DDC-UDC 616.314-083:528.315-38 DOI:10.19221/201919

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THE STUDY OF MORPHOMETRIC CHARACTERISTICS OF THE CAPILLARY NETWORK AT THE EARLY TERMS OF THE POROUS FIBROSE MATRIX SUBCUTANEOUS IMPLANTATION

Abstract. *From year to year medicine and bioengineering have become more and more interested in biopolymers. The materials applied in bioengineering to make biografts should possess a spectrum of special properties and provide engineering or microengineering constructions with characteristics peculiar for living tissues, that is: ability to self-reproduction, ability to change structure and properties in response to environmental factors. The study was conducted on 20 laboratory animals (rabbits) divided into two groups. The first group included 10 animals operated on with the formation of "pocket" in the subcutaneous tissue followed by applying stitches. The second group included 10 animals which underwent subcutaneous implantation of biopolymer matrix in the back area between the shoulder-blades. 9 segments were taken for examination: one located in the center and each 4 segments from the paracentral and peripheral areas. The results obtained are indicative of the lack of both acute and chronic response inflammatory infiltration, as well as acute or chronic graft rejection as a foreign substance in the implanted area of the animal. The fibrous matrix created by us due to its hygroscopicity and porosity creates a distinctive bridge for emergence and development of the capillary network.*
Key words: *biopolymer, biograft, capillary network.*

Introduction. Nowadays medicine and bioengineering have become more and more interested in biopolymers. The materials applied in bioengineering to make biografts should possess a spectrum of special properties and provide engineering or microengineering constructions with characteristics peculiar for living tissues, that is: ability to self-reproduction, ability to change structure and properties in response to environmental factors. [1]. The problem arising in front of tissue engineering is to optimize isolation, reproduction and differentiation of cells, to construct the frames (matrix) or systems of supply promoting maintenance, coordination of tissue regeneration in three dimensions [2, 3]. One of the important criteria that should be considered in matrix construction is its ability to maintain stable hemodynamics inside and around of the frame. Hemodynamic stability and, respectively, tissue vitality first of all will depend on the character of the capillary network development inside of the scaffold [4, 5].

Objective: to assess the character of the capillary network development at the early terms of subcutaneous implantation of biopolymer fibrous matrix in the experiment.

Materials and methods. We have developed fibrous matrix made of the granules of 100% pure polylactide. This matrix was applied in conducting the research. The matrix was developed by means of polymer phase division method. The fibrous matrix was on an average 30 mm thick. The diameter of its fibers was from 4 mcm to 10 mcm (Fig. 1, Fig. 2).

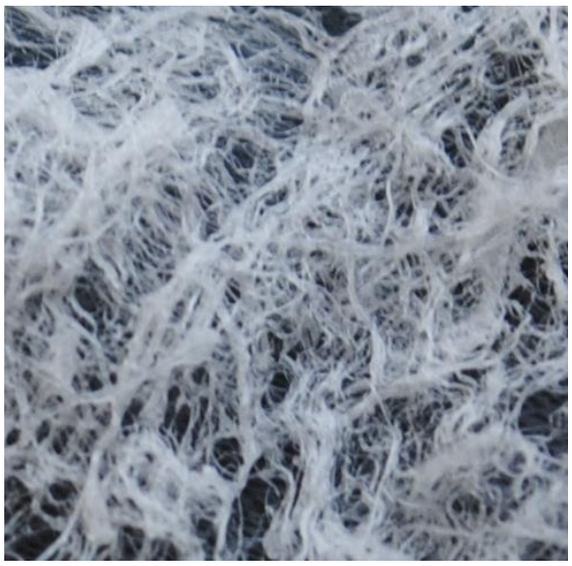

Fig. 1 Fibers of biopolymer matrix (macrograph).

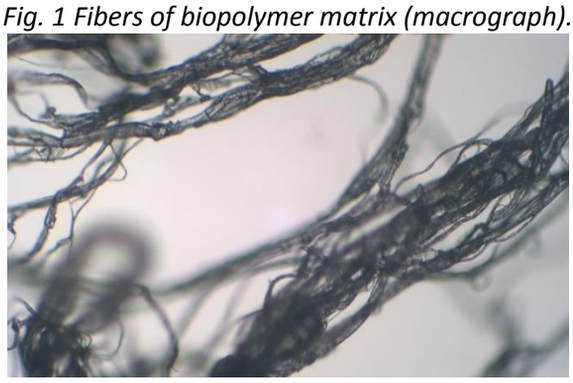

Fig. 2 Fibers of biopolymer matrix (micrograph, magnification: x100).

The above matrix was exposed to gamma sterilization. The scaffolds hermetically double packed for sterilization were evenly distributed under electron beam with the energy of particles 4 mega electron volts (MeV) and duration of impulses 4,5 microseconds (mcs). Every package «Medicom» standardized EN 868-5, ISO 11140-1, ISO 11607-1, in which the polymer was packed was 0,6 mm thick. On radiation the number of impulses varied from 4 to 70.

Sterilization occurred by the following parameters: frequency of the accelerator work was 250 Hz, maximum energy of electrons was 5 MeV, maximum beam power was 5 kW, duration of impulses was 4,5 mcs, pulse current 1,5 A, suppressive radiation power at the distance of 1 m from the target was – 104 R/sec. The dose of radiation of the object was up to 50 Gy.kg on the basis of the volume and density of the material. According to the norm, the maximum tolerance dose of 50 Gy.kg was with maximum electron power of 5 MeV. Processing with the electrons with the power less than 10 MeV did not cause nuclear transmutations, that is it did not lead to the occurrence of radioactive isotopes and did not create residual radiation background of the object.

After sterilization biopolymer matrix was surgically implanted under the skin of a laboratory animal. The study was conducted on 20 laboratory animals (rabbits) divided into two groups. The first group included 10 animals operated on with the formation of “pocket” in the subcutaneous tissue followed by applying stitches. The second group included 10 animals which underwent subcutaneous implantation of biopolymer matrix in the back area between the shoulder-blades.

1 month later the matrix was surgically removed from the animal body together with adjacent tissues. To make general histological examination the matrix together with the adjacent tissues was dissected perpendicular in 25 similar segments. 9 segments were taken for examination: one located in the center and each 4 segments from the paracentral and peripheral areas. The obtained parts of the graft were fixed in 10% neutral formalin solution (Ph-7,0). The time of fixation was 24 hours. Then the pieces of the examined organs were placed into the ascending battery of alcohols for dehydration, followed by placement into chloroform, chloroform-paraffin mixture (1:1), paraffin (at the temperature of 37°C). After paraffin preparation the pieces were coated with paraffin. Series paraffin sections were made 4-6 mcm thick on the sliding microtome. The specimens were stained with hematoxylin and eosin [6].

Histological specimens were examined by means of the light microscope Leica DME under different magnifications of the lens and eyepiece. Morphometric parameters were determined by means of the system to obtain microscopic images of histological specimens (microscope Leica DME and digital camera "Nikon P5100") and the program of image analysis ImageTool 2.0 for Windows at the Department of Pathomorphology and Forensic Medicine, Ivano-Frankivsk National Medical University.

The results were statistically processed by means of the computer programs Microsoft Excel and Statistica 5.5 (MultipleRegression) applying the methods of variation statistics and correlation.

To examine blood vessels of the fibrous matrix

the ether-chloroform mixture of French blue was used (10 g of the stain per 100 ml of the solvent consisting of ether and chloroform in the ratio 3:1). This mixture was injected into the thoracic region of the aorta. 3-4 hours after the blood vessels were filled in with the above mixture, and the graft together with the adjacent tissues was removed and fixed in 10 % neutral formalin solution during 14 days.

On a freezing microtome sections were made 30-50 mcm thick. They were dehydrated in the alcohols of an increasing concentration, clarified in salicylic acid methylene ether and placed in polysterol. After that the sections were examined under the binocular microscope МПС-6 with different magnification.

Results. Pathomorphological examination of the peripheral graft areas of the 1-month term determined development of the connective tissue and capillaries in the space between matrix fibers. The vessels in the connective tissue are located unevenly (Fig. 3 exposition 1_025), on an average 49345,18±4,22 mcm^2 of the square per one vessel. An average gauge is 697,61±3,99 mcm^2, with the norm 597,24± 3,12 mcm^2. The vessels are mainly of a capillary type, with thin walls, an average thickness of 3,2±0,14 mcm, and clear basal membrane. Endotheliocytes with clear oval elongate nuclei line the space inside. The groups of erythrocytes are available in the vascular lumen.

Pathomorphological examination of the paracentral areas of grafts determined found thin vessels of a capillary type, arterioles, and venules in the connective tissue (Fig. 4 exposition 1_028). An average thickness of the walls of these vessels is 3,09±0,17mcm, the square of the transverse section is 739,56±4,32 mcm^2, in comparison with 2,97±0,13mcm and 728,45±3,98 mcm^2 in the control group. According to the morphometric examination there is 37698,96 ±4,32 mcm^2 of the connective tissue matrix per one vascular section.

In certain areas on the borders with the graft fibers the groups of leukocytes are found in the connective tissue. The centers of angiomatosis in the form of transverse sections of vessels are found around the parts of matrix fibers in the connective tissues (Fig. 5 Exposition 1_020).

Pathomorphological examination of the central areas of grafts found that spaces between the graft fibers are filled with the connective tissue in which small blood vessels full of blood are found – an average square of the transverse vascular section is 1321,23±4,75 mcm^2, wall thickness – 2,65±0,16 mcm (Fig. 6 Exposition 1_004). In the intact animals these parameters are 934,23±4,05 mcm^2 and 2,44±0,14 mcm respectively. The amount of vessels per the unit of square is a little larger of the norm in cases of loose location of fibers constituting 20915,92 mcm^2 of the

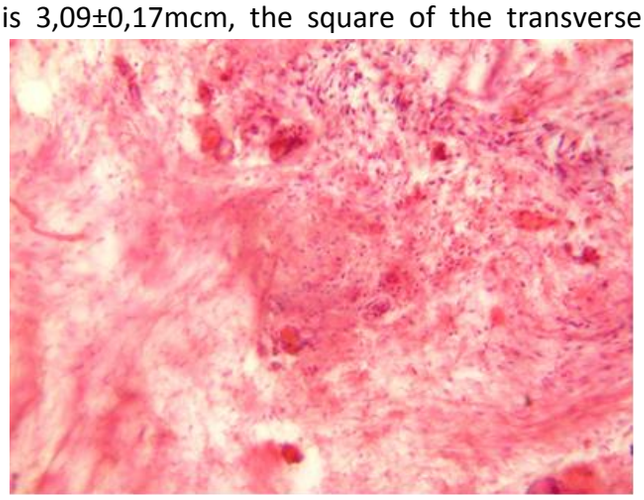

Fig. 3 Exposition 1_025 Staining: hematoxylin and eosin. (micrograph, magnification: x100).

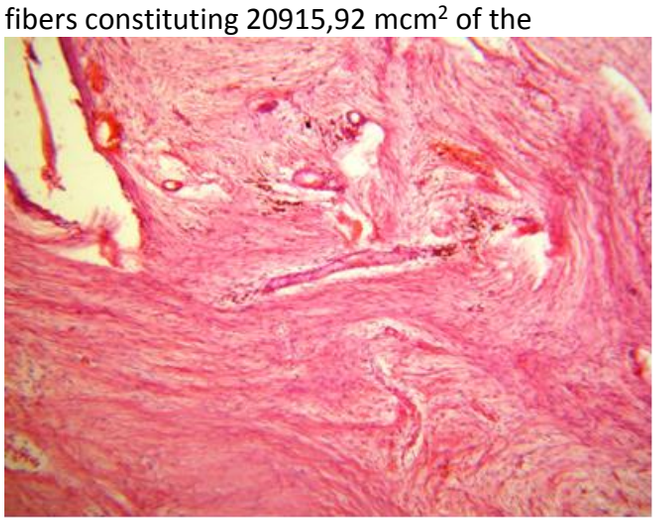

Fig. 4 exposition 1_028 Staining: hematoxylin and eosin. (micrograph, magnification: x100).

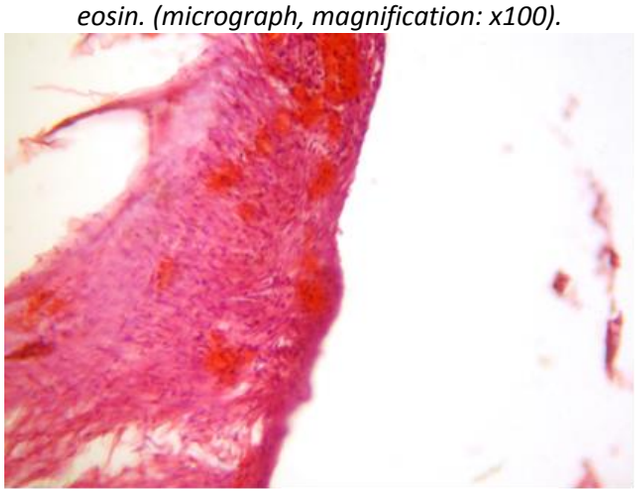

Fig. 5 Exposition 1_020 Staining: hematoxylin and eosin. (micrograph, magnification: x100).

connective tissue per one transverse section of the vessel. In other cases there is 24797,47±3,33 mcm^2 of the connective tissue per one transverse section of the vessels. By means of the morphometric and statistical analyses an increased density of the vascular bed in the areas around synthetic fibers was found (19872,94±4,32 mcm^2 of the connective tissue square per one vascular section) concerning the connective tissue deeper located, which is described in the loose and denser connective tissues.

The areas with slightly marked segmental leukocyte infiltration are determined around the part of the graft fibers.

Injection of vessels determined that the blood stream of the adjacent tissues to the matrix and inside of the polymer matrix consists of the links interrelated between themselves: arteries, arterioles, pre-capillary arterioles, hemocapillaries, extra-capillary venules, venules and veins (Fig. 7).

The arteriole (20-30 mcm in diameter) is divided dichotomically or extends 10-12 pre-capillary arterioles (10-20 mcm in diameter), mainly from its one side. Smaller in the diameter (10 mcm) extremity of the pre-capillary arteriole in certain areas empties into the venule, but more often the final part of the pre-capillary arteriole is divided dichotomically into the hemocapillaries or united into venules. Pre-capillary arterioles (10-20 mcm) in the majority of cases are dichotomically divided into hemocapillaries consisting of arterial (5-10 mcm) and venous parts (10-20 mcm). Hemocapillaries forming the blood stream in the form of network empty into the extra-capillary venules (15-25 mcm), which join together and form venules (25-40 mcm). Arterial branches and arterioles emerge from the arteries penetrating into the whole thickness of the subcutaneous tissue.

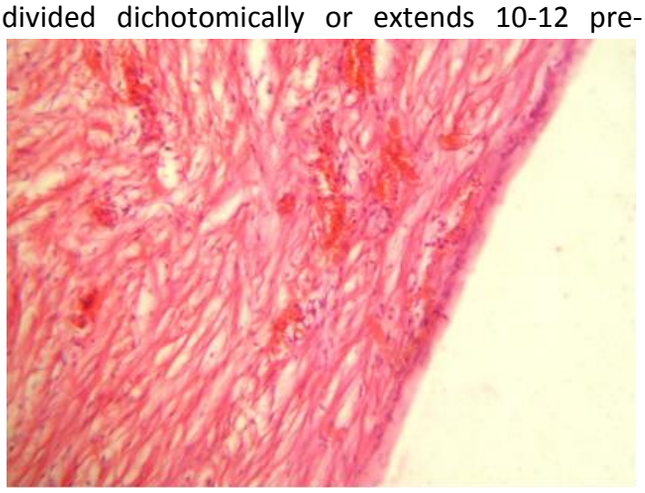

Fig. 6. Exposition 1_004 Staining: hematoxylin and eosin. (micrograph, magnification: x200)

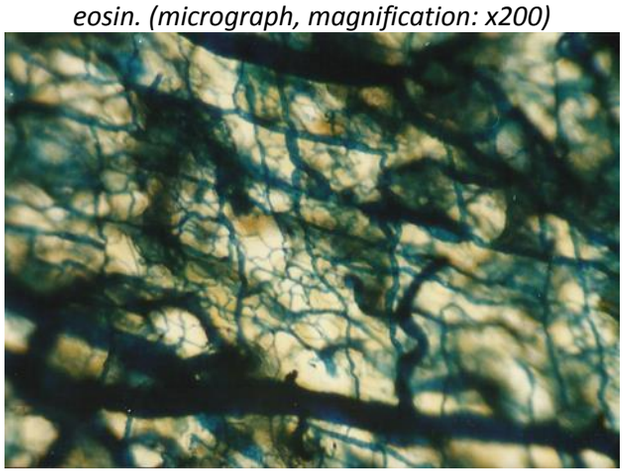

Fig. 7 Arteries, arterioles, pre-capillary arterioles, гемокапіляри, hemocapillaries, extra-capillary venules, venules and veins

Results and discussion. Therefore, on the basis of the conducted research it was found that the state of the capillary network surrounding fibers of the biopolymer matrix does not differ from the animals in the control group, except a small amount of leukocytes available. Inconsiderable amount of leukocytes and macrophages available can be indicative of the process of material hydrolysis, which mechanism is associated with the activity of the enzymatic systems of cells. Availability of the blood vessels not only in the periphery of the matrix, but inside of it as well is indicative of a developed blood network providing not only supply of nutrients deep into the matrix, but active elimination of waste products, providing active development of biosynthetic processes in the tissue. Vascular plethora is indicative of it. It is evidence of thin wall capillaries available with slightly seen lumen and fibers located loosely, which is indicative of neoangiogenesis. The process of neoangiogenesis continues with development and maturation of the connective tissue.

Conclusions: 1. The lack of a great number of neutrophils, increased amount of macrophages and lymphocytes are indicative of the lack of both acute and chronic response to inflammatory infiltration, as well as acute and chronic response to graft rejection as a foreign substance in the implanted area of animals.

2. The fibrous matrix, developed by us, due to its hydroscopic and porous features, creates a specific bridge for the growth and development of

the capillary network.

Prospects of further studies include the use of the porous fibrous material in the treatment of granulomas, cystogranulomas, cysts and assessment of clinical results, cytological and morphological changes in the area of implantation of the given material.

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