

The preliminary studies of a structure and electrospinning of new polyurethanes based on synthetic atactic poly[(*R*, *S*)-3-hydroxybutyrate]

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Abstract. Novel polyurethanes based on synthetic, atactic poly[(*R*, *S*)-3-hydroxybutyrate] (a-PHB) and polycaprolactone (PCL) or polyoxytetramethylene (PTMG) diols were synthesized. It was shown that the presence of a-PHB within soft segments reduces crystallinity of PUR. Because of the low melting temperature for polyurethanes with PCL in soft segments, at this stage of work, electrospinning was limited to polyurethanes containing PTMG and a-PHB. Polyurethane containing 80% of PTMG and 20% of a-PHB was electrospun at various parameters from hexafluoro-2-propanole solution, resulting in formation of fibers with the average diameter ca. 2 μm . The fiber diameter decreased with decreasing polymer concentration in a solution and was practically insensitive to the needle-collector distance in the applied range of distances.

Key words: polyurethane, polyhydroxybutyrate, electrospinning, scaffolds.

1. Introduction

Taking into account limited accessibility of organs for transplantation the reconstruction of damaged fragment of soft tissue is the very effective way of its treatment, like in the case of myocardial infarction healing. There are a few ways of using biomaterials in tissue engineering: as biodegradable synthetic or natural scaffolds with pre-formed three-dimensional structures, or as materials with undefined structures, or as injectable biomaterials for *in situ* regeneration, or as temperature-responsive biomaterials that act as a substrate for cell sheet formation [1].

Biocompatibility of scaffolds depends on many parameters related to the material per se as well as to the specific structure formed in technological processes. A material used in making the scaffolds cannot be toxic, allergenic, cancero- and mutagenic. Its physical properties (solubility, thermal stability) ought to facilitate the processing into scaffolds, which can be made for instance by electrospinning. Electrospinning is a unique technology that can produce non-woven fibrous articles with fiber diameters ranging from tens of nanometers to microns. The fiber thickness and morphology can be controlled by many parameters, like concentration, viscosity, elasticity and surface tension of polymer solution, applied voltage, distance between the spinneret and the collector, temperature and humidity [2, 3]. The electrospun material should possess the appropriate physical, chemical, mechanical and degradation properties in order to promote cell attachment, proliferation,

organization and integration with surrounded tissues. So, using an appropriate material and conditions of electrospinning is the key to the success of this tissue engineering method.

In tissue engineering, some natural (collagen, gelatin, elastin, fibrinogen, silk fibroin, hyaluronic acid and alginate) and synthetic materials like aliphatic polyesters, polylactones, and polyurethanes are used [2, 4, 5].

Polyurethanes (PUR) are very promising material for the cardiac application as temporary scaffolds because of their high compatibility to living tissues, low induction of blood cell coagulation and their physical properties: softness and flexibility. The rate of polymer degradation can be controlled by using appropriate, degradable substrates for their synthesis. Whereas biocompatibility of polyurethane can be achieved by applying the natural or looks-like-natural substrate for its production, like vegetable oils, polylactide acids or polyhydroxyacids [6, 7].

Polyhydroxybutyrate is a biodegradable polyester, naturally synthesized by many microorganisms as a resource of carbon and energy. The product of its degradation – 3-hydroxybutyric acid - a common metabolite in human blood, is produced in ketone bodies of mammals during the prolonged starvation [8]. 3-hydroxybutyric acid belongs to short-chain fatty acids and reveals antibacterial activity [9]. The chemically synthesized substitute of natural PHB is atactic poly[(*R,S*)-3-hydroxybutyrate] (a-PHB). a-PHB is almost completely amorphous, which is close to its original state in the cell. Biocompatibility of its oligomers is proved [10].

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Preliminary investigations of polyurethanes based on a-PHB indicated that it is the biocompatible, biostatic and degradable material [11–13].

Electrospinning of polyurethanes using various solvents is described in literature [e.g. 14–17]. It should be noticed however, that all up to date results were obtained on polyurethanes without a-PHB in a structure. Up to date there were no attempts to electrospin new polyurethanes containing a-PHB in soft segments.

The aim of this work is to obtain preliminary information on a structure of novel polyurethanes containing atactic polyhydroxybutyrate as well as to verify at the first time possibility of electrospinning of this new polymer, synthesized for possible soft tissue engineering applications.

2. Materials

Telechelic, atactic poly[(*R,S*)-3-hydroxybutyrate], containing hydroxyl groups at both polymer chain ends (a-PHB) ($M_n=1730$) was obtained by anionic ring-opening polymerization of (*R,S*)- β -butyrolactone (Aldrich) in the presence of 3-hydroxybutyric acid sodium salt/18-crown-6 complex at room temperature and terminated with bromoethanol [18]. Before the synthesis, a-PHB, polycaprolactonediol (PCL) ($M_n=1900$) (Aldrich) and polyoxytetramethylenediol (PTMG) ($M_n=2000$)

(Aldrich) were dried by heating at 60–70°C under reduced pressure (1,4 hPa). 4,4'-methylene dicyclohexyl diisocyanate (H_{12} MDI) (Alfa Aesar), was purified via vacuum distillation. Chain extender 1,4-butanediol (1,4-BD) (Aldrich) was dried by an azeotropic distillation from benzene solution prior to use. Solvent dimethylformamide (DMF) (Labscan Ltd) was dehydrated over P_2O_5 and distilled under low pressure before synthesis. Catalyst: Stannous octoate (OSn) (Alfa Aesar) was used as received.

Synthesis of polyurethanes. Synthesis of polyurethanes was carried out in a two-step reaction at the vacuum reactor, according to procedure described previously [19]. Soft segments, which total concentration in polyurethane was 75%, were built of commercially available polyols: PCL or PTMG, blended with a-PHB (with 10 or 20% concentration of a-PHB in soft segments, see composition in Table 1). First, the prepolymer was prepared from polyols and H_{12} MDI, in a presence of catalyst (OSn), at reduced pressure (1,4 hPa) at molar ratio of NCO:OH in all prepolymers as 2:1. The reaction was carried out for 3 h at temperature range 60–70°C.

The prepolymer was then dissolved in DMF to solid mass concentration of 40% and the chain extender (1,4-BD) was added. Stoichiometric molar ratios of hydroxyl and isocyanate groups were kept at 1:1 to obtain linear polymer. Reaction was continued at 60°C for 2 h (Fig. 1).

Table 1
Composition of polyurethanes

PUR	Soft segments	Hard segments	Molar ratio of NCO:OH in prepolymer
PTMG80/PHB20	80% PTMG+20% a-PHB	H_{12} MDI+1,4-BD	2:1
PCL80/PHB20	80% PCL+20% a-PHB		
PCL90/PHB10	90% PCL+10% a-PHB		

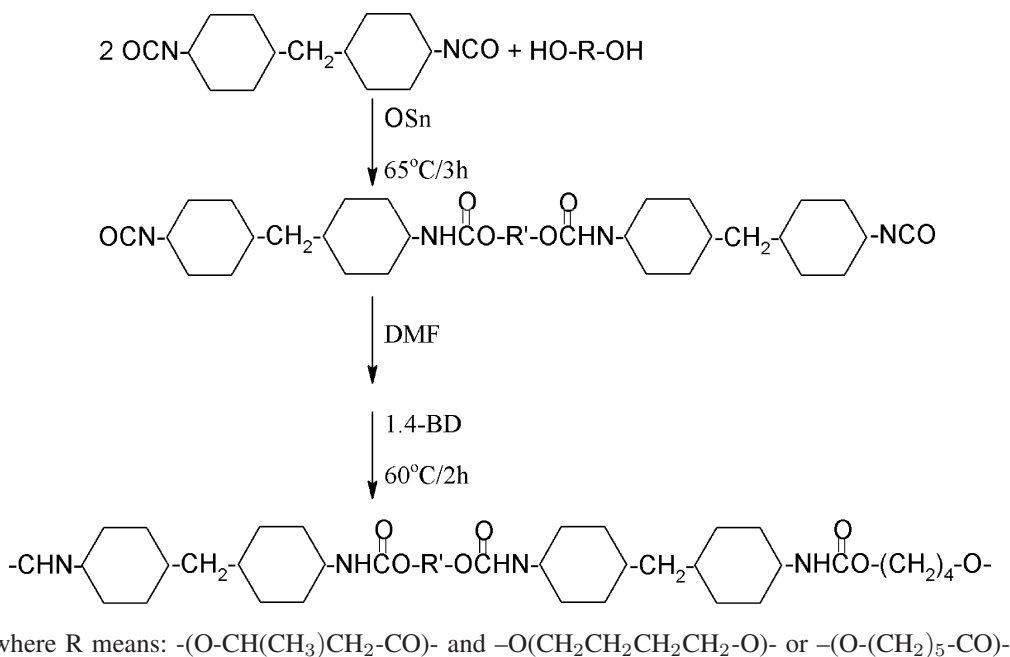


Fig. 1. Scheme of synthesis of polyurethane

Solution of polyurethane was poured on Teflon plates and heated at 80°C for solvent evaporation. Next, the foils were heated at 105°C in a vacuum dryer for 5 h.

Both types of polyurethanes, i.e. poly(ester urethane)s and poly(ester ether urethane) were investigated. In the case of poly(ester urethane)s, samples differing in ratio of PCL to a-PHB were obtained (sample PCL80/PHB20 with PCL/a-PHB = 80/20; sample PCL90/PHB10 with PCL/a-PHB = 90/10) while in the case of poly(ester ether urethane) the ratio of PTMG/a-PHB was 80/20 (sample PTMG80/PHB20).

3. Methods

The general scheme of experiments consisted of three stages:

- determination of polymer structure after synthesis in order to choose material for further electrospinning and planned biological experiments in vitro conditions,
- electrospinning,
- preliminary analysis of morphology of electrospun fibers.

3.1. DSC and WAXS analysis. The differential scanning calorimetry (DSC) and wide angle X-ray scattering (WAXS) analysis of PUR films was performed before electrospinning. In the case of DSC, Perkin-Elmer Pyris-1 instrument calibrated with indium was used. All scans were registered for samples weighted between 4 and 6 mg, during heating at 10°C/min, starting from 20°C. Samples were purged with gas nitrogen.

WAXS measurements were performed using the Bruker D8 Discover diffractometer. CuK α radiation, 1.5418 Å was used at the applied voltage 40 kV, and current 40 mA. All measurements were done in reflection mode using Bragg-Brentano geometry with 0.6 mm slit and two Soller collimators applied on both sides. The angular range of measurements, 2 θ , was between 10 and 30 deg, with a step of 0.02 deg and time of data accumulation at particular angular point 0.2 s.

3.2. Electrospinning. The electrospinning equipment in horizontal mode, consisting of a programmable single syringe pump (New Era Pump Systems Inc., model NE-1000), the grounded plate collector and the high voltage generator (self-constructed), connected with a stainless steel needle (Terumo Neolus 23G, 0.6 mm outer diameter \times 26 mm length) was used. The schematic diagram of electrospinning apparatus can be found in many publications [e.g. 3]. Electrospinning was performed from hexafluoro-2-propanole solution at concentrations ranging between 7.5 and 12.5% w/w, voltage of 10 kV, flow rate 500 μ l/h, needle to collector distance of 11 and 17 cm, resulting in nonwovens with random architecture, typical for plate collectors.

3.3. SEM analysis. Morphology of fibres was determined by scanning electron microscopy (SEM) (Hitachi TM3000 analytical tabletop microscope) using 5 kV accelerating voltage at 1650 mA filament current and 11.2 μ A emission current. Application of relatively low voltage allows to reduce induced

area, achieve higher resolution and gather more information from fibers surface. A mean fibre diameter was estimated from SEM images by averaging 20 measurements.

4. Results and discussion

The endothermic peaks related to crystal melting are observed on DSC scans of non-spun material (Fig. 2). The common peak for all samples is a high temperature peak with temperatures of extreme being 104.2, 106.2, and 107.2°C for samples PCL80/PHB20, PCL90/PHB10, and PTMG80/PHB20, respectively. The melting enthalpy, related to this peak is relatively low: 2.63, 3.15, and 2.42 J/g, for samples PCL80/PHB20, PCL90/PHB10, and PTMG80/PHB20, respectively. Much larger is the low temperature peak, being registered only for samples containing PCL in soft segments. The temperature of extreme of this low temperature peak is 42.5 and 46.1°C for PCL80/PHB20, and PCL90/PHB10, respectively with corresponding melting enthalpy of 21.8, and 28.76 J/g. This peak is completely absent for polymer with PTMG in soft segments.

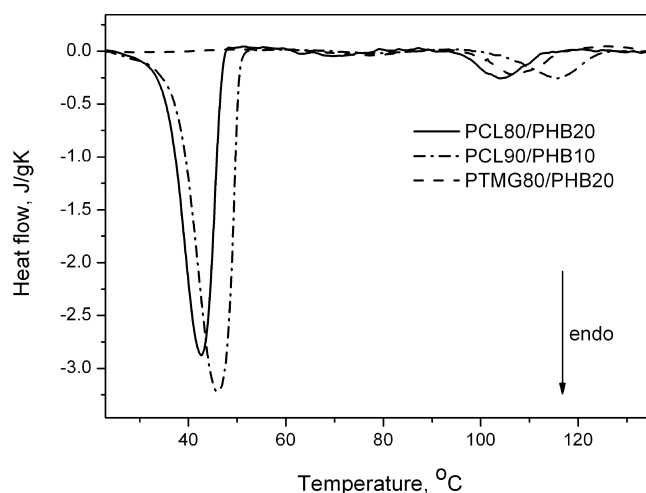


Fig. 2. DSC scan during heating of PUR films at 10°C/min

Figure 3 shows WAXS radial profiles of investigated samples. It is seen that in the case of samples containing PTMG there is broad amorphous halo only, while for samples with PCL there is additional narrow peaks attributed to diffraction from crystal phase of PCL. Crystallinity determined from the ratio of the crystal peaks area to the total scattering in the registered range of angles is 0.24 for PCL80/PHB20, and 0.36 for PCL90/PHB10. The shape of amorphous part of scattering indicates some bumps, particularly on the lower angle side for the sample with PTMG, indicating possibility either some inhomogeneity within amorphous phase or contribution of very low crystallinity being on the border of sensitivity of the method, which is evidenced by DSC (higher temperature melting peak). Additionally, it is seen small shift in angular position of crystal peaks between PCL80/PHB20 and PCL90/PHB10 samples. It can be due to disturbance of the PCL crystal lattice caused by various amount of PHB molecules.

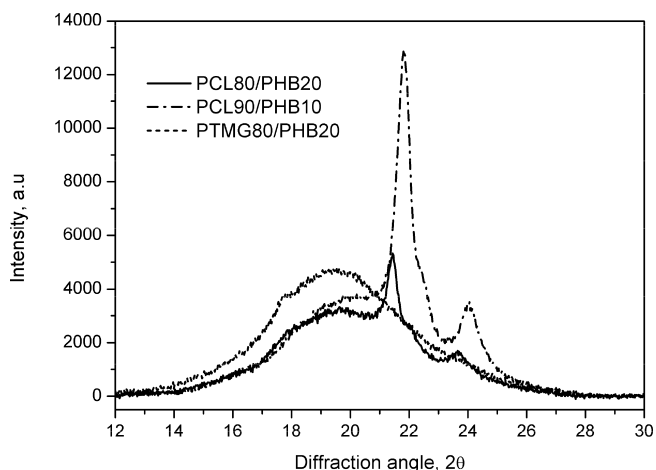


Fig. 3. WAXS radial profiles of PUR films

Without going into details of interpretation at this stage of work, the general conclusion from DSC and WAXS results is that polyurethanes containing PCL in soft segments have much higher ability for crystallization than those having PTMG in soft segments. Additionally, it is evident that an increase of α -PHB content results in reduction of crystallinity. Taking into account relatively low temperature of crystal melting for polyurethanes having PCL in soft segments we decided at this stage of work to focus on electrospinning of polyurethanes containing PTMG and α -PHB.

Figure 4 illustrates SEM images of nonwovens formed by electrospinning from PTMG80/PHB20 at various conditions while Table 2 shows an average diameter of obtained fibers.

Solution concentr., % w/w	Needle – collector distance, cm	
	11	17
7.5	 TM3000_4469 2012-09-27 10:57 F SD4,9 x1,0k 100 um	 TM3000_4461 2012-09-27 10:46 F SD4,9 x1,0k 100 um
10	 TM3000_4440 2012-09-27 10:15 F SD4,8 x1,0k 100 um	 TM3000_4447 2012-09-27 10:24 F SD4,8 x1,0k 100 um
12.5	X	 TM3000_4475 2012-09-27 11:08 F SD4,8 x1,0k 100 um

Fig. 4. SEM images of electrospun PTMG80/PHB20 fibers (concentration of polymer in solution and needle to collector distance, indicated)

Table 2

Mean fiber diameter estimated from SEM images (averaging from 20 measurements)

Solution concentration, % w/w	Fiber diameter, μm	
	11 cm	17 cm
7.5	2.17	2.022
10	2.403	2.269
12.5	x	2.273

Electrospinning of PTMG80/PHB20 from hexafluoro-2-propanole solution results in formation of relatively thick fibers with average diameter ca. $2 \mu\text{m}$. It is evident that the thickness increases with increasing polymer concentration in solution, that is expected as an effect of increasing viscosity [e.g. 14, 16], while fiber diameter is practically insensitive to the needle-collector distance, at least in the applied range of distances. At the highest applied concentration, 12.5% w/w, the viscosity is very high resulting in non-stable, impulsive electrospinning, resulting often in a non-fiber structure. Therefore, at this polymer concentration it was impossible to obtain fibers at 11 cm needle – collector distance, and those electrospun at 17 cm distance should not be considered as a reference result. Observations of morphology of nonwovens shows rather round shapes of contact points between fibers suggesting that the fibers approaching collector still contain solvent.

5. Conclusions

Applying of two-step reaction of polyurethane synthesis allowed to obtain the linear elastomeric material. It was shown for the first time that the use of atactic PHB results in reduction of crystallinity of PUR. Additionally it is evident that crystallinity of PUR containing PCL within soft segments is much higher than for material with PTMG. Our investigations allowed to select the promising material synthesized by us for electrospinning and further studies on its application possibility in tissue engineering. According to our results, polyurethanes containing PTMG and a-PHB in soft segments are spinnable in an electric field, showing at the same time thermal stability (no phase transitions) in the temperature range up to 95°C . Attempts of electrospinning allowed to determine a processability window for the process with hexafluoro-2-propanole as a solvent. Electrospinning of PTMG80/PHB20 from hexafluoro-2-propanole solution results in formation of fibers with an average diameter ca. $2 \mu\text{m}$. Further attempts of electrospinning using other solvents and instrument parameters offer potential possibility to reduce a fiber diameter.

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REFERENCES

- [1] I.C. Parrag, "The development of elastomeric biodegradable polyurethane scaffolds for cardiac tissue engineering", *PhD Thesis*, University of Toronto, Toronto, 2010.
- [2] D. Liang, B.S. Hsiao, and B. Chu, "Functional electrospun nanofibrous scaffolds for biomedical applications", *Advanced Drug Delivery Reviews* 59, 1392–1412 (2007).
- [3] N. Bhardwaj and S.C. Kundu, "Electrospinning: a fascinating fiber fabrication technique", *Biotechnology Advances* 28, 325–347 (2010).
- [4] S.A. Sell, M.J. McClure, K. Garg, P.S. Wolfe, and G.L. Bowl-in, "Electrospinning of collagen/biopolymers for regenerative medicine and cardiovascular tissue engineering", *Advanced Drug Delivery Reviews* 61, 1007–1019 (2009).
- [5] S. Agarwal, J.H. Wendorff, and A. Greiner, "Use of electrospinning technique for biomedical applications", *Polymer* 49, 5603–5621 (2008).
- [6] A.E. Hafeman, K.J. Zienkiewicz, A.L. Zachman, H.-J. Sung, L.B. Nannay, J.M. Davidson, and S.A. Guelcher, "Characterization of the degradation mechanisms of lysine-derived aliphatic poly(ester urethane) scaffolds", *Biomaterials* 32, 419–429 (2011).
- [7] W. Ou, H. Qiu, Z. Chen, and K. Xu, "Biodegradable block poly(ester-urethane)s based on poly(3-hydroxybutyrate-co-4-hydroxybutyrate) copolymers", *Biomaterials* 32, 3178–3188 (2011).
- [8] L.J.R. Foster and B.J. Tighe, "Centrifugally spun polyhydroxybutyrate fibres: accelerated hydrolytic degradation studies", *Polymer Degradation and Stability* 87, 1–10 (2005).
- [9] T. Defoirdt, N. Boon, P. Sorgeloos, W. Verstraete, and P. Bossier "Short-chain fatty acids and poly- β -hydroxyalkanoates: (new) biocontrol agents for a sustainable animal production", *Biotechnology Advances* 27, 680–685 (2009).
- [10] V. Piddubnyak, P. Kurcok, A. Matuszowicz, M. Głowała, A. Fiszler-Kierzkowska, Z. Jedliński, M. Juzwa, and Z. Krawczyk, "Oligo-3-hydroxybutyrate as potential carriers for drug delivery", *Biomaterials* 25, 5271–5279 (2004).
- [11] J. Brzeska, H. Janik, M. Kowalczyk, and M. Rutkowska, "Preliminary investigations of biocompatibility of polyurethanes based on synthetic polyhydroxybutyrate", *Engineering of Biomaterials* 106–108 (XIV), 65–72 (2011).
- [12] J. Brzeska, H. Janik, M. Kowalczyk, and M. Rutkowska, "Influence of polyurethanes based on synthetic poly([R,S]-3-hydroxybutyrate) on microorganisms growth", *Engineering Biomaterials* 106–108 (XIV), 73–78 (2011).
- [13] J. Brzeska, P. Dacko, A. Heimowska, H. Janik, M. Kowalczyk, and M. Rutkowska, "Flexibility to degradation of polyurethanes with synthetic polyhydroxybutyrate in oxidizing and hydrolytic environment", *Protection Against Corrosion* 1, 8–14 (2012), (in Polish).
- [14] M.M. Demir, I.Yilgor, B.Yilgor, and B.Erman, "Electrospinning of polyurethane fibers", *Polymer* 43, 3303–3309 (2002).
- [15] P.C. Caracciolo, V. Thomas, Y.K. Vohra, F. Buffa, and G.A. Abraham, "Electrospinning of novel biodegradable poly(ester urethane)s and poly(ester urethane urea)s for soft tissue-engineering applications", *J Mater Sci: Mater Med* 20, 2129–2137 (2009).
- [16] P.N. Shah, R.L. Manthe, S.T. Lopina, and Y.H. Yun, "Electrospinning of L-tyrosine polyurethanes for potential biomedical applications", *Polymer* 50, 2281–2289 (2009).

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- [17] S.A. Theron, E. Zussman, and A.L. Yarin, “Experimental investigation of the governing parameters in the electrospinning of polymer solutions”, *Polymer* 45, 2017–2030 (2004).
- [18] H. Arslan, G. Adamus, B. Hazer, and M. Kowalczyk, “Electrospray ionisation tandem mass spectrometry of poly[(R,S)-3-hydroxybutanoic acid] telechelics containing primary hydroxy end groups”, *Rapid Commun. Mass Spectrom.* 13, 2433–2438 (1999).
- [19] J. Brzeska, P. Dacko, H. Janik, M. Kowalczyk, and M. Rutkowska, “Biodegradable polyurethanes and the way of their production”, Patent No 212763, (2012), (in Polish).