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MATERIALS FOR ENSURING HUMAN LIFE ACTIVITY AND ENVIRONMENT PROTECTION

Biodegradable Porous Scaffolds for the Bone Tissue Regeneration

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Abstract—Scaffolds made of recombinant spidroin and fibroin of *Bombix mori* silk were produced by the salt leaching technique. The regenerative properties of scaffold were evaluated in experiments with rats by implantation into bone wounds. According to the X-ray tomography data, the use of both types of biocompatible materials provides the restoration of the integrity of a bone. By analyzing the dynamics of regeneration, it was found that the use of spidroin leads to more rapid regeneration of bone tissue in the defect area as compared to silk fibroin.

Keywords: silk fibroin, recombinant spidroin, biodegradable scaffolds, bone tissue regeneration

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INTRODUCTION

An important problem of regenerative medicine is the choice of materials for the design of artificial organs, tissues, and bioengineering structures. The list of necessary requirements imposed on such materials is rather long. The most important of them are as follows: the material must form a porous carcass and be nontoxic to cultured cells; it must be characterized by a sufficient mechanical strength and elasticity necessary for surgical procedures; it must possess biocompatibility with the body; it must be biodegradable so as to avoid additional invasions for withdrawal of the structure at the final stage of regeneration; the material must preserve its medical and chemical properties upon sterilization using the standard techniques.

The search for optimal polymers that meet the above criteria as much as possible is not simple and it faces a number of limitations. This is related to the fact that each of the materials has both positive and negative properties that are revealed in the process of design and then in the process of treatment. Biomaterials applied in medicine must degrade at a rate comparable with the formation of new tissue to permit cells to synthesize the new extracellular scaffold and recover functionally active tissue. The most promising materials for design of carriers in tissue engineering are polymers of biological origin. The degradation products of such polymers do not have a toxic reaction and sometimes they can serve as highly efficient biostimulants [1–3]. The materials meeting all the above requirements are silk fibroin and spidroin.

Fibroin is the basic silk protein which is obtained from *Bombyx mori* cocoons or related species. It is a heterodimer consisting of two chains covalently linked by disulfide bridges: a light Fib-L chain with a mass of 26 kDa (262 amino acids) and a heavy Fib-H chain with a mass of 350 kDa (5263 amino acids), as well as glycosylated protein P25 with a mass of 30 kDa bonded to Fib-L and Fib-H by hydrophobic bonds [4–6]. The primary sequence of fibroin is composed of (in wt %) 43% glycine, 30% alanine, 12% serine [7], 5% tyrosine, 2% valine, aspartate, glutamate, and cysteine, the latter performing the main integrating role in uniting the subunits into one molecule. Fibroin is an amphiphilic protein with a significant domination of hydrophobicity and is insoluble in water and in dilute solutions of many acids and bases. Owing to the ability of fibroin to form both α-helixes and β-pleated sheets, it occurs in several structural forms: (1) a fluffy, globular form; (2) an amorphous form enriched in α-helixes (silk I); and (3) a strong crystalline β-form (silk II). The form saturated with β-structures is the one that determines and supports the shape of a biostructure on its basis and provides its integrity and stability in aqueous solutions close to the physiological medium of the organism for a long period of time [8]. Thus, the structures made of fibroin should be exposed to β-crystallization before application in order to preserve their integrity and to avoid their destruction in a culture medium or under in vivo conditions. Fibroin is a thermostable protein, the temperature of its denaturation is higher than 127°C, its elastic modulus is 15–17 GPa, and it possesses a high tensile strength (610–690 MPa) [9].

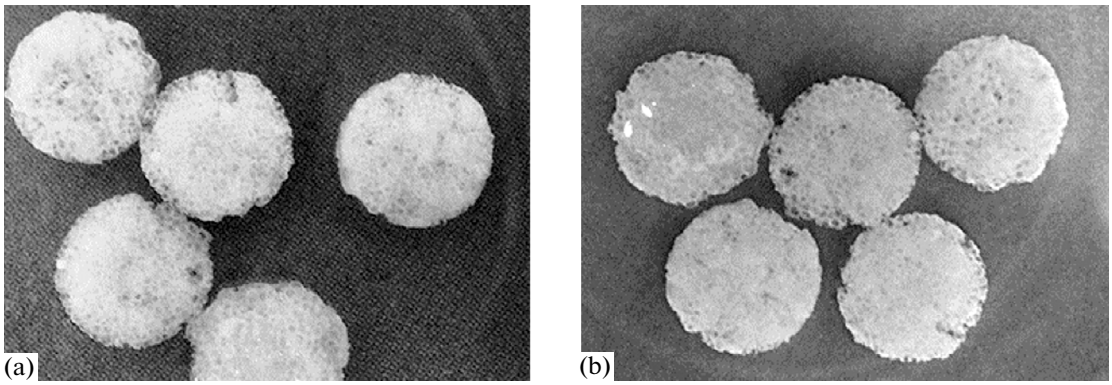

Fig. 1. Macrophotograph of the porous scaffolds made of recombinant spidroin (a) and fibroin (b) of *Bombyx mori* silk. The scaffolds are porous disks with a diameter of 10 mm and height of 2 mm.

Carcass spider silk is silk produced by spiders [10]. Carcass spider silk threads are very elastic, they are characterized by high tensile strength comparable with Kevlar and exceeding the strength of steel, which makes this polymer unique among other natural and most artificial materials [11]. Spider silk is resistant to the conditions of the environment, and it possesses a high biocompatibility and ability for biodegradation. Such properties are explained by its structure. The composition of a carcass thread contains two covalently linked proteins: spidroin 1 encoded by the MASP1 gene (which forms crystalline β-pleated sheets structures) and spidroin 2 encoded by the MASP2 gene (which forms the "amorphous scaffold"). The crystalline parts of the macromolecule are responsible for the high tensile strength while the "amorphous scaffold" provides elasticity. The molecular mass of the isolated spidroins is 300–350 kDa, and for the dimeric forms, it is 550–650 kDa [12]. Carcass spider silk threads possess a high thermal stability (up to 230°C). There are recombinant analogs of natural spidroins characterized by lower mechanical strength, which are synthesized in *Pichia pastoris* and *Saccharomyces cerevisiae* yeast cells by using animal cells as culture producers and in transgenic animals and plants [13, 14].

The aim of this work is to study the possibility of regeneration of bone tissue in an experimental model of mechanical damage in a bone upon the implantation of porous biodegradable scaffolds made of silk fibroin or recombinant spidroin.

MATERIALS AND METHODS

Preparation of Porous Scaffolds Based on Regenerated Silk and Recombinant Spidroin

Lyophilized recombinant spidroin 1f9 [15] was kindly provided by V.G. Bogush, leading researcher of the Laboratory of Protein Engineering of the State Research Institute of Genetics and Selection of Industrial Microorganisms.

Fibroin was isolated from cocoons of the *Bombyx mori* silk moth provided by V.V. Bogoslovskii, head of the Republican Sericulture Research Station of the Russian Academy of Agricultural Sciences (Zheleznovodsk, Stavropol krai). For washing away sericin, the cocoons were boiled in 0.03 M $NaHCO_3$ for 1 h and washed with distilled water.

The porous scaffolds were obtained by leaching. A sample of each polymer with mass of 15 mg was solved at 40°C for 30 min in 50 μL of a 10% lithium chloride solution with 90% formic acid. The resulting solution was centrifuged for 10 min at 11300 *g*, and then 50 μL of supernatant liquid was mixed with 110 mg of sodium chloride; the salt particle size was 200–400 μm. After that, a disk with a diameter of 10 mm and height of 2 mm was formed. At room temperature (20–25°C), the disk was dried and treated with 96% ethanol for 2 h, after which it was washed with distilled water to remove sodium chloride particles. The obtained scaffolds were degassed and stored in 70% ethanol (Fig. 1). The pore size of the scaffold was 100–600 μm, and the porosity was 85%.

In vivo Experiments

All the procedures of the care and work with the animals were performed in accordance with protocols approved by the Animal Care and Use Committee in accordance with the Guidelines of the Ministry of Health and Social Development of the Russian Federation (Order no. 755 of August 12, 1977) and the Declaration of Helsinki of the World Medical Association (2000). In the process of the experiment, 60 Wistar rats underwent surgery; all the animals were divided into the test groups, which is shown in Table 1. A bone defect was created by the following technique: a through hole with a diameter of 2 mm was made in the projection of the diaphysis of the femur bone in the anteroposterior direction. From the obtained scaffold disks, cylinders with a diameter of 2 mm and length of 5 mm were formed to fill the defect. Before the

implantation, the scaffolds were washed in sterile saline solution.

In all the terms of observation (1–8 weeks), blood samples were collected from the animals from the tail vein; then five animals of each group were killed by ether overdose inhalation. By disarticulation of joints, femur bones with surrounding soft tissues were withdrawn, soft tissues were eliminated, and the bones were immersed in a 10% neutral formalin solution.

The results of the application of scaffolds of the two types were evaluated by biochemical and X-ray studies of the region of interest in terms of 1, 2, 4, and 8 weeks after the operation. The studies were performed using a Light Speed VCT 64-slice multidetector computed tomography scanner (General Electric (GE)). The scanning parameters were as follows: 120 kV, 80 mA, and the thickness of reconstruction of 0.6 mm. The obtained series of images were processed using an AW VolumShare 4 expert-class workstation with multiimage viewing by 3D ("three-dimensional") and VR ("virtual reality") protocols. The quantitative evaluation was performed by the fracture callus index (FCI) on the Hounsfield scale. For this purpose, the dynamic measurements of the densitometric indices were performed in the intermediate zone of the regenerated tissue and the intact cortical layer by helical computed tomography; the FCI was calculated according to the formula

$$\mathrm{FCI} = \frac{\mathrm{IR}}{\mathrm{CL}} \times 100\%,$$

where IR is the density of the intermediate zone of the regenerated tissue in Hounsfield units (HU) and CL is the density of the intact cortical layer of the adjacent parts of the bone, HU.

Hounsfield units evaluate the degree of absorption of the X-ray radiation by anatomical structures of the organism as compared to water. The degree of absorption of X-ray radiation by water on the Hounsfield scale is taken equal to 0. Air and fat have negative values; bone tissue has positive values.

On the obtained images, a region was selected in the interactive mode where the area and the density of the region of interest in HU was calculated automatically. The measurements were performed to evaluate the shape and the ratio of a bone defect and the material for the substitution of bone tissue and the bone structure and to identify the presence of dystrophic changes in the bone and soft tissue components and the character of changes in surrounding tissues. The evaluation of the above parameters were performed in two stages. At the first stage, the obtained axial images were viewed in the bone and soft tissue windows, the condition of the bone and soft tissue structures in the defect region were evaluated, and the areas and the densities of the bone regenerate were measured. At the second stage, the measurement was performed in a series of multiplanar reconstructions in the bone and soft tissue modes. The qualitative evaluation of the osteoregeneration was performed according to the following criteria:

—the decrease in the clarity of the border of a bone defect and

—the increase in the optical density of a fracture callus due to the formation of mineralization fragments.

Table 1. Test groups of rats depending on the terms of observation and different types of biopolymers

Groups	Number of rats in experiments at the time of observation, weeks			
	1	2	4	8
Spidroin	5	5	5	5
Fibroin	5	5	5	5
Control	5	5	5	5

Determination of Biochemical Indices and Blood Count

The levels of total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine were measured using a Verno flow automated biochemical analyzer (Electa Lab., Italy). The alkaline phosphatase was determined using a Sapphire 350 biochemical analyzer (Audit Diagnostics, Ireland).

The blood count was calculated using a Nikon Eclipse 50i laboratory microscope (Nikon Instruments, USA) after the hematoxylin–eosin staining of blood smears by the standard technique. The blood samples were collected from live animals through the tail vein.

Statistical Analysis

The evaluation of the results was performed by methods of variational statistics using the Microsoft Excel XP standard statistical software packages and Statistica for Windows Version 6.0 (StatSoft Inc., USA). The mean value (X), the standard (root-mean-square) deviation (SD), and the probability value (p) were determined. For the analysis of the available data samples, the hypothesis about a normal distribution was used (Kolmogorov–Smirnov test). To evaluate the significance of the difference between samples not normally distributed, the nonparametric Mann–Whitney test (U test) was used. The difference between the two compared values was considered statistically significant if the probability of their identity was less than 5% ($p < 0.05$).

RESULTS AND DISCUSSION

In the postoperative period, during the first two days, an expressed hypodynamic reaction and swelling of the surgically operated area were observed both in

Table 2. Average values of the hematological indices of the blood of rats before and after surgery in 1, 2, 4, and 8 weeks, $X \pm SD$

Group	Time after surgery, weeks	Indicators				
		leukocytes, 10^9/L	lymphocytes, 10^9/L	segmented neutrophils, 10^9/L	eosinophils, 10^9/L	monocytes, 10^9/L
	Before surgery	4.4 ± 0.3	6.43 ± 0.4	2.8 ± 0.3	0.2 ± 0.01	0.15 ± 0.02
Spidroin	1	6.3 ± 0.6	3.95 ± 0.7	5.08 ± 0.1	2.66 ± 0.07	4.05± 0.02
	2	4.8 ± 0.7	4.56 ± 0.9	4.2 ± 0.2	1.59 ± 0.02	2.08 ± 0.01
	4	3.9 ± 0.4	5.85 ± 0.6	3.4 ± 0.14	0.9 ± 0.13	0.58 ± 0.02
	8	4.0 ± 0.5	6.35 ± 0.7	1.95 ± 0.15	0.1 ± 0.08	0
Fibroin	1	6.9 ± 0.9	3.15 ± 0.7	5.64 ± 0.3	3.16 ± 0.07	4.25 ± 0.02
	2	5.3 ± 0.8	3.56 ± 0.9	4.4 ± 0.2	2.59 ± 0.02	2.16 ± 0.01
	4	4.9 ± 0.6	4.95 ± 0.6	3.6 ± 0.2	1.0 ± 0.1	0.73 ± 0.02
	8	4.6 ± 0.7	6.15 ± 0.7	2.15 ± 0.1	0.2 ± 0.03	0
Control	1	6.6 ± 1.0	4.56 ± 0.5	4.53 ± 0.21	1.2 ± 0.05	0.15 ± 0.01
	2	5.9 ± 0.8	4.30 ± 0.7	4.31 ± 0.19	0.77 ± 0.12	0.1 ± 0.02
	4	4.7 ± 0.6	5.36 ± 0.5	3.56 ± 0.12	0.46 ± 0.01	0
	8	4.3 ± 0.4	6.58 ± 0.4	2.4 ± 0.3	0.27 ± 0.06	0

the experimental animals and in the control group. Then, in the period of up to 1 week, the above effects decreased, and moderate swelling remained in soft tissues of femur. After 4 weeks of the postoperative period, a full regeneration was observed.

In the analysis of the dynamics of the indicators of peripheral blood leukocyte in rats, changes were observed, which are shown in Table 2.

The increase in the leukocyte level, especially the leukocyte shift toward the increase in the content of segmented neutrophils and monocytes, indicates the inflammation process, which gradually decreases over a period of 2 weeks. Two weeks after the introduction of the material, the content of neutrophils did not differ from the physiological norm. The changes in the leukocyte count indicating an allergic reaction in the early postoperative period are more expressed in the group with the implanted fibroin scaffolds as compared to the group where the spidroin scaffolds were used and to the control group. Such a reaction gradually disappeared in the observed dynamics and fully disappeared 2 weeks after surgery.

The postoperative recovery period in animals proceeded satisfactorily, and a rather rapid decrease in all the postoperative effects was observed, as well as the absence of local and systemic complications, which was proved by the study of the levels of biochemical parameters in the blood serum of animals. The levels of total protein content, ALT, AST, and creatinine during the entire observation period were within the physiological norm in all the terms of observation, which indicates the normal functional state of the main organs of animals.

The dynamic observation of the change in the content of alkaline phosphatase in blood serum showed its insignificant increase by the 14th day at the application of both spidroin and fibroin during the operation. Since the effects of inflammation completely disappear by the second week of observation, which is proved at the analysis of the hematological indices, it can be said that higher level of alkaline phosphatase as compared to the control group, probably, reflects the activation of osteoblasts and, thus, the bone tissue regeneration processes. The data of the biochemical study are shown in Table 3.

The data on the X-ray study also prove more active processes of the reparative regeneration of bone tissue in the case of the application of spidroin as compared to fibroin and the control group. In the course of the experiment, bones after surgery in each group of five animals were studied in preassigned terms after surgery. At the densitometric analysis of the multiplanar tomograms, the absolute values of the density in the defect region were obtained. The results of the studies are shown in Fig. 2 and in Table 4.

The formation of a fracture callus manifests itself by the increase in the density of the artificially created hole in a bone, which is related with the calcification process and the bone tissue regeneration. These processes are more pronounced in animals of the test groups, especially in the group with the application of spidroin. Starting from the second week, a higher regeneration index of bone tissue was observed in rats at filling of the defect with the studied materials as compared to the control group. The advantage of spidroin as compared to fibroin and the control group was shown in all the terms of observation after surgery (Fig. 3).

The introduction of silk fibroin and recombinant spidroin creates the conditions for the regeneration of

Table 3. Average values of the biochemical indices of the blood of rats before and after surgery in 1, 2, 4, and 8 weeks, $X \pm SD$

Group	Time after surgery, weeks	Indicators					
		total protein, g/L	alkaline phosphatase, units/L	total bilirubin, μmol/L	creatinine, μmol/L	ALT, nmol/L	AST, nmol/L
Spidroin	Before surgery	86.7 ± 1.8	70.0 ± 1.2	3.6 ± 0.4	105.3 ± 6.5	72.7 ± 5.5	101.0 ± 8.4
	1	81.3 ± 1.6	94.9 ± 1.5	3.3 ± 0.1	103.7 ± 7.2	73.4 ± 6.8	99.6 ± 8.5
	2	84.1 ± 1.5	90.0 ± 1.3	3.2 ± 0.2	101.9 ± 8.1	75.1 ± 5.9	96.8 ± 7.9
	4	84.9 ± 1.6	84.8 ± 1.4	3.4 ± 0.14	102.7 ± 7.3	73.8 ± 6.2	98.9 ± 8.0
	8	87.5 ± 1.5	75.3 ± 1.3	3.7 ± 0.15	105.8 ± 6.0	72.4 ± 5.6	102.8 ± 8.5
Fibroin	1	79.9 ± 1.8	91.5 ± 1.7	3.4 ± 0.3	101.6 ± 7.7	75.6 ± 5.8	97.6 ± 7.5
	2	82.3 ± 1.9	89.6 ± 1.5	3.1 ± 0.2	104.8 ± 7.5	74.4 ± 6.1	99.8 ± 7.9
	4	84.9 ± 1.5	84.9 ± 1.6	3.6 ± 0.2	107.3 ± 7.1	74.9 ± 5.6	103.1 ± 8.5
	8	86.6 ± 1.8	71.5 ± 1.3	3.5 ± 0.16	106.5 ± 6.9	73.3 ± 5.2	104.5 ± 8.5
Control	1	82.3 ± 1.5	74.6 ± 1.4	3.5 ± 0.2	103.4 ± 8.3	74.2 ± 5.1	97.4 ± 7.6
	2	85.4 ± 1.6	74.3 ± 1.6	3.3 ± 0.15	104.9 ± 7.5	75.3 ± 6.3	99.4 ± 8.1
	4	84.7 ± 1.8	73.7 ± 1.5	3.5 ± 0.2	105.5 ± 7.0	76.1 ± 5.8	105.9 ± 9.2
	8	86.3 ± 1.7	71.8 ± 1.4	3.4 ± 0.3	107.3 ± 7.3	74.2 ± 5.5	102.4 ± 8.7

bone tissue in the region of surgery. At the application of the two types of materials, the integration of bone tissue with an implant followed by the subsequent restoration of the integrity of the bone is provided.

In the analysis of the dynamics of the bone tissue formation, it was revealed that the use of spidroin for its substitution leads to faster regeneration of bone tissue in the defect zone as compared to silk fibroin.

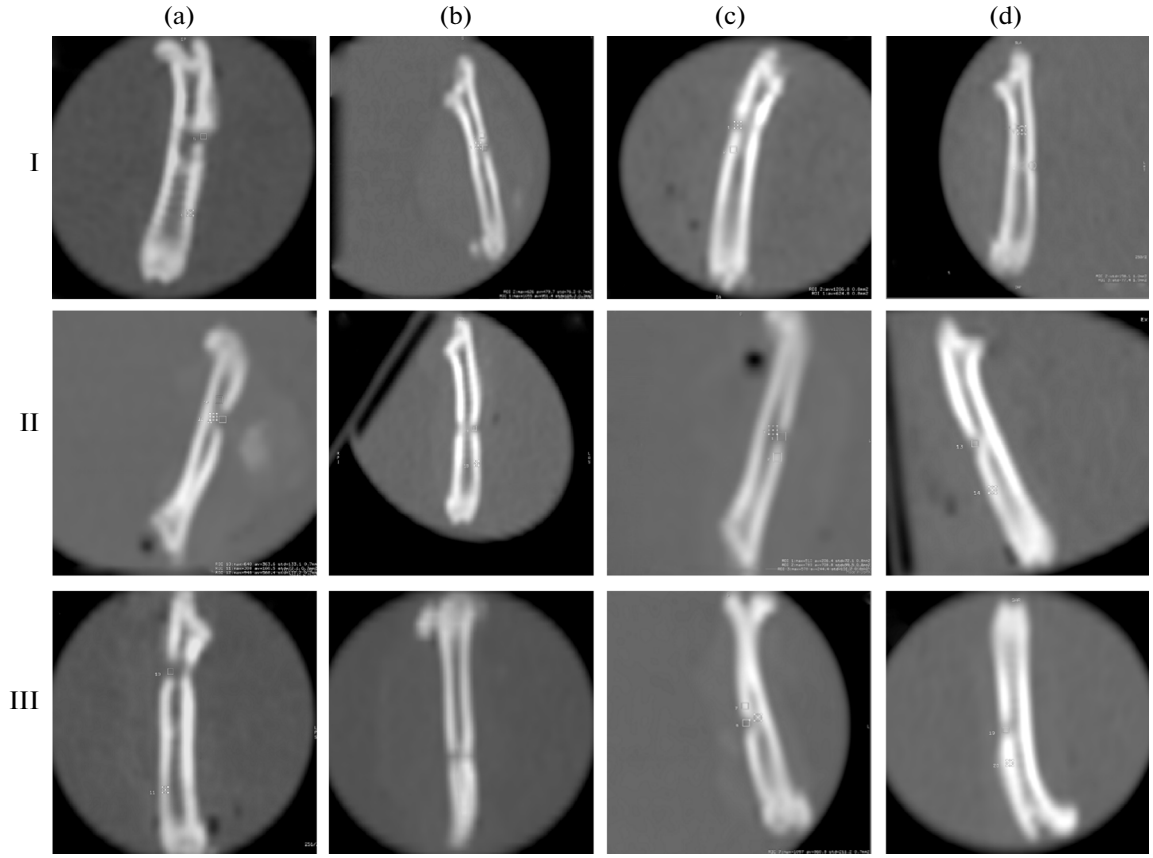

Fig. 2. X-ray photographs of bones of animals after surgery. X-ray photographs of one of five rats from each group are presented. Regeneration terms, weeks: (a) 1, (b) 2, (c) 3, (d) 8. (I) Spidroin, (II) fibroin, (III) control group (without scaffold).

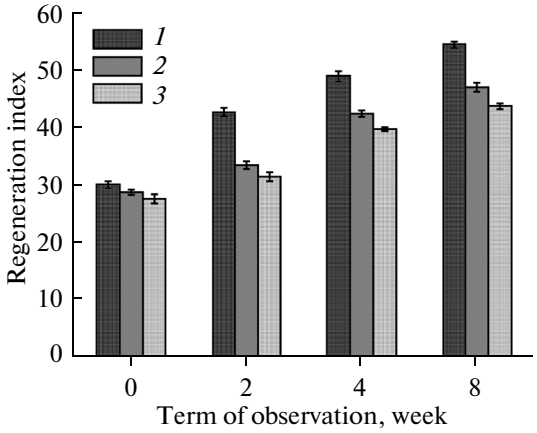

Fig. 3. Changes in the Hounsfield index in the dynamics of observation at the use of different materials for filling the defects in bone tissue: (*1*) spidroin, (*2*) fibroin, (*3*) control group. Significant differences were revealed between the groups 2, 4, and 8 weeks after surgery ($p < 0.05$).

Probably, this result of the regeneration can be affected by the differences in the amino acid sequences of fibroin and spidroin, their molecular masses, and the distinct nano- and microstructures of the synthesized scaffolds, which requires further study.

CONCLUSIONS

Silk fibroin and recombinant spidroin are promising materials for porous bioengineering structures that contribute to the regeneration of bone tissue.

The use of artificial scaffolds made of recombinant spidroin for the substitution of bone tissue leads to faster recovery of tissue in the defect zone as compared to silk fibroin.

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Table 4. X-ray data of the femur bone samples with different types of implanted materials in different terms of observation, $X \pm SD$

Material	Terms of observation, weeks	Density of the intermediate zone of regenerated tissue, HU	Density of the intact cortical layer, HU	Regeneration index
Spidroin	1	290.7 ± 21.0	953.1 ± 12.7	30.5 ± 0.6
	2	451.9 ± 9.2	1048.7 ± 19.4	43.1 ± 0.7
	4	560 ± 12.2	1129.1 ± 21.8	49.6 ± 0.9
	8	862.3 ± 10.3	1565.1 ± 22.5	55.1 ± 0.5
Fibroin	1	268.1 ± 0.8	921.4 ± 15.6	29.1 ± 0.4
	2	292.7 ± 10.2	863.4 ± 16.1	33.9 ± 0.6
	4	423.8 ± 0.9	990.3 ± 19.1	42.8 ± 0.5
	8	731.5 ± 10.0	1364 ± 20.2	47.5 ± 0.7
Control	1	431.2 ± 13.2	1540 ± 12.1	28.0 ± 0.8
	2	239.5 ± 10.3	753.0 ± 0.89	31.8 ± 0.7
	4	387.7 ± 0.95	964.5 ± 12.6	40.2 ± 0.4
	8	435.5 ± 11.5	983 ± 14.9	44.3 ± 0.6

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REFERENCES

1. Sevastyanov, V.I. and Perova, N.V., Bio-polymer heterogenic hydrogel Sphero®GEL—an injection biodegradable implant for substitutive and regenerative medicine, *Prakt. Medits.*, 2014, no. 8, pp. 110–116.
2. Kirsanova, L.A., Baranova, N.V., Bubentsova, G.N., Skaletskaya, G.N., Perova, N.V., Sevastyanov, V.I., and Skaletsky, N.N., Influence of microstructured collagen hydrogel on pancreatic islet cell cultures, *Vestn. Transplantol. Iskusstv. Organov,* 2014, no. 1, pp. 29–33.
3. Sevastyanov, V.I. and Kirpichnikov, M.P., *Biosovmestimye materialy* (Biocompatible Materials), Moscow: MIA, 2011.
4. Wadbua, P., Promdonkoy, B., Maensiri, S., and Siri, S., Different properties of electrospun fibrous scaffolds of separated heavy-chain and light-chain fibroins of *Bombyx mori*, *Int. J. Biolog. Macromolec.*, 2010, vol. 46, no. 5, pp. 493–501.
5. Tanaka, K., Inoue, S., and Mizuno, S., Hydrophobic interaction of P25, containing Asn-linked oligosaccharide chains, with the H-L complex of silk fibroin produced by *Bombyx mori*, *Insect Biochem. Molec. Biol.*, 1999, vol. 29, no. 3. pp. 269–276.
6. Ho, W., Single-molecule chemistry, *J. Chem. Phys.*, 2002, vol. 117, no. 24, pp. 11033–11061.
7. Vepari, C. and Kaplan, D.L., Silk as a biomaterial, *Progr. Polym. Sci.,* 2007, vol. 32, nos. 8–9, pp. 991–1007.
8. Scherer, M.P., Frank, G., and Gummer, A.W., Experimental determination of the mechanical impedance of atomic force microscopy cantilevers in fluids up to 70 kHz, *J. Appl. Phys.*, 2000, vol. 88, no. 5, pp. 2912–2920.
9. Smith, R.K., Lewis, P.A., and Weiss, P.S., Patterning selfassembled monolayers, *Progr. Surf. Sci.*, 2004, vol. 75, nos. 1–2, pp. 1–68.
10. Shen, Z.Q., Hu, J., Wang, J.L., and Zhou, Y.X., Comparison of polycaprolactone and starch/polycaprolactone blends as carbon source for biological denitrification, *Int. J. Environ. Sci. Technol.*, 2014, vol. 12, no. 4, pp. 1235–1242.
11. Kluge, J.A., Rabotyagova, O., Leisk, G.G., and Kaplan, D.L., Spider silks and their applications, *Trends in Biotechnol.*, 2008, vol. 26, no. 5, pp. 244–251.
12. Sponner, A., Schlott, B., Vollrath, F., Unger, E., Grosse, F., and Weisshart, K., Characterization of the protein components of *Nephila clavipes* dragline silk, *Biochemistry*, 2005, vol. 44, no. 12, pp. 4727–4736.
13. Yang, J., Barr, L.A., Fahnestock, S.R., and Liu, Z.B., High yield recombinant silk-like protein production in transgenic plants through protein targeting, *Transgenic Res.*, 2005, vol. 14, no. 3, pp. 313–324.
14. Wen, H., Lan, X., Zhang, Y., Zhao, T., Wang, Y., Kajiura, Z., and Nakagaki, M., Transgenic silkworms (*Bombyx mori*) produce recombinant spider dragline silk in cocoons, *Mol. Biol. Rep.*, 2010, vol. 37, no. 4, pp. 1815–1821.
15. Bogush, V.G., Sokolova, O.S., Davydova, L.I., Klinov, D.V., Sidoruk, K.V., Esipova, N.G., Neretina, T.V., Orchanskyi, I.A., Makeev, V.Yu., Tumanyan, V.G., Shaitan, K.V., Debabov, V.G., and Kirpichnikov, M.P., A novel model system for design of biomaterials based on recombinant analogs of spider silk proteins, *J. Neuroimm. Pharmacol.: The Official J. Soc. on Neuroimm. Pharmacol.*, 2009, vol. 4, no. 1, pp. 17–27.

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