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COMPOSITES

Properties of Film Materials Based on Composite Nanofibers from Aliphatic Copolyamide and Carbon Nanotubes for Tissue Engineering

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Abstract—The investigation of the dependence of effective viscosity on shear rate for a water-alcohol solution of an aliphatic copolyamide and its mixtures with single-wall carbon nanotubes reveals that additives of the nanoparticles in the amount of 0.5 wt % lead to a substantial reduction in the effective viscosity as the shear rate rises. The measurement of the surface tension and electrical conductivity of the solutions bearing 0.1–2.0 wt % of the nanotubes allows one to choose an optimal mode for electrospinning of the composite nanofibers based on the aliphatic copolyamide. The introduction of carbon nanofibers reduces the specific resistance of the material to 8.9×10^9 Ω m, but increases the elastic modulus. The lack of cytotoxicity of the resulting materials and the high proliferative activity of human dermal fibroblasts on their surface allow one to use the film materials based on the composite nanofibers in cell technologies and as matrices for tissue engineering.

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INTRODUCTION

Porous polymer films are used for filtration of liquid and gaseous media and separating membranes in different technological processes [1, 2]. Recently, these materials have come into use for culturing of stem and somatic cells [3]. There are several methods for formation of a porous structure of film materials: irradiation of the initial film with heavy ions followed by etching with alkaline solutions [4] and a coagulation method where the polymer solution is spinned through a slitlike nozzle on a substrate and then is placed into a precipitator. The displacement of the solvent with the precipitator results in the formation of pores of different shapes and sizes.

Currently porous film materials are produced by electrospinning of nanofibers with the diameter ranging from several tens to hundreds of nanometers [5, 6]. The film materials based on nanofibers feature low density, high porosity, and water and gas permeability and have pore sizes from tens to hundreds of microns. It was shown [3] that an alcohol-soluble aliphatic copolyamide possesses a complex of properties which allows it to be used in two-dimensional matrices based on nanofibers. Scanning electron microscopy studies revealed good adhesion and high proliferative activity of mesenchymal stem cells on the surface of film materials based on nanofibers from a copolyamide [6].

A promising trend in the use of porous films includes advanced materials of medical purpose, in particular, wound coverings and tissue-engineered preparations. Besides the required mechanical characteristics and the optimal porous structure, these materials must possess bioinertness, must not contain residual solvents and other toxic impurities, must provide the required gas and moisture exchange with the ambient environment, and must hamper the penetration of pathogenic microbial flora into the wound surface. Their chemical and porous structure must also facilitate the adhesion of stem or somatic cells on the surface and provide the exchange processes required for effective cell proliferation and differentiation. All the mentioned properties are characteristic of the materials based on nanofibers from a copolyamide [6].

One of the main problems of tissue engineering is the improvement of adhesion of stem and somatic cells to a polymer matrix as well as the creation of conditions for their intense proliferation. These processes and the matrix material resorption give rise to a tissue that is identical to the native tissue of a human or animal organ. There are data [7–9] on the effect of elec-

trical field on the rate of tissue regeneration; in particular, the high efficiency of electrostimulation in tissue engineering was noted upon regeneration of bone, nerve, and heart tissues. The improvement of cell proliferation and gene expression of collagen protein and calcium tones in the extracellular matrix was observed upon electrostimulation of osteoblasts on a substrate made of a polylactide with single-wall carbon nanotubes (SCNT) [10].

Nowadays, single-wall carbon nanotubes find growing application in the creation of composite materials. Nanocomposites containing SCNT possess improved strength and elastic characteristics compared to the items from a neat polymer [11, 12]. Moreover, single-wall carbon nanotubes are added to increase the electrical conductivity of a polymer material and impart antistatic properties to it [13].

The goal of the present work is to obtain a porous film material based on composite nanofibers from copolyamide bearing SCNT by electrospinning, to investigate their electrical conductivity, strength, and elastic characteristics, as well as the proliferative activity of human fibroblasts on these materials.

EXPERIMENTAL

The investigation was concerned with composite nanofibers based on the following copolyamide: a copolymer of poly-ε-caprolactam ($-NH-(CH_2)_5-CO-$) and hexamethylenediamide ($-NH(CH_2)_6NHCO(CH_2)_4CO-$) with the component ratio of 40 : 60, $M_w = 20$ kDa, $T_{mp} = 188°C$ (OOO ANID, Russia). The solvent for the copolyamide was a water-alcohol mixture, which made the spinning process environmentally benign. The investigation of the process of phase separation of the copolyamide solution in a binary solvent, the effect of the solution concentration on its rheological properties, and the peculiarities of the formation of nanofiber structures by electrospinning were reported earlier [3]. The materials from this polymer did not contain cytotoxic solvent residues and retained the structure and properties in aqueous media for a long time. The results of investigation of the structure and properties of the films from the copolyamide of variable copolymer composition obtained by the authors of the present work are published in a monograph [6].

The filler for the composite nanofibers was carboxylated single-wall carbon nanotubes in the form of dispersion in deionized water. The particle concentration in the dispersion was 2.5 wt %. Single-wall carbon nanotubes with the diameter of ~1.5 nm and the length of ~1.5 μm were synthesized by the arc method at OOO Uglerod ChG (Russia).

To obtain a mixture of the copolyamide and SCNT in water-alcohol solvents, firstly, the solution of the copolyamide was prepared [14]. Then an aqueous dispersion of SCNT was added to the solution, and the mixture was stirred at 60°C for 1 h. The solutions were deaerated at 0.1 atm for 2 h. The copolyamide concentration in the solution was 18 wt %. The concentration of SCNT was 0.1–2.0 wt % relative to the copolyamide mass. The ethanol : water ratio in the solvent was 80 : 20 vol %.

The rheological properties of the solutions were studied on a Physica MCR-301 rheometer (Anton Paar, Austria). The measurements were carried out at 20°C by the cylinder-in-cylinder method in a dynamic mode (sinusoidal loading with the constant strain) at the frequencies from 0.1×10^4 s^{-1} and in the shear flow mode at the shear rate ranging from 1×10^{-2} to 10^4 s^{-1}.

The surface tension coefficient was measured on a DSA30 unit (Kruss, Germany) by the hanging drop method at 20°C. To measure the electrical conductivity of the solutions, a Seven Multi electronic pH meter was used (Mettler Toledo, USA). The values of specific resistance of the composite film materials were measured on a laboratory unit at the Institute of Macromolecular Compounds, Russian Academy of Sciences, by the four-point method.

The electrospinning of the fibers was performed on a Nanon-01A laboratory unit (MECC Co., Japan). The flow rate of the copolyamide and SCNT solutions through a nozzle electrode with the diameter of 1.2×10^{-3} m was 2.8×10^{-6}–7.0×10^{-4} cm^3/s. The precipitation of nanofibers on a receiving electrode in the form of a metallic plate proceeded in the electrical field with the intensity of 1.4×10^5–2.0×10^5 $V\,m^{-1}$ at the distance between the electrodes of 0.1–0.2 m. A difference in the parameters of electrospinning is caused by different concentrations of SCNT in the copolyamide solution.

The structure of the resulting nonwoven materials was studied using a Supra 55 VP scanning electronic microscope (Carl Zeiss, Germany). For this purpose, the material was coated with a gold layer with the thickness of ~25 nm using an Eiko-IB3 unit (Ioncoater) at the ionic current of 6 mA and interelectrode voltage of 1.5 kV.

The strength-strain properties of the nonwoven materials at 20°C were determined in the mode of monoaxial tension using an Instron 5943 tensile machine (UK). The thickness of the tested samples was 0.5 ± 0.1 μm, the width was 2 mm, and the working length was 20 cm. The samples were loaded at the rate of 0.1 cm/min. The mechanical characteristics of the samples were determined by statistical averaging of the measurements for at least twenty samples.

The cytotoxicity of the resulting materials was studied on human dermal fibroblasts, which were cultured in a DMEM medium (Biolot, Russia) bearing 10% of fetal bovine serum from HyClone (USA) in 5% CO_2 atmosphere at 37°C. The cells were used after 4–6 culturing passes, and the nutritional medium for them was changed every three days. The proliferative activ-

ity of the cells was estimated using a tetrazolium dye, namely, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay).

RESULTS AND DISCUSSION

Upon production of nanofibers by electrospinning, it is necessary to take into account the properties of a polymer solution, such as the dynamic viscosity η, surface tension, molecular mass, composition and nature of the solvent, and its electrical conductivity [15].

For an optimal electrospinning process, the solutions with the polymer molecular mass of 10–100 kDa are used. Changing the electrospinning parameters, such as the electrical field intensity, the solution flow rate, and the distance between the feeding and receiving electrodes, one can affect the properties of the resulting material [5].

On the basis of the published data [3, 14], the optimal concentration of a copolyamide solution in a water-alcohol solvent for production of high-quality nanofibers by electrospinning was chosen to be 18 wt %. The rheological investigations showed that this solution of the copolyamide is a Newtonian liquid with the viscosity $\eta = 0.23$ Pa s in the range of shear rate ($\dot{\gamma}$) from 0.1 to 2500 s^{-1} (Fig. 1). The introduction of SCNT in the amount of 0.5 wt % from the copolyamide mass leads to a substantial change in the character of the dependence of η on the shear rate; as $\dot{\gamma}$ increases, the viscosity of the mixture decreases, which indicates the formation of a percolation network from SCNT in the polymer solution (Fig. 2); i.e., the introduction of SCNT into the polymer solution leads, on one hand, to a significant increase in the effective viscosity of the solution at low shear stresses and, on the other hand, to a reduction of this value at high shear rates.

It can be assumed that, in the shear field, the anisodiametrical SCNT nanoparticles orient, which can facilitate, in turn, the orientation of the copolyamide macromolecules. From Fig. 2, it is obvious that the effective viscosity of a mixture of the copolyamide and SCNT at low values of shear rate (~0.01 s^{-1}), which are close to the rate of polymer flow through the nozzle electrode, is over 10^3 Pa s; at the same time, the value of the solution viscosity for the copolyamide which does not contain the nanoparticles is only 0.23 Pa s.

The value of the solution viscosity and its dependence on the shear stress allow one to determine an optimal flow rate of a solution of the polymer or its mixture with the nanoparticles through the nozzle. The comparison of the rheological properties of the solutions and their mixtures with the anisodiametrical nanoparticles featuring composite fiber structure showed that the chitin nanofibrillae as well as the chrysotile nanoparticles are oriented along the fiber

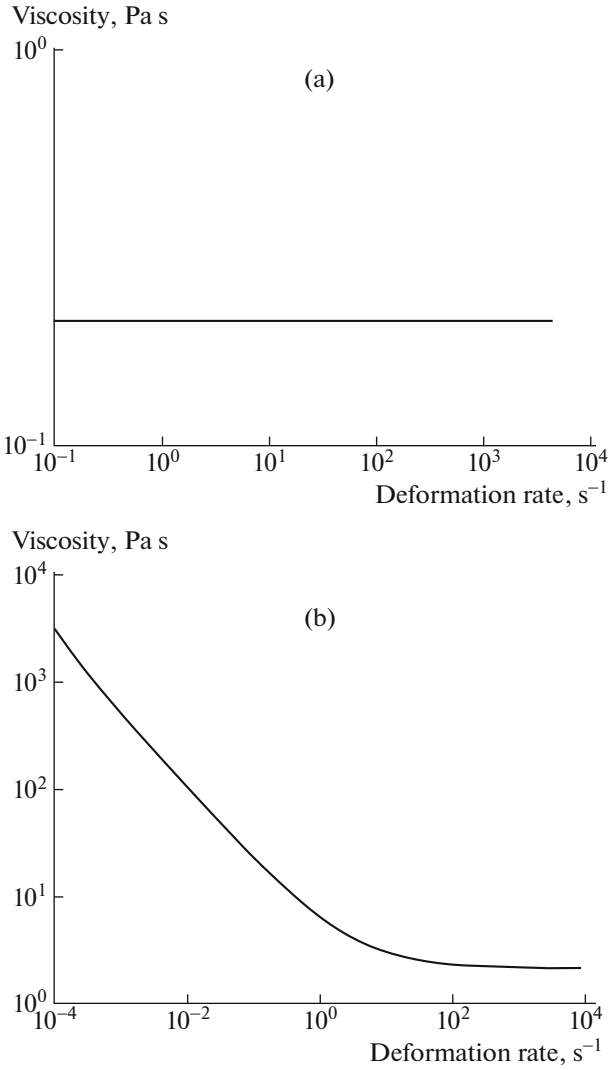

Fig. 1. Dependence of the effective viscosity η on the shear rate $\dot{\gamma}$ for the copolyamide solution (a) and the mixture bearing 0.5% of SCNT (b).

axis [16]. The orientation of the nanoparticles occurs in the nozzle during passing of the composite solution through it. The tensions of the fibers—plasticizing and thermal ones—hardly affect the orientation of macromolecules and nanoparticles of the filler. The oriented structure of the composite fibers is predetermined in the nozzle under action of the shear field, which is characterized by the value of shear rate that corresponds to a reduction in the effective viscosity of the mixture. The electrospinning results in the predominant orientation of structural fragments of the polymer, which is not characteristic of the films obtained from the solution by the casting method (dry spinning), which is indicated from the ratio of the intensities of reflexes on the X-ray patterns of the nanofibers and polymer films [17].

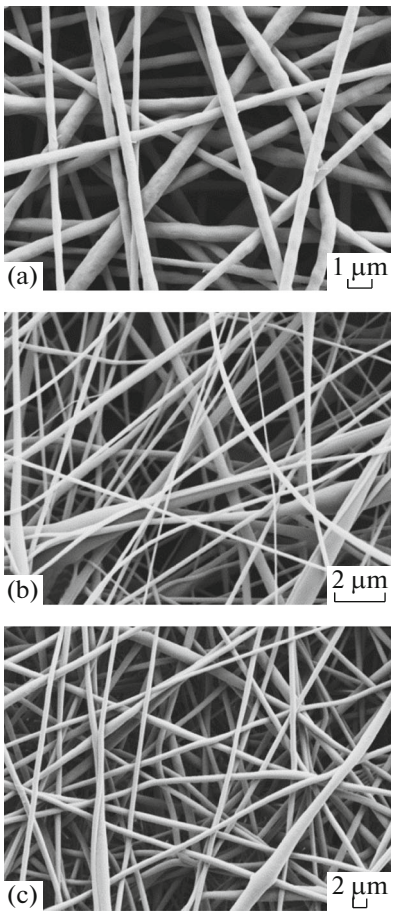

Fig. 2. Porous film materials based on composite nanofibers from the copolyamide bearing (a) 0.5, (b) 0.8, and (c) 2 wt % of SCNT.

On this basis, it was concluded that, in the shear field, the initial polymer structure decomposes and a new one is formed, which features the improved orientation of the filler nanoparticles.

Another parameter that affects the process of electrospinning is the surface tension of a solution or its mixture. The value of the surface tension coefficient is one of the most important properties that define the efficiency of the impact of electrical field on the formation of a fiber structure. The lower the surface tension coefficient of a spinning solution, the steadier the liquid stream, which, in turn, defines the process stability and the diameter and uniformity of the resulting nanofibers [18]. A high surface tension coefficient hampers the formation of a steady solution stream [1], whereas a low value hampers fiberization and facilitates formation of a film on the receiving electrode [5, 14].

Table 1. Surface tension coefficients of mixtures of the copolyamide solution and mixtures bearing single-wall carbon nanotubes

Composition	Surface tension coefficient, N/m
Copolyamide solution	24.56 ± 1.13
Copolyamide solution + 0.5 wt % of SCNT	25.48 ± 0.97
Copolyamide solution + 0.8 wt % of SCNT	26.05 ± 1.26
Copolyamide solution + 2.5 wt % of SCNT	14.70 ± 1.03

It should be noted that the competition between the forces of surface tension and electrostatic forces results in capillary surface instability, which affords changes in the morphology of the stream surface and violation of its cylindricity and gives rise to spherical and drop-shaped defects on a fiber.

The values of the surface tension coefficient of the copolyamide solution and its mixtures bearing SCNT are presented in Table 1. It is obvious that the introduction of a small amount (0.5–0.8 wt %) of SCNT hardly affects the value of the surface tension. However, upon introduction of more than 2 wt % of SCNT, the surface tension of the mixture drastically decreases. It can be assumed that the nature of this phenomenon is connected with the formation of a cluster structure of the filler, which significantly modifies the structure and properties of the copolyamide solution, in particular, the character of intermolecular interactions: it violates the intermolecular bonds of the copolyamide and forms new bonds between the macromolecules and nanoparticles. An increase in the content of SCNT in the copolyamide solution leads to a substantial reduction in the surface tension and hampers the formation of fiber structures in the electric field.

Figure 2 depicts the micrographs of nanofibers from the copolyamide bearing different amounts of SCNT. It is apparent that the fiber diameter changes from 200 to 500 nm, the pore sizes range from 1 to 10 μm, and these values only insignificantly depend on the content of nanotubes. This structure is optimal for adhesion and proliferation of cells. It is identical to the collagen structures which constitute most of the native tissues of animals and humans: muscular, vascular, tendinous, and cartilaginous tissues. The diameter of fibers facilitates the maximum contact of cell pseudopodia with the matrix, and its porous structure provides for exchange processes which are necessary for efficient proliferation of cells. The matrices based on the copolyamide do not contain residual solvents and other chemical compounds which were used in the polymer synthesis. This conclusion was made earlier upon investigation of cytotoxicity and genotoxicity [3], where the biocompatibility of the tested material was demonstrated.

One of the goals of this work was to determine the maximum concentration of SCNT in the polymer solution which enables the electrospinning of fibers

Table 2. Properties of the porous film materials from composite nanofibers based on the copolyamide and single-wall carbon nanotubes

Content of SCNT, %	Specific resistance, Ω m	Elastic modulus, MPa	Strength, MPa	Deformation at rupture, %
0	$(1.8 \pm 0.2) \times 10^{11}$	54.5 ± 7.1	7.1 ± 0.5	259 ± 15
0.5	–	87.6 ± 12.3	8.1 ± 1.3	142 ± 15
0.8	–	85.7 ± 9.0	5.8 ± 0.8	113 ± 13
2.0	$(8.9 \pm 0.2) \times 10^{9}$	57.3 ± 11.7	4.0 ± 0.6	98 ± 11

free from defects. For this purpose, we performed a series of experiments on production of the solutions with different amounts of SCNT. It was established that, at the SCNT concentration of 0.1–2.0% of the copolyamide mass, the nanofibers are formed without defects. At the SCNT concentration over 2 wt %, the formation of a fiber structure does not occur in a wide range of the polymer solution flow rate. Drops of variable sizes are formed, which is likely connected with the low value of surface tension at high (over 2 wt %) SCNT concentrations (see Table 1).

The introduction of the carbon filler into the solution does not strongly affect the value of specific conductivity of the solution. For the solution of neat copolyamide, this value is 47.5 μS/m, and for the solution bearing 2 wt % of SCNT, it is 61.3 μCm/m.

The values of specific resistance of the films based on composite nanofibers and nanofibers from the copolyamide showed (Table 2) that this value for the material bearing 2 wt % of SCNT is lower by two orders of magnitude than that for the analogous film from neat copolyamide.

Figure 3 demonstrates the load–strain diagrams of the film materials based on nanofibers from the copolyamide and composite nanofibers bearing SCNT. As it can be seen, the character of the curve for the material based on the composite nanofibers drastically differs from the analogous curve for the film from nanofibers of neat copolyamide. The introduction of SCNT into nanofibers increases considerably the elastic characteristics of the materials and reduces the deformation at rupture.

The mechanical properties (elastic modulus, strength, and relative elongation at break) of the film samples based on nanofibers from the copolyamide bearing different concentrations of SCNT are presented in Table 2.

It is apparent that the introduction of SCNT into nanofibers from the copolyamide leads to an increase in the elastic modulus and a decrease in the deformation at rupture. This is connected with the reinforcing effect of the filler and formation of a macrocluster structure. The value of the percolation barrier R_p (vol %) was obtained using the following equation: $R_p = (0.6/r) \times 100\%$, which was published earlier [19], where $r = L/d$ is the axial ratio, L is the length of nanoparticles, and d is the diameter. The calculations showed that the percolation barrier for SCNT is 0.2%. An increase in the SCNT content over 2 wt % leads to a reduction in the elastic modulus, which is likely connected with the formation of defects in the fiber.

At the present time, the problem of interaction of carbon nanotubes with proteins inside and outside the cell matrix is still controversial. There are data [20] on the penetration of nanotubes through a cell membrane and their accumulation in cytoplasm without toxic effect on a cell. At the same time, a series of papers mention that the nanotubes are toxic, and the toxic effect depends on their length and is higher in the case of long tubes [20–22].

The matrix for cell technologies must possess biocompatibility and must not exert a negative action on biological objects; therefore, the current work implies investigation of the proliferative activity of cells on the resulting composite materials and estimation of their cytotoxicity. For this purpose, human dermal fibroblasts were cultured on the surface of porous films based on nanofibers from the copolyamide bearing different amounts of SCNT. The analogous investigations were carried out on the surface of a standard bottle.

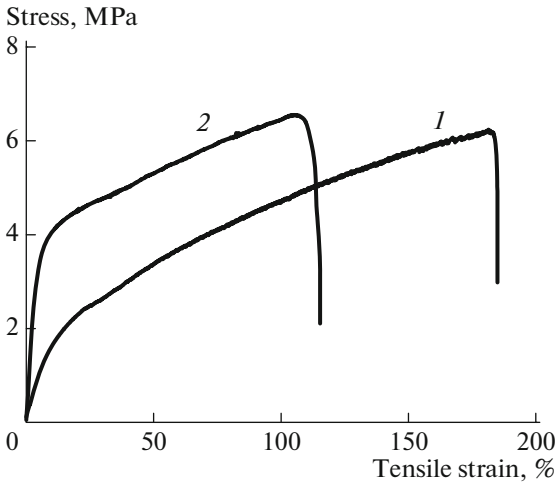

Fig. 3. Stress–strain diagrams for the film materials based on nanofibers from the copolyamide (*1*) and composite nanofibers bearing 0.8 wt % of SCNT (*2*).

Table 3. Density of human fibroblasts after 5 days of culturing on the matrices from composite nanofibers based on the copolyamide and single-wall carbon nanotubes

Matrix type	Cell density 10^3, units/cm^2
Standard bottle	7.2 ± 0.2
Copolyamide nanofibers	4.6 ± 0.2
Nanofibers from copolyamide + 0.2% of SCNT	4.3 ± 0.2
Nanofibers from copolyamide + 0.5% of SCNT	4.7 ± 0.2
Nanofibers from copolyamide + 2.0% of SCNT	4.6 ± 0.2

Table 3 presents the values of surface density of human fibroblasts after five days of culturing on the matrices from the composite nanofibers based on the copolyamide and SCNT. The measurements performed using the MTT method showed that the density of cells fixed on the tested materials hardly depend on the presence of carbon nanotubes and their amount. This value is very close to the density of cells cultured on the surface of a standard bottle. Therefore, it can be concluded that the presence of SCNT in nanofibers from the copolyamide does not affect the proliferative activity of cells.

The number of dead cells upon culturing of fibroblasts on a nonwoven material was also estimated. It was found that, on the material from the copolyamide, 11% of dead cells were observed, and on the materials with the addition of the carbon component, there were 14% of dead cells. Hence, it can be concluded that the introduction of SCNT into the polymer matrix does not cause cytotoxicity of the samples.

Figure 4 demonstrates the micrographs of the porous film materials based on nanofibers from the copolyamide with the addition of 0.5 wt % of SCNT, on which the cells were cultured for three days. It is obvious that the cells uniformly occupy the matrix area. The resulting data indicate the high proliferative activity of cells on the porous materials based on CPA nanofibers bearing SCNT. The materials obtained can be used as matrices for cell technologies.

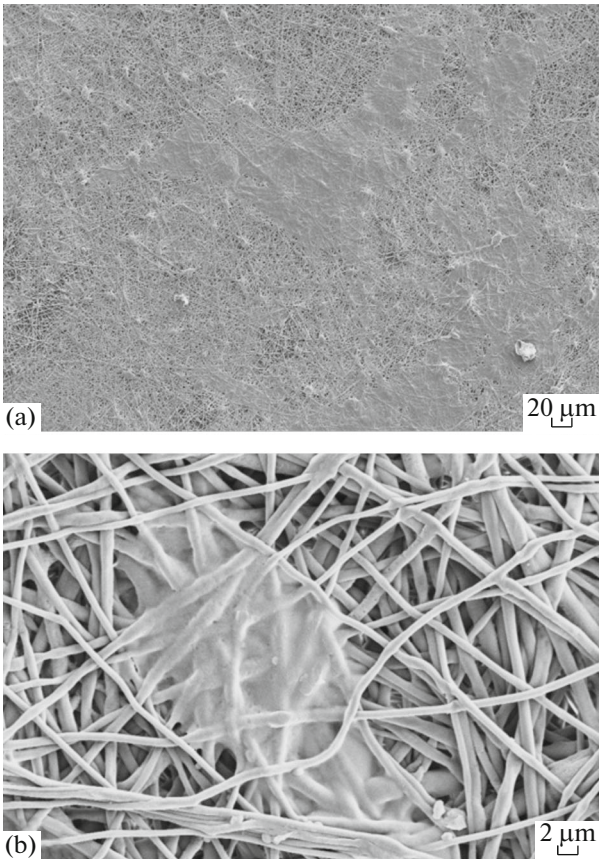

Fig. 4. Micrographs of the tissue-engineered preparation based on the film porous material from composite nanofibers of the copolyamide with the addition of 0.5 wt % of SCNT after three days of cell culturing obtained at (a) low and (b) high magnification.

CONCLUSIONS

On the basis of the results of investigations on the rheological properties of the copolyamide solution and its mixture bearing single-wall carbon nanotubes, as well as their electrical conductivity and surface tension coefficient, we developed a method for electrospinning of composite nanofibers based on the copolyamide and single-wall carbon nanotubes. The strength and strain characteristics of the materials based on the composite nanofibers, the value of their specific electrical resistance, and the high proliferative activity of fibroblasts on the resulting films enable their use as two-dimensional matrices for tissue engineering.

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