, pH 7.1–7.2. Fixation was carried out for two weeks, with the fixative solution changed every three days. After fixation, the material was washed in running water for 12 hours, followed by immersion in a 5% trichloroacetic acid solution for decalcification. The solution was changed every 3 days, with an exposure period of 7 days. After decalcification, the material was washed in running water for 2 hours; the operative site was excised with a razor blade, and transverse bone sections were cut and subsequently placed into histological cassettes. Dehydration of the material was performed in an ascending series of alcohols, with the concentration increased by 10%, beginning with 70° ethyl alcohol. Exposure lasted 12 hours in each alcohol. After the material was brought to 96% ethyl alcohol, it was placed into two portions of absolute ethyl alcohol for 12 hours in each. It was then transferred into a 1:1 mixture of absolute alcohol and xylene for 1 hour, followed by transfer into two portions of xylene for 1 hour in each. The material was then placed into a 1:1 mixture of xylene and paraffin at 37°C for 1 hour, followed by immersion in two portions of paraffin at 54–55°C for 1 hour in each. After paraffin impregnation, the bone fragments were removed from the histological cassettes and placed into preheated metal molds, oriented in the required plane, filled with pure heated paraffin, and covered from above with a plastic histological cassette to form the base of the block. The material was cooled, and the obtained blocks were removed from the mold and fixed in block holders. Serial sections 7 μm thick were obtained using an MS-2 microtome. They were transferred into warm distilled water at 40°C, straightened by immersing the slide under the section floating on the surface, collected onto the slide, and dried in a thermostat for one day at 37°C. The histological sections were deparaffinized by passing them through two portions of xylene, with an exposure time of 2 min in each. Rehydration of the sections was performed by passing them through a descending series of alcohols, beginning with two portions

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of 96% ethyl alcohol for 1 min in each, followed by 80% alcohol for 1 min, 70% alcohol for 1 min, and 60% alcohol for 1 min. After being brought to distilled water, the sections were washed for 1 min, stained with Ehrlich's hematoxylin for 2 min, washed with running water for 2 min, and immersed in a 0.1% aqueous eosin solution for 1 min. Dehydration of the sections was performed beginning with 70% alcohol, followed by transfer into 80%, 90%, 96%-1, and 96%-2 alcohol for 5 seconds each, and then into 96%-3 alcohol for 1 min. The sections were then transferred into two portions of xylene for 2 min in each. The obtained specimens were mounted with coverslips after preliminary application of a synthetic mounting medium. After drying and secure attachment of the coverslip to the slide, photodocumentation was performed using a Leica DM2500 optical microscope and a DFC450 C digital camera with Leica Application Suite Version 4.4.0 software. Statistical analysis of the study results was performed using Student's t-test with computer software.

RESULTS AND DISCUSSION

Ten days after open sinus lift operations, in three rabbits of the control group, microscopic examination revealed morphological manifestations of an acute inflammatory reaction in the areas of SM perforation, in which cellular infiltration with predominance of polymorphonuclear leukocytes and vascular congestion was visualized. In two animals of this group, hemorrhages into the maxillary sinus were observed. In the third animal from this group, no hemorrhage into the maxillary sinus was observed; however, against the background of acute reactive inflammation, destruction of the surface ciliated epithelium of separate areas of the Schneiderian membrane occurred, with deposition of leukocytic-necrotic detritus on its surface. The periosteal inflammatory infiltrate did not extend beyond the bone tissue of the anterior wall of the sinus that was damaged during surgery. At the same time, in the nasal passages of the animals on the ipsilateral side, pronounced serous edema of the mucosa was observed, with a moderate number of leukocytes present on its surface and entering the lumen of the nasal passage (Figure 1).

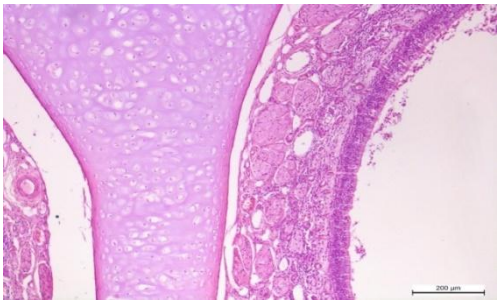

Figure 1. Pronounced serous inflammatory reaction of the mucosa of the inferior nasal meatus on the injured side of the upper jaw. Control group rabbits. Observation duration: 10 days. Magnification x 100.

Thirty days after sinus lift procedures, histological examination of the experimental animals revealed traces of hemorrhages or parietal hematomas in the lumen of the maxillary sinuses, where remnants of neutrophilic leukocytes were still present; however, reactive edema of the mucosa in the inferior nasal meatus was no longer present. Inflammatory polymorphocellular infiltration of the Schneiderian membrane persisted, with fibroblasts, lymphocytes, connective-tissue macrophages, and plasma cells present in the structure of its regenerate. Areas of osteolysis of the bone graft were detected and replaced by granulation tissue (Figure 2).

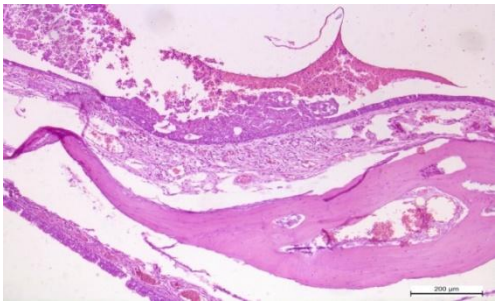

Figure 2. Replacement of the eroded edges of the bone graft by granulation tissue.
Control group rabbits. Observation duration: 30 days. Magnification x 100.

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Ninety days after the performed sinus lift procedures with bone augmentation of the anterior walls of the maxillary sinuses, the resorbed areas of the bone graft by osteoid were replaced in rabbits; along its periphery, isolated giant multinucleated cells were encountered. Separate areas of the bone defect were replaced by mature fibrous connective tissue (delayed osteogenesis) (Figure 3).

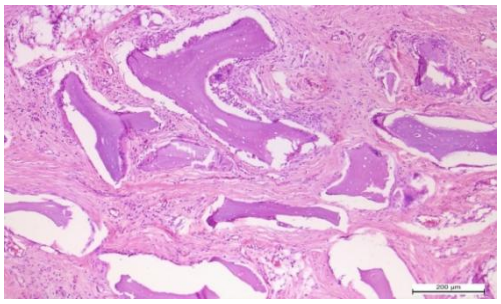

Figure 3. In the areas of maxillary augmentation, replacement of the bone graft by osteoid is visualized; individual areas are still filled with mature fibrous connective tissue.
Control group rabbits. Observation duration: 90 days. Magnification x 100.

In rabbits of the main group, on postoperative day 10, moderate inflammatory infiltration of the Schneiderian membrane mucosa with hyperplasia of mucus-producing glands was found in biopsy specimens. Structures of the lymphoid-follicle type (immune reaction) formed in the infiltrate, and no necrosis of cells of the covering ciliated epithelium was detected. At the same time, in the ipsilateral inferior nasal meatus, the mucosa was of normal structure without morphological signs of inflammation (Figure 4).

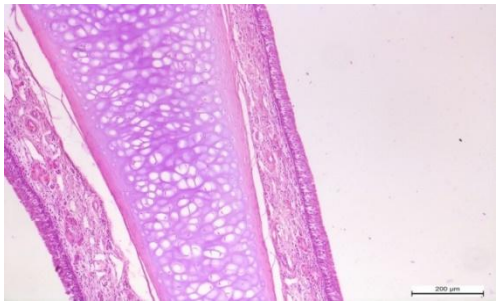

Figure 4. The mucosa of the inferior nasal meatus is of normal structure without morphological signs of inflammation. Main group rabbits. Observation term: 10 days. Magnification x 100.

After 30 days, in animals of the main group, microscopic examination revealed morphological signs of reduction of the inflammatory process in the injured Schneiderian membrane and its active regeneration. Complete restoration of its anatomical integrity occurred in the perforation area. In the regenerate, cellular elements of connective tissue and newly formed vessels predominated; these vessels grew between the lamina propria of the maxillary sinus mucosa and the cortical layer of the maxillary bone. During this period, in the augmentation areas of the anterior wall of the maxillary sinus, active lysis of the bone xenograft and its diffuse replacement by mature, vessel-rich connective tissue occurred (Figure 5).

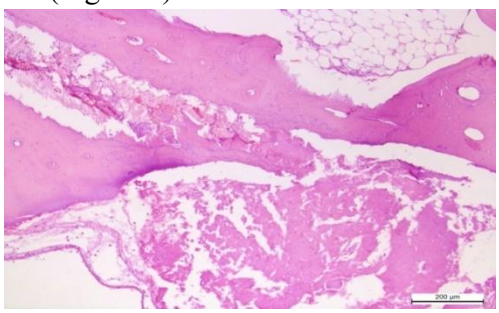

Figure 5. Lysis of the xenograft in the augmentation areas and its active replacement by connective tissue. Erosion (thinning) of the edges of the maxillary bone defect.
Main group rabbits. Observation term: 30 days. Magnification x 100.

After 90 days, in the augmentation areas of the anterior wall of the maxillary sinus, formed osteoid was detected, which in individual zones was already being replaced by mature bone tissue (Figure 6).

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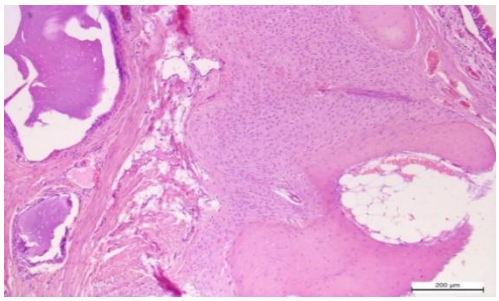

Figure 6. In the areas of maxillary augmentation, the beginning of formation of mature bone tissue from osteoid, filling the jaw defect, is visualized. Main group rabbits. Observation term: 90 days. Magnification x 100.

Thus, in rabbits of the main group, under the influence of PRF, a more active process of reparative osteogenesis occurred in the maxillary bones in the areas of augmentation; this was confirmed by morphological signs of transformation of the non-mineralized organic matrix, osteoid, into mature bone tissue, detected 3 months after the osteoplastic procedures. At the same point, in animals of the control group, only osteoid was detected in the bone regenerate of the maxillae, and isolated areas of mature fibrous connective tissue were still observed. Under the action of the autologous fibrin adhesive in the areas of SM injury, the acute reactive inflammatory process was less pronounced than in the control group of animals and had a localized character. The anti-edematous and hemostatic properties of this bio adhesive [15], together with complete sealing of the perforation areas, prevented blood from seeping from the bone wound into the maxillary sinus. Restoration of the morphological structure of the maxillary sinus mucosa occurred within one month after its mechanical injury. Fibrin glue induces blood coagulation. Thrombin converts fibrinogen into a fibrin clot, which, due to its hemostatic properties, promotes wound sealing and supports regeneration of damaged tissues. The clot adheres to adjacent tissues, and the resulting structure becomes a natural scaffold for progenitor and effector cells, as well as hematopoietic growth factors that actively promote wound healing [16]. Scientific studies confirm that the SM, especially its connective-tissue base, the lamina propria, has osteogenic properties similar to those of the periosteum and is rich in mesenchymal stem cells [17]. Slowing of the SM regeneration process in animals of the control group may be related to the fact that the use of dense collagen membranes, especially those with a long resorption period, can create a physical barrier that prevents nutrient diffusion and migration of osteogenic cells directly from the SM into the regeneration area, which may slow new bone formation in the sub antral cavity [18]. Effective bone regeneration requires modulation of three key processes: regulation of inflammation, formation of a vascular network, and osteogenic differentiation of progenitor cells at the defect site [19]. The immunohistological advantages of PRF are associated with the presence of many pro-inflammatory and anti-inflammatory cytokines in its composition, which, together with the fibrin clot, stimulate bone wound healing through the slow release of growth factors [11, 12, 20]. Platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF), bone morphogenetic protein 2 (BMP-2), and epidermal growth factor (EGF), released by PRF, have a therapeutic role in bone formation and remodeling [21]. They induce chemotactic activity and promote cellular differentiation and proliferation [22, 23], substantially influencing the volume and rate of new bone tissue formation and its maturation [24].

CONCLUSIONS

In the injured Schneiderian membrane during open sinus lifting in rabbits, morphological manifestations of an acute inflammatory process arise, which may spread ipsilaterally into the maxillary sinus and to the mucosa of the nasal meatus. Plastic closure of Schneiderian

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membrane perforation with a collagen membrane does not always prevent blood from entering the maxillary sinus from the bone wound. Fibrin autoglue ensures tight sealing of the Schneiderian membrane perforation area, stable hemostasis, and an anti-edematous effect, and accelerates its regeneration. Under the influence of platelet-rich fibrin (PRF), in the maxillary augmentate of rabbits, 3 months after osteoplastic operations, osteoid formation is completed and mature bone tissue is formed more actively, which is confirmed histomorphologically.

Prospects for further research. In further clinical studies, based on the obtained experimental results, methods for treating Schneiderian membrane perforations during open sinus lift operations in dental patients will be optimized.

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