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Morphofunctional Rearrangement of the Fibrous Structures of the Rat Dermis under the Conditions of Implantation of 3D Scaffold Based on Polyprolactone

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Abstract

Background: the use of various scaffolds allows us to model the future fibrous framework of the newly formed regenerate, and also serves as a substrate for the settlement of the cellular component. The development of tissue engineering in regenerative medicine demands an understanding of the more specific mechanisms of the formation of the connective framework at the site of the defect. The aim of this research was to study the morphofunctional rearrangement of the fibrous structures of the rat dermis in response to the implantation of a 3D scaffold based on polyprolactone.

***Methods and Results*:** The experiment was performed on 30 white male Wistar rats. The object of the study was a skin fragment together with an implantable 3D scaffold based on polyprolactone, taken on Days 3, 7 and 14 after implantation. Biomaterial with implantable scaffold was studied using light and scanning electron microscopy. The results of the study indicate that the 3D scaffold based on polyprolactone has good biocompatibility, causing a weak inflammatory reaction, and contributes to the formation of the connective tissue framework by Day 14.

Conclusion: The results of the study can be used to develop new scaffolds or modify existing ones, as a "framework" for populating the cellular component and creating tissue-engineering structures.(**International Journal of Biomedicine. 2021;11(3):376-378.**)

Key Words: skin • 3D scaffold • regeneration • collagen fibers

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Introduction

Recently, a large number of studies have appeared on the development of scaffolds for the replacement of skin defects. This is primarily due to the need to improve the effectiveness of treatment of burns and chronic wounds, as well as re-epithelization of large areas of damaged skin during surgical operations. The created dermal scaffolds should contribute to the improvement of healing indicators (reduction of the inflammatory response, formation of granulation tissue, stimulation of angiogenesis, acceleration of wound epithelization, etc.) and reduction of complications.[1,2] Currently, there is no doubt that the effectiveness of artificial extracellular matrices in stimulating tissue regeneration is associated with providing sufficient temporary mechanical support for the formation of a new fibrous backbone.[3-5]

To assess the prospects of scaffolds, morphological studies of tissue response to the implantation of scaffolds in vivo and the study of the features of the formation of the collagen framework are necessary.[6,7] Such studies are not numerous in the scientific literature. Based on the above, the aim of our research was to study the morphofunctional rearrangement of the fibrous structures of the rat dermis

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in response to the implantation of a 3D scaffold based on polyprolactone

Materials and Methods

The experiment was performed on 30 white male Wistar rats, weighing 150±15 g. The object of the study was a skin fragment together with an implantable 3D scaffold based on polyprolactone, taken on Days 3, 7 and 14 after implantation. The biomaterial was taken from the lateral surface of the back, by excision of the skin of the specified size, to the fascia of the subcutaneous muscle.

In vivo experiments were carried out in accordance with the legislation of the Russian Federation, in strict compliance with the European Convention for the protection of animals used for experimental and other purposes (Strasbourg, France, 1986), the provisions of Directive 210/63/EU of the European Parliament and the Council of the European Union of 22 September 2010 on the protection of animals used for scientific purposes (Article 27), and approved by the Regional Ethics Committee of Kursk State Medical University (Protocol No. 4 dated June 10, 2019).

For light microscopy, the material was fixed in a 10% aqueous solution of neutral formalin. Microtomization and filling in with paraffin were carried out according to standard prescriptions. Sections with a thickness of 5-7 microns were stained by H&E. For scanning electron microscopy (SEM), the skin was fixed with 10% buffered neutral formalin, dehydrated in a frozen state in alcohols of increasing concentrations. The samples prepared in this way were mounted on a special aluminum table with a conductive carbon glue, sprayed with gold or platinum-paladium alloy in a Quorum Q150TS spray unit (GaLa Gabler Labor Instrumente. Handels GmbH, Germany) and viewed in an SEM S 3400N (Hitachi, Japan).. Next, the scanned image was processed using the ImageJ program, where the thickness of the collagen fibers surrounding the threads of the polyprolactone matrix was measured in each case in 50 fields of view.

Statistical analysis was performed using the STATISTICA software package (v.12.0, Stat-Soft Inc., USA). For descriptive analysis, results are presented as median (Me), first quartile (*25th percentile*) and third quartile (*75th percentile*). The Mann-Whitney U Test was used to compare the differences between the two independent groups. A probability value of P<0.05 was considered statistically significant.

Results

On Day 3 of the experiment, the fibrous structures were disorganized in the dermis. At the same time, on the cross sections, their shape varied from flattened to rounded. Large inter-fibrous gaps were determined between the fibers, which may indirectly indicate the preservation of interstitial edema by this time. Also, in the field of view, were a large number of individual fibrils, as well as some combined into fibers (Figure 1).

By Day 7, we observed active cell migration at the implantation site. The cellular composition is mainly represented by lymphocytes, numerous macrophages and single fibroblastic cells. When studying the fibrous component around the scaffold threads, we found collagen fibers strictly oriented in one direction, the thickness of which was 0.13[0.09;0.16] microns. The orientation of the collagen structures followed the shape of the implant structures – they spread both circularly around the scaffold threads, and in parallel, sprouting between the polyprolactone threads (Figure 2).

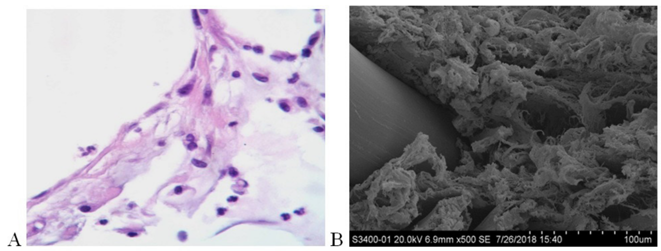

Fig. 1. *Microphotography of the rat skin dermis on the site of the implanted 3D scaffold based on polycaprolactone on Day 3 of the experiment. A - H&E staining (× 400). B - SEM (× 500)*

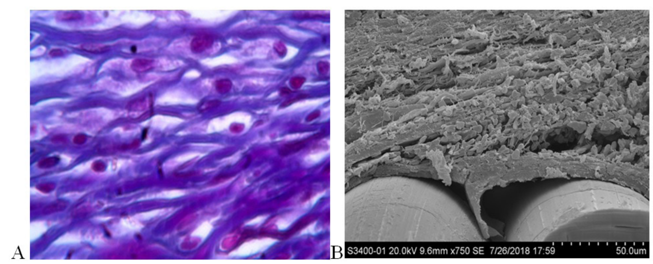

Fig. 2. *Microphotography of the rat skin dermis on the site of the implanted 3D scaffold based on polycaprolactone on Day 7 of the experiment. A - H&E staining (× 400). B - SEM (× 1000)*

On Day 14 of the experiment, an increase in the number of collagen structures was observed around the matrix structures. The fiber thickness increased to 0.24[0.18;0.84] microns. At the same time, a certain spatial pattern was noted – sagittal directed fibers were located more compactly, and had practically no inter-fiber gaps. The frontal fibers were more loosely arranged; they were thin and had a large number of branches (Figure 3).

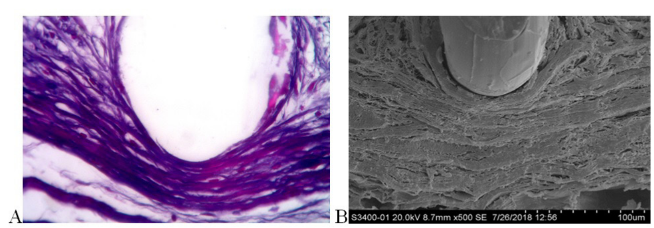

Fig. 3. *Microphotography of the rat skin dermis on the site of the implanted 3D scaffold based on polycaprolactone on Day 14 of the experiment. A - H&E staining (× 200). B - SEM (× 500)*

This organization of fibrous structures is the basis for the construction of the connective tissue capsule and the germination of blood vessels into it; in addition, it allows us to judge the completion of the stage of adaptation and reconstruction of the dermis for the further formation of the connective tissue capsule that separates the scaffold from the surrounding tissue.

Tissue engineering is currently considered the leading field in regenerative medicine. Tissue-engineered structures consisting of scaffolds (matrices) and cells cultured on them are used in reconstructive operations in various fields of medicine. These properties, as well as the possibility of modeling a three-dimensional porous structure similar to natural extracellular matrices, allow us to create conditions for organotypic regeneration. The results of the study indicate that the 3D scaffold based on polyprolactone has good biocompatibility, causing a weak inflammatory reaction, and contributes to the formation of the connective tissue framework by Day 14.

Comparing our data with that of other authors, the optimal time for the beginning of resorption of such materials should be the period from 10 to 14 days, which is necessary for maintaining normal metabolism, proliferative activity and differentiation of cells and, as a result, for determining the possibility of vascularization and remodeling of regenerating tissue.[8-10]

In conclusion, our experimental study showed that the use of a 3D scaffold based on polyprolactone contributes to a faster formation of the collagen framework at the implantation site. The results of the study can be used to develop new scaffolds or modify existing ones, as a “framework” for populating the cellular component and creating tissue-engineering structures.

Competing Interests

The authors declare that they have no competing interests.

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