

Effect of chitin nanofibrils on electrospinning of chitosan-based composite nanofibers

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ABSTRACT

Electrical conductivity, surface tension and viscosity of chitosan-based composite nanofibers are reported. 20 wt. % of chitin nanofibrils introduced into a chitosan solution leads to increase in viscosity of the mixture; the effect of shear rate becomes more pronounced. This phenomenon is caused by the formation of cluster structures involving filler particles, and by orientation of chitin nanofibrils under the action of shear stresses in electro-magnetic field. Presence of chitin facilitated formation of nanofibers in electric field and led to significant decrease in the amount of defects.

1. Introduction

Bioresorbable materials based on natural (Muzzarelli et al., 2007; Naseri et al., 2014; Yang et al., 2010; Zhou et al., 2008) or synthetic (Fabbri et al., 2016; Schiffman & Schauer, 2008) polymers have been successfully used as matrices in tissue engineering. Biocompatibility and non-toxicity of the initial polymers and of the products of resorption (Shoichet, 2010) are prerequisites for that. Stem or somatic cells can be cultivated on such matrices; proliferation and differentiation of the cells leads to formation of tissues identical to those of a recipient. It has been shown that presence of pores ranging in their sizes from tens or hundreds of micrometers in a matrix is required for that (Dobrovolskaya, Kasatkin, Yudin, & Ivan'kova, 2015; Rahmanian-Schwarz et al., 2014). Porous matrices can be prepared by irradiation of polymer films with high-energy particles, by phase separation of polymer solutions, or by introduction of certain additives into the polymer (Mulder, 1996). Several authors (Dobrovolskaya, Popryadukhin et al., 2015; Dobrovolskaya et al., 2016; Li, Laurencin, Catterson, Tuan, & Ko, 2000) observed good adhesion and high rate of proliferation of mesenchymal stem cells and fibroblasts on the surface of nanofiber-based materials.

Electrospinning is the most efficient way for preparation of films with pore sizes varying from tens to hundreds of micrometers (Baumgartner, 1971; Filatov, 1997). Solution of a polymer is fed through a metallic spinneret, and a jet is formed just behind it due to

the action of high voltage electric field, where the jet drawing direction coincides with the electric field gradient. The direction of the jet changes on a certain distance from the outlet, and it becomes dispersed over a conical surface. Macromolecules or their associates that have the same surface charges are repulsed, and this leads to splitting of the jet into thin microjets. Solvent evaporates as the solution moves from the feeding electrode; as a result, the microjets form a network of nanofibers on the collector electrode. Diameters of these nanofibers range from 50 to 500 nm.

It is known (Naseri et al., 2014) that the rate of spinning, the fiber diameter and the amount of defect structures are affected by the conductivity, viscosity and surface tension of solution. Electrospinning processes also depend heavily on chemical composition of macromolecules, molecular mass, chain rigidity, and the presence of additives in polymer solution (Filatov, 1997). However, the effects of these parameters need to be studied in more details.

The biocompatible and biodegradable polymer chitosan, a derivative of the polysaccharide chitin, is widely used in preparation of materials for biomedical applications (Muzzarelli et al., 2007). Chitosan macromolecules consist of β -(1–4)-D-glucosamine and N-acetyl-D-glucosamine units. The products of the chitosan biodegradation are non-toxic, and they participate in the natural biochemical reactions occurring in a living organism. Electrospinning of chitosan nanofibers is very difficult due to the high electrical conductivity of its solutions in comparison with the solutions of other polymers. This phenomenon is

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explained by the presence of ionogenic NH_3^+ groups in the chitosan molecules. To facilitate electrospinning of the chitosan-based nanofibers, water-soluble polymers, such as poly(ethylene oxide) (PEO), poly(vinyl alcohol), methyl cellulose, polyvinylpyrrolidone, are introduced into the spinning solutions (Bizarria, d'Ávila, & Mei, 2014; Duan, Dong, Yuan, & Kangde Yao, 2004; Schiffman & Schauer, 2008;). It is necessary to add up to 50% of such polymers to produce a defect-free fiber based on chitosan. However, additives of the flexible-chain water-soluble polymers deteriorate the mechanical properties of the resulting materials and increase their hygroscopicity. In the present work, the effect of PEO additives on the properties of chitosan solutions and the process of nanofibers spinning has been studied.

(Homayoni, Abdolkarim, Ravandi, & Valizadeh, 2009) have shown that treatment with alkali, which hydrolyzed chitosan chains and so decreased their molecular weight, can also decrease viscosity of chitosan solutions. Chitosan nanofibers hydrolyzed for 48 h display a moisture regain which is 74% greater than that of either treated or untreated chitosan powders. The diameters of the nanofibers are strongly affected by the electrospinning conditions and by the solvent concentration.

Both chitin and chitosan polymers were found to be hard to electrospun, however, it was possible to produce nanofibers using special solvents; for example, 90% acetic acid was able to reduce the surface tension thus making the electrospinning feasible (Maher, Elsabee, Nagui, & Elsayed, 2012). Mixtures of organic acids were also tested for preparation of homogenous and uniform fibers. A considerable attention was paid to electrospinning of their soluble derivatives such as dibutyl and carboxymethyl chitin. It was revealed, that the obtained nanofibers have much better qualities than the normal chitosan fibers. Several polymer blends of chitin/chitosan with many commercial polymers are capable of producing uniform beads-free fibers by electrospinning (Bizarria et al., 2014; Duan et al., 2004).

The authors of (Yudin et al., 2014) have obtained composite fibers based on chitosan with the chitin nanofibrils by wet spinning and demonstrated enhanced mechanical strength and elasticity of the resulting materials. Introducing chitin nanofibrils into chitosan solution facilitates orientation of the macromolecules in the shear stress field that arises during the flow of suspension through the spinneret. Moreover, introducing chitin into chitosan solution stabilizes the process of fiber formation by providing a laminar flow of the jet during its passage through the spinneret hole. It is assumed that the presence of chitin will also lead to more efficient electrospinning of composite nanofibers with smaller content of water-soluble polymers.

In this work we studied the effect of chitin additives on electrical conductivity of chitosan solutions in acetic acid (AA), on their surface tension and viscosity in shear stress field. Analysis of these relationships and of the structure of composite nanofibers will help to optimize electrospinning process and to decrease contents of water-soluble polymers in the spinning solution.

2. Experimental

2.1. Preparation of composite nanofibers by electrospinning

In preparation of composite nanofibers by electrospinning, chitosan with a molecular mass of about (M_w) 120 kDa, and deacetylation degree of 92% was used ("Bioprogress OOO", Russia). Molecular mass of chitosan samples were determined by a method based on sedimentation and diffusion analysis in their dilute solutions in 0.33 M CH_3COOH + 0.2 M CH_3COONa . Velocity sedimentation was investigated using Beckman XLI analytical ultracentrifuge. The deacetylation degree was determined by conductometric titration method, using OKI02/I Radelkis. The measurement accuracy was 0.1 μS .

Chitin nanofibrils (SRL "Mavi Sud", Italy) were used as a filler. Concentration of chitin in aqueous dispersion was 31.9 mg/mL, electrical conductivity of the dispersion (σ) was equal to 116 $\mu\text{S}/\text{cm}$,

pH = 4.87. The structure of chitin nanofibrils was studied by SEM and X-ray diffraction in articles (Dobrovolskaya, Popryadukhin et al., 2015; Yudin et al., 2014), it is shown that the diameter is 20 ± 4 nm and the length is 700 ± 100 nm. Glacial AA was purchased from "Vekton ZAO" (Russia). PEO with a molecular mass (M_w) of 900 kDa was purchased from «Union Carbide Corp» (USA).

It is known (Yudin et al., 2014) that the optimal concentration of chitosan for wet spinning of fibers is 4 wt.%. In this paper, the effect of chitin and PEO additives on the properties of chitosan solutions with the concentrations of 3–5 wt.% is reported.

Different amounts of chitosan powder were dispersed in water. The mixtures were stirred for 40 min at 200 rpm until chitosan has become partially swollen. Then glacial AA was introduced during continuous stirring at 1000 rpm to obtain a 70 vol.% solution. The mixture was further stirred for 40 min at 500 rpm. Prior to spinning, the obtained solution was exposed for 3 days at 4 °C. Dispersion of chitin nanofibrils was prepared with the use of an UZV-1.3 ultrasonic disperser (Russia) at a frequency of 40 kHz; the power input was 1.3 kW.

Composite nanofibers based on chitosan and chitin nanofibrils were spun from the mixture prepared as follows. Aqueous dispersion of chitin nanofibrils was subjected to ultrasound treatment for 40 min. The dispersions contained nanoparticles in the concentrations ranging from 1.0 to 30 wt.% with respect to chitosan. Chitosan and/or PEO were introduced into the obtained dispersion; then, the solution was prepared according to the technique described above. In order to prepare a mixture of chitosan solution and PEO, the latter was dissolved in water, after which it was added to the aqueous dispersion of chitosan.

Electrospinning of fibers was carried out using a NANON-01A setup (MECC Co., Japan). The solution was fed by an injection pump through a spinneret electrode with a radius of 0.3 mm into the electric field with a voltage of 18 kV. The distance between the spinneret electrode and the collector electrode (where fibers were deposited) was 0.2 m.

2.2. Rheological studies

Rheological studies were done with the use of a Physica MCR-301 concentric cylinder rheometer (Anton Paar, Austria) at 20 °C, at shear rates $\dot{\gamma}$ ranging from 0.01 to 400 s^{-1} . For the measurements, 5 mL of solution was poured into a cuvette, and the dependences of the viscosity on shear rate were obtained subsequently at the shear rate decreasing from 4000 s^{-1} .

2.3. Conductivity and surface tension of chitosan solutions

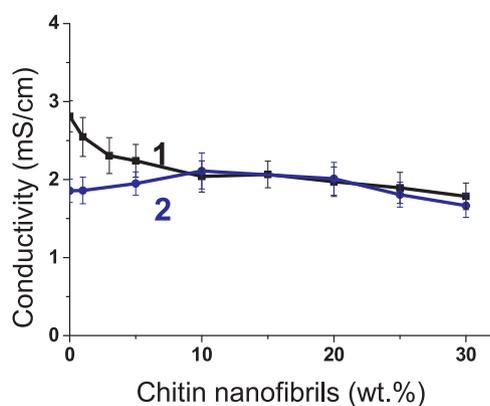
Conductivities of chitosan solutions and their mixtures were measured 10 times for each point using a Seven Multi electrometer (pH-meter) (Mettler Toledo Co., Switzerland). The standard deviation was less than 10%.

Surface tension was measured by the pendant drop technique with a Drop Shape Analysis (DSA 4) tensiometer (KRÜSS). Densities of solutions were determined by the pycnometry method. Surface tension were measured 10 times for each point. The standard deviation was less than 12%.

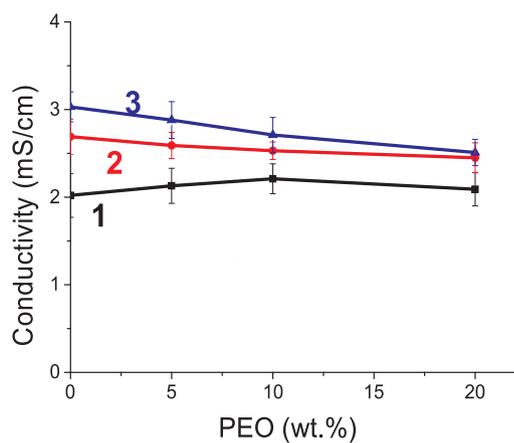
2.4. Structural studies

Films consisting of unidirectional nanofibers and containing 30 wt. % of chitin nanofibrils were prepared. In our earlier work (Popryadukhin et al., 2015) it was shown that nanofibers became oriented along the direction perpendicular to the axis of the cylindrical collector electrode during its rotation at a rate exceeding 1200 rpm. In this work the film was obtained at 3000 rpm. After removing from the metallic substrate it was compacted and additionally drawn.

Scanning electron microscopy (SEM) studies were performed using a Supra-55 VP instrument (Carl Zeiss, Germany). The samples were covered by a thin Pt layer.



a



b

Fig. 1. a – Dependences of conductivity of 4 wt.% chitosan solution (curve 1) and of the solution containing 10 wt.% of PEO (curve 2) on the concentration of chitin nanofibrils; b – conductivities of 3, 4, and 5 wt.% chitosan solutions with different PEO contents (curves 1, 2, 3, respectively).

Wide-angle X-ray diffraction (WAXD, Bruker D8 DISCOVER instrument) was used for studying the fine structure of the composite fibers. To reveal orientation of the chitin nanofibrils with respect to the axis of the composite nanofibers we measured intensities of the 013 peak for different directions from the equator to the pole of a diffractogram with a step of 10° .

3. Results and discussion

3.1. Conductivity of chitosan solutions and their mixtures with chitin nanofibrils and PEO

Preparation of chitosan nanofibrils is virtually impossible without addition of other polymers in amounts from 10 to 50 wt.% (Rošic et al., 2012; Schiffman & Schauer, 2008). At the same time, electrospinning of composite nanofibers based on chitosan and PEO is stable; the resulting films do not usually contain defects in the form of drops of various shapes and sizes. In this work the effect of PEO on the conductivity of chitosan solutions was studied.

It is seen in Fig. 1a that chitin nanofibrils introduced into a 4 wt.% chitosan solution in the amount of up to 30 wt.% (that corresponds to their concentration of 1.5–2.0 vol.%) lead to a slight decrease in the electrical conductivity of the mixture. A mixture of 4 wt.% chitosan solution with 10 wt.% PEO is less conductive than a pure chitosan solution. Additions of chitin nanofibrils affect weakly the electrical

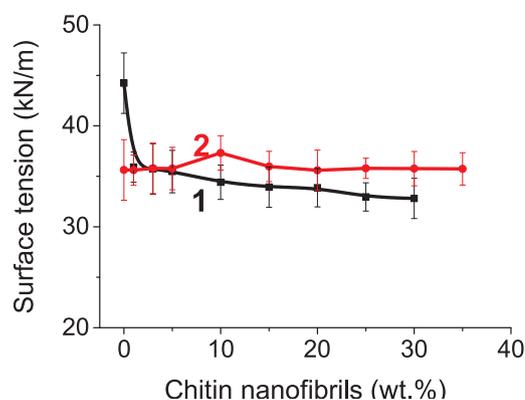


Fig. 2. Effect of chitin nanofibrils on surface tension of the 4 wt.% of chitosan solution with and without 10 wt.% of PEO (curves 2 and 1, respectively).

conductivity of the three-component mixture.

Fig. 1b shows an increase in conductivity of solutions from 2 mS/cm to 3 mS/cm with increasing chitosan concentration from 3 to 5 wt.%. Addition of PEO does not change considerably the initial solution conductivity. The values are by 3–4 orders of magnitude higher than those of PEO solutions and of some other polymers used in electrospinning. Electrospinning of fibers from those polymers is highly stable; the films and tubular samples do not normally contain any defects (Dobrovolskaya, Popryadukhin et al., 2015; Popryadukhin et al., 2015).

The small effect of PEO on the electrical conductivity of chitosan solutions is probably due to the small amount of the additive: 10 wt.% PEO concentration with respect to chitosan corresponds to 1 vol.% of the polymer in the solution. Another possible reason is the absence of negatively charged fragments in PEO molecules which could be able to bind to chitosan ionogenic groups ($-\text{NH}_3^+$).

3.2. Surface tension of the chitosan solutions containing PEO and chitin nanofibrils

Surface tension of the 4 wt.% chitosan solution (Fig. 2) decreases rapidly with introduction of a relatively low (about 1 wt.%) amount of chitin nanofibrils (curve 1). The molecular dynamics calculations (Yudin et al., 2014) demonstrated that a parallel orientation of chitosan molecules and chitin nanofibrils was the most energetically favorable configuration. Accordingly, the chitosan solutions gain a higher degree of ordering under the action of anisometric particles that leads to a decrease in free surface energy. Further increase in the concentration of chitin nanofibrils produced only a small effect on the surface tension of solutions (Fig. 3).

Introducing 10 wt.% of PEO into a chitosan solution decreases its

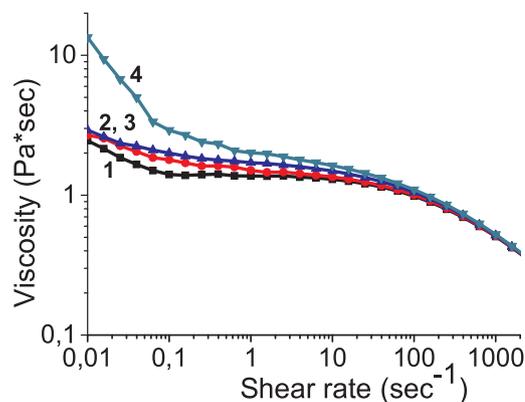


Fig. 3. Viscosity as a function of shear rate for 4 wt.% chitosan solution (curve 1) and for solutions containing 5, 10, and 20 wt.% of chitin nanofibrils (curves 2, 3, and 4, respectively).

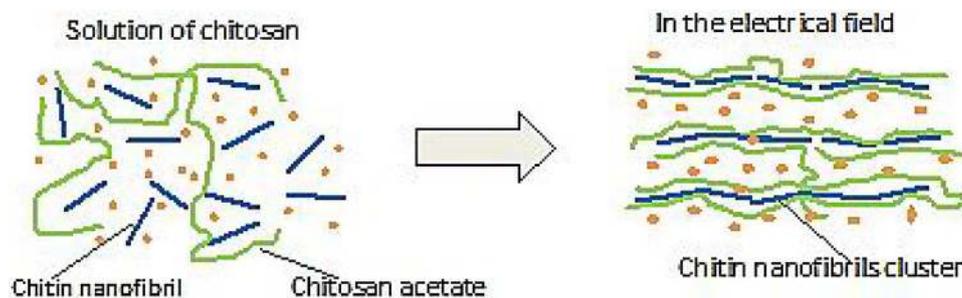


Fig. 4. Schematic representation of the formation of a cluster structure from chitin nanofibrils in chitosan solution in shear field.

surface tension (curve 2). Like all aliphatic polymers, PEO demonstrates high chain flexibility and has a globular structure. It can be assumed that PEO macromolecules are localized between the chitosan molecules whose chains are considerably more rigid. This weakens the interaction between chitosan molecules, and the surface tension of the mixed solution decreases. Addition of chitin nanofibrils in the mixed chitosan/PEO solution does not virtually change its surface tension.

3.3. Rheological properties of chitosan solutions and their mixtures with chitin nanofibrils

The results of earlier studies (Dobrovolskaya, Popryadukhin et al., 2015; Filatov, 1997) indicate that viscosity of a polymer solution affects considerably the electrospinning process, the structure and properties of the resulting nanofibers. It has been demonstrated (Yudin et al., 2014) that solutions of chitosan and their mixtures with chitin nanofibrils are non-Newtonian liquids, and their viscosity depends on shear rate. Chitosan concentrations above 4 wt.% increase the solution viscosity significantly, especially when nanoparticles are additionally introduced, even in small amounts. This makes pressing the mixture through a die impossible. In Fig. 4 viscosities are plotted against the shear rate for the 4 wt.% chitosan solution and for the solutions containing 5, 10, and 20 wt.% of chitin nanofibrils.

Viscosity of chitosan solutions and of their mixtures with chitin nanofibrils decreases with increasing shear rate, but depends weakly on the concentration of nanofibrils until it reaches a certain value. At the filler concentration of 20 wt.% viscosity starts rising and does it most strongly at low shear rates (curve 4).

The decrease in solution viscosity at increasing shear rate is obviously related to a transformation of the solution structure and to appearance of mutually oriented chitosan macromolecules and chitin nanofibrils. In our previous work (Yudin et al., 2014) the chitin nanofibrils were oriented along the axes of the composite fibers prepared by wet spinning. The trends in viscosity observed in this work indicate an effective interaction between the chitin nanofibrils and chitosan macromolecules.

The enhanced viscosity of the chitosan solutions containing chitin nanofibrils may be explained by the formation of cluster structures involving the filler particles. It is known that the properties of composite polymer materials depend nonlinearly on the concentration of anisometric filler particles (Gordeyev, Macedo, Ferreira, van Hattum, & Bernardo, 2000). At a certain concentration of the filler (the percolation barrier), a cluster structure appears due to the contacts formed between the particles, and the properties of the composite material change sharply. Supposedly, the filler particles form a cluster structure in the chitosan solutions studied in this work. Simultaneously, the chitin nanofibrils become oriented along the fiber axis.

It is possible to estimate the percolation threshold R_p (vol.%) theoretically (Garboczi, Snyder, Douglas, & Thorpe, 1995) using the equation for a cylinder: $R_p = 0.6/r \cdot 100\%$, where $r = L/d$ is the aspect ratio, L is the length, and d is the diameter of the cylinder. It was shown by SEM and X-ray diffraction that for chitin nanofibrils, the L value is

about 800 nm, and the d value is ~ 20 nm (Dobrovolskaya, Kasatkin et al., 2015). This results in $r \sim 40$, and $R_p \sim 1.5$ vol.%.

The content of chitin nanofibrils of 10–25% relative to the mass of chitosan corresponds to their concentration of 0.8–1.5 vol.% in solution. The values obtained agree reasonably well with the concentration of chitin nanofibrils at which the viscosity of the mixed solution begins to rise. This suggests that the increased viscosity of the chitosan solution containing 20 wt.% of chitin nanofibrils is caused by the formation of a percolation cluster in the studied solution. The nanofibrils are able to orient in shear field that leads to a decrease in the viscosity of the whole system. The cluster formation is illustrated schematically in Fig. 4.

3.4. Rheological properties of chitosan solutions and their mixtures with chitin nanofibrils and PEO

Fig. 5 shows viscosity vs. shear rate dependences obtained for the 4 wt.% chitosan solution with and without 10 wt.% PEO, and for the ternary mixtures containing 5, 10 and 25 wt.% of chitin nanofibrils. Addition of 10 wt.% PEO to the pure chitosan solution results in an increased viscosity. Introduction of chitin nanofibrils further increases the viscosity of the mixture.

Above it was shown that an addition of less than 20 wt.% PEO did not change considerably the conductivity of chitosan solutions and their surface tension. It should be noted that the measurements were carried out in the absence of shear or electromagnetic field. At the same time, it is known that presence of PEO additives facilitates spinning of chitosan-based nanofibers. Supposedly, exposure to high-voltage electric field leads to orientation of PEO macromolecules and to appearance of anisotropic structure in the mixture, similarly to the case of chitin nanofibrils. High rate and high stability of electrospinning of PEO-based nanofibers support that.

When the pure chitosan fibers are prepared by wet spinning, the

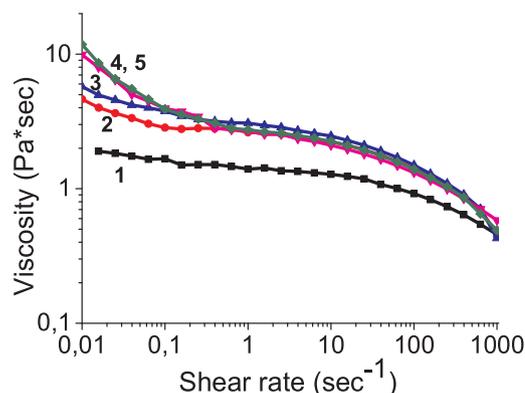


Fig. 5. Viscosity vs. shear rate curves measured for the 4 wt.% chitosan solution (curve 1), for the mixture containing 10 wt.% PEO (curve 2), and for the ternary mixtures containing 5, 10 and 25 wt.% of chitin nanofibrils (curves 3, 4, 5, respectively).

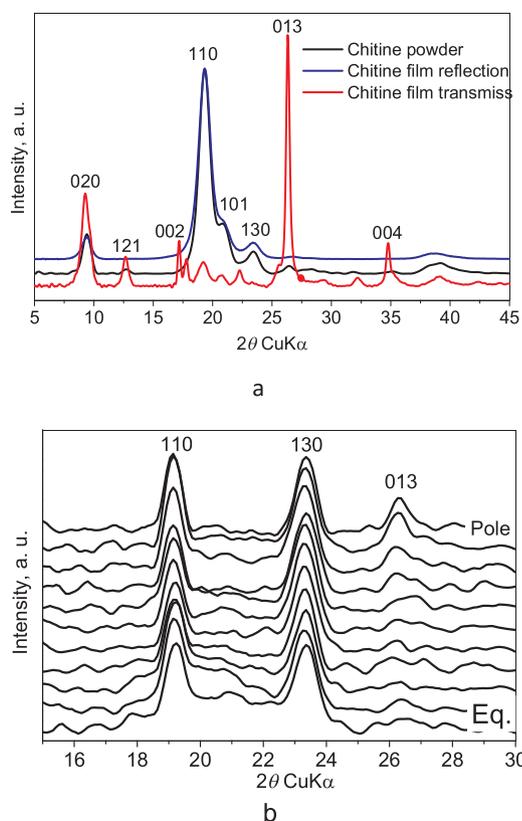


Fig. 6. a – Diffractograms of chitin nanofibrils; b – XRD patterns taken with different angles of inclination of the fiber (from equator to pole with a step of 10°).

value of shear rate observed during passage of the solution through a spinneret varies from 10^1 to 10^2 s^{-1} . It is in this range where the highest slope of the viscosity vs. shear rate curves is observed. Spinning of these fibers occurs at rather high rate ($Q = 0.1 \text{ mL/min}$). During electrospinning, the chitosan solution feed rate is considerably lower ($Q = 3\text{--}8 \times 10^{-4} \text{ mL/min}$), and the shear rate value is equal to $\dot{\gamma} = 0.1\text{--}0.3 \text{ s}^{-1}$ (calculated by the formula $Q = \pi R^3/2$, where $R = 0.3 \text{ mm}$ is the spinneret hole radius).

After passing through the spinneret hole, the mixture containing PEO and chitin nanofibrils enters the electric field. High voltage exerts a certain influence on the ternary mixture, and this effect is most significant in the case of chitin nanofibrils and PEO macromolecules which demonstrate high dielectric properties. As will be shown later, the orienting action of electric field leads to the formation of nanofiber structure.

3.5. Structure of the composite nanofibers

The results obtained with XRD confirm that the chitin nanofibrils are preferentially oriented with respect to the axes of the composite nanofibers. To study the structure of chitin nanofibrils, films were prepared by drying an aqueous dispersion of nanoparticles on a glass substrate, while the powder was obtained by its lyophilization. Fig. 6a shows diffractograms of the film and of the powdered chitin nanofibrils. The diffractograms were taken in transmission and reflection modes to reveal texture. It can be concluded that the [001] axes of the chitin crystallites lie in the film plane. The crystal structure of α -chitin with the following unit cell parameters: $a = 0.473 \text{ nm}$, $b = 1.899 \text{ nm}$, $c = 1.025 \text{ nm}$ agrees well with the literature data (Sikorski, Hori, & Wada, 2009). The macromolecules are packed in antiparallel orientation in the unit cell of this crystalline modification. The unit cell parameter b is substantially larger than the a -parameter that is,

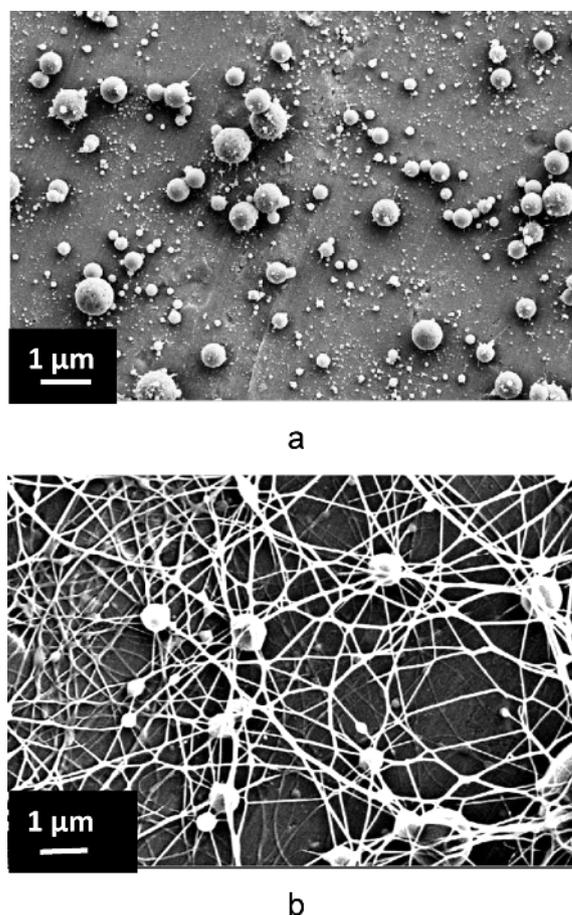


Fig. 7. Microphotographs of the materials obtained by electrospinning from 4 wt.% chitosan solutions containing 5 and 20 wt.% of PEO (a, b).

apparently, caused by the presence of the lateral group $-\text{NH}-\text{CO}-\text{CH}_3$ in the macromolecule.

The strongest diffraction peak at the Bragg angle $2\theta \approx 20^\circ$ is an overlap of the 110 and the 022 chitin maxima and of chitosan reflexes; changes in its intensity may lead to erroneous results when directly estimating the orientation of chitin nanofibrils. Texture of the composite fibers was studied using the azimuthal distribution of the chitin 013 peak intensity. The (013) planes are parallel to the x-axis and intersect the z-axis, which coincides with the axes of the chitin macromolecules.

Fig. 6b shows an increase in the 013 peak intensity in the series of XRD patterns taken with different angles of inclination of the fiber (from equator to pole with a step of 10°) with respect to the diffraction vector. This proves the preferential orientation of the chitin nanofibrils along the axes of composite nanofibers.

Thus, it can be concluded that the composite nanofibers based on chitosan and chitin nanofibrils have an anisotropic structure. When the content of filler in chitosan solution reaches approximately 20 wt.%, the particles form anisotropic clusters in electric field. This process is similar to that observed during wet spinning of composite fibers containing chitin nanofibrils when shear stresses occur during passage of the mixture through the spinneret. In the case of electrospinning, the polymer solution is drawn under the action of electromagnetic field; the resulting shear stress facilitates orientation of chitin nanofibrils and chitosan macromolecules.

Anisotropic structure of the bulk solution is inherited by the jet. In the transverse direction denser clusters of chitin nanofibrils alternate with smaller density areas with higher solvent content (Fig. 4); consequently, the strength of the jet in the transverse direction decreases, and its strength in the lengthwise direction increases. This promotes its separation into microjets in electric field. This observation is in

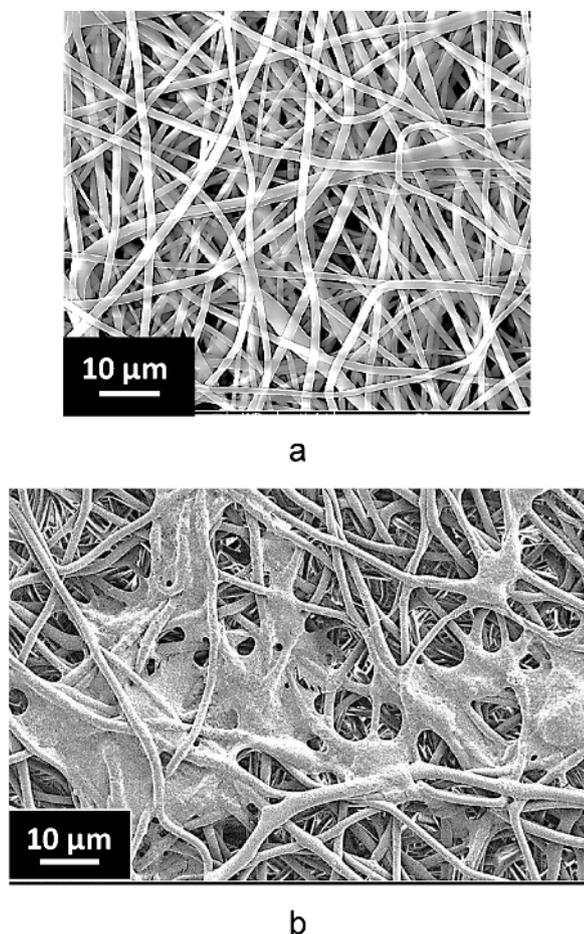


Fig. 8. Microphotographs of the materials obtained by electrospinning from the 4 wt.% chitosan solution containing 10 wt.% of PEO and 20 wt.% of chitin nanofibrils (a) and proliferation of stem cells (ASCs) for 3 days on the composite nanofiber matrix (b).

agreement with the data published in (Filatov, 1997); it has been shown that orientation of macromolecules leads to anisotropy of cohesion strength of a primary jet, i.e. to an increase in longitudinal strength and a decrease in cross-wise strength.

The fundamental distinction between the wet spinning and electrospinning is the difference between concentrations of chitin nanofibrils in the solutions and their role in the formation of structure and properties of the composites. In the first case, the content of chitin nanofibrils is very low (less than 0.5 wt.%) that is insufficient to overcome the percolation barrier. At this concentration the filler particles and chitosan macromolecules orient in the shear field, but their (re) orientation during plasticization drawing remains possible. At higher concentrations clusters of nanofibrils appear and make the structure rigid.

On the contrary, electrospinning requires high concentrations of nanofibrils (about 20 wt.%), that facilitates formation of clustered structures of the filler particles. Stretching of a primary jet in electric field leads to formation of microjets and, correspondingly, to spinning of composite nanofibers.

In the micrographs of Fig. 7 it is seen that electrospinning of the mixture containing 5 wt.% of PEO produced spheres with diameters varying from 0.5 to 2.0 µm (Fig. 7 a), but no nanofibers were obtained. The fibers with diameters ranging from 10 to 200 nm were formed during electrospinning of the mixture containing 20 wt.% of PEO; some 80–200 nm spheres (Fig. 7 b) were also observed. The nanofibers were possible to spun only at very low feed rate ($Q = 3 \times 10^{-4}$ mL/min); an increase in the feed rate resulted in the formation of drop-like defects

and in significant decrease in the amount of nanofibers.

As illustrated in Fig. 8a, electrospinning of the mixture containing 20 wt.% of chitin nanofibrils and 10 wt.% of PEO allowed preparation of defect-free composite nanofibers. Fig. 8b shows SEM micrographs of the composite material after exposure in cellular matrix for 3 days. One can see that the material surface is covered with cells that testify acceptable adhesion of the stem cells to the matrix and its non-cytotoxicity. This opens perspectives for the use of the materials based on the composite nanofibers as medical supplies, particularly as wound dressings, and as matrices for cellular technologies.

4. Conclusions

Electrical conductivity of chitosan solutions and of the mixtures containing chitin nanofibrils and/or PEO is by 3–4 orders of magnitude higher than the corresponding parameters of the polymers with high rates of electrospinning. Surface tension of chitosan solutions decreases with introducing relatively small amounts of chitin nanofibrils (about 1 wt.%). Addition of PEO into a chitosan solution containing chitin nanofibrils does not change the surface tension considerably. 20 wt.% of chitin nanofibrils increase the viscosity and strengthen its dependence on shear rate. This is caused by the formation of cluster structures involving filler particles, and by orientation of chitin nanofibrils under the action of shear stresses appearing in electromagnetic field. Chitin nanofibrils tend to orient along the axes of composite nanofibers. Presence of chitin nanofibrils facilitates formation of nanofibers in electric field and leads to significant decrease in the amount of defects.

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