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# DETERMINATION OF HISTOLOGICAL COMPOSITION AT THE LOCUS OF INSTALLATION OF ALLOGENEIC DURA MATER IMPLANT AN IN VIVO MODEL. LABORATOR-HISTOMORPHOLOGICAL RESEARCH

Nosova M.A., Sharov A.N., Nefedova I.F., Volova L.T., Trunin D.A.

## ОПРЕДЕЛЕНИЕ ГИСТОЛОГИЧЕСКОГО СОСТАВА В МЕСТЕ УСТАНОВКИ АЛЛОГЕННОГО ИМПЛАНТАТА ТВЕРДОЙ МОЗГОВОЙ ОБОЛОЧКИ НА МОДЕЛИ IN VIVO: ЛАБОРАТОРНО-ГИСТОМОРФОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ

Носова М.А., Шаров А.Н., Неведова И.Ф., Волова Л.Т., Трунин Д.А.

## IN VIVO MODELDA ALLOGEN DURAL IMPLANT O'RNATISH JOYIDA GISTOLOGIK TARKIBINI ANIQLASH: LABORATORIYA VA GISTOMORFOLOGIK TADQIQOT

Nosova M.A., Sharov A.N., Nefedova I.F., Volova L.T., Trunin D.A.

Federal State Budgetary Educational Institution of Higher Education "Samara State Medical University" of the Ministry of Health of the Russian Federation, Limited Liability Company «Dental Shop HAMOMILLA»

**Цель:** определение гистологического состава тканей в месте имплантации твердой мозговой оболочки и оценка изменений структуры имплантата и тканевой реакции окружающих тканей в результате операции на клеточном уровне. **Материал и методы:** лабораторно-гистоморфологическое исследование выполнено на 60 лабораторных крысах, которым проведена операция, адекватная хирургической методике лечения рецессии десны: в контрольной группе без пластики, в основной группе – с аллогенной твердой мозговой оболочкой. Макропрепараты получали на 3-7-14-28-90-107-е сутки после операции. **Результаты:** во всех случаях комплекс тканей формируется повторно, реакция на операцию одинаковая. Замена пластического материала происходит в те же сроки. При поднадкостничной инсталляции пластический материал замещается костной тканью, при внутридесневой – соединительной тканью. Утолщение десневого биотипа происходит в большей степени из-за травмы от операции, в меньшей степени от пластического материала. Аллогенная твердая мозговая оболочка стимулирует оксификацию раньше по сравнению с контролем. **Выводы:** восстановление/создание костного объема замыкающей альвеолярной пластинки вестибулярно обеспечивает поддержку мягких тканей десны; определяет стабильность результата хирургического лечения рецессии десны и хороший прогноз в отдаленном периоде: без осложнений и рецидивов.

**Ключевые слова:** рецессия десны, гистологический тканевой состав, аллогенная твердая мозговая оболочка.

**Maqsad:** dura mater implantatsiyasi joyidagi to'qimalarning gistologik tarkibini aniqlash va hujayra darajasida operatsiya natijasida implantatsiya tuzilishidagi o'zgarishlarni va atrofdagi to'qimalarning to'qimalarga reaksiyasi baholash. **Material va usullar:** 60 ta laboratoriya kalamushlarida laboratoriya gistomorfologik tadqiqoti o'tkazildi, ularda gingival retsessiyani davolashning jarrohlik usuliga mos keladigan operatsiya o'tkazildi: nazorat guruhida plastik jarrohliksiz, asosiy guruhda allogen dura mater bilan. Operatsiyadan keyingi 3-7-14-28-90-107-kunlarda yalpi tayyorgarlik olindi. **Natijalar:** barcha holatlarda to'qimalar majmuasi qayta shakllanadi, operatsiyaga reaksiya bir xil bo'ladi. Plastik material bir vaqtning o'zida almashtiriladi. Subperiostal instilatsiya bilan plastik material suyak to'qimasi bilan, intragingival instilatsiya bilan - biriktiruvchi to'qima bilan almashtiriladi. Gingival biotipning qalinlashishi ko'proq operatsiyadan olingan travma, kamroq darajada plastik material tufayli sodir bo'ladi. Allogenik dura mater ossifikatsiyani nazoratdan oldinroq rag'batlantiradi. **Xulosa:** alveolyar plastinkaning yopilishining suyak hajmini tiklash / yaratish vestibulyar ravishda milklarning yumshoq to'qimalarini qo'llab-quvvatlaydi; gingival retsessiyani jarrohlik davolash natijasining barqarorligini va uzoq muddatli davrda yaxshi prognozni aniqlaydi: asoratlar va relapslarsiz.

**Kalit so'zlar:** gingival retsessiya, to'qimalarning gistologik tarkibi, allogen dura mater.

Gingival recession is a frequently encountered dental pathology, both in Russia and worldwide [6]. There are effective and safe treatment methods offering almost complete elimination of single and multiple gingival recession (100% closure of the tooth root) [1,8]. All modern techniques in the last 30 years give qualitatively positive clinical result that is stable in the long term [2].

The problem of gingival recession treatment complications is also relevant due to the high incidence of early and remote terms. Most often, complications are associ-

ated with: errors in surgical treatment planning; erroneous choice of strategy, tactics and surgical treatment protocol; inadequate choice or application of a particular surgical technique in the personalized clinical situation of the patient [11,15]. When complying with modern scientifically proven treatment protocols, complications are practically absent when any common surgical technique is performed: Bjorn (1963), De Sanctis and Zuccelli (2000), Homayoun H Zadeh's "VISTA" (2006). The frequency of complications and their nature are de-

terminated by the condition of the tissue complex formed at the surgical site and the condition of surrounding tissues: bone, connective, soft gum tissues, tooth tissues; in the condition of the superficial and/or marginal epithelium in the early period [4,14].

Recurrence with an adequate choice of strategy and tactics for the treatment of gingival recession, compliance with the surgical protocol; with appropriate pre-, intra- and postoperative patient's management is practically uncommon [5,10,12]. A detailed analysis of the histological composition of tissues formed in the process of biodegradation and replacement of allograft with new tissues in a human being can be found neither in scientific Russian literature nor in foreign ones: English, Italian, Spanish, French, Finnish, German.

There is mention of a tissue reaction to the allogeneic dura mater in rhinoplasty: it is encapsulated and not resorbed for 2 months. This may be due to the type of preservation of the dura mater with aldehydes and thymol, whereby additional lateral cross-linking bridges in the collagen fibers, which prevents resorption, biodegradation, and regeneration [3].

Evidence of the result of formation of a specific tissue species or complex after dura mater subperiosteal or into the split mucosal-periosteal flap (MPF), that is, into the thickness of soft tissues of the gingiva are also not found in the literature [13].

The allogeneic dura mater set into the subcutaneous tissue in 60-90 days is replaced by the connective tissue of the same volume, the dura mater placed on the bone instead of the removed periosteum induces the formation of a bone callus within 30-60 days [3].

There is an experience of the clinical application of a dura mater implant and an autograft in the same protocol of the same patient. The same response to surgery and comparable clinical result in all indices [9].

There is an experience in a laboratory study with an in vivo simulation of an adequate surgery technique of surgical treatment of recession with a single-layer technique or a double-layer technique: with an autograft and/or allogeneic dura mater implant [7].

There is an experience with the use of allogeneic dura mater in the treatment of gingival recession of orthodontic patients with existing gingival recession or the risk of recession during orthodontic treatment. X-ray Computer tomography analysis shows that in the area of all operated recessions, there is a vestibular increase in bone mass volume [8].

It is assumed that at the locus of the dura mater implant subperiosteal insertion, bone structure is formed in the entire volume, and therefore gingival recession stops or is eliminated completely. If connective tissue is formed, there is a high risk of recurrence, because the bone support for the volume of the soft gum tissue in this area is absent.

It is interesting to assess the histological composition of tissues formed at the locus of the subperiosteal and intragingival dura mater implant placement, the process of new tissue formation as well as tissue response.

The aim of the study was to determine the histological composition of tissues at the site of plastic material placement: allogeneic dura mater implant in comparison with the control without it; to estimate the change of the implanted structure in dynamics and tissue reaction of the surrounding tissues as a result of the operation at the cellular level.

## Materials and methods

**Preparation of rat's dura mater allogeneic implant.** Production technology of dura mater allogeneic implants «LYOPLAST»® includes primary mechanical cleaning of the dura mater fragments, application of ultrasound under vacuum in a liquid phase, eliminates the chemical component as a reagent or catalyst, using it as a solvent for cleaning and active physical, physical-chemical and colloidal leaching of proteins, lipids and nuclear acids from the spaces and intercellular matrix.

After purification the bioimplant is gently dried with organic solvents and lyophilized by low temperature method. The hermetically packaged material is sterilized by the radiation method.

**Subjects and methods of the study.** The study was performed on 60 laboratory rats - mature animals of both sexes age 5-6 months Wistar, the average weight of which was - 190 grams (range 180-200 grams).

The criteria for selecting animals for the experiment were the same age and the absence of any diseases. Before the experiment, the animals were kept in isolation for 14 days, treated for ecto- and endoparasites. The animals were kept on a balanced light regime, free access to water and standard food for laboratory animals. All animals were operated within a period of 15 days.

When performing surgical interventions on animals, as well as keeping them in the vivarium of the IEMB the European Convention for the Protection of Vertebrate Animals Used for Experimental or Scientific Purposes (ETS No. 123) Strasbourg, 18.03.1986); "Principles of Good Laboratory Practice" National Standard of Russian Federation GOST № 33044-2014, introduced from 01.08.2015; by the order of the Ministry of Health of Russia 01.04.2016 № 199n "Approval of the Rules for Good Laboratory Practice", "Sanitary and Epidemiological Requirements for the Design, Equipment and Maintenance of Experimental and Biological clinics (vivariums)" (SP 2.2.1.3218-14).

The samples of the tested materials were checked for sterility by the methods regulated by the current State Standard GOST 28085-89 "Biological preparations. Method for bacteriological control of sterility".

All surgical interventions on animals were carried out under intramuscular anesthesia of the mixture anesthetics "Zoletil 100" (Virbac C.A., France) in a dosage of 15 mg/kg weight and "Rometar" (Bioveta, Czech Republic) at a dosage of 6 mg/kg of weight. Levomekol was applied to the cornea of the test animal's eye to exclude its drying out. All manipulations were performed in compliance with the aseptic and antiseptic rules. Withdrawal of the animals from the experiment was carried out using an overdose of anesthesia drugs by intracardiac injection.



**All subjects were combined into 2 groups:**

1. Control group – gingival recession was surgically simulated according to the developed model and the wound was sutured according to the protocol of the simulated operation [7];

2. Study group – gingival recession was surgically simulated, plastic material (allogeneic dura mater) were placed, the wound was sutured according to the protocol of the simulated operation [7].

**Surgical protocol for each group.** Twenty-four hours before surgery, food was removed from the animal and free access to water was maintained.

**In the control group:**

Incision design: intrasulcular incision design parallel to the tooth root;

Modeling off the mucosal-muscular flap;

Creation of a “pocket” in the submucosal space;

Medication treatment of the operating wound with «Fitodent» solution with aspen bark and chlorophyll copper complex (Russia);

Suturing of the operating wound with double twisted “purse” sutures;

Fixation of the MPF with a cross-shaped vertical clamping suture.

In the study group (Fig. 1 a-j):

Incision design: intrasulcular incision design parallel to the tooth root;

Modeling off the mucosal-muscular flap;

Creation of the “pocket” in submucosal space;

Medical treatment of the surgical wound with «Fitodent» solution with aspen bark and copper chlorophyll complex (Russia);

Preparation of the dura mater allograft: perforation, rehydration with physiological solution;

Medical treatment of the operating wound with «Fitodent» solution with aspen bark and copper chlorophyll complex (Russia);

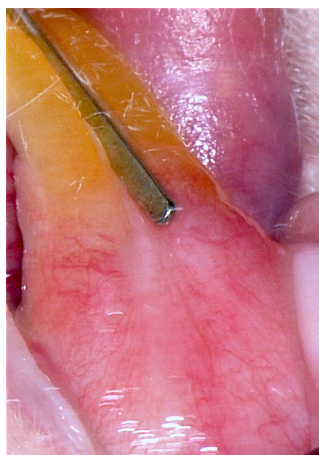
Fixation of the allograft in the operating area with knotted sutures along the perimeter;

Suturing of the surgical wound with double twisted “purse” sutures;

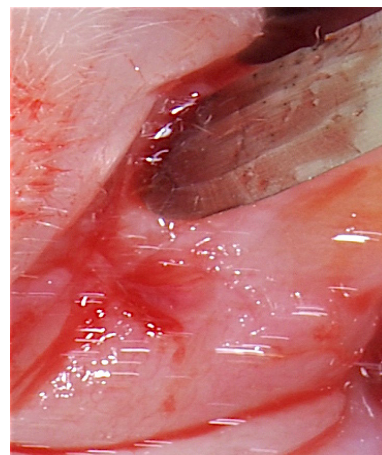
Fixation of the MPF with cross-shaped vertical clamping sutures.



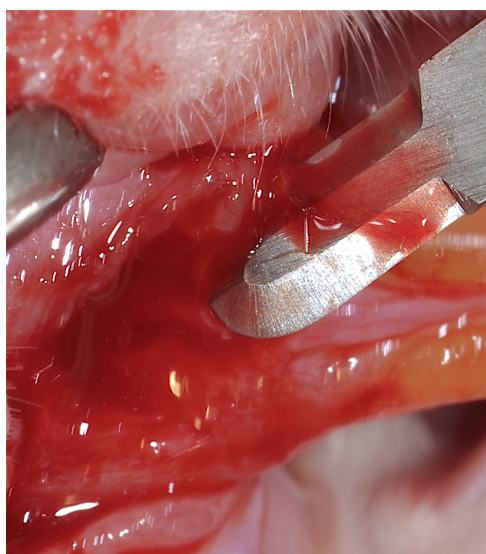
a



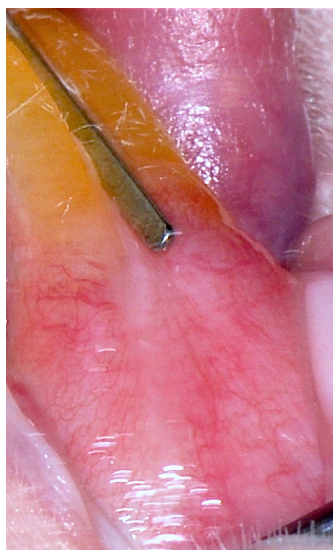
b



c



d

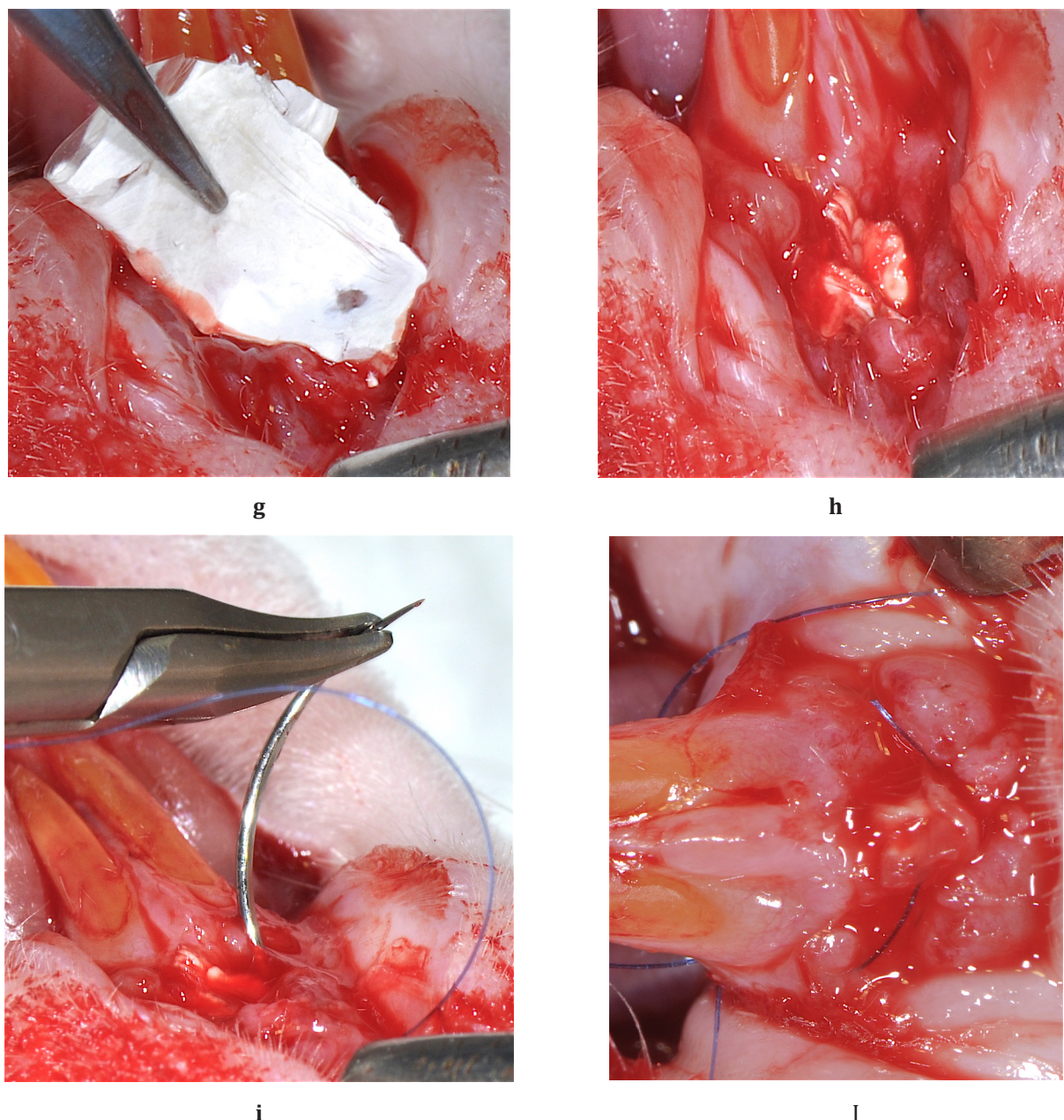


e



f





**Fig. 1.** Experimental operation on the laboratory rat. *a. The animal is put under anesthesia. b. Selection of the incision start area and incision design. c. Incision with a double-edged scalpel blade. d. Peeling off the full-layer SNL. e. Formation of full-layer SNL, imitation of gingival recession. f. Transplant of allogenic rat dura mater: g. Introduction of a folded doubled dura mater graft into the defect. h. Fixation of the dura mater in the area of the defect was performed. i. Suturing of the surgical wound. j. The edges of the flaps were fixed to the teeth with an overlapping suture.*

**Obtaining macroobjects.** In all animals after euthanasia, the condition of tissues in the area of surgery was evaluated: mucosa, gingiva, teeth; signs of inflammation. Macro-objects were taken using the sharp saw method: a fragment of the jaw with the teeth of the operation.

#### Results and discussion

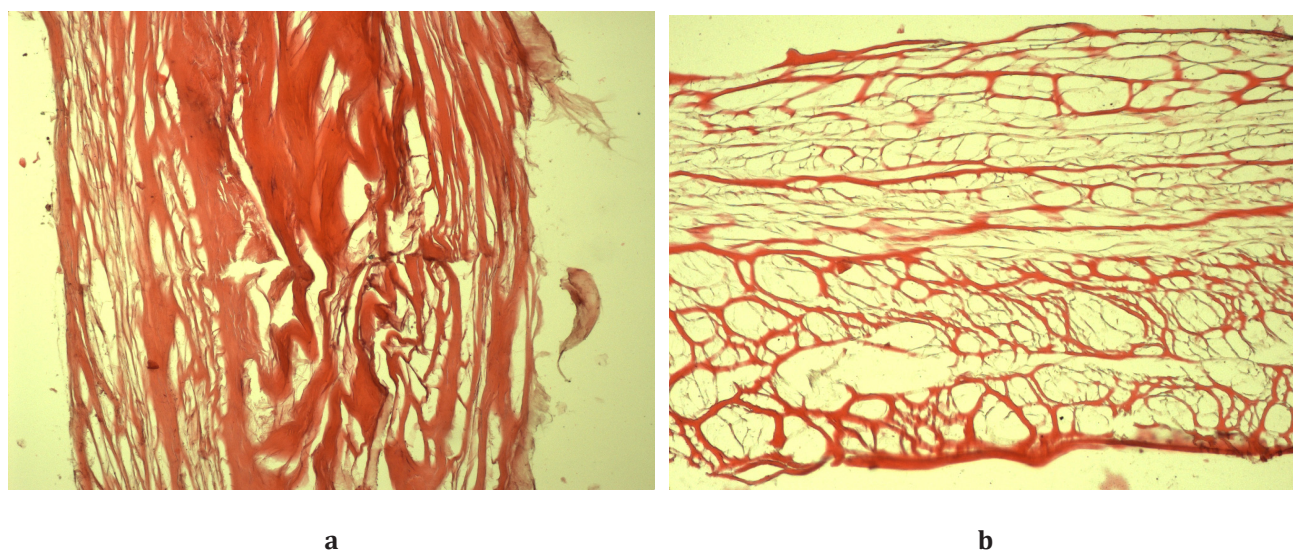
##### Description of histo-morphological prepares.

Histological prepares of allogenic rat's dura mater before treatment with «LYOPLAST» technology (Fig. 2 a, b), few cells are visualized. The collagen fibers are dense, unidirectional and mature. During processing (ultrasound, vacuum, lyophilization) mature collagen fibers

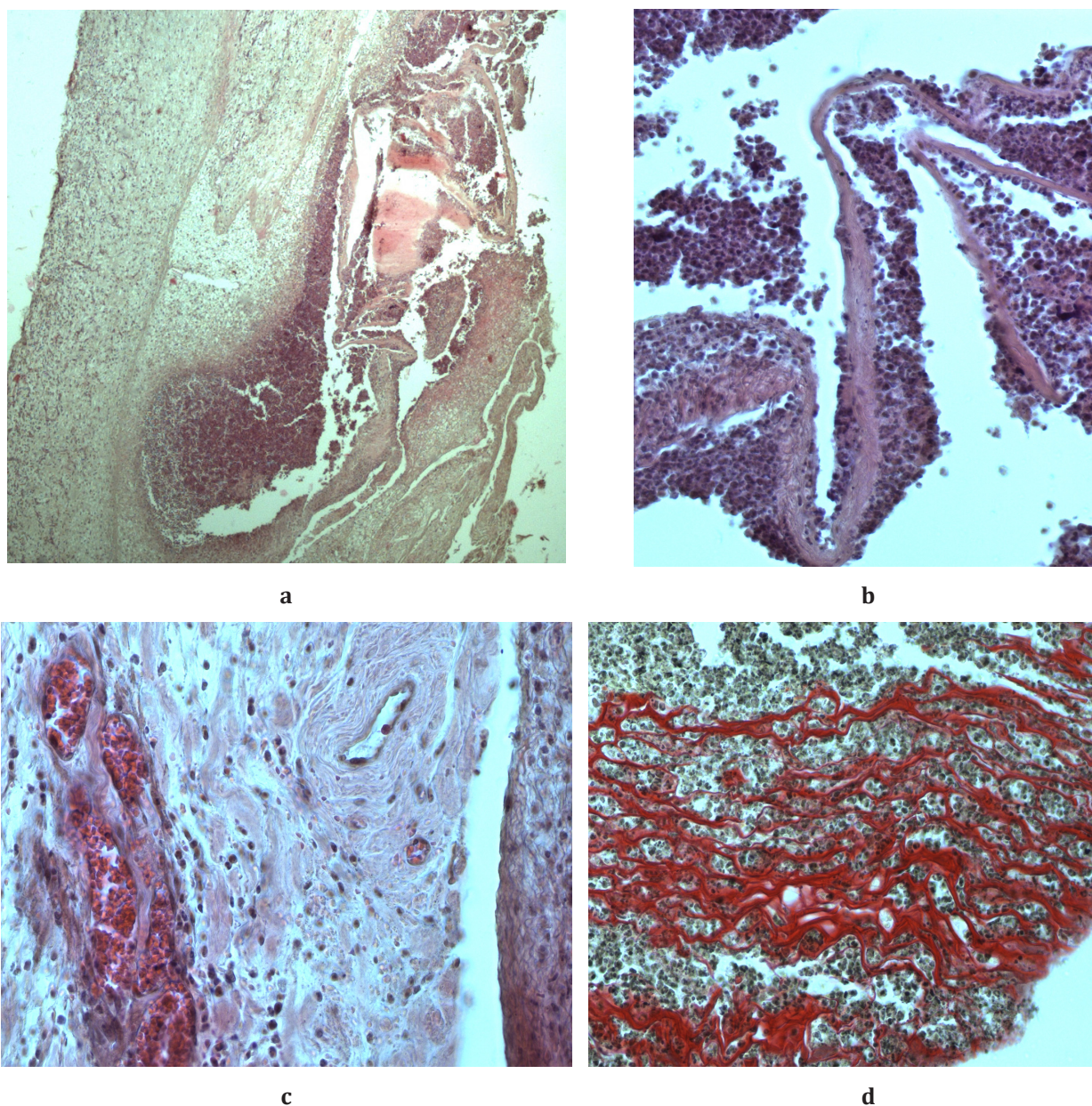
are thinning, the structure becomes looser. There are no cells in the prepare, the fibers uniform unidirectional, thin. The structure of the material is reticular.

After 3 days, a local inflammatory reaction to the dura mater insertion locus of the implanted material was observed (Fig. 3 a-d). The graft is clearly visualized. The tissues distant from it are without any morphological changes. The area of implantation is separated from the surrounding tissues by a clear demarcation line, is represented by a fragment of dura mater surrounded by a large number of segmented and bacilliform lymphocytes, as well as macrophages.





**Fig. 2. Histological preparation of rat's dura mater. a. Before treatment. Hematoxylin and eosin staining. Magnification x100. b. After treatment. Staining with hematoxylin and eosin. Magnification x100.**



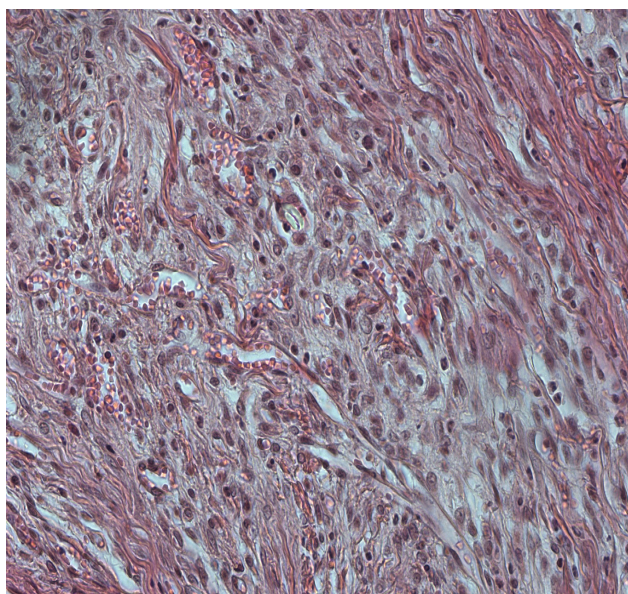
**Fig. 3. Soft tissue preparation 3 days after surgery at the locus of dura mater insertion. a. Staining with hematoxylin and eosin. Magnification x40. b. Staining with hematoxylin and eosin. Magnification x400. c. Staining with hematoxylin and eosin. Magnification x400. d. Van Gizon microfuchsin staining. Magnification x400.**



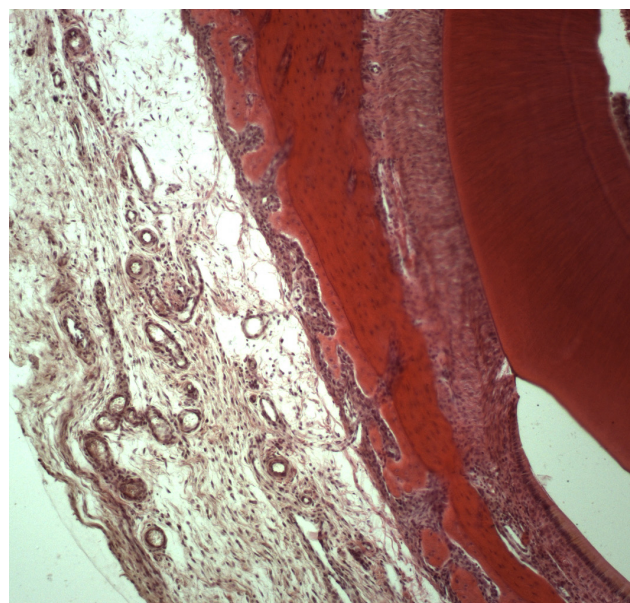
In the border area are observed dilated capillaries with signs of full-blooded hemorrhage. A large number of erythrocytes of different shapes, tightly adhering to each other are visualized tightly adjoining each other. A pronounced neutrophilic reaction on the dura mater; macrophages are present, which degrade the dura mater. At X400 magnification, active aggregation of cells on the dura mater surface and many macrophages on all sides of the dura mater. Vessels of the microvasculature are sharply dilated, stasis (marginal standing of erythrocytes) is observed. Many macrophages around the vessels are visualized. The dura mater fibers are also

visible, with detritus mass around and between them: which is biodegraded by macrophages.

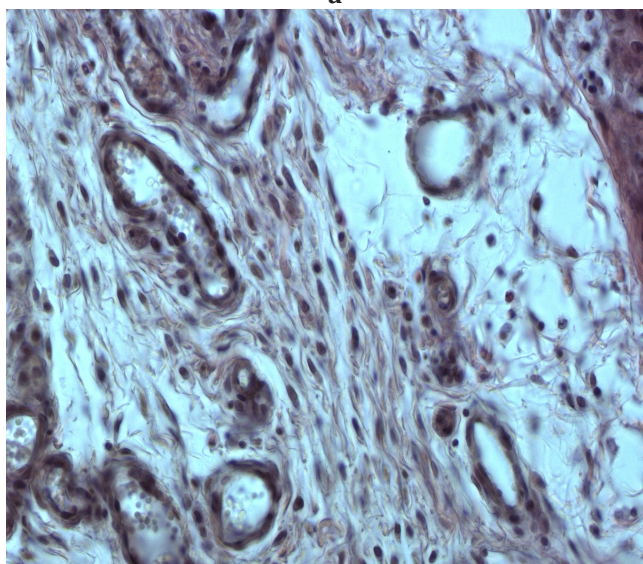
After 7 days, dura mater fragments undergoing biodegradation (Fig. 4 a-c). Active vascularization in the soft tissues, a large number of newly formed vessels. There are no signs of inflammation, young connective tissue is formed. Fragments of suture material were visible. On the preparation of transverse section of the tooth (Fig. 4 b), ligament of the tooth, alveolar bone and soft tissues the new spongy bone is seen.



a



b



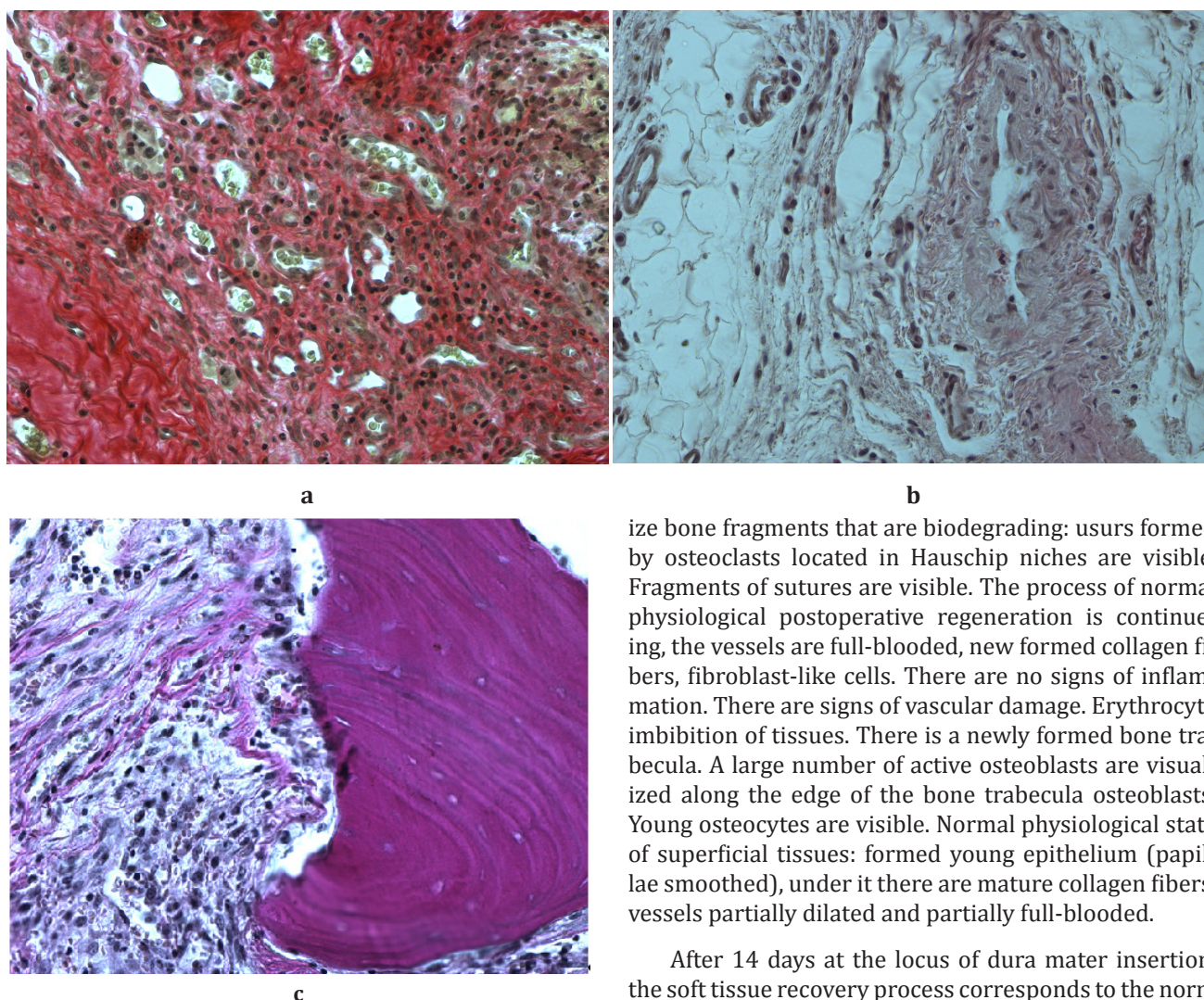
c

**Fig. 4. Soft tissue preparation 7 days after surgery at the locus of dura mater insertion. a. Hematoxylin and eosin staining. Magnification x400. b. Staining with hematoxylin and eosin. Magnification x100. c. Staining with hematoxylin and eosin. Magnification x400.**

The line separating mature compact bone from newly formed spongy bone is clearly visualized, thickened periosteum; cellular and fibrous layers of the periosteum are clearly distinguished. Also fibroblast-like cells and collagen fibers are seen. Active vascularization of soft tissues, with many young vessels of the microvasculature, - the process of soft tissue regeneration after surgery. Multiple formation of vessels, not all of them full-blooded. The fibers of newly formed collagen is thready. At this stage after the operation, the process of soft tissue regeneration corresponds to the norm.

After 7 days at the locus of the operation without plastic material installation (control) there are visualized collagen fibers of different degrees of maturity, as evidenced by their thickness and intensity staining (Fig. 5 a-c). There are a lot of young formed vessels of the microvasculature, in stasis is observed in some of them. Formation of collagen fibers in the surgical area. At this term after the operation, the histological picture corresponds to the norm. On the surface of the acellular bone fragments formed during the modeling of the defect, osteoblasts are visible. Lacunae fragments are empty, there are no cells. On the whole surface there are in large numbers osteoclasts, which biodegrade bone intercellular substance in the whole volume. New collagen fibers are formed. Fibroblast-like cells are observed.



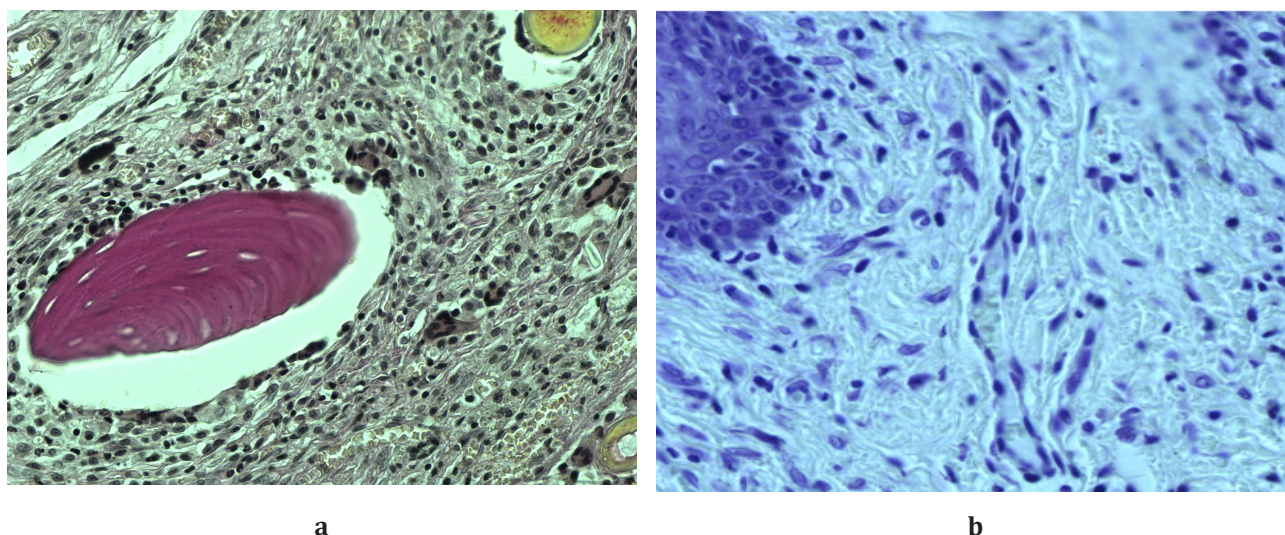


**Fig. 5. Soft tissue preparation 7 days after surgery in the control group. a. Staining with Van Gieson picrofuchsin. Magnification x400. b. Staining with hematoxylin and eosin. Magnification x400. c. Van Gieson microfuchsin staining. Magnification x400.**

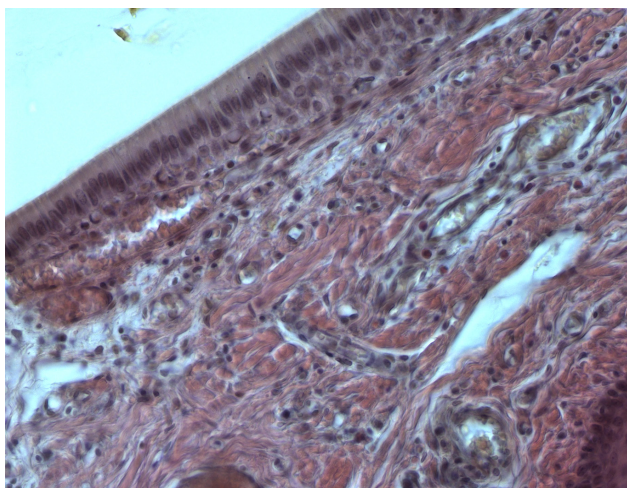
After 14 days (Fig. 6 a-c) at the locus of the operation without plastic material (control) we can visual-

ize bone fragments that are biodegrading: usurs formed by osteoclasts located in Hauschip niches are visible. Fragments of sutures are visible. The process of normal physiological postoperative regeneration is continuing, the vessels are full-blooded, new formed collagen fibers, fibroblast-like cells. There are no signs of inflammation. There are signs of vascular damage. Erythrocyte imbibition of tissues. There is a newly formed bone trabecula. A large number of active osteoblasts are visualized along the edge of the bone trabecula osteoblasts. Young osteocytes are visible. Normal physiological state of superficial tissues: formed young epithelium (papillae smoothed), under it there are mature collagen fibers, vessels partially dilated and partially full-blooded.

After 14 days at the locus of dura mater insertion, the soft tissue recovery process corresponds to the norm (Fig. 7). The new cancellous bone, the line separating the mature compact bone from the newly formed, the cellular and fibrous layers of the periosteum are clearly distinguished. Also fibroblast-like cells and collagen fibers are visible. Active vascularization of soft tissues vestibularly, with many young vessels of the microvasculature, not all of them full-blooded. Fibers of newly formed collagen are filamentous.



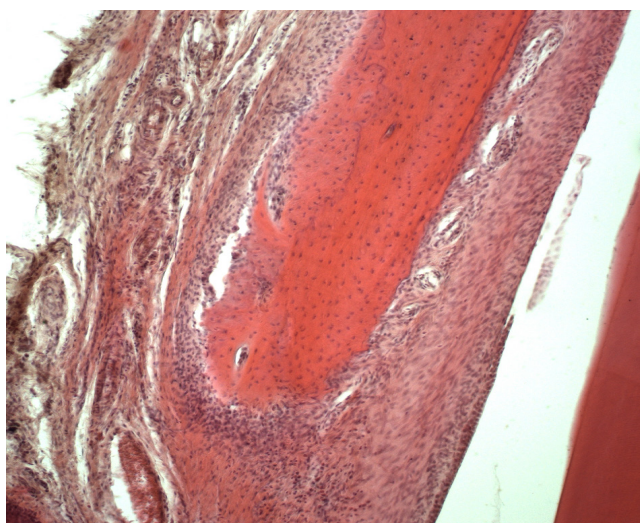




c

**Fig. 6. Soft tissue preparation 7 days after surgery in the control group. a. Van Gieson microfuchsin staining. Magnification x400. b. Staining with cresyl violet. Magnification x400. c. Staining with hematoxylin and eosin. Magnification x400.**

After 28 days at the locus of the operation (Fig. 8) without installation of plastic material (control) the process of soft tissues restoration corresponds to the norm: the new cancellous bone, the line separating the mature compact bone from the newly formed bone, thickened periosteum; cellular and fibrous layer of the periosteum are clearly distinguished. Fibroblast-like cells and collagen fibers are also visible.



**Fig. 7. Soft tissue preparation 14 days after surgery at the locus of dura mater insertion. Hematoxylin and eosin staining. Magnification x100.**

Active vascularization of soft tissues vestibularly is observed, with many young vessels of the microvasculature, not all of them full-blooded. The newly formed collagen fibers are filamentous. Fibers collagen fibers are thickened.

### Results

In all study groups the tissue complex is re-formed: in the control group in the empty cavity in the stroma, in the study group - by biodegradation and replacement of plastic material with new tissues: bone and connective.

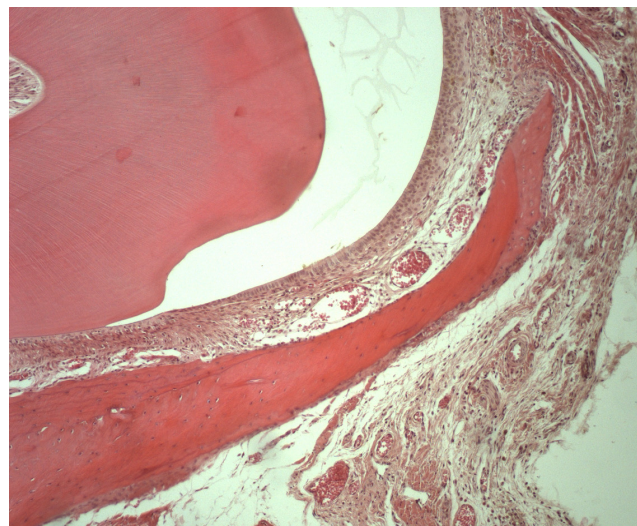
Wherever the plastic material was placed subperiostally - bone tissue was formed, and where it was placed in a place surrounded by soft tissues - connective tissue.

Thickening (change) of the gingival biotype is largely due to the trauma from surgery, and also partially due to the plastic material in the area without periosteum.

The timing of regeneration and replacement with new tissue of the surgical area is similar in both groups.

Reaction to surgery is comparable in all groups, related to surgical intervention.

The allogeneic dura mater implant stimulates ossification and bone formation occurs earlier compared to controls.



**Fig. 8. Soft tissue preparation 28 days after surgery in the control group. Hematoxylin and eosin staining. Magnification x100.**

### Conclusions

1. In all cases of the use of plastic material in the surgical treatment of recession it is justified to place it subperiostally, forming a full-layer MPF using an acute method (scalpel) to preserve the cambial layer of the periosteum on the flap.
2. The use of dura mater is preferable because of the induction of ossification.
3. Restoration/creation of the bone volume of the alveolar closure plate vestibularly provides support to the soft gingival tissues of the newly formed tooth ligament and prevents the formation of recurrence of recession.
4. The formation of a bone and connective tissue complex in the area of dura mater placement determines the stability of the surgical treatment of gingival recession and a favorable prognosis in the without complications and recurrences in the long term.
5. Crushed dura mater can also be used as a plastic material in bone defects and in directed tissue regeneration.

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## DETERMINATION OF HISTOLOGICAL COMPOSITION AT THE LOCUS OF INSTALLATION OF ALLOGENEIC DURA MATER IMPLANT AN IN VIVO MODEL. LABORATOR-HISTOMORPHOLOGICAL RESEARCH

Nosova M.A., Sharov A.N., Nefedova I.F., Volova L.T., Trunin D.A.

**Objective:** To determine the histological composition of tissues at the site of implantation of the dura mater and to assess changes in the structure of the implant and the tissue response of surrounding tissues as a result of surgery at the cellular level. **Material and methods:** Laboratory histomorphological study was carried out on 60 laboratory rats, which underwent an operation adequate to the surgical method for treating gingival recession: in the control group without plastic surgery, in the main group with allogeneic dura mater. Gross preparations were obtained on days 3-7-14-28-90-107 after the operation. **Results:** In all cases, the complex of tissues is re-formed, the reaction to the operation is the same. The plastic material is replaced at the same time. With subperiosteal instillation, the plastic material is replaced by bone tissue, with intragingival instillation - by connective tissue. Thickening of the gingival biotype occurs to a greater extent due to trauma from the operation, to a lesser extent from the plastic material. The allogeneic dura mater stimulates ossification earlier than controls. **Conclusions:** Restoration/creation of the bone volume of the closure of the alveolar plate vestibularly provides support for the soft tissues of the gums; determines the stability of the result of surgical treatment of gingival recession and a good prognosis in the long-term period: without complications and relapses.

**Key words:** gingival recession, histological tissue composition, allogeneic dura mater.

