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Structure and properties of porous films based on aliphatic copolyamide developed for cellular technologies

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Abstract The effect of concentration and viscosity of the copolyamide (copolymer of ε-caprolactam and hexamethylendiaminadipate) solutions in aqueous/alcoholic solvents on its phase state was studied. The films obtained by the coagulation method were characterized by monodisperse pore distribution with an average pore size of 1.3 μm. The films processed by electrospinning from copolyamide solutions were characterized by a bimodal distribution of macropores with one peak of pore radius at 2.0 μm and second peak of pore radius at 20 μm. The adhesion and proliferation of mesenchymal adhesion stem cells (ASCs) stem cells to copolyamide matrix were studied. With the help of scanning electron microscopy it was shown that both tapes porous films were characterized by good adhesion of mesenchymal ASCs stem cells. It was shown that the porous structure, transport and mechanical properties of these copolyamide films allow their use as two-dimensional matrices for cellular technology.

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1 Introduction

Porous films based on polymers of aliphatic and aromatic structures are used for filtering liquids and gases, pervaporation, electrodialysis [1, 2]. In recent years, porous materials are used as matrices for the adhesion and proliferation of stem and somatic cells [2–5]. To create a porous structure, various methods are used, in particular, the irradiation of a polymer film by high-energy ions, the phase separation of the polymer solution [1, 6], the deformation of the initial film [7, 8]. The films processed by such methods can be characterized by porous structure with porous sizes in the range of 0.1–10 μm and more. To obtain polymeric materials with a high porosity the electrospinning (ESP) method can be used also [9]. This method consists of the applying polymer solution through a die-electrode high-voltage electric field to process nonwoven fabric from fibers with a diameter in nanometer range.

The promising trend of using the porous films is obtaining the materials of the medical designation [10, 11], which must possess biocompatibility, contain no the remainders of the solvents and other toxic admixtures, ensure necessary gas and moisture exchange with the environment, prevent the penetration of pathogenic microflora into the wound surface. Recently, porous films found their use as the matrices for the cellular technologies, scaffold materials. Its chemical and porous structure should promote the adhesion of stem or somatical cells on the surface, to ensure metabolic processes for the effective proliferation and differentiations of cells, biodegrade at a controllable rate under the culture conditions, and provoke a minimal degree of inflammation or toxicity in vivo [12–16]. Developing scaffolds with the optimal characteristics, such as their strength, porosity and microstructure are more

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readily and reproducibly controlled in polymeric scaffolds [17].

Scaffold materials can be synthetic or biologic, degradable or nondegradable, depending on the intended use [18]. The properties of polymers depend on the composition and structure of macromolecules. Naturally occurring polymers, synthetic biodegradable, and synthetic nonbiodegradable polymers are the main types of polymers used as biomaterials

The porous materials based on nanofibers processed by ESP [19–21] are of great interest for cellular technologies. ESP can be distinguished as a versatile technique for the preparation of polymer fibers from a few nanometers to microns in diameter, depending on the processing conditions. ESP uses a high voltage to create an electrically charged jet of polymer solution or melt which can lead to fibers formation [22–24]. The nanofibers mat or films from PE, PP, PVA, PEO, PVP, PA, PMMA, polylactide, cellulose derivatives and other polymers are processed by this method [25–27]. These materials are characterized by low density, high porosity and water-permeability.

Scaffold materials based on nanofibers obtained by ESP processed from biodegradable (collagen, elastin, fibrinogen, fibrin) and non-biodegradable (PET, PU, polyglycolic acid, polyethylene, polyamides, PTFE, and mixtures thereof) polymers are described in literature very well [28], but as the solvent there were used toxic substances are hexafluoroisopropanol, dimethylformamide, acetone, acetonitrile.

In this connection, obtaining the porous films from aliphatic copolyamide (CoPA) or copolymer of ε-caprolactam (–NH–$(CH_2)_5$–CO–) and hexamethylendiaminadipate (–NH$(CH_2)_6$NHCO$(CH_2)_4$CO–) can be of special interest [29]. Really, CoPA is easily soluble in the mixture of alcohol and water, making the molding process of films very friendly for environment. Materials made this polymer do not contain cytotoxic residues of solvent and retain their characteristics in aqueous media for a long time.

Porous structure and properties of materials based on the nanofibers processed by ESP and porous films obtained by coagulation of CoPA solution are studied insufficiently. There is not enough information about the influence of the mixed solvent and spinning conditions (temperature, polymer concentration, etc.) on the porous structure of the films from CoPA and their mechanical characteristics.

The purpose of the work is a study of porous structure and properties of CoPA films obtained by the traditional method of the coagulation from the solution and also the porous films or mats from CoPA nanofibers processed by ESP, as well as an investigation of adhesion and proliferation of stem cells on the matrices from CoPA.

2 Materials and methods

2.1 Materials

The nanofibers processed by ESP and porous films obtained by coagulation of CoPA solution were prepared from a copolymer of ε-caprolactam and salt of adipic acid with a ratio of components as 40:60 (LTD "Anid," Russia), $M_w = 20$ kDa, $T_m = 188$ °C. The CoPA was dissolved in a mixture of ethanol/water = 80/20 at T = 80 °C for 1 h with permanent stirring.

Cultures were used of ASCs-1 adipose tissue mesenchymal stem cells obtained in the course of standard procedures [13]. The ASCs-1 cells were cultivated in the MEM Alpha Modification nutritive medium containing 15 % calf fetal serum and the same antibiotics. All reagents were from Gibco (United States). The cells were cultivated in a CO_2 incubator at 37 °C in atmosphere of 5 % CO_2 and increased humidity.

2.2 The porous films processed by coagulation method

The porous films were processed by coagulation method from a solution onto a glass substrate using a slit die; the height of the gap was 400 μm. After exposure in the air the glass substrate with polymer solution on its surface was placed in the water, where the coagulation occured for 10 min, after which the film was washed in distilled water and air dried.

2.3 Processing of nanofibers by ESP method

ESP of CoPA nanofiber mat was processed at laboratory equipment. The anode of the voltage power supply was connected with the tip of the syringe and the cathode attached to the plate covered by aluminum foil on which fibers were deposited. The solution was ejected through a syringe using a flow pump at a feed rate of 0.1 mL/min and applying a voltage of 30 kV and tip-target distance of 20 cm.

2.4 Mechanical properties

Mechanical properties (Young modulus E, tensile strength σ and deformation at break ε) of the porous films were determined with the aid of testing machine UTS-10 (Germany). The samples for testing had the form of strips with the following sizes: 2 mm in width, ≈100 μm in thickness and base length of 25 mm. The tension speed was 10 mm/min. Data represent the average of 10 sample's measurements of E, σ and ε .

2.5 Estimation of phase separation

To study the CoPA solution phase state, the light scattering method was used. The start of the phase separation was estimated by the determination of the turbidity point of the solution with the aid of spectrophotometer SPECORD (Germany). As known [30], when the light with a wavelength λ passes through the optically inhomogeneous medium, its intensity decreases. The intensity of the transmitted light depends on the size of the scattering particles, their polydispersity, concentration, layer thickness. All these factors cause a decrease in the intensity of the incident light. The intensity in the wavelength range of 350–850 nm was measured in order to estimate the turbidity point.

Figure 1a shows the dependence of the intensity of the transmitted light as a function of the wavelength for CoPA solutions with concentration 18 wt%; ratio of ethanol/water = 70/30. Before measuring the light scattering solution was kept at T = 20 °C for various times (5–70 min).

Figure 1b shows a typical dependence of the scattered light intensity with λ = 440 nm on the exposure time solution. The growth of the light scattering intensity with the solution exposure time allows to assume the beginning of the gel phase formation. The inflection point in the dependence was chosen to assess the start of the phase separation of the CoPA solution. Similar curves were obtained for the solutions with various concentrations (C wt%) as well as the solutions with various ratios of ethanol/water. The presence of an inflection point in light scattering allows one to construct the phase diagram of CoPA solutions with various concentrations and the ratio of ethanol/water from 40/60 to 100/0.

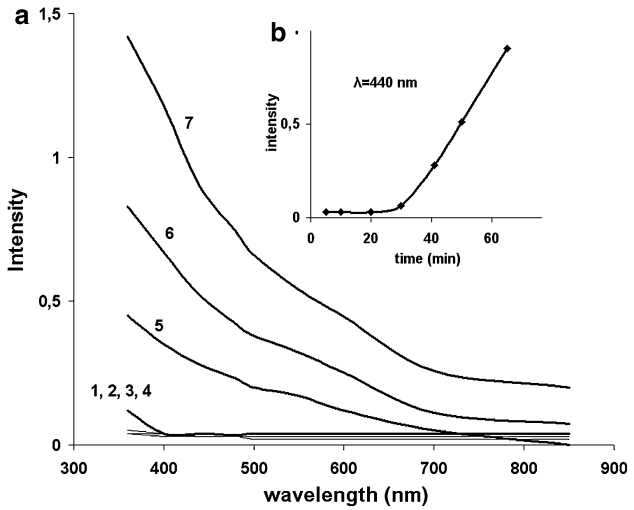

Fig. 1 The intensity of the light scattering of wavelength λ; the 18 wt% CoPA solution; the exposure time t = 5, 10, 20, 30, 40, 50 and 70 min (curves *1*, *2*, *3*, *4*, *5*, *6*, *7*)—(**a**); the dependence of the light intensity (λ = 440 nm) on the exposure time of CoPA solution—(**b**)

The transition of the solution into the gel state was determined by the change in viscosity. The viscosity measurements of the polymer solutions were carried out on MCR-301 rheometer (Anton Paar, Austria) at 20 °C. Dependence of the viscosity on the shear rate was determined by the unit CC17 as "cylinder–cylinder".

2.6 Measuring of the water permeability

Water permeability of the porous films was measured at the laboratory equipment at the pressure of 1 atm. Samples had a shape of discs with a diameter of 20 mm and a thickness of 150 μm. The vapour permeability of the films was determined with respect to a change in the weight of the bottle filled the water and covered by the investigated film; the distance from the film to the water surface was 10 mm.

2.7 Measuring of the porosity

The specific surface areas of porous films were calculated from the nitrogen adsorption isotherms measured at 77 K according to standard BET method [31] using SORPTOMATIC 1990 Carlo Erba apparatus. The samples were evacuated at 353 K for 2 h prior to analysis and then were again evacuated inside the instrument until a stable pressure was reached.

The method of high-pressure mercury porosimetry was used for determination of the total pore volume V_t within a given range of pore radii, their surface area S_t for an assumed model pore shape, modus of radii r_{mode}, bulk density d_{Hg} and porosity Por. The samples were measured using coupled porosimeters Pascal 140 + 240 by Thermo Electron-Porotec. The Pascal 140 porosimeter works as a filling device and is utilized for low-pressure measurements below 100 kPa. The Pascal 240 porosimeter works within the pressure range of 0.1–200 MPa.

2.8 Cultivation of stem cells

White adipose tissue is obtained by lipectomy, it was subjected to homogenization, enzymatic treatment with a solution of 0.2 % collagenase (Sigma, USA) and centrifuged. The cells were cultured in medium MEM Alpha Modification (Gibco, USA) supplemented with 15 % fetal calf serum (Gibco, USA) and antibiotics (100 units/ml penicillin, 100 μg/ml streptomycin (Gibco, USA). Culturing of stem cells held in CO_2-incubator in an atmosphere of high humidity of 70 % at T = 37 °C, the CO_2 content is 5–7 % when the 80 % of the monolayer produced subcultured cell-free plastic vials. The cultures of mesenchymal stem cells of adipose tissue (ASCs) were used for

cytological studies of CoPA films. The cultivation of stem cells was carried out in a CO_2 incubator at 37 °C in dump atmosphere with humidity 70 %, the content of CO_2 was 5–7 %. For evaluating the degree of the adhesion of cells to different substrata, the board with the square holes (Nunc, USA) was used, into which the investigated materials were placed.

The stem cells are precipitated into a matrix from liquid nutrient medium containing the cells. Exposure time of the test sample in the matrix ranged from 1 h to 2 weeks. After incubation, cells were fixed on the surface of the matrix by treatment with 0.25 % solution of glutaraldehyde (Sigma, USA) followed by dehydration with ethanol.

2.9 Structural investigation of porous film and the materials containing stem cells

The structure of the porous film and the materials containing stem cells was examined using the scanning electron microscope Supra 55VP (Carl Zeiss, Germany). The samples were sputter-coated with gold to prevent charging during SEM imaging.

The precipitation of the stem cells on the CoPA matrix was carried out from the liquid nutrient medium, which contained stem cells; the time of holding the sample in the matrix various from 1 h to 2 weeks. In order to study the adhesion of stem cells and their proliferation by the SEM, the cells were fixed on the surface of the CoPA matrix using a glutaraldehyde solution. After the necessary time from the moment of seeding the cells, the samples were washed by 0.1 M PBS (phosphate buffer solution) with pH = 7.4, after which the cells were fixed during 40 min by 0.25 % solution of glutaraldehyde in PBS at T = 4 °C. After the removal of the fixative solution, the samples were washed by PBS and the dehydration of the material was conducted by the ascending concentrations of ethanol; then the samples were placed for 30 min into hexamethyldisilazane and air dried.

3 Results

3.1 Characteristic of CoPA solutions

The start time of the phase separation was determined by the increase in the intensity of turbidity spectrum. The dependence of the intensity versus the wavelength is shown for the CoPA solution in the alcohol-water mixture in Fig. 1a. Figure 1b shows a dependence of the scattered light with λ = 440 nm on the exposure time of the solution.

The dependence of the solution phase composition on concentration and exposure time at T = 20 °C was determined using SEM method. Figure 2 shows SEM micrographs of the films obtained from 10 wt% CoPA solution; the holding time before precipitation 60 s.

Figure 3 shows the state of the solution on the content of the CoPA (C %); solvent is the ethanol/water with the ratio as 80/20.

The viscosity of the CoPA solutions in the ethanol–water mixtures having different concentration has no dependence on the shear rate (Fig. 4a). Figure 4b shows the dependence of the CoPA solution viscosity (solvent is the ethanol/water with the ratio as 80/20) on the concentration of CoPA.

The analysis of the phase diagram of the solutions containing 18 wt% CoPA in the mixtures with various content of ethanol (Fig. 5) gives the possibility to determine the optimal content of the solvent.

3.2 Characteristic of porous films processed by coagulation method

Data on the water permeability of the films processed by coagulation method indicate the most developed film porous structure with through-porosity (Table 1). The vapor

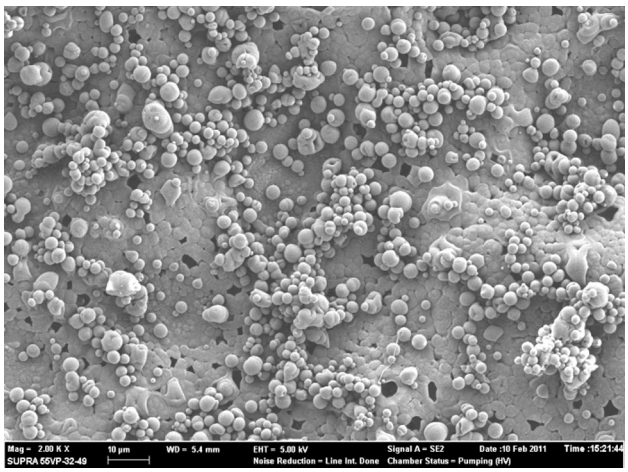

Fig. 2 SEM micrographs of the film obtained from 10 wt% CoPA solution

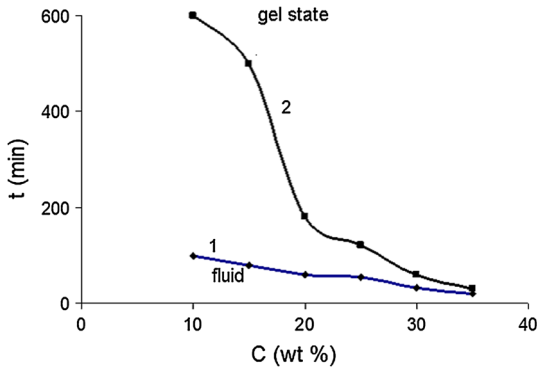

Fig. 3 State diagrams of the CoPA solution, *C* the CoPA concentration

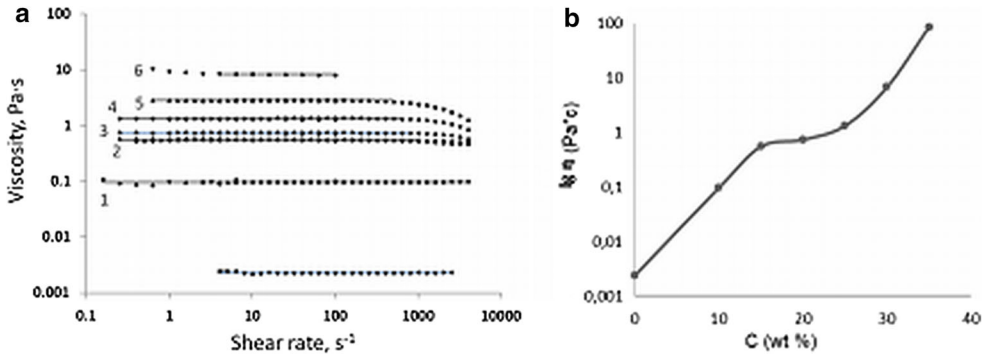

Fig. 4 The viscosity dependence of CoPA solutions of various concentrations (0, 10, 15, 20, 25, 30, 35 wt% curves 1, 2, 3, 4, 5, 6 respectively) on shear rate (**a**); and on CoPA concentration (**b**). Solvent is a mixture of ethanol/water = 80/20 vol%

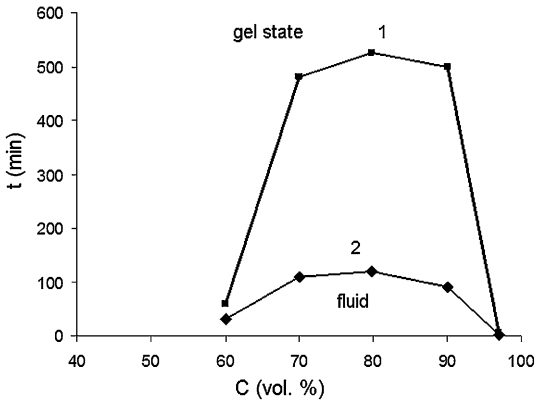

Fig. 5 The phase diagram of the 18 wt% CoPA solution; *t* (min) is exposure time at T = 20 °C; *C* (vol%) is ethanol content in the mixture

Table 1 Water permeability of the CPA films obtained with exposure time *t* before the deposition

t (min)	1	2	4	6	20
Flow [kg/(h m^2)]	187 ± 10	233 ± 14	171 ± 14	46 ± 7	0

permeability is an important characteristic of porous films too (Table 2).

Porous structure of the films processed by the coagulation method was studied by SEM method. Figure 6a–d shows the various structure of the CoPA films processed by the coagulation method from the 18 wt% solutions and exposed prior to deposition for t = 1, 2, 4, 20 min.

The pore size distribution for the CoPA films processed by coagulation method shown in Fig. 7a.

3.3 Characteristic of porous materials processed by ESP method

The SEM micrographs of the nanofibers processed by ESP from 18 wt% concentration of CoPA solution (mixture ethanol/water = 80/20), adhesion single stem cells (ASCs)

Table 2 Mechanical characteristics of the CPA films processed by the coagulation method

t (min)	E (MPa)	σ_p (MPa)	ε_p (%)
1	91 ± 10	3.2 ± 0.2	23 ± 2
2	129 ± 18	4.4 ± 0.2	52 ± 5
4	287 ± 17	8.4 ± 0.2	123 ± 3
6	465 ± 37	12 ± 1	212 ± 1
20	829 ± 79	48 ± 4	382 ± 46

and their proliferation for 3 and 7 days are shown in Fig. 8a, b, c, d.

3.4 Analysis of porous structure of the films

The porous structure of the film based on CoPA was investigated by BET method [31] and mercury porosimetry. The results of these measurements are shown in Table 3.

The pore size distribution for the CoPA films processed by ESP method shown in Fig. 7b.

3.5 Analysis of mechanical properties of the films

Table 4 demonstrates the mechanical characteristics of the investigated porous films. The porous film obtained by coagulation characterized by a somewhat lower strength (Table 2), However, the materials having such porous structure and mechanical properties can be used in dry or wet conditions in medicine and biology.

3.6 Adhesion and proliferation of stem cells on the surface of CoPA porous films

After the fixation of cells on the matrix with the aid of glutaraldehyde the sample were investigated with the help of the SEM. The CoPA films processed by coagulation and

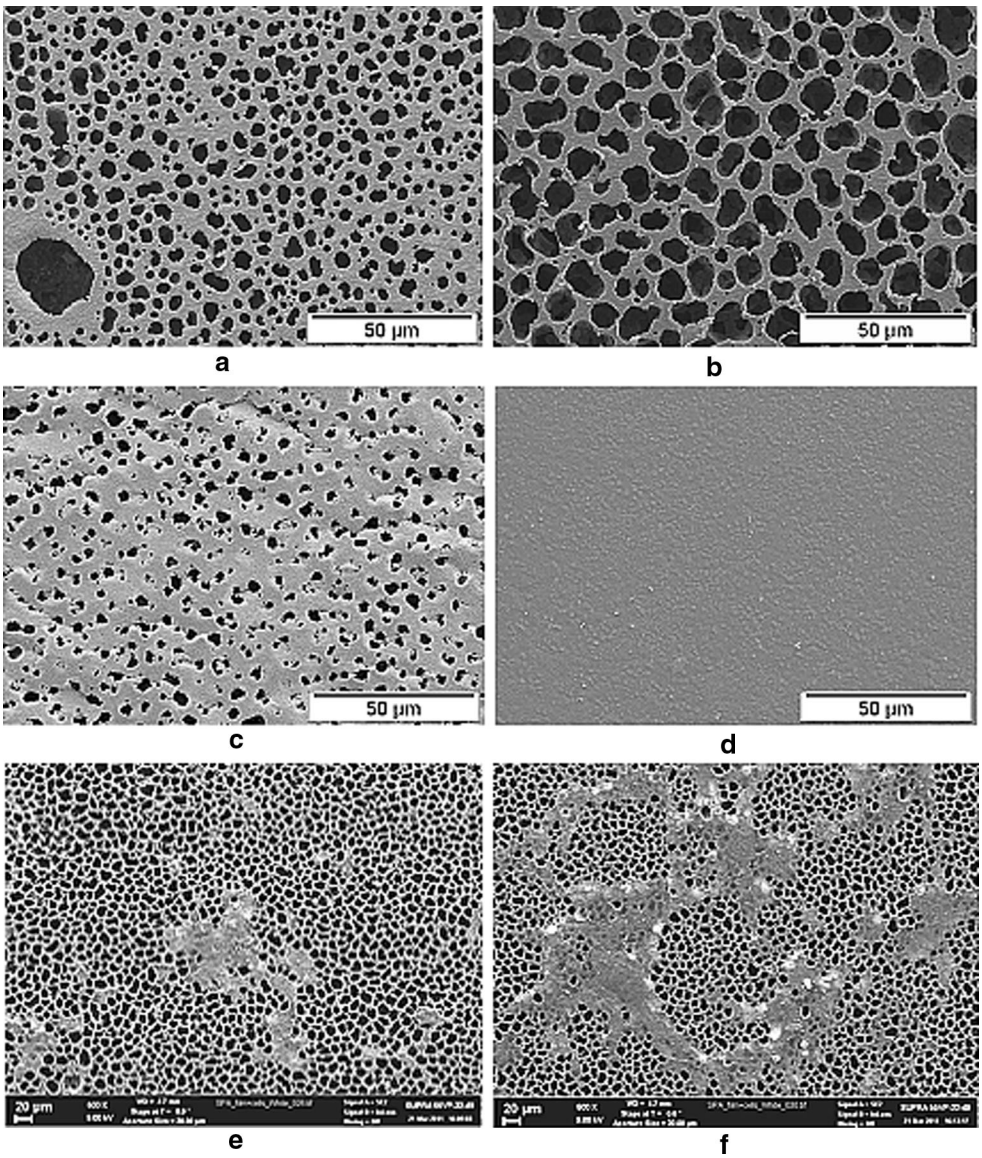

Fig. 6 Micrographs of the CoPA films processed by the coagulation method from the 18 wt% solutions and exposed prior to deposition for t = 1, 2, 4, 20 min (**a**, **b**, **c**, **d**); adhesion stem cells (ASCs) on the on porous CoPA films and their proliferation for 3 (**e**) and 7 (**f**) days

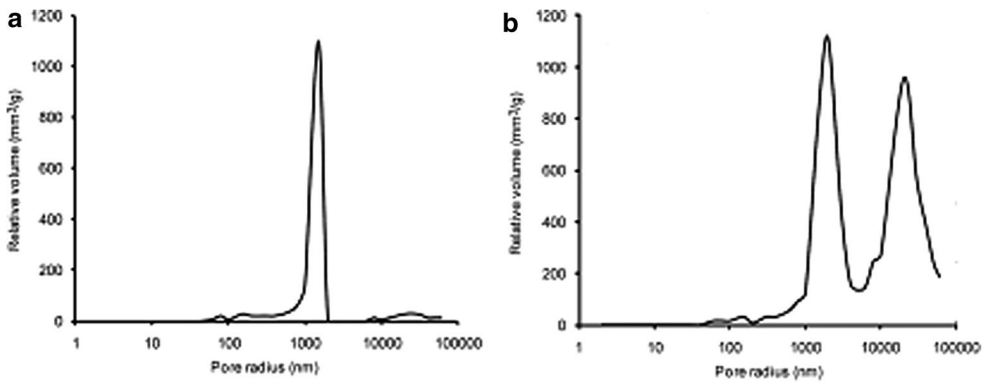

Fig. 7 The pore size distribution for the CoPA films processed by coagulation method (**a**) and ESP method (**b**)

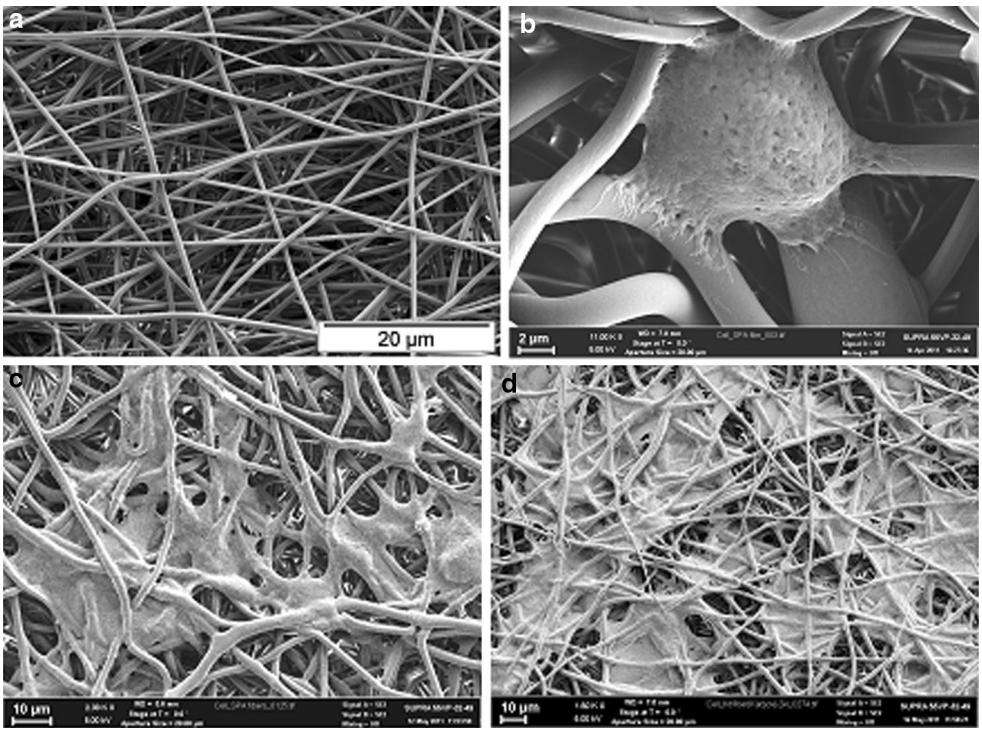

Fig. 8 The SEM micrographs of the CoPA nanofibers matrix (**a**); adhesion single stem cells (ASCs) (**b**) and their proliferation for 3 (**c**) and 7 (**d**) days

Table 3 Analysis of the porous structure of the CoPA films

CoPA (%)	S_{BET} (m^2/g)	V_t (cm^3/g)	$r_{mode,}$ (μm)	Por (%)	d_{Hg} (g/cm^3)
ESP					
15	4.9	3.275	1.27	80.6	0.25
20	4.6	5.922	1.56	94.0	0.16
25	3.2	4.854	2.72	84.7	0.17
Coagulation					
18	3.7	1.597	1.26	69.8	0.44

S_{BET} Specific surface area according to BET method, V_t the total volume of pores, r_{mode} modus of radii, *Por* porosity, d_{Hg} bulk density

Table 4 Mechanical properties of CoPA films processed by the ESP methods and tested in dry and wet conditions

Samples	Thickness (мкм)	Strength (MPa)	Modulus (MPa)	Elastic deformation (%)	Deformation at break (%)
ESP dry	150 ± 8	6.9 ± 0.5	45.5 ± 0.6	5 ± 0.5	144 ± 9
ESP wet	150 ± 10	5.1 ± 0.4	7.8 ± 0.4	8 ± 0.6	248 ± 12

ESP methods exhibit good adhesion with respect to stem cells (Figs. 6e, f and 8c, d).

4 Discussion

It is known [1] that the process of forming porous structure of the polymers from the solution includes the following stages: the solution phase separation with the isolation of the gel particles, the substitution of the solvent by the precipitator with the formation of the pores. Phase separation of the polymer solution, the formation of gel particles with high density accompanied by increased turbidity of solution. Interaction of high heterogeneity density system with precipitator leads to the formation of pores of various shapes and sizes in the low density regions. Structural elements providing the strength properties of the porous material are formed from the high concentration regions.

The phase separation in the solution with following formation of gel particles was investigated using a light scattering method (Fig. 1). It is seen that the light

scattering curves have monotonous character, indicating a polydisperse distribution of the scattering particles. A main feature of these dependencies is a sharp increase in scattering intensity at the solution exposure time over 30 min (Fig. 1a). The sharp increase in the scattering intensity with the growth of the solution exposure time at T = 20 °C is caused by the formation of areas with high density. These scattering centers were gel particles that formed during phase separation.

The existence of two phases in the CoPA solution and their effect on the film porous structure is confirmed by the data of SEM studies. The coagulation of 10 wt% CoPA solution after its exposure on a glass substrate within 60 s leads to the formation of two kinds of structural elements having planar and spherical shape (Fig. 2). It is possible to assume that the particles of the planar form are the result of the coagulation of gel phase with reduced solvent content, but the spherical particles are formed during the coagulation of the solution with the reduced polymer content.

It follows from the phase diagram shown in Fig. 3 that below the curve 1 the solution is in a single-phase liquid state. Increasing the exposure time leads to the phase disintegration and formation of sol state (region between curves 1 and 2). Above the curve 2 the whole system of colloidal solution goes into gel state whose shear stress is $\tau = 440 \pm 42$ Pa. This value is much higher than similar values for both solution CoPA $\tau = 8.7 \pm 1.2$ Pa.

The CoPA solution of 20 % concentration and above it characterized by a narrow time interval of biphasic system existence. This makes it difficult to form a porous structure with the necessary parameters such as total porosity, average pore size, pore size distribution. The optimal time interval of the two phase existence corresponds to the solution concentration of 15–20 wt%.

The results described above are confirmed by the data of rheological measurements. The viscosity of the solution and its dependence on the shear rate, polymer concentration, temperature and, as shown by the studies of the phase separation, time is very important characteristic for obtaining the porous film material. The CoPA solutions in the ethanol–water mixtures having different concentrations can be described by Newton's equation for the liquids and there is no dependence of viscosity on the shear rate for them (Fig. 4a). From the results of measuring the viscosity of the CoPA solutions of various concentrations (Fig. 4b) is follows that the viscosity varies a little in the concentration range 15–20 %. It allows producing the films and fibers from the CoPA solutions.

The important factor influencing both the dissolution of polymer and the formation of films porous structure is the composition of solvent namely the ratio of ethanol/water. The dependence of the scattering intensity on time for solutions containing different proportions of alcohol-water makes it possible to construct a phase diagram of CoPA—solvent (ethanol content of the solvent) (Fig. 5). The homogeneous CoPA solution exists below the curve 2; gel is above the curve 1. The CoPA solution on the basis of the ethanol/water mixture, which contains 80 vol% of ethanol, is characterized by the maximum time interval in which two phases exist simultaneously: the gel particles and solution with the low polymer content. With an increasing exposure time t of the solution in the range $t_1 \leq t \leq t_2$ the gel phase fraction increases whereas the solution concentration decreases. The change of the relationship between phases can predict the pore volume and their average sizes.

It follows from the data shown in Fig. 5 that solutions CoPA, containing the mixture of alcohol–water in the ratio 70/30–90/10 as the solvent are more stable in time. These solutions have a maximum time of gel transition state; they are more suitable for the film production.

The films obtained from the 18 % solution and aged before precipitation over 1 min do not contain isolated spherical particles (Fig. 6). At the curing time less than 2 min, the film porous structure is heterogeneous and there is a wide pore size distribution from 1 to 50 μm. When the films are exposed for 2 min, the pores cover uniformly the entire surface of the film that is characterized by monodisperse pore sizes distribution (Fig. 7a) with an average pore size value of 1.3 μm. The films aged during 1–6 min in air and then placed in a precipitator (H_2O) have the well-developed porous structure (Fig. 6a, b, c). The size and number of pores decreases until complete disappearance by increasing the expose time up to 20 min or more (Fig. 6d). Prolonged exposure of the solution on a substrate in air leads to the formation of the gel over the entire volume and prevents the formation of pores in contact with the precipitant. As it follows from the Table 2 the strength, modulus and elongation at break increase with increasing the time of aging before precipitation due to the decreasing of total porosity of the films.

The water permeability of porous films depends on the exposure time of solution on a substrate before precipitation and the time of gel formation. The maximal water permeability is found for the films aged for 2 min prior to the precipitation and the lowest is for the films aged for 20 min (Table 1). The measurements have shown that an average value of vapor permeability for most CoPA porous films is 0.25 g/cm^2 per day. This value is close to values of vapor permeability of the materials used as wound dressings [26, 29].

The capability of CoPA solutions for phase separation, the dielectric characteristics of polyamides, environmental friendliness and a volatility of ethanol/water solvent make it possible to use them for processing the fibers by an ESP. During spinning the solution with 10–20 wt % CoPA concentration a bundle of defects in the form of the

extended drops is arise. Probably, in this case, the CoPA solutions of relatively low concentration do not possess dielectric characteristics necessary for the polarization of polymer in the electric field in order to form the accurate microjets. Precipitating out on the electrode, these microjets can form nanofibers. Incomplete solvent evaporation from the polymer solution during its deposition on the receiving electrode is the second possible reason for the presence of the defects at the nanofiber mat.

As it is seen in Fig. 8a, the CoPA concentration of 18 wt% in the solution (mixture ethanol/water = 80/20) is optimal to process uniform fibers without defects by ESP. The smallest fiber diameter about 200 nm is observed during forming the fibers from a solution of CoPA concentration as 10 wt%. The nanofibers of diameter about 4.0 μm are obtained from the solution of concentration as 30 wt%.

We have compared mechanical properties (Tables 2,4) and porous structure (Table 3) of the films processed by the coagulation and ESP methods. It follows from these data that the films based on nanofibers which were obtained from a 20 wt% solution have a maximum pore volume V_t, maximum value of porosity Por and minimum value of density d_{Hg}. The porous film obtained by the coagulation method has a lower porosity and higher density. An analysis of functions of the pore size distribution shows that the materials processed by ESP are characterized by a bimodal distribution (Fig. 7b). According to the method of mercury porosimetry the radius of the smaller pores (r_{mode}) is in the range of 1.3–2.7 μm. At the same time, the presence of the large pores with sizes from tens to hundreds μm is also typifying for these materials. This pore structure is clearly demonstrated in the SEM micrographs of the materials based on the nanofibers (Fig. 8a). It can be assumed that the smaller pores are caused by free volume between the separate layers of fibers, but large pores by interfiber space inside the individual layer.

Mechanical characteristics of two types porous materials as σ, E and ε including their standard deviations are shown in the Tables 2 and 4. The tests were carried out in dry condition and after aging the films in the physiological solution during 15 min—this simulates film behavior in a biological environment. The porous films demonstrate relatively low strength and Young modulus (Table 4). However, these mechanical characteristics give the possibility to manipulate successfully the porous films in a dry state or in a liquid medium. The films are flexible both in dry and wet condition and can duplicate well surface morphology. So the materials with these mechanical properties can be used as wound dressings.

The study of adhesion and proliferation of stem cells on the surface of CoPA porous films is of particular interest for cell technologies. CoPA macromolecule contains amide, carbonyl, carboxyl chemical bonds. The material based on CoPA in contrast to the fluorine-containing polymers and polylactides possesses some hydrophilic properties. Other side, the CoPA materials as compared with water soluble polymers (chitosan, PEO, PVA, etc.) retain their dimensions and properties in prolonged contact with aqueous media. These properties and the absence of solvent (ethanol) in the CoPA material after its processing makes it possible to assume a good adhesion and the high speed of the proliferation of cells on the surface and in the volume of the CoPA based materials.

The cultivation of the mesenchymal stem cells of adipose tissue (ASCs) was carried out in liquid nutrient medium. The holding time of the investigated sample in the matrix was varied from 1 h to 2 weeks. One can see (Fig. 6e, d) that the matrix surface is covered with cells, that testifies acceptable adhesion of stem cells to the matrix and the absence of its cytotoxicity. Thus, porous film materials based on CoPA obtained by coagulation method can be used as medical supplies, particularly wound dressings, as well as the matrices for cellular technologies.

According to SEM micrographs (Fig. 8a, b) it is evident by seen that the fiber diameter is 500–700 nm and the value of the interfiber space ensure a good contact of the cells pseudopodium with the fibers and the spreading of cells in the interfiber space (Fig. 8b). The cell surface contacts with liquid nutrient medium containing amino acids and ions. It is necessary for the cellular processes.

The optimum transport characteristics of material provide conditions for the cell proliferation are the high gas and water permeability, that are necessary for the occurrence of metabolic processes in the cell for its normal functioning. The SEM micrographs (Fig. 6e, f and 8c, d) demonstrate the CoPA matrix after its exposure in the cultural medium for 3 and 7 days. It is clearly seen that after 7 day of exposure the large part of the matrix surface and its near-surface layer are coated with stem cells. This indicates a high rate of the cell proliferation on the CoPA matrices processed by coagulation and ESP methods. The results on the study of adhesion and proliferation of stem cells on the CoPA porous films allow one to conclude the perspective of using these films for cellular technologies.

5 Conclusion

Summariseng the results described above, one can conclude that the porous structure and the transport properties of the film type materials processed from CoPA by the coagulation or ESP methods make it possible to use them as the two-dimensional matrices for the cellular technologies.

The porous structure of the film processed by the coagulation method depends on the concentration of the

CoPA solution, the time of exposure of the solution on a substrate and the composition of the solvent. The phase composition of the solution and its rheological characteristics determine the porosity of the films and their average pore size.

The films based on CoPA nanofibers processed by the ESP are characterized by the bimodal pore size distribution.

The porous film obtained by coagulation and ESP provide good adhesion and high proliferation rate of the mesenchymal stem cells.

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