Biological bases of biomaterials application in regenerative surgery

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Abstract

The development rate of modern surgery doesn't allow all biological potential of an organism to be realized in reparative regeneration. Now it has become urgent to develop regenerative surgery i.e. surgery that provides valuable restoration of tissue and organs owing to the effective regeneration. Realization of the regenerative potential can be carried out on the basis of tissular grafting as a biological method of stimulation of regeneration.

The analysis of kinds of tissue transplantation (auto-, allo-, xeno) shows that the most prospective one is tissular allografting when tissue contains no cellular elements and dosed extraction of glycosaminoglycans. This principle realized with Alloplant biomaterials, allows to substitute grafted materials with a structurally perfect regenerate. It is shown that one can regulate the structure formed in the place where a regenerate is implanted and effectively prevent such a negative phenomenon as scar tissue development if in the capacity of the forming-up material there will be tissues with different fibro-architectonics and histochemical composition. The mechanism to increase the efficacy of regeneration is the best cell-matrix inter-correlation and intercellular cooperation with the macrophages playing the key role.

Key words: regeneration, allogenous biomaterials, biological bases, surgery.

The development rate of technically equipped modern surgery allows to keep organs preserved in many kinds of operations but does not realize to a full extent such biological potential of an organism as reparative regeneration. Regenerative aspects do not dominate in surgery so far, that is why an operation resulting in scar tissue healing is thought to be a normal outcome. The problem of effective tissular regeneration of an adult organism still remains unsolved. It is about time to develop regenerative surgery which would provide of full value structural and functional restoration of tissue and organs thanks to the effective regeneration.

Tissular grafting as a biological method to stimulate reparative regeneration can be one of the trend of the regenerative surgery development (1, 2, 3, 4, 5).

Biological bases of tissular grafting.

At present there are three kinds of grafting which depend on an origin of grafted tissue; they are auto-, allo-, and xenografting.

Autografting differs from other kinds of grafting because it always deals with authentic engrafting. As a result of the fact that the operation is carried out within one organism there is no need to overcome any tissue incompatibility. Autografting can result in: 1) authentic engrafting (6, 7); 2) substitution by newly formed tissue with the subsequent fibrosis (8, 9); 3) postoperative necrosis of a transplant because of lack of blood supply (6, 7). The latter can be avoided if transplantation is carried out by microvascular anastomoses if vascular continuity is rapidly reestablished (10, 11, 12).

The considerable shortcomings of autografting lie in limited tissue sampling and extra trauma a patient receives with manipulation (13, 14).

Allografting gives a good possibility to tissue sampling and its preservation that helps to provide to keep it for a long time in special tissue banks. The factor that limits the use of the allogenic grafts is the difficulty to prognose the postsurgery results because of the tissue reaction incompatibility which takes place on a different level immediately after transplantation (13, 4).

The basic advantage of xenografting is the possibility to harvest big amounts of tissue which can grow into serial production. Reaction of tissue incompatibility however reveals itself more with xenogenic grafts than with allotransplants and is characterized by an acute immune inflammation which leads to a rapid lysis of a graft (8). Xenografts can become less immunogenic with the help of conservation in aldehydes but in this case transplants cause rapid cellular infiltration with swelling and fiber bundles splitting (15). In the postoperative follow-up xenografts will be wrapped with a dense fibrous capsule (16), as aldehyde processing makes tissue better resist fermentative lysis (17).

In that way, these are antigenous characteristics of grafts and the level of their reductions by means of conservation that influence on the results of an operation with the use of allo- and

xenografts. From this point of view the use of conserved allotransplants should be considered as the most promising. At present in spite of the fact that tissue allografting is well studied morphological results of an operation noticeably vary for they depend on a number of factors that influence on the final results of an operation. The main problem why the allografts cannot be used on a large scale is tissue incompatibility. As a rule native grafting causes immune inflammation which leads to a rapid lysis of grafted tissue followed by scarring (3, 8, 18, 19). There is no evident immune reaction if grafted tissue is conserved; in this case transplants are substituted by newly formed tissue or encapsuled at different rate depending on the method of conservation.

Methods of conservation and the way they influence antigenous characteristics of allografts. One can divide methods of conservation into two groups according to the way they influence on tissue: physical and chemical.

The purpose of the physical ones (deep freezing, lyophilization) is to maximum preserve native structure of tissue and to provide its postoperative engrafting (13, 20, 21, 22). In deep freezing and lyophilization there occurs some reduction of the antigenous characteristics of grafts, but antigens of the main histocompatibility complex are preserved (23, 24). That is why cryoconserved tissue grafting often leads to the formation of the rough fibrous cicatricial regenerate as the result of the immune inflammation of different degree (25).

Methods of chemical conservation allow to effectively reduce antigenous tissular characteristics and prevent bacterial insemination of grafts due to the antiseptic properties of the used reagents (26, 27). These methods do not presuppose keeping the grafted tissue viable as after chemical conservation the transplants serve as a kind of skeleton where newly formed tissue substitutes transplants (13, 19, 25, 28, 29, 30).

Mostly antigen properties are reduced by tissue conservation.

In aldehydous solutions (formalin, glutarous aldehyde) but in most cases grafting ends in encapsulation of transplants which serve as biological prostheses (8, 16). It is known aldehydous tissue processing forms extra transversal intermolecular suturings in collagenous fibers, which make the latter ones more stable against lysis after implantation (17, 31).

There is another mechanism to reduce antigenous properties of a graft which takes place while the process of their conservation in cytolysing solutions (mertyolathos, cyalithos). Many researchers came to the conclusion that antigenous properties of a graft conserved in cytolysing solutions reduce because of destruction of cellular elements as major immunogenous components of grafted tissue (8, 19, 32, 33, 34). The collagen that represents the basis of fibrous stroma of connective tissue causes a postoperative insignificant immune reaction (8, 31, 35). Taking into consideration all the given facts and ideas some researchers made the following significant

conclusion: in the course of optimal conservation the allografts should have such a fibrous structure free of cells (19, 32, 34, 36).

Russian Eye and Plastic Surgery Centre worked out an original approach to reduce antigenous properties of a graft (3). There was a hypothesis that told that antigenous properties free of cellular elements are conditioned by proteglycans contained in fibrous framework. The grounds for this hypothesis is an experiment: electronomicroscopic examination of various allografts showed that within a short period of time after experimental transplantation proteglycans and glycozaminoglycans left collagenous fibers which preceded the most cellular reaction to the transplant. By that time it was defined with the help of immunohistochemistry methods that proteglycans of extra-cellular matrix of connective tissue possess epitope properties (37, 38).

Proceeding from that we developed a method how to extract proteglycans out of collagenous fibers in doses; this method allows to reduce antigenic properties of allografts and surrounding tissue reaction to the optimal minimum. The given method lies in the basis of an original technology which presupposes physicochemical graft processing, different kinds of step-by-step control and radiation sterilization. Connective tissue allografts produced according to that technology can be reasonably called biomaterials because tissular structure undergoes partial modification in the course of processing as a result of cellular elements destruction and dosed extraction of proteglycans.(4, 39).

Morphological regularities of resorption and allograft substitution. At present there is a well accepted fact that preserved allografs undergo gradual resorption and are replaced by newly formed tissue (13, 19, 25, 28, 30, 40, 41, 42). However one can interpret this process differently. This happens because of different theoretical approaches, the absence of morphological analysis of grafted tissues and the basic factors which define the nature and period of transplants resorption and their substitution. P.P. Kovalenko (13) was the first to give generalized characteristics of morphological transformations in case of allografting; he divided this process into four periods. The first period is characterized by polymorphocellular reaction to a graft which begins with recipient cells invasion along the fibers and vessels of a graft. The second period is characterized by differentiation of cells which get into the graft and by formation of new vessels. The third period is a period of tissular differentiation. The fourth period is characterized by a final tissular differentiation and formation of a new tissue – regenerate.

Graft resorption is thought to be caused by the cellular immunity reaction together with mononuclears and lymphocytes due to which the transplant is destructed and a regenerate is formed (13, 15). Thus the structure of a regenerate which is formed at the place of the allograft mostly depends on the level of the immune inflammation (4). The given above dependence can

explain different results of an operation the extreme of which are: a) fibrosis (scarring) as a result of evident immune inflammation and rapid graft lyses, b) formation of rough fibrous capsule around transplants which do not undergo lysis (for example, preserved in aldehydes).

Is there any other mechanism of allograft resorption and substitution? The investigations performed with the use of Alloplant biomaterial (ABM) give grounds for positive answer to this question. ABM implanted in tissular defects **in all cases got resorped by macrophages and substituted by the newly-formed tissue.** The examination of cellular infiltrate showed that macrophages were revealed in different quantities during all the period of ABM resorption and substitution (5, 43). Depending on the structural peculiarities of ABM and place of implantation the speed of resorption, and the nature of biomaterial, the level of different tissular components varied in a regenerate (3, 4, 43, 44).

Generalized morphological dynamics of Alloplant biomaterial resorption and replacement. It is proved (3, 4, 43, 44) that within a short period after ABM implantation one could see slight cellular infiltration in the surrounding tissues with polymorphonuclear leucocytes and macrophages prevailing. There was a serous impregnation of collagenous fibrous bundles and slight swelling around ABM. Later the homogenization area of ABM collagenous fibers enlarged and in the peripheric areas there was a macrophages invasion. In the same area of ABM one could notice an accumulation of fibroblasts migrating along the collagenic fibers and signs of new capillaries formation. An inflammatory reaction slightly reduced in the surrounding tissue, there were only single lymphocytes that showed inconsiderable cellular immune reaction.

Further collagenic fibers of the biomaterial gradually degraded; it happened because macrophages provided their enzymatic lysis followed by phagocytosis. The given process effected all the biomaterial layers and large ABM area was involved in in the process of resorption. Accordingly the size of the regenerate, which began to be formed in the place of the resorped biomaterial elements, was increasing too. New collagenous fibers with distinctive tinctorial properties were found in the mentioned fragments. In some areas degrading colagenous fibrils were synchronously substituted by the new ones. On the whole a grafted biomaterial was fragmented with new tissue layers, which differed in more density of the cellular elements and new vessels. This told us about a peculiar intimate adhesion of biomaterial and surrounding tissue: it was already hard to differentiate the borderlines between the implanted biomaterial and newly formed tissue. Most part of ABM was replaced by new connective tissue, in which one can see mature collagenous fibers. Fibroblasts, macrophages, network of new blood capillaries were found in spaces between the collagenous fibers.

After some time since ABM has been implanted, in a new tissue which had replaced the biomaterial there were observed cases of all structural elements differentiation (reduction of part

of blood capillaries and collagenous fibers); the latter can be characterized as remodeling of the newly-formed tissue. By its structure the regenerate did not practically differ from the surrounding tissue.

Regeneration and differentiation of micro-circular channel vessels as an index of the full value structural regenerate. The plasty of bulbar conjunctiva which needs the use of ABM as a film (44) became a convenient model for experimental study of blood vessels regeneration. On the total preparations of conjunctiva taken at a different moment within postoperative period one could follow all the stages of vascular growth and differentiation. These stages went on in a newly-formed tissue that replaced the biomaterial.

The primary stage of microvessels regeneration represented the formation of new capillaries formed out of preceding capillaries and venules by means of proliferation of endotheliocytes. Growing capillaries represented blindly pointed endothelinous outgrowths, consisting at first of one, and then of several cells. The longer growing capillaries were the more loops they made, there appeared basal membrane in their wall and their canalization to place. Later some capillary loops got anastomosed and formed a local capillary network.

The fusion of several local capillary networks led to the formation of one common microvascular channel. In the central parts of the newly-formed network there appeared signs of microvascular differentiation: some capillaries enlarged in diameter, then in the structure of their wall one could find sleek-muscled or adventitial cells depending on what kind of vessel was formed – an arteriole or venule. Later there were two parallel processes: growth of new capillaries in the peripheric zone of the newly-formed network and vessels differentiation in central zones. There is a factor that influences the differentiation of newly-formed vessels; this is increasing of hydrostatic blood pressure because of enlarging of sanguiferous channel and peripheric resistance (45, 46, 47).

By the moment of a complete biomaterial resorption and its replacement by a regenerate a newly-formed microvascular network developed to its maximum. By this period all sections of the microcircular channel had normal structure. Later arterioles and venules magistralised due to the reduction of part of the capillaries; that is why the density of the capillary channel gradually reduced till it became normal.

Together with circulatory regeneration one managed to define the basic stages of lymphatic microvessels development (44). New-formation of lymphatic capillaries took place owing to the proliferation of endotheliocytes that prolonged from the wall of the preceding lymphatic capillary. Within longer follow-up there was observed the union of several capillaries as well as the formation of slightly differentiated lymphatic network, in the vessels of which there

were no valves. Then together with differentiation, the valves began to appear in the walls of the newly-formed lymphatic vessels and the network architectonics began to seem to be definitive.

The given findings show that the microvascular channel develops in the newly-formed tissue which in its turn replaces the biomaterial; parameters of the channel tells us about the effective development of cicatrical and lymphatic vessels of the regenerate.

Variants of resorption and biomaterial substitution depending on their structure. The experiments with the use of the biomaterial implantation of different density of collagenous fibers packing and thus with physicomechanical properties (3, 43) show that there are variations both in speed and in the nature of resorption and ABM replacement. The material with more density caused less reaction within a short period of time. The speed of resorption and the subsequent replacement was lower than it was with the implantation of the biomaterial with a friable packing of collagenous fibers and bundles. The density of the regenerate correlated with that of the implanted biomaterial.

The nature of differences in the formation of the regenerate differed in the course of the biomaterials implantation with different spatial organization of the collagenous fibers. The biomaterial substitution with the orientation of fibers in one direction had an even linear character from periphery to centre. If ABM bundles had three directions to develop they were substituted by fragments which were divided by bands of newly-formed fibrous tissue (44).

A comparative analysis of fibroarchitectonics of the implanted biomaterial and substituted tissue showed that an optimal structure of the regenerate is formed at 70% of accordance (3).

In plasty of tissue defects which have epithelial cover (eyelid plasty or conjunctiva plasty), the surface of the biomaterial was covered with regenerative epithelium long time before its complete resorption and substitution (44).

A comparative analysis of morphological findings and clinical observation allowed to bring forward a **theory of selective growth of tissue on the basis of Alloplant biomaterial application** (3). According to this theory it is possible to stimulate regeneration of dense and loose connective tissue, richly vascularized tissue, epithelial tissue, transparent tissue of cornea if the biomaterials with prevailing content of different kinds of glycozaminoglycans (hyaluronic acid, heparin sulfate, chondroitin sulfate, keratan sulfate) are selected.

Some facts about the mechanism of regeneration of connective tissue in case of Alloplant biomaterial implantation.

Thus, allogenic Alloplant biomaterials differ from traditional allotransplants not only in low antigenic properties but also in the fact that these biomaterials are resorped exclusively by tissular macrophages. From this point of view the process of ABM resorption is more similar to that one which takes place in the course of collagen implantation; degradation of a collagen

occurs with the help of collagenase secreted by macrophages and with the help of phagocytosis (31). It is proved that products of exogenic disintegration exert a stimulating influence on connective tissue regeneration due to the increase of macrophagal activity (31), stimulation of fibroblast proliferation , synthesis of collagen and extra-cellular fibrillogenesis (31, 48, 49).

ABM resorption and substitution however go slower after implantation than those of collagenic preparations. There can be several reasons for that. Firstly, ABM, which contains collagen and structurally connected with the latter proteglycans and glycoproteins, are the materials that better resist fermentative lysis. This fact is proved by the slowest speed of resorption of those kinds of ABM, which were harvested from dense formed connective tissue (43). Secondly, proteglicans and glycoproteins, which get free in case of degradation of the biomaterial collagenic fibers, can reduce a proliferative activity of fibroblasts and thus – the speed of synthesis of collagen and fibrillogenesis (43). It is well known that proteglycans and glycoproteins, which take part in structural stabilization of mature collagenic fibers, are considered by scientists as components of extra-cellular matrix which influence proliferation and differentiation of different cell populations (50, 51). There is a statement that together with collagen glycosaminoglycans, proteoglycans and glycoproteins are short distant regulators of fibrillogenesis (48).

It is known that products of collagenic destruction play a role of regeneration stimulators of the fibrous part of the connective tissue i.e. fibrogenesis at the first stage of the local autoregulation of connective tissue growth (48). Evidently, it is connected with rapid resorption of an implanted collagen and stimulation of fibroblasts activity by the products of its disintegration. As a logical stage with such a healing of the wound is the formation of a scar which further is to be remodeled with the resorption of part of the collagenic fibers by the fibroblasts (48).

The studies of ABM implantation give grounds to suppose that there is a bit different mechanism of the connective tissue growth in the place of the resorbed ABM, when the speed of resoption and fibrillogenesis is balanced. Among other confirmations, this hypothesis is proved by synchronic new-formation of collagenic fibrils following the biomaterial fibrils breakdown; this fact was defined with the help of electronic microscopy. According to this logic collagenic synthesis is supposed to form structurally valuable collagenic fibers with the adequate architecture and prevents from scarring as this synthesis is prolonged in time and connected with biomaterial resorption; the idea of scarring shows that fibrous components of a regenerate prevails over cellular ones. It is possible that fibroclasia passes more actively in the phase of regenerate remodeling in case of ABM implantation as fibroclasts undergo an active influence of macrophages which are found in the place of implantation up to the complete resorption of biomaterial fibers. It is known that macrophages enforce phagocytous activity of fibroblasts with

the help of the secreted cytokins – inductors of collagenase production (52). One should keep in mind the mechanism of fibrillogenesis contact inhibition (48) by means of the biomaterial collagenic fibers. Possible correlations of tissular and cellular factors in regenerative process, caused by the Alloplant biomaterial, can be represented by the scheme (Fig.)

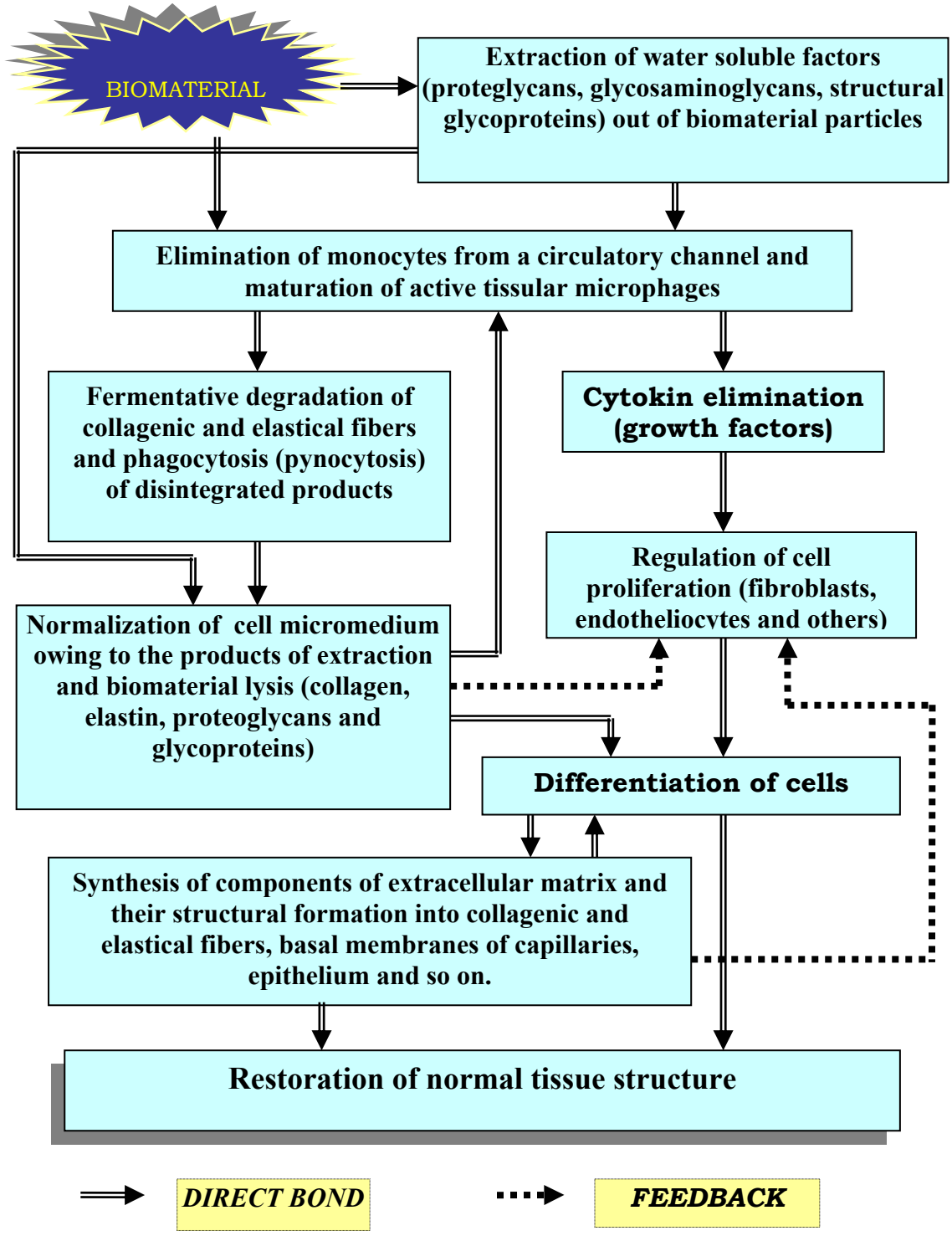

Fig. Possible correlation of tissular and cellular factors in regenerative process initiated by Alloplant biomaterial introduction

To judge properly about the effectiveness of the formed regenerate one needs corresponding assessing criteria, that is why a special point assessing system was developed to evaluate morphological and morphometric properties of the regenerate scarring, which is formed in the place of the resorbed biomaterial (4). Morphological regenerate assessment made according to these criteria (pic.) showed that a relatively quick biomaterial resorption provides a substitution of the scarring type regenerate. For example, one could watch complete substitution of conjunctiva allograft in case of bulbar conjunctiva 4 months after an operation. In the newly-formed tissue there were avascular zones with incomplete fibrillogenesis and large amount of fibrocytes. The substitution of the transplanted tissue goes slower with the use of ABM accompanied by the formation of a regenerate with differentiated conjunctiva elements: adequate microvascular channel, valuable epithelial cover, the presence of sensitive neural endings etc (4).

Thus, biomaterials with enumerated above characteristics are replaced by the newly-formed tissue with the development of all differentiated elements. Now one can make the following conclusion about the possibility of **valuable reparative regeneration** with the help of allogenic biomaterials. In other words, properly prepared biomaterials can be considered as an instrument of **regulated tissular regeneration.**

Realization of this principle in clinical practice gives prospect to the use of biomaterials with the aim to replace different kinds of anatomic defects and to prevent and correct postinflammatory cicatricofibrous process with the restoration of valuable tissue structure thanks to regeneration.

REFERENCES

1. Von Versen R., Matthes G., Schimmack. Verfahren zur Herstellung von Weichteilpreparaten fur die Klinische Anwendung //3rd International meeting of Tissue Bank Specialists. – Rostock, 1990. – Abst. № 46.
2. Frank K., Muller C. Die Entwicklung des Sortiment und der Anforderungen an die Gewebebank des BI BT Leipzig //3rd International Meeting of Tissue Bank Specialists. – Rostock, 1990. – Abst. № 3.
3. Muldashev E.R. Theoretical and applied aspects of "Alloplant" allografts formation for facial plasty: Abstracts of doctoral dissertation. – Saint-P-g., 1994. – 40 p.
4. Muldashev E.R., Muslimov S.A., Nigmatullin R.T et al. Basic research conducted on Alloplant biomaterials // eur. J. Ophthalmol. – 1999. – Vol.9, №1. – p. 8-13.
5. Muslimov S.A. Morphological aspects of regenerative surgery. Ufa: Bashkortostan, 2000. – 168 c., Illustrated, 48 p.
6. Berlin L.B. Post skin burn morphology and free transplantation. – Len-d.: Medicine, 1966. – 223 p.
7. Limberg A.A. Biological and pathophysiological problems of free grafting of autoskin. – Len-d: Nauka, 1971. – 18 p.
8. Tauro J.C., Parson J.R., Ricci J. et al. Comparison of bovine collagen xenograafts to autografts in the rabbit // Clin. Orthop. – 1991. – Vol. 266. – p. 271-284.
9. Vrabec M.P., Weinsenthal R.W., Elsing S.H. Subconjunctival fibrosis after conjunctival autograft. Cornea. – 1993. – Vol. 12.- p. 181-183.
10. O'Brien B. Microvascular reparative surgery: Translated from English.- M.: Medicine, 1981. – 422 p.
11. Krylov V.S., Nerobeyev A.I., Milanov N.O. The use of microvascular technics in plastic elimination of soft tissues defects by means of free grafting of musculocutaneous flaps //Vestn. Hirurgii. – 1982. – Vol.129, №7. – p. 8-12.
12. Nerobeyev A.I. Restoration of cranial and cervical tissues.- M.: Medicine, 1988. – 272 p.
13. Kovalenko P.P. Principals of transplantology. – Rostov-on-Don: Rostov university edition. – 1975.-180 p.
14. Muldashev E.R., Gabbasov A.G., Nigmatullin R.T. and others. Some ways of new allografts selection for ophthalmosurgery // Topical questions on organs and tissues grafting / Works of 2-nd MOLGMI named for N.I. Pirogov. – Moscow, 1979. – Vol. 113. – Part 23. – p. 21-22.

15. Johnson K.A., Rogers G.J., Roe S.C., Howlett C.R., Clayton M.K., Milthorpe B.K., Schidhelm K. Nitrous acid pretreatment of tendom xenografts cross-linked with glutaraldehyde and sterilized with gamma irradiation // Biomaterials. – 1999. –Vol.20, №11. – p. 1003-1015.

16. Schmidt T., Leipert K.P., Fellbaum C. Tarsusplastik mit hondroplast // Fortschr Ophthalmol. – 1991. – Vol. 88. – p. 279-282.

17. Jorge-Herreri E., Fernandez P., Tunay J., Olmo N., Calero P., Garcia R., Freile I., Castillo-Olivares J.L. Influence of different chemical cross-linking treatments on the properties of bovine pericardium and collagen //Biomaterials. – 1999. – Vol 20, №6. – p. 539-545.

18. seiffert K.E. Biologische grundlagen der homologen transplantation konservieter bindegewebe. – Springer – Verlag, Berlin et al. – 1967. – p. 2-3, 58 – 118.

19. Seiffert K.E. Biological aspects of collagenous homografts // Acta Oto – rhino-laryngol. – Belg. – 1970. – Vol. 24, №1. – p. 27-33.

20. Matskeplishvilli T.Y. Transplantation of lyophilized homo- and heterological tendons in experiment //Sterilization, conservation and transplantation of tissues. – Volgograd, 1975. – p. 143-152.

21. Imamaliev A.S. Biological assessment of grafted tissues. – M.: Nauka, 1975. – 184 p.

22. Zaykova M.B. Plastic ophthalmosurgery. – 2nd edition. – M: Medicine, 1980. – 208 p.

23. Tojo T., Kitamura S., Gojo s., Kushibe K., Nezu K., Taniguchi S. Epithelial regeneration and preservation of tracheal cartilage after tracheal replacement with cryopreserved allograft in the rat // J. Thorac Cardiovasc. Surg. - !998. – Vol. 166, №4. – p. 624-627.

24. Goble E.M., Kohn D., Verdonk R., Kane S.M. Meniscal substitutes- human experience // Scand J. Med. Sci. Sports. – 1999. – Vol. 9, №3. – p. 146-157.

25. Salamon A., Hamori J. Development of collagenous fibers in autologous and preserved homologous tendon grafrts // Acta. Morpol. Acad. Sci. Hung. - 1976 (1977). – Vol. 24, №1-2. – p. 11-22.

26. Klement A.A. Experimental and clinical study of preserved homografts of cranial dura mater: Abstract of doctoral dissertation. – Len-d., 1967. – 20 p.

27. Kunitsyn Y. V. The use of homologous osseous grafts harvested in bacterial conditions, sterilized and preserved in solution of **dyocyde:** Abstract of a thesis for a master's degree.- M., 1973. – 24 p.

28. Marzo J.M., Bowen M.K., warren R.F., Wickiewisz T.L., Altchek D.W. Intraarticular fibrous nodule as a cause of loss of extention following anterior cruciate ligament reconstruction //Arthroscopy. – 1992. – Vol. 8, №1. – p. 10-18.

29. Zaykova M.V., Molokova N. F., Gurina O.Y., et al. Morphological examination of umbilikovazoallografts in experiment //Topical problems of ophthalmology: Theses of scientific conference reports. – Izhevsk, 1995. – p. 189-190.

30. Shino K., Oakes B.W., Horibe S et al. Collagen fibril populations in human anterior crutiate ligament allografts. Electron microscopic analysis // Am. J. Sports. Med. – 1995. Vol. 23, №2. – p. 203 -208.

31. Collagen and its application in medicine / Hilkin A.M., Shehter A.B., Istranov L.P., et al. – M.: Medicine, 1976. – 228 p.

32. Beigel A., Wottge H.U., Muller-Ruchholtz W. immunologische Reaktion gegen konservierte Tracheal transplantate? Untersuchungen bei Ratteninzuchtstammen // Laryngorhinootologie.- 1991. Vol.70, №11. – p. 630-634.

33. Bujia., Wilmes E., Bartual-Pastor J., Hammer C. comparative study of the effect of different chemical procedures on the antigenicity of allogenic transplants of the human trachea // Acta Otorrinolaringo. Esp. – 1993. – Vol. 44, №3. – p. 209-216.

34. Allaire E., Guettier C., Bruneval P., Plissonnier D., Michael J.B. Cell-free arterial grafts: morphologic characteristics of aortic isografts, and xenografts in rats //J. Vasc. Surg. – 1994. – Vol.19, №3. – p. 446-456.

35. Timpl R. Immunological studies on collagen // Biochemistry of collagen. – New york. – 1976. – p. 319-365.

36. Davies A.H., Parums D.v. storage of donor long saphenous vein // cardiovasc. Surg. – Torino. – 1992. –Vol. 33., 31. p. 92-97.

37. Hagiwara H. Immunoelection microscopic study of proteoglycans in rat epiphyseal growth plate cartilage after fixation with ruthenium hexamine trichloride (RHT) // histochemistry. – 1992. Vol. 98, №5. – p. 305-309.

38. Iwata M., Carlson S. S. A large chodroitin sulfate proteoglycan has the characteristics of a general extracellular matrix component of adult brain //J.Neurosci. – 1993. – Vol. 13, №1. – p. 195-207.

39. Patent RF №2189257 “alloplant biomaterial for regenerative surgery”

40. Longmire W.R. et al. General Surgical Problems of Tissue Transplantatuion//Preservation and Transplantation of Normal Tissues: ACIBA Foundation Symposium. _ London. – 1954. – p. 23-43.

41. Falk I.G. Mucopolysaccharides and proteins in regeneration and grafting of heel tendon in experiment: Abstract of thesis for master’s degree. – Barnaul, 1996. – 25 p.

42. Messner K. Meniscal regeneration or meniscal transplantation // scand. J. Med. Sci. Sports. – 1999. – Vol 9, №3. – p. 162-167.

43. Nigmatullin R.T. Morphological aspects of transplantation of connective tissue allografts. Abstract of doctoral dissertation. – Novosibirsk, 1996. – 40 p.

44. Muslimov S.A. Microcirculatory channel of normal bulbar conjunctiva and in experimental alloplasty: Abstract of a thesis for a master's degree. – Yaroslavl, 1984. – 20 p.

45. Clark E.R. Clark E.L. Microscopic observations on the extraendothelial cells of living mammalian blood vessels. – Amer. J. Anat., 1940. – Vol. 66, № 1. – p. 1-49.

46. Kupriyanov V.V. Morphological peculiarities of the ways of microcirculation and their formation in pre- and postnatal ontogenesis. – in the book: Morphological principals of microcirculation: 2nd MOLGMI. – 1978. – Vol. 65, №4. – p. 3-16.

47. wagner R.C. Vascular endothelium and basement membranes. Ed. By Altura B.M. – Karger, Basel e.a., 1980. – p. 345 -349.

48. Serov V.V., Shehter A.B. Connective tissue (functional morphology and general pathology). – M.: Medicine, 1981. – 312 p.

49. Postlethwaite A.F., Kang A.H. Fibroblast // Inflammation basic principles and clinical correlates / Ed. J Gallin . – N.Y.: Raven Press, 1998. – p. 577-597.

50. Sorgente O.N., Dorey C.K. Inhibition of endothelial proteglycans immobilized on extracelluar substrates // J.Biol. Res. – 1980. – Vol. 128. – p. 63-71.

51. Yamagata M., Suzuki S., Aiyama S.K., Yamada K.M., Kimata K. regulation of cell-substrate adhesion by proteglycans immobilized on extracellular substrates // J.Biol. Chemistry. – 1989. – Vol 264. – p. 8012-8018.

52. Arlein W.J., Shearer J.D., Caldwell M.D. Continuity between wound macrophage and fibroblast phenotype; analysis of wound fibroblast phagocytosis // Am. J. Physiol. 1998. – V. 275/ - p. 1041-1048.