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Abstract: Silk fibroin (SF) isolated from *Bombyx mori* cocoons is a biocompatible and biodegradable polymer. It is promising for preparation of matrices for tissue engineering and regenerative medicine or for development of drug delivery systems. Regenerated fibroin, which can be obtained from natural silk after removing sericin is water-soluble. Its aqueous solutions can be processed into scaffolds of various forms, for example fibrous matrices using electrospinning method. In the current study, we have studied correlation between concentration of fibroin aqueous solutions and their properties, in order to obtain electrospun mats for tissue engineering. A slowdown in a viscosity enhancement of fibroin solutions at concentrations above 30% (w/v) and a decrease in solution transparency were found. This could be explained by conformational rearrangements, namely fibroin random coils occurring in dilute solutions transformed into an anisotropic helix conformation that resulted in formation of β-pleated sheets. Fibrous mats were obtained from 10 and 20% (w/v) solutions of regenerated fibroin. Two methods were used to prevent solubility of fibroin-based matrices: conversion fibroin to the β-conformation by treatment with an ethanol solution and chemical crosslinking with genipin (Gp). The structures of the fibrous mats were studied by AFM and confocal laser scanning microscopy. The composition of the molding solution for preparation of fibrous matrices from regenerated fibroin by electrospinning as well as conditions for their modification were optimized. The composition of fibroin with chitosan were shown to contribute to a decrease in the solubility of electrospun fibrous matrices due to increase of a number of amino groups available for modification with genipin, and resulted in a decrease in the fiber diameter. The electrospun fiber matrices based on regenerated fibroin modified by crosslinking with genipin in water-alcohol solutions were shown to promote cell adhesion, spreading and growth and therefore could be promising for tissue engineering.

Keywords: silk fibroin; electrospinning; fibrous matrices; tissue engineering; chitosan; crosslinking; genipin

1. Introduction

Biodegradable materials based on biopolymers are widely used in the creation of products for medical and biological purposes: absorbable sutures, implants for plastic surgery, matrices for cell and tissue engineering, and regenerative medicine [1–5]. The creation of materials for innovative medical technologies requires the use of biopolymers and adequate technologies for their processing, which ensure their effective functioning in the human body.

Electrospinning has the greatest promise as such a technology, since it allows fabrication of a nanofibrous matrix with desired fiber diameter and porosity which should be identical to native ECM fibers and allow high adhesion, rates for cell infiltration and mass transport [6]. Biocompatible fiber-forming polymers are used as spinning solutions - most often proteins and polysaccharides. Synthetic

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biodegradable polyesters are sometimes added to improve fiber formation [7], but this is best avoided. Fibrous materials derived from polysaccharides and proteins such as chitosan and fibroin mimic the body's natural environment and thus provide optimal conditions for tissue growth and regeneration [8–12]. The possibility of using chitosan substrates in cellular technologies as a matrix for tissue engineering was studied in a number of works, which is a consequence of its biological and physicochemical properties, as well as the presence of amino groups, which determine the possibility of its simple chemical modifications [13–16]. SF is interesting in that it's not immunogenic, has good biocompatibility, it's nontoxic, and it's capable of biodegradation without causing side effects [1,3]. Important advantages of SF include the ability to process through aqueous solutions, and transfer to a water-insoluble form without the use of chemical cross-linking reagents, as described in articles [17,18]. As the properties of silk fibroin are being studied, it is increasingly being used in various fields of biomedicine, mainly due to its mechanical strength, elasticity, biocompatibility and controlled biodegradability [19]. These properties are especially important for tissue engineering. Quite a lot of works have been published on the processing of SF by electrospinning from solutions in various solvents, including from aqueous and mixed solutions [12,20–22]. For this purpose, regenerated SF from the cocoons of the silkworm Bombyx mori is used.

Materials from regenerated SF are soluble in water and require additional hydrophobization. A well-known method for chitosan to prevent solubility, improve water resistance and mechanical properties - chemical cross-linking with non-toxic cross-linking reagents, such as genipin - may not be effective enough for SF due to the low content of primary amino groups in this protein [23], however, the addition of chitosan can contribute to the effectiveness modifications with bifunctional reagents. In addition, given that several possible conformational states are known for SF: water-soluble α-helices or statistical coil conformations and insoluble β-folded structures, creating conditions for the $\alpha \rightarrow \beta$ conformational transition in the process of modifying fibrous materials from regenerated SF will allow one to influence on the solubility of the polymer material.

Thus, in order to obtain SF-containing biopolymer matrices from aqueous solutions, in addition to the molding process itself, it is necessary to study the conditions of SF modification leading to the loss of its solubility in water. The aim of this study was to develop an optimal method for obtaining water-insoluble fibrous matrices intended for use in regenerative medicine and tissue engineering, combining chemical cross-linking with a naturally occurring agent Gp and conformational transition in SF, as well as to evaluate in vitro the biocompatibility of fibrous matrices during cell cultivation.

2. Materials and Methods

2.1. Chemicals

Chitosan (Mw 190 kDa, degree of deacetylation 87%) was purchased from «Roeper» (Germany). Raw silk in the form of cocoons and threads was purchased in China. Genipin was purchased from Sigma (St. Louis, MO, USA).

Preparation of regenerated fibroin. To obtain fibroin solutions, raw silk was treated in 0.02M sodium carbonate for 1 hour to purify the fibers from contamination, remove sericin and residual fats. Next, the silk was washed three times in distilled water, then kept in distilled water at a temperature of 90-100°C for up to 2 hours, and left to dry at room temperature for a day. The purified silk was dissolved in 9M lithium bromide at a temperature of 70°C for 1 hour. The resulting solution was centrifuged and filtered to get rid of the insoluble conglomerates, then dialized against water for three days with a change of medium every 3-4 hours to remove salts, freeze-dried and a water-soluble fibroin powder was obtained.

2.2. Preparation of fibroin solutions and mixed solutions of fibroin and chitosan

SF solutions were prepared in water using exact weights of dry polymer (±0.0002 g). The dissolution was carried out on a magnetic stirrer in flasks of the required volume.

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To prepare mixtures containing SF and chitosan with a ratio of 5:1 an accurate weight of chitosan was dispersed in distilled water, an accurate weight of fibroin was added, stirring was continued in water for 30 minutes, then the calculated amount of glacial acetic acid was added.

2.3. Dynamic viscosity of fibroin solutions

Dynamic viscosity was determined using vibroviscometer SV-10 (AND, Japan), this device after calibration shows values with an accuracy of 0.1%.

2.4. Measurement of turbidity

Turbidity is assessed by measuring the absorbance of aqueous solutions of SF. Absorbance was measured at λ = 400 nm using a Spectronic Genesys 10UV (Spectronic Instruments, Inc. Rochester, N.Y., USA).

2.5. Electrical conductivity of fibroin solutions

The determination of electrical conductivity was carried out on an "Expert-002" conductometer manufactured by Ekoniks (Russia) using a submerged electrode. 5 ml of the test solution was taken and poured into the measuring cell, the electrode was immersed, and the electrical conductivity of the solution was measured 3-4 times until converging results were determined.

2.6. Thermogravimetric analysis

The materials were analyzed by the dynamic method (TDM) (when the furnace temperature changed over time at a constant heating rate) on a TA Instruments TDM Q50 analyzer in the temperature range from 0 to 600°C at a heating rate of 10°C/min in a nitrogen atmosphere.

2.7. Study of gel formation in fibroin solutions when cross-linked with genipin

Gelation in the systems chitosan/SF solution - Gp was studied at different molar ratios of the crosslinking agent per chitosan amino group. The point of gelation in the system was taken as the time at which the mixture of chitosan with fibroin stopped flowing under its own weight. The results obtained were presented as the dependence of the gelation time on the ratio of the crosslinking agent Gp/NH_2.

2.8. Obtaining fibrous matrices by electrospinning

The fibrous material was obtained by electrospinning method utilizing the free surface of a polymer solution-coated electrode in a strong electrostatic field on a Nanospider NS-Lab (Elmarco, Czech Republic). Electrospinning was carried out from aqueous solutions of fibroin from the electrode surface onto substrates in the form of non-woven material.

2.9. Studying of fibroin matrix morphology by confocal laser scanning microscopy

The structures of the fibrous samples were analyzed by confocal laser scanning microscopy (CLSM) using Nikon TE-2000 inverted microscope equipped with an EZ-C1 confocal laser (Nikon, Tokyo, Japan). The fibrous samples were treated with 96% ethanol for 10 minutes and stained with fluorescamine. A solution of fluorescamine (0.3 mg/ml in acetone) was added to the samples and incubated for 10 min at room temperature and then washed with saline. The excitation wavelength was 408 nm and fluorescence signals were collected at 515±30 nm.

2.10. Studying the structure of fibrous matrices by AFM.

The images of the fiber surface and their diameters were obtained using an atomic force microscope (AFM) based on the NtegraPrima micro-console system (NT-MDT, Russia) in the cantilever semi-contact mode. The obtained data were subjected to processing and comparative analysis in the Nova SPM control program based on the INTEGRA platform and Solver.

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2.11. Cell culture

In the current study ASC52telo, mesenchymal stem cells immortalized with human telomerase (hTERT) (hTERT-MSC) were used. The cells were kindly provided by Prof. Efimenko from Moscow State University. The cells were cultured in αMEM in a 5% CO_2 humidified atmosphere at 37°C (CO_2 incubator Heraeus B5060 EK/CO2, Hanau, Germany). We used media supplemented with 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 50 µM 2-mercaptoethanol, 100 µg/mL streptomycin and 100 U/mL penicillin.

2.12. Morphology of cells after 3 days of cultivation in fibrous matrices

The fibrous samples were sterilized by incubation in 96% ethanol for 1 hour. After that, the samples were washed 3 times with saline and incubated in 1 ml of DMEM + 10% FBS for 1 hour. Cell suspension (20 µl, 10^6 cells/ml) in culture medium containing 10% serum was added to the fibrous samples. In 1 hour, another 100 µl of medium was added to each well. Next, the plate was placed in a CO_2 incubator and cultivated under standard conditions for 3 days. After 3 days of cell culture on the matrices, the samples were incubated in 100 µl of culture medium (without serum) containing Calcein AM (1 µg/ml) and DAPI (10 µg/ml) for 30 minutes at 37°C. Further, the samples were studied using confocal laser scanning microscope (Nikon TE-2000, Tokyo, Japan) with excitation and emission at 408/515±30 and 488/590±50 nm for DAPI and Calcein AM, respectively.

3. Results

3.1. Effect of SF Solution concentration on fibrous matrices from regenerated fibroin electrospinning

It is advisable to use non-toxic solvents to obtain SF fibrous matrices for tissue engineering by electrospinning [24,25]. When using aqueous solutions, the most important factor determining the possibility of molding is the concentration of the solution [25,26]. Regenerated SF obtained using concentrated LiBr solutions according to the procedure described in 2.1 takes the form of random tangles and α-helices in aqueous solutions [23,27,28], which interact weakly with each other. The formation of the mesh required for fiber formation is possible only at high concentrations of SF. Figure 1 shows the concentration dependences of the dynamic viscosity of an aqueous solution of fibroin.

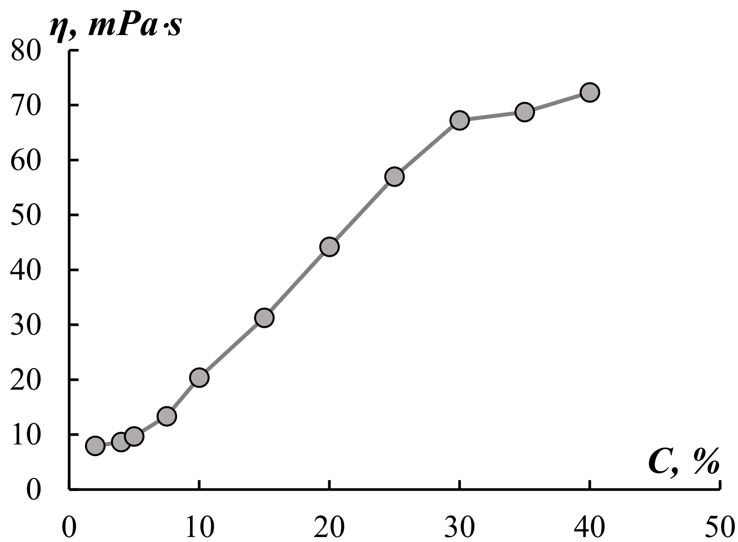

Figure 1. Dependence of dynamic viscosity on SF solution concentration.

The curve η=f(C) has several sections with different concentration dependency. An intensive increase in viscosity corresponding to the formation of the fluctuation grid is observed in the

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concentration region above 5%, which is typical for proteins. This type of dependence cannot be simply due to an increase in the number of ionizing groups, since the electrical conductivity of the solution changes monotonously, and the pH does not exceed 0.1 units with a change in concentration by 10% (Table 1).

Table 1. Properties of fibroin solutions.

Fibroin solution concentration,%	pH	Conductivity ϰ, mS/cm	Dynamic viscosity η, mPa·s
10	6,8	3,6	20,4
20	6,9	6,4	44,2
30	6,93	9,8	67,2

A sharp slowdown in viscosity growth after 30% concentration (Figure 1) may be associated with the beginning of conformational rearrangements: the transition of fibroin from the globular conformation characteristic of dilute solutions to the anisotropic conformation of the α-helix and the appearance of β-folded structures. This assumption is confirmed by the results of nephelometric studies (Figure 2) – a sharp increase in the optical density of the solution in this concentration region.

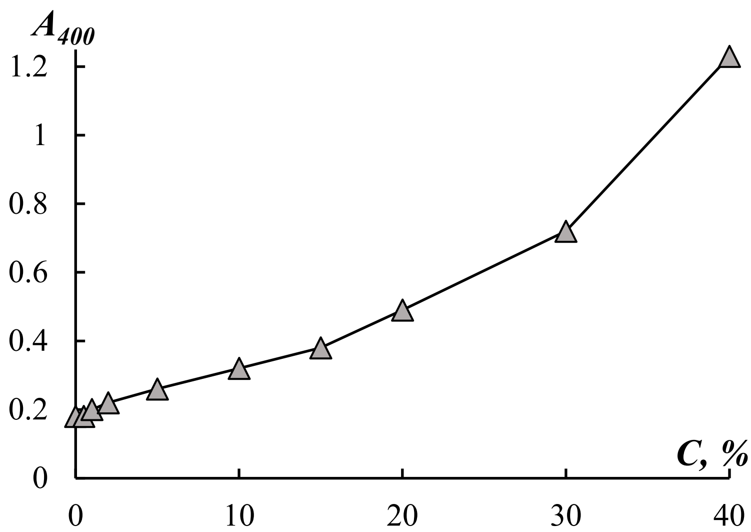
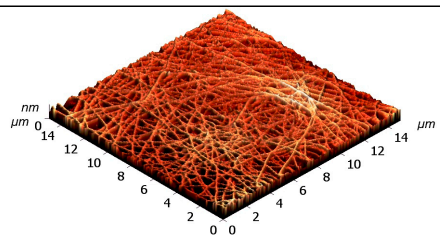


Figure 2. Dependence of absorbance on concentration of SF solution, λ 400 nm.

Electrospinning was performed by method utilizing the free surface of a polymer solution-coated electrode. Table 2 shows AFM-images of electrospun fibers from SF solutions with different concentrations or viscosities, ranging from about 10% (w/w) to about 30% (w/w).

Table 2. Effect of aqueous fibroin solution concentration on electrospinning process parameters.

Fibroin solution concentration ,% w/w	Electrospinning voltage E, kV	Characteristics of the electrospinning process	AFM-image	Fiber diameter , µm
10	22,2-25,8	Stable		0,61±0,22

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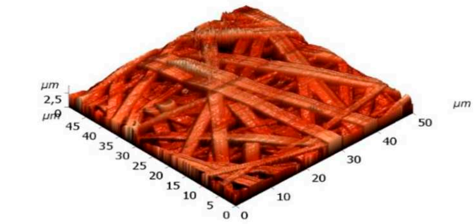
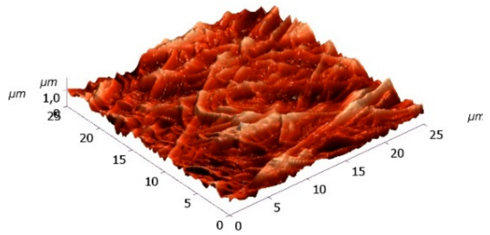
20	23,0-26,4	Stable		2,80±0,20
30	24,1-28,0	Unstably		2,20±0,80

From solutions with a concentration below 10%, fiber formation did not occur even at an electrospinning voltage of 30 kV, only splashing of the solution was observed. AFM-images showed that SF fibers are arranged randomly, intersect with each other, forming numerous pores, the size of which increases with increasing fiber thickness. As shown in Table 2, at concentration of 10%, an electrospun fibrous material consisting of fibers with an average diameter of 0.61 μm is formed. An increase in the concentration of the solution leads to a sharp increase in the diameter of the fibers. Non-woven mats at the voltage of 23.0-26.4 kV with a diameter of 2.80±0.2 μm were obtained from a solution with a concentration of 20%. The fibers had a belt-like morphology instead of the usual wire morphology. This is usually due to incomplete evaporation of a solvent with low vapor pressure from fibers of large thickness. At a SF solution concentration of 30%, the morphology of the resulting material loses its pronounced fibrous structure (Table 2): the fibers probably stick together due to incomplete evaporation of water from a thicker jet of a viscous SF solution. Fibrous matrices obtained from a solution with a concentration of 10% were not strong enough and were destroyed during subsequent manipulations associated with the modification of SF in order to reduce solubility. The solution with a concentration of 20% (w/w) was chosen to fabricate non-woven mats, their subsequent modification and to evaluation in vitro biocompatibility of fibrous matrices during cell cultivation.

3.2. Hydrophobization of electrospun with SF fibers with ethanol solution

The resulting electrospun SF fibers are water soluble and require additional hydrophobization. The conversion of fibroin to insoluble form can be accomplished by a post-treatment to change the random coil conformation to β-sheet which is more stable and insoluble in water. Such post-treatment by treating the formed fibers of silk fibroin with methanol or ethanol [29,30]. Methanol is a toxic solvent, therefore, a method of forming water-resistant materials from fibroin with ethanol is used.

The change in the structure of the protein during the conformational transition occurs as a result of the redistribution of hydrogen bonds in favor of intermolecular bonds, which maintain a β-stacked conformation and give the material resistance to water. The conformational stability of silk fibroin in an aqueous-alcoholic solution is determined by interactions of amino acid residues with each other and with a solvent. Aggregation of hydrophobic side chains is disrupted by breaking the hydrogen bond network of water with alcohol and binding the alcohol to the hydrophobic group [27,29]. The formation of a β-stacked conformation is a process of rearrangement of hydrogen bonds, with their formation between peptide units of different macromolecules.

Based on preliminary experiments to study the solubility of electrospun fibers from SF and the results described in [31], a 80% aqueous ethanol solution was selected for the treatment of the fibers, in which the material was held for 2 hours. Structural changes in fibroin induced by ethanol treatment confirmed by FTIR spectroscopy (Figure 3).

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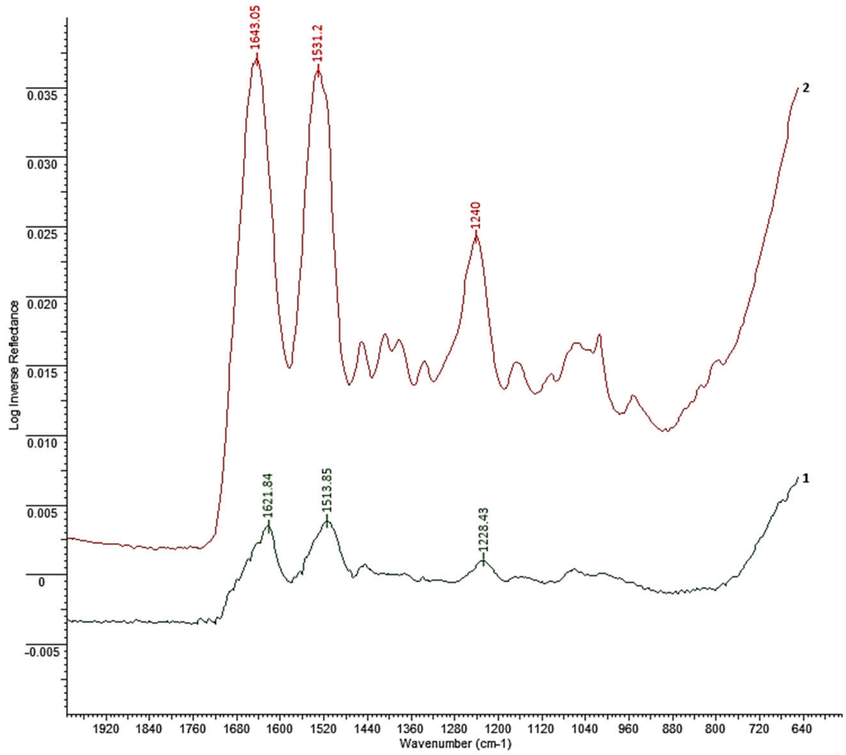

Figure 3. FTIR spectrum correction for internal reflectance, log 1/R, of 80% alcohol treated electrospun fibrous matrices from regenerated SF (**1**) and the untreated electrospun fibrous matrices (**2**).

As stated previously, fibroin can exist as random coil/globules (low solute concentration) with α-structure (silk I), and β-form (silk II, ordered as antiparallel β-folded sheets, formed by physical shear or exposure to solvents such as ethanol). Figure 3 shows the FTIR spectra of electrospun fibrous fibroin mats: initial and treated with 80% ethanol solution, which confirm the effect of ethanol on the conformational transformation of regenerated silk fibroin. Fibroin fibrous materials initially have a mainly amorphous structure with the characteristic structure of silk I. The untreated sample (curve 2) shows strong bands at 1531.2 cm^{-1} (amide II), and 1643.05 cm^{-1} (amide I), characteristic of the α-helix and random coil conformation [32]. The material treated with 80% ethanol (curve 1) shows bands shifting and appearance at 1513.85 and 1621.84 cm^{-1} characteristic of the β-sheet conformation [33]. These changes indicate the transformation of random helices into an ordered β-sheet upon treatment with aqueous ethanol solution. Actually, water might act as swelling agent towards the compact and dense silk fibroin [34], promoting ethanol penetration and rearrangements of inter- and intramolecular hydrogen bonds. The data obtained are consistent with those described in the literature earlier [30–35].

In this work, the untreated and 80% alcohol treated electrospun fibrous matrices were analyzed by the method of thermogravimetric analysis (TGA). The method of thermogravimetric analysis is based on continuous recording of the change in the mass of the sample depending on the temperature under conditions of its programmed change.

The untreated and 80% alcohol treated electrospun fibrous matrices showed a trend of weight loss with increase in temperature in the TGA plots (Figure 4). The degradation of the untreated untreated and alcohol treated matrices when heated to 150°C was attributed to the loss of bound water. In this region, the fibrous matrix sample lost mass more slowly than the untreated sample. It is manifested that the water molecules are much stronger bonded with the β-sheet (silk II) structure of fibroin. The main weight loss both for the untreated and treated samples was observed from 150°C to 450°C and was attributed to the degradation of silk structure.

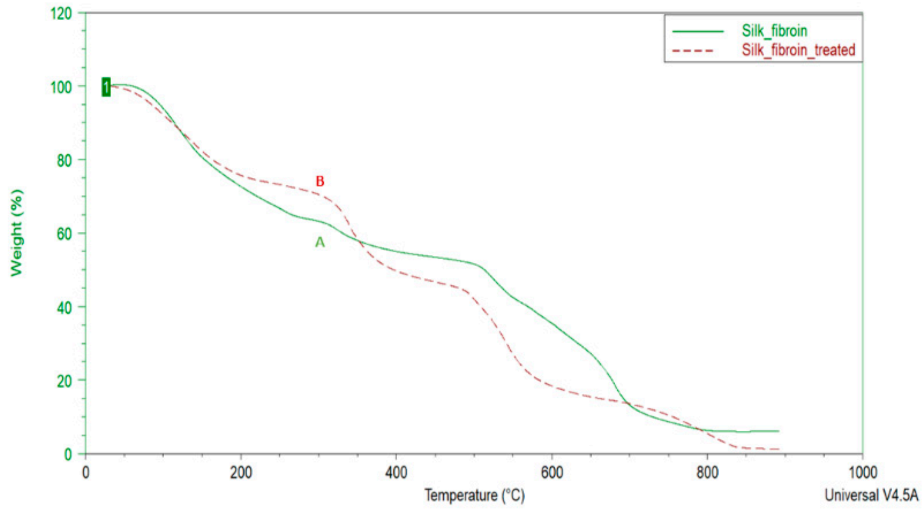

Figure 4. TGA plots of the untreated (A) and 80% alcohol treated(B) electrospun fibrous matrices.

The observed reduction in the rate of mass loss resulting from treatment with 80% ethanol solution along with FTIR-spectroscopy results indicate that that treatment with aqueous-ethanol solution electrospun fibrous matrices causes the conditional transition of fibroin into β-folded conformation and results in hydrophobization of the material.

3.3. Use of genipine crosslinking reagent to obtaining water-insoluble fibrous matrices from regenerated fibroin

Another way to prevent solubility, improve water resistance and mechanical properties is chemical crosslinking with non-toxic crosslinking reagents. It is known that chemical crosslinking of chitosan with a natural-origin crosslinking reagent, genipin, causes gelation in its solutions, and during post-treatment leads to the production of water-insoluble fibrous, film materials and porous cryogels based on [15,16,37]. There are different ideas about the mechanism of reaction of amino-containing biopolymers with Gp [37,39–41]. Summarizing the literature and considering that the interaction of SF with Gp not only leads to an increase in viscosity, but also the appearance of absorption in various regions of the spectrum, including the visible region (Figure 5) and the staining of the material in blue, scheme of the reaction of crosslinking fibroin with genipin can be represented by Figure 6.

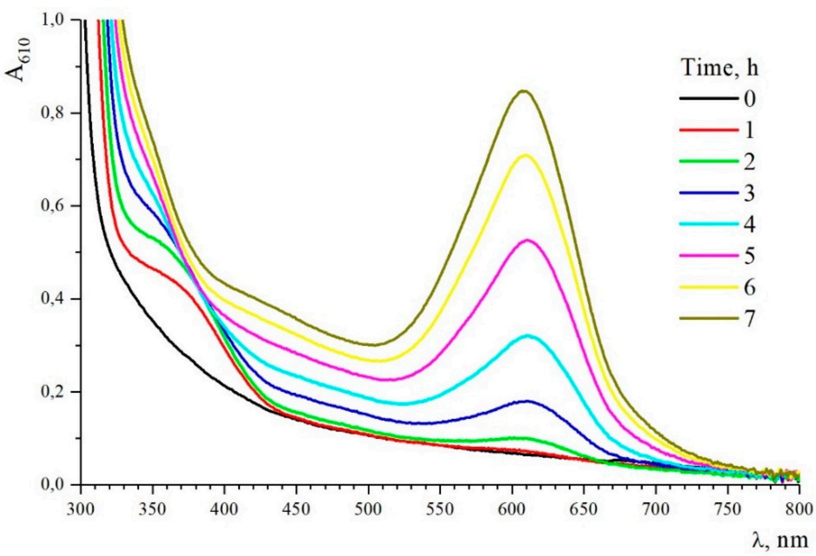

Figure 5. UV spectra of fibroin solutions during reaction with genipin. Fibroin concentration 25 mg/mL, Gp 7.8% of fibroin weight.

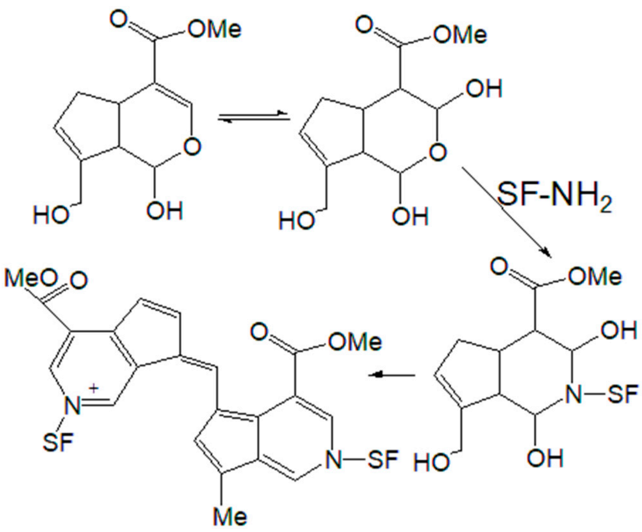

Figure 6. Scheme of the reaction of crosslinking fibroin with genipin.

The gelation process in equiconcentrated fibroin and chitosan solutions was investigated. Gelling time dependence in 2% SF solutions at pH 7.3 and chitosan at pH 5.6. in the process of crosslinking with Gp, the content of crosslinking agent is shown in Figure 7.

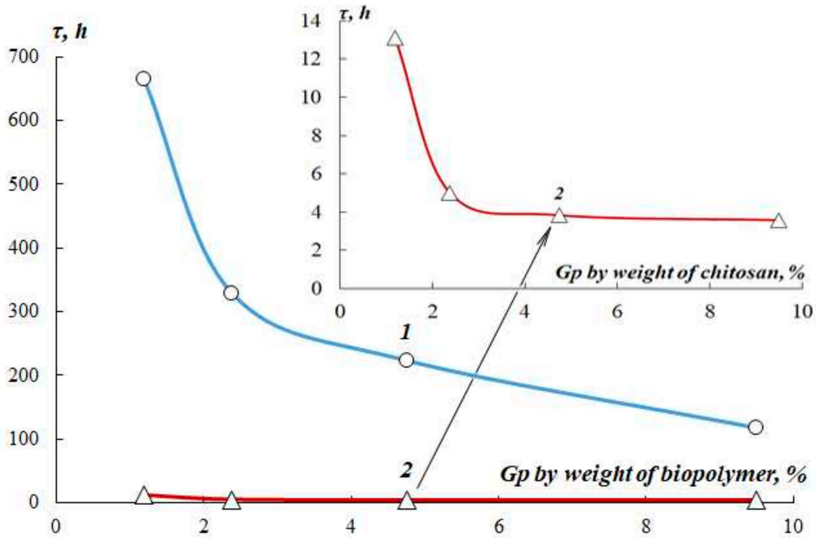

Figure 7. Dependence of gelling time (τ) in 2% solution of fibroin pH 7.3 (**1**) and chitosan pH 5.6 (**2**) on genipin content.

As can be seen from the data obtained, the gelation time in a 2% SF solution, even with a high content of Gp and a pH of 7.2, is 20-50 times higher than in an equiconcentrated chitosan solution and is several days. This is due to the low content of primary amino groups in the fibroin molecule. Protein crosslinking using Gp occurs primarily with the participation of amino groups of basic amino acids and, above all, lysine, whose content in regenerated fibroin is only 0.3-0.5% mol [23,42]. The terminal amino groups of the protein are not always available for modification, therefore, it is natural that under conditions leading to the gelation of chitosan, the addition of Gp to an equi-concentrated solution of SF does not lead to gelation, although it causes the appearance of a blue color.

On Figure 8 kinetic curves of change in viscosity of SF solutions in the process of crosslinking with Gp at the same content of crosslinking agent 10.9% of fibroin weight in a wide range of SF concentrations obtained on a vibration viscometer are given. Within 24 hours, a marked change in viscosity was observed only starting with the 10% concentration. During measurements (42 hours) in the presence of Gp, even 40% SF solutions did not lose their flow ability. However, the color change to green or blue (depending on the concentration of the solution) occurred, which indicates a secondary reaction with the formation of a product absorbing in the λ=600 nm region.

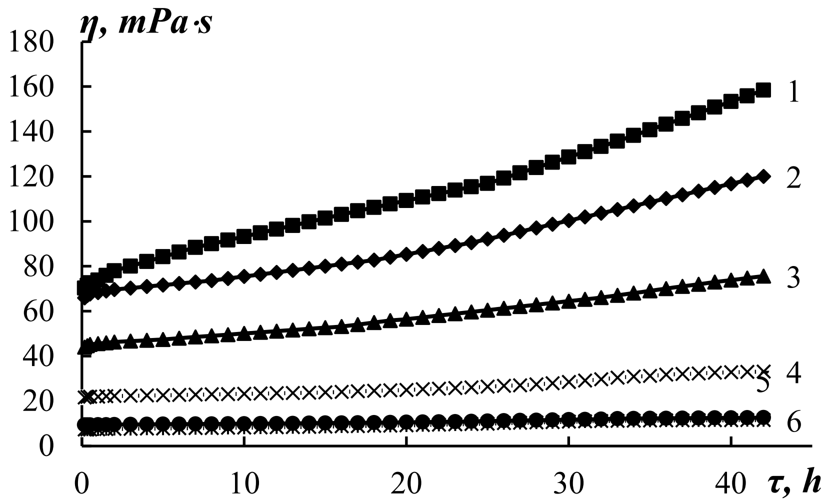

Figure 8. Kinetics of viscosity change in solutions of fibroin with genipin, pH 7.3, fibroin concentration: 1-40 wt%; 2-30 wt%; 3-20 wt%; 4-10 wt%; 5-5 wt%; 6-2 wt%.

To accelerate the intensification of crosslinking leading to the formation of water-insoluble biomedical materials, the present paper proposes the use of chitosan-containing systems based on SF solutions. Dehummed and regenerated fibroin is soluble throughout the pH range. Chitosan is soluble in water only in an acidic environment when its primary amino groups protonate and the macromolecule acquires a positive charge. Mixed solutions of chitosan and fibroin in dilute acetic acid prepared according to the procedure described in 2.2 were used to obtain fibrous matrices by electrospinning.

The concentration of SF in the solution was 10%, such a concentration was chosen based on the fact that the addition of chitosan leads to a sharp increase in viscosity, which prevents droplet formation on the free surface of the solution and the formation of extended fibers on the receiving electrode. For the electrospinning of fibrous mats from a mixture of SF and chitosan, solutions with the physicochemical characteristics given in Table 3 were used. Electrospinning took place at the voltage of 26 kV.

Table 3. Composition and properties of molding compositions based on fibroin solutions.

Concentration,%		Fibroin:Chitosan ratio, g/g	pH	Conductivity ϰ, mS/cm	Dynamic viscosity η, mPa·s
Fibroin	Chitosan				
20	-	1:0	6,9	6,4	44,13
10	2	5:1	4,84	27,3	62,21

Gp is soluble in both water and ethanol, and this fact allows the use of solutions of Gp in 80% aqueous ethanol solution to modify the electrospun fibrous matrices from SF and chitosan in order to prevent their dissolution during cell culture. Conditions for redistribution of hydrogen bonds accompanying the formation of β sheets are realized in 80% aqueous ethanol solution, and the presence of chitosan in the material contributes to intensification of the process of crosslinking with Gp and inclusion of macromolecules of SF in the three-dimensional grid of the crosslinked biopolymer. The fibrous matrices were kept in 0.95% Gp solution at 2 μl/mg for 72 hours, then washed with phosphate buffer solution (pH 7.4).

Morphology of received electrospun fibrous matrices treated with 80% ethanol and crosslinked with Gp showed in Table 4.

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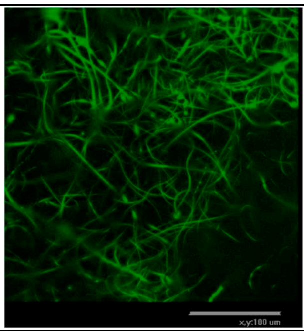
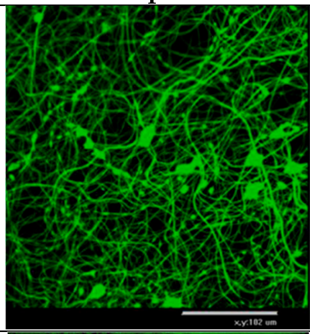
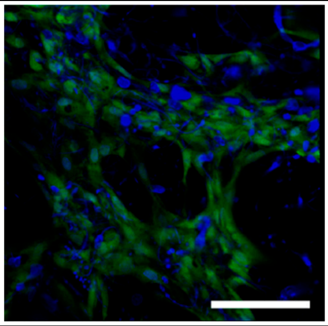
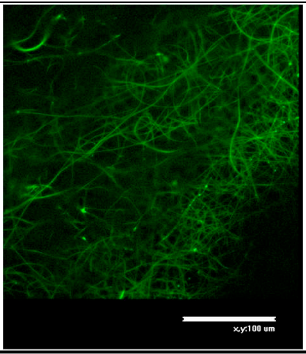
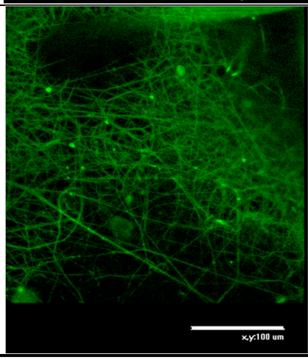
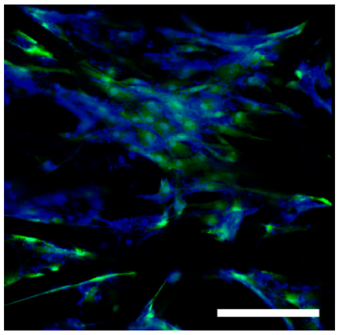
Table 4. Morphology and biocompatibility of electrospun fibrous matrices treated with ethanol and crosslinked with Gp.

Fibrous matrix composition	Source electrospun fibrous matrices morphology	Morphology of electrospun fibrous matrices washed with PBS (pH 7.4)	Material morphology after 3 days of cultivation hTERT-MS
100% fibroin			
Fibroin: Chitosan 5:1			

According to the procedure described in 2.9, of morphology of source electrospun fibrous matrices and after washing with phosphate buffer solution (pH 7.4) was studied by confocal laser microscopy. Morphology of electrospun fibrous matrices based on 100% fibrin after washing with phosphate buffer solution showed the presence of defects, due to partial dissolution of the material, despite the fact that visually the material retained its integrity. That is, hydrophobization of SF fibers when treated with a Gp solution in 80% ethanol solution was insufficient, probably due to the low content of Gp amino groups available for crosslinking in SF. The introduction of chitosan, which in the process of crosslinking with Gp promotes the inclusion of SF in the three-dimensional network of crosslinked biopolymers, improve water resistance and reduce defective material (Table 4).

Polysaccharide and protein-derived fibrous materials mimic the body's natural environment and thus must provide optimal conditions for tissue growth and regeneration. To evaluate the biocompatibility of the fibrous matrices hTERT-MSCs were seeded on fibers and cultured for 3 days. Before use the fibrous samples were treated with 96% ethanol for 10 minutes. Cell morphology and distribution were monitored using transmission light and confocal microscopy. In order to assess the viability of hTERT-MSCs cultivated in the fibrous matrices, cells were stained with vital dye Calcein AM and DAPI after three days of cultivation (see Table 4). The fluorescence micrographs showed that the cells penetrated through the fiber matrix and adhered and spread on the fibers. Moreover, conversion of non-fluorescent Calcein AM to green fluorescent dye Calcein, which occurs in living cells only, indicated that those labelled hTERT-MSCs were viable after 3 days of cell culture within the fibers.

4. Conclusions

Fibrous mats from SF were obtained from water solutions with a concentration of 10 and 20% using the electrospinning method. Materials from regenerated SF are soluble in water and require additional hydrophobization. Two methods were studied to prevent solubility of fibroin-based matrices: conversion of fibroin to the β-conformation by treatment with an ethanol solution and chemical cross-linking with genipin. Using the TGA method, a stronger binding of water to the material was found after its treatment with 80% ethanol solution, which, along with the results of FTIR-spectroscopy, indicate that that treatment with aqueous-ethanol solution electrospun fibrous matrices causes the conformational transition of fibroin into β-folded conformation and leads to hydrophobisation of material. The interaction of Gp with SF leads to the appearance of a characteristic blue color, but does not call for the gelation of solutions. To speed up the crosslinking reaction with Gp, it is proposed to use chitosan-containing systems and modify fibrous materials by treating with a solution of Gp in 80% ethanol. At the same time, on the one hand, conditions are created for redistribution of hydrogen bonds accompanying the formation of β sheets, and the presence of chitosan in the material contributes to intensification of the process of crosslinking with jenipin and the inclusion of macromolecules of fibroin in the three-dimensional network of the crosslinked biopolymer. Thus, the method of producing water-insoluble fibrous matrices for use in regenerative medicine and tissue engineering is optimized, combining chemical crosslinking with a naturally occurring crosslinking reagent genipine and $\alpha \rightarrow \beta$ a conformational transition in fibroin. Electrospun fiber matrices based on regenerated SF, modified by cross-linking with Gp in water-alcohol solutions, promote cell growth and proliferation and may be promising for tissue engineering.

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