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Novel electrospun poly(ε-caprolactone)-based bicomponent nanofibers possessing surface enriched in tertiary amino groups

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Abstract

For the first time preparation of electrospun poly(ε-caprolactone) (PCL) based nanofibers possessing surface enriched in tertiary amino groups is shown. For that purpose the pair PCL and poly(ε-caprolactone)-*b*-poly[(2-dimethylamino)ethyl methacrylate] (PCL-*b*-PDMAEMA) diblock copolymers was used. PCL-*b*-PDMAEMA copolymers were synthesized using a combination of ring-opening polymerization and atom transfer radical polymerization (ATRP). Nanofibers with mean diameters ranging from 400 to 800 nm were obtained. Their morphology was evaluated by scanning electron (SEM) and atomic force microscopy (AFM). It was found that the morphology of the fibers depended on the weight ratio between the partners and the length of the PDMAEMA-block in the copolymers. The enrichment of the fiber surface in tertiary amino groups was studied by X-ray photoelectron spectroscopy (XPS). Increasing the copolymer content and the length of the PDMAEMA-block led to increase of the amount of tertiary amino groups on the fiber surface. The AFM analyses of the mechanical properties of the fiber surface showed that increasing the copolymer content led to decrease of the surface stiffness. The increase of the copolymer content led also to decrease of the melting temperature and the crystallinity degree in respect to PCL from the (co)polymer as determined by differential scanning calorimetry.

Keywords: Block copolymers; Electrospinning; Nanocharacterization; Nanofibers; Poly(ε-caprolactone)

1. Introduction

The electrospinning is one of the most promising techniques for preparation of micro- and nanofibers of polyesters like poly(ε-caprolactone) (PCL), polylactide and polyglycolide [1–3]. Such fibers gained significant interest due to the possibility to be used as scaffolds for cell and tissue engineering. PCL is approved as a biodegradable material compatible with soft and hard tissues, and it has found application for design of various devices used in the biomedical field [4]. During the last years special attention has been paid onto the preparation of fibers with specific surface chemistry [5,6]. The modification of the surface of polyester materials is of special interest in terms of their biological behavior.

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It is known that the hydrophobic nature of the polyesters is the reason for the adhesion of pathogenic microorganisms onto implants prepared from them [7]. In consequence, formation of a bacterial biofilm which can lead to implant failure is possible. Very recently some of us have shown an easily achieved method for surface modification of polyester fibers by their coating with a thin chitosan film [8]. It has also been shown that the physical coating of the fibers with chitosan leads to preparation of new materials possessing hemostatic and antibacterial properties.

Poly[(2-dimethylamino)ethyl methacrylate] (PDMAEMA) and its quaternized derivatives have inherent antibacterial activity [9,10]. These properties have been recently considered for surface modifying of polyolefinic materials by grafting of PDMAEMA polymer chains in a controlled manner using atom transfer radical polymerization (ATRP) [10]. The preparation of PDMAEMA-based fibers by electrospinning of its aqueous solution in presence of NaCl [11] as well as of commercially available methacrylate DMAEMA-based copolymers (known under the trade name Eudragit®) by electrospinning of their ethanol solutions [12] has already been reported. However, in this case only water-soluble mats are obtained. It might be expected that the electrospinning of PCL and its block copolymers with PDMAEMA using their blend solutions in a common solvent is an easily feasible way for preparation of hybrid water-insoluble fibrous materials. This would lead to combination of the beneficial physico-mechanical properties of PCL and the biological behavior of PDMAEMA in a one-step procedure. The preparation of electrospun fibers of polyelectrolytes may be facilitated by electrospinning of mixed solutions of the polyelectrolyte with a non-ionogenic polymer [13–17].

The possibility for preparation of novel PCL-based bicomponent nanofibers with surface enriched in tertiary amino groups by electrospinning of mixed solutions of PCL and its block copolymers with PDMAEMA is shown in the present study. The morphology, the surface composition and stiffness, and the thermal behavior of the fibers were investigated.

2. Experimental section

2.1. Materials

ε-Caprolactone (CL, Acros, 99%) was dried over calcium hydride (CaH_2) for 48 h at room temperature and then distilled under reduced pressure before use. Aluminum triisopropyloxide ($Al(O^iPr)_3$, Acros, 98%) was distilled under vacuum, quenched in liquid nitrogen, rapidly dissolved in dry toluene, and then stored under nitrogen atmosphere. The 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, Aldrich, 97%) and copper bromide (CuBr, Fluka, 98%) were used without further purification. (2-Dimethylamino)ethyl methacrylate (DMAEMA, Aldrich, 98%) was passed through a column of basic alumina to remove the stabilizing agents and then stored under nitrogen atmosphere at −20 °C. Toluene (Labscan, 99%) was dried by refluxing over CaH_2 and then distilled before utilization. Tetrahydrofuran (THF) was passed through a column of basic alumina. Poly(ε-caprolactone) (PCL, CAPA 6800) was purchased from Solvay Interox, UK; dichloromethane (CH_2Cl_2) and fluorescein disodium salt – from Fluka, and used as received.

2.2. Synthesis and characterization of PCL-*b*-PDMAEMA diblock copolymers

PCL-*b*-PDMAEMA diblock copolymers were synthesized by three-step procedure described in details elsewhere [18]. In a first stage the PCL block was prepared by ring-opening polymerization using $Al(O^iPr)_3$ as an initiator leading to the formation of PCL—OH. The second step was the quantitative conversion of PCL—OH into PCL—Br. The last step was ATRP of DMAEMA initiated by PCL—Br. Adjusting the initial amount of monomer in the reaction medium allowed the modification of the length of PDMAEMA-block. The polymerization was performed in THF at 60 °C in the presence of a CuBr/HMTETA catalytic system under nitrogen. The composition of the diblock copolymers was determined by ^1H NMR (BRUKER 500 MHz in $CDCl_3$ using 0.03% tetramethylsilane as a standard). The number-average molar mass of the diblock copolymers was calculated using the relative intensity of —NCH_2-protons in the DMAEMA residues at 2.6 ppm and that of the —CH_2-protons of the PCL block at 1.65 ppm. Size exclusion chromatography (SEC) analyses of the macroinitiator PCL—Br- and PCL-*b*-PDMAEMA-block copolymers were performed in THF (1 ml/min) using a PL-Gel® 10 μm (50 mm × 7.5 mm) precolumn and two PL-Gel® 10 μm mixed-B (300 mm × 7.5 mm) gradient columns. The molar mass was calculated with reference to polystyrene standards. The SEC chromatograms of the PCL—Br macroinitiator showed unimodal peaks with a polydispersity index lower than 1.2.

2.3. Preparation of fibrous mats from PCL and PCL/PCL-b-PDMAEMA by electrospinning

PCL fibers were prepared by electrospinning of its solutions in CH_2Cl_2 (PCL concentration – 7.5 or 11 wt.%). PCL/PCL-*b*-PDMAEMA fibers at different $(PCL^{homo} + PCL^{block})/PDMAEMA^{block}$ weight ratios (Table 2) were prepared by electrospinning of their mixed solutions in CH_2Cl_2 at a constant total PCL concentration – both from the homo- (PCL^{homo}) and copolymer (PCL^{block}), of 7.5 wt.%. Typically, PCL or PCL/PCL-*b*-PDMAEMA solutions were placed in a syringe (5 ml) equipped with a conical nozzle. The nozzle was connected to an electrode via an alligator clip. The electrode was connected to a high-voltage power supply generating positive DC voltage up to 30 kV. For the easier collecting of the electrospun mats aluminum plates with size 10 × 10 mm were placed on the collector. The spinning solution was delivered at a controlled feed rate of 1.84 ml/h at a constant value of the applied voltage (15 kV) and at a constant distance between the tip of the conical nozzle and the collector (15 cm). The electrospun samples were placed under vacuum for 72 h to remove any solvent residues.

2.4. Characterization of the electrospun PCL and PCL/PCL-b-PDMAEMA fibers

The morphology of the electrospun fibers was observed by scanning electron (SEM) and atomic force microscopy (AFM). For the performance of the SEM analyses the mats were vacuum-coated with carbon and gold and analyzed using SEM Philips 515. The fiber morphology was evaluated in terms of the criteria for complex evaluation of electrospun mats reported elsewhere [19] using Image J software [20] by measuring at least 20 fibers from each SEM image.

The AFM analyses were performed with a Nanoscope IIIa from Digital Instruments operating in tapping mode at room temperature. The imaged fibers were transferred on slightly humidified mica. A commercial silicon tip-cantilever was used, with a stiffness of about 40 N/m, a measured frequency of 180,066 Hz and a quality factor of 470. All the images were recorded "as is" without any filter or image treatment. Height images were recorded at 90° scanning direction at a given setpoint and a fixed frequency.

The stiffness of the fiber surface was evaluated by AFM recording approach-retract curves using a procedure described by Kopp-Marsaudon et al. [21]. When recording approach curves, the sample was moved up and down (in the Z direction) at a fixed X, Y location of the surface. The amplitude variation was recorded versus the vertical displacement of the piezoactuator holding the sample to demonstrate the stiffness variation of the fiber surface. Typical experimental conditions were 0.5 Hz for the vertical movement frequency and 20 nm for the vertical extension. All the curves shown in this work have been normalized on the mica approach curves to remove the various effects as the dimension tip. For comparison, films of different composition were prepared by casting on mica of 30 μl of concentrated solution (0.5 wt.%) in dichloromethane. The thickness of these films was more than 200 nm and thus was not affected by the mica nature.

For detection of the tertiary amino groups on the fibers surface the fibrous mat of PCL or PCL/copolymer was immersed in 10 ml 0.02 wt.% aqueous solution of fluorescein for 7 h and then washed repeatedly with distilled water.

The surface chemical composition of the fibers was determined quantitatively by X-ray photoelectron spectroscopy (XPS) using VG ESCALAB 220iXL spectrometer with monochromatic Al Kα X-ray source ($h\nu = 1486.6$ eV) of 250 W. Quantitative elemental conditions were determined from peak areas using experimentally determined sensitivity factors and the spectrometer transmission function. The high-resolution spectra were dissected by means of special deconvolution software package. The free parameters of component peaks were their binding energy, height, full width at a half-maximum, and the Gaussian/Lorentzian ratio.

The differential scanning calorimetry (DSC) was performed on DSC Q100 equipment in the temperature range from −80 to 100 °C for a heating rate of 10 °C/min under nitrogen flow.

3. Results and discussion

In the present study diblock copolymers composed of PCL and PDMAEMA blocks (PCL-*b*-PDMAEMA) were synthesized in a controlled manner using a three-step procedure described in details elsewhere [18]. Briefly, in a first stage PCL was prepared by ring-opening polymerization of ε-caprolactone and then converted into α-isopropoxyloxy-ω-bromoisobutyrate PCL (PCL—Br) which served at the next step as a macroinitiator for the atom transfer radical polymerization (ATRP) of DMAEMA. PCL homo- and block copolymers are presented in Scheme 1.

The macromolecular characteristics of PCL and the copolymers used in the present study are listed in Table 1.

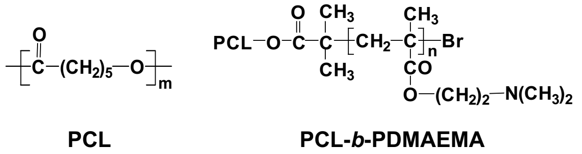
The copolymers consisted of 58 wt.% and 68 wt.% PDMAEMA are further denoted as B60 and B70, respectively.

3.1. Preparation of PCL and PCL/PCL-b-PDMAEMA fibers by electrospinning of their solutions in dichloromethane

The preliminary experiments on fiber formation using 20 wt.% solutions of PCL-*b*-PDMAEMA alone (B60 and B70, Table 1) in dichloromethane (CH_2Cl_2) by electrospinning were unsuccessful. It is known that the molar mass of the polymers is one of the main parameters that significantly affect the possibility of fiber formation by electrospinning as well as the morphology of the obtained fibers. Recently, it has been shown that fibers can be prepared by electrospinning of solutions with polymer concentration equal to or higher than the entanglement concentration [23,24]. Using the dependence of the solution entanglement number $(n_e)_{soln}$, the weight-average molar mass $\overline{M}_w$ and the entanglement molar mass (M_e, according to the literature data) for moderately concentrated and concentrated solutions presented elsewhere [24] the polymer concentration at which fibers are obtained by electrospinning of a certain polymer can be predicted. It is known that the M_e value of PCL is equal to 3000 g/mol [25]. Taking into account that $\overline{M}_w$ of PCL—Br used as macroinitiator in this work was 6000 g/mol the polymer concentration in respect to PCL-block at which the formation of fibers from the copolymers by electrospinning is possible is equal to 100 wt.%. That is why the electrospinning of the presented in Table 1 block copolymers alone practically cannot be achieved. Recently, some of us have shown that fullerene-containing fibers can be prepared by simultaneous electrospinning of chloroform solutions of both PCL and fullerenes modified with PCL or polyoxyethylene chains [26,27]. In the present study we applied the same approach for preparation of PCL fibers containing DMAEMA-units by electrospinning of the homopolymer PCL with $\overline{M}_w = 120{,}000$ g/mol together with the block copolymer B60 or B70 using mixed PCL/copolymer solutions. The data obtained for the mean fiber diameter and mean defects sizes depending on the spinning solution composition and total polymer concentration are summarized in Table 2.

SEM and 3D- and height AFM micrographs of PCL fibers prepared by electrospinning of its solutions in CH_2Cl_2 at polymer concentration of 11 wt.% and 7.5 wt.% are shown in Fig. 1.

As far as PCL with $\overline{M}_w = 120{,}000$ g/mol is concerned the threshold concentration at which continuous fibers can be formed by electrospinning is 5 wt.%. Depending on the initial PCL concentration fibers differing significantly in their morphology and mean diameters are obtained. The electrospinning of 11 wt.% PCL solutions resulted in defect-free porous fibers. The pores along the fibers had elliptic shape. The higher the PCL fiber diameter the higher was the pore size. For example, the mean pore length along the axis of the fiber with the highest diameter presented in Fig. 1A is ca. 970 nm and the mean pore width – 420 nm. As seen from the 3D image of a PCL fiber it has a rough surface as a result of the presence of pores. The electrospinning of 7.5 wt.% PCL



Scheme 1. Formulae of the (co)polymers used for preparation of fibers by electrospinning

Table 1
Macromolecular characteristics of PCL (from Solvay Interox, UK) and PCL-*b*-PDMAEMA used in the present study

(Co)polymer	PDMAEMA[a]			PCL$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$
	F_w [%]	$\overline{DP}$	$\overline{M}_n$		
PCL	—	—	—	69,000[b]	1.74[b]
B60	58	44	6900	5000[a]	1.26[c]
B70	68	69	10,800	5000[a]	1.24[c]

[a] Weight fraction (F_w), degree of polymerization ($\overline{DP}$) and number-average molar mass ($\overline{M}_n$, g/mol) of PDMAEMA and PCL blocks as determined by ^{1}H NMR spectroscopy in $CDCl_3$.

[b] According to Solvay specification [22].

[c] Polydispersity index of the block copolymers as determined by SEC in THF (+2 wt.% NEt_3) using polystyrene standards.

Table 2
Mean fiber diameter and mean defects sizes depending on the spinning solution composition and total polymer concentration

Spinning solution composition	Total polymer concentration [wt.%]	$(\mathrm{PCL}^{\mathrm{homo}} + \mathrm{PCL}^{\mathrm{block}})/\mathrm{PDMAEMA}^{\mathrm{block}}$ weight ratio [w/w][b]	Mean fiber diameter [nm ± SD]	Defects sizes: length/width [μm ± SD]
PCL	11.0	–	4000 ± 2000	No defects
PCL	7.5	–	615 ± 196	20 ± 7/8 ± 2
PCL/B60[a]	10.5	2.5/1	470 ± 196	11 ± 2/5.7 ± 1.4
PCL/B60[a]	15.0	1/1	850 ± 584	26 ± 5/16 ± 5
PCL/B70[a]	11.2	2/1	390 ± 163	9.6 ± 3/4.7 ± 2
PCL/B70[a]	15.0	1/1	635 ± 360	12 ± 2.5/5.6 ± 1.2

[a] Total PCL concentration (from the homo- and copolymer) is equal to 7.5 wt.%.

[b] Weight ratio of the total PCL weight (calculated as the sum of the weights of PCL homopolymer and of PCL-block) in respect to the weight of the PDMAEMA-block.

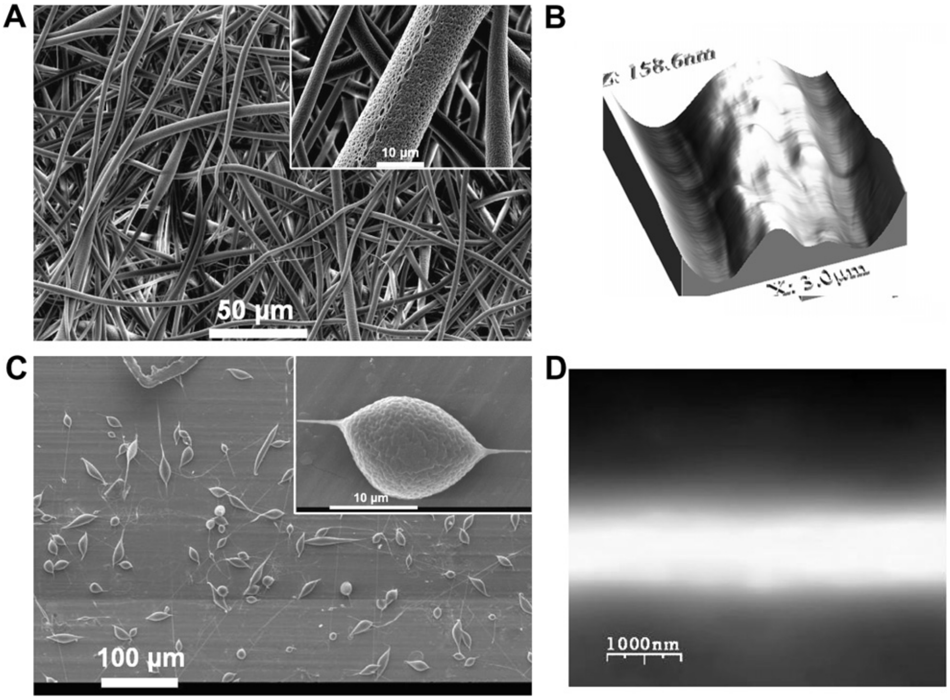


Fig. 1. SEM (A, 500× and 2500×; C, 200× and 5000×) and 3D (B) and height (D) AFM micrographs of electrospun PCL fibers prepared at polymer concentration: 11 wt.% (A, B) and 7.5 wt.% (C, D).

solutions led to fibers with lower mean diameters and spindle-like defects along their axis. As seen from the SEM and height AFM micrographs the fibers in the defect-free part had a smooth surface while the defects were highly porous similarly to the PCL fibers prepared at higher polymer concentration. The obtained results are in good agreement with the dependence of the morphology and the mean diameters of the electrospun fibers on the polymer concentration [24] as well as with the data for electrospinning of PCL in CH_2Cl_2 [28]. The electrospinning of PCL solutions at polymer concentration close to the threshold one for fiber formation yielded nanosized fibers with defects along their length. By increasing the polymer concentration defect-free fibers with mean diameters in the microscale, broad diameter distribution and porous surface were obtained.

SEM and height AFM micrographs of fibers prepared by electrospinning of PCL/B60 mixed solutions at weight ratios of the total PCL weight (calculated as the sum of the weights of PCL homopolymer and of PCL-block) in respect to the weight of the PDMAEMA-block $(\mathrm{PCL}^{\mathrm{homo}} + \mathrm{PCL}^{\mathrm{block}})/\mathrm{PDMAEMA}^{\mathrm{block}} = 2.5/1$ or 1/1 (w/w) are shown in Fig. 2.

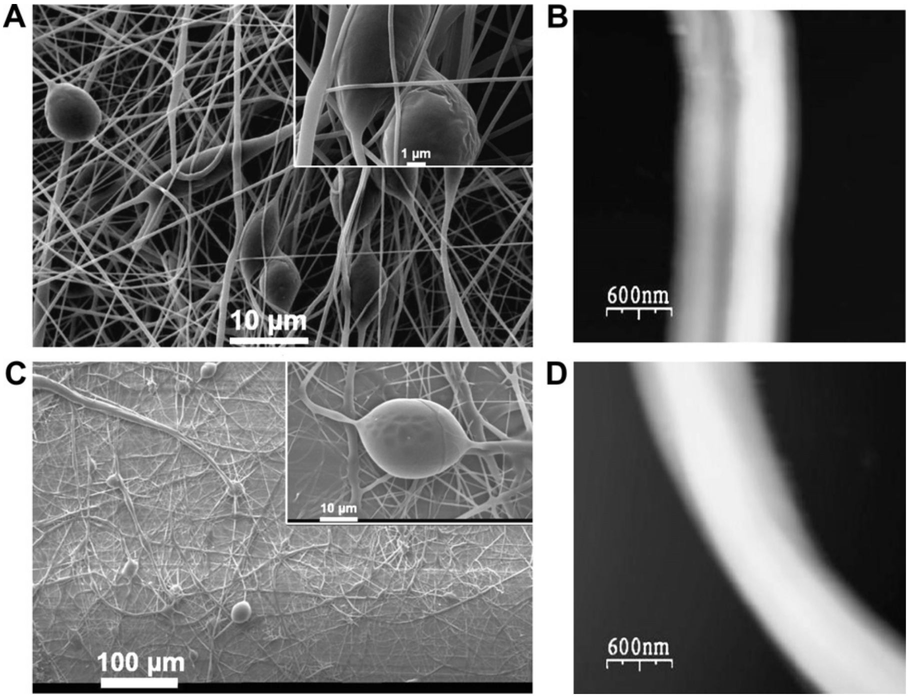


Fig. 2. SEM (A, 2000× and 10,000×; C, 200× and 2000×) and height AFM (B, D) micrographs of PCL/B60 fibers electrospun at weight ratios: (A, B) PCL/B60 = 1/1 [($PCL^{homo} + PCL^{block}$)/$PDMAEMA^{block}$ = 2.5/1 (w/w); total polymer concentration – 10.5 wt.%]; (C, D) PCL/B60 = 1/6 [($PCL^{homo} + PCL^{block}$)/$PDMAEMA^{block}$ = 1/1 (w/w); total polymer concentration – 15 wt.%].

Comparing the obtained fibers at weight ratio of 2.5/1 and total polymer concentration 10.5 wt.% with the PCL ones (polymer concentration – 11 wt.%) it is seen that the presence of PDMAEMA-segments leads to non-porous fibers with lower mean diameter (Table 2). However, they have spindle-like defects along their length. As seen from the AFM image (Fig. 2B) of a fiber with diameter of 1100 nm the fibers in their defect-free section have smooth surface. Noteworthy, in contrast to the electrospinning of PCL alone at polymer concentration of 7.5 wt.%, the defects along the PCL/B60 fiber length were with smooth surface and their mean sizes are lower (Table 2). These differences may be attributed to the presence of PDMAEMA in the fiber composition and the higher total polymer concentration of the spinning solution. The electrospinning of PCL/B60 pair at weight ratio ($PCL^{homo} + PCL^{block}$)/$PDMAEMA^{block}$ = 1/1 resulted in fibers with mean diameters and defects sizes higher than those obtained at weight ratio of 2.5/1. This is due to the higher total polymer concentration of the spinning solution containing the polymer partners at weight ratio of 1/1.

In a next series of experiments the effect of the PDMAEMA-block length in PCL-*b*-PDMAEMA on the morphology of PCL/copolymer fibers was evaluated using a copolymer with almost twice higher length of the PDMAEMA-block (B70). SEM and height AFM micrographs of the fibers prepared by electrospinning of PCL/B70 at weight ratio ($PCL^{homo} + PCL^{block}$)/$PDMAEMA^{block}$ = 2/1 or 1/1 (w/w) are presented in Fig. 3.

Non-porous spindle-like defects along the length of the non-porous fibers were observed again. The mean fiber diameter prepared at weight ratio of 2/1 (w/w) was slightly lower compared to fibers containing the copolymer B60 at similar weight ratio (Table 2). The maximum measured value of the mean diameter is ca. 740 nm. An AFM image of a fiber with such diameter is shown in Fig. 3B. Successful preparation of PCL/B70 fibers at ($PCL^{homo} + PCL^{block}$)/$PDMAEMA^{block}$ = 1/1 (w/w) was achieved as well (Fig. 3C and D). The mean diameter of the fibers defect-free section is twice higher as compared to those prepared at weight ratio of 2/1 due to the higher total polymer concentration used for preparation of the fibers at weight ratio of 1/1.

Comparing the fibers of PCL/B70 and PCL/B60 prepared at weight ratio ($PCL^{homo} + PCL^{block}$)/$PDMAEMA^{block}$ = 1/1 and total polymer concen-

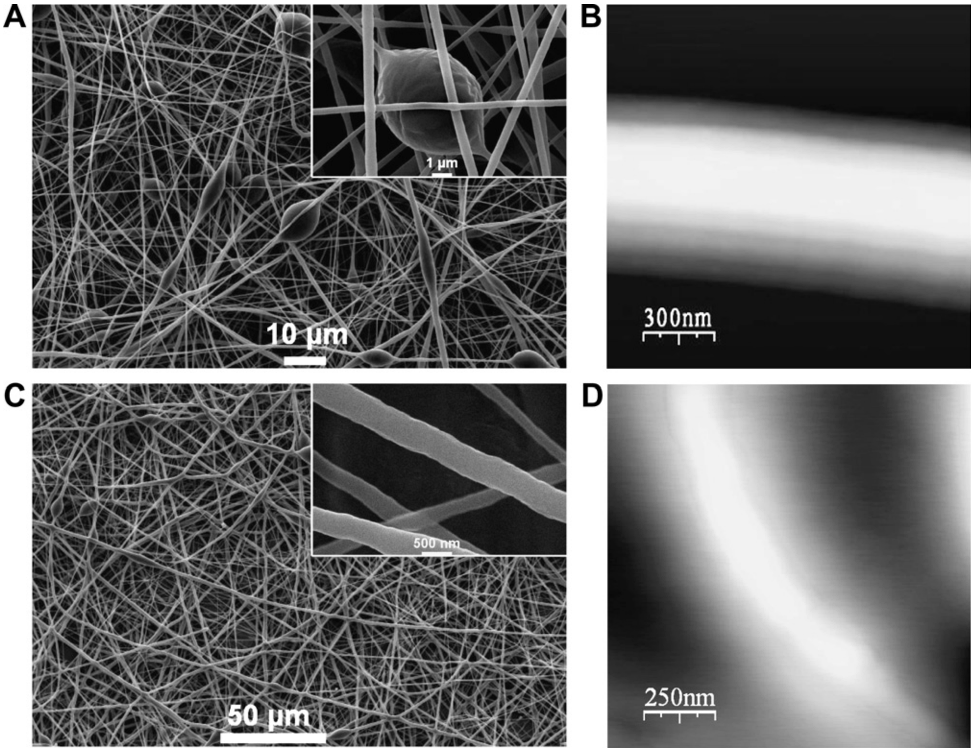


Fig. 3. SEM (A, 1000× and 10,000×; C, 500× and 30,000×) and height AFM (B, D) micrographs of PCL/B70 fibers electrospun at weight ratios: (A, B) PCL/B70 = 1/1 [(PCL^{homo} + PCL^{block})/PDMAEMA^{block} = 2/1 (w/w); total polymer concentration – 11.2 wt.%]; (C, D) PCL/B70 = 1/3 [(PCL^{homo} + PCL^{block})/PDMAEMA^{block} = 1/1 (w/w); total polymer concentration – 15 wt.%].

tration of 15 wt.% it is seen that the former display lower mean diameters and defects sizes (Table 2). This is evidence that the higher length of PDMAEMA-block in B70 provokes diminution of the fiber diameter and the defects sizes. Furthermore, the preparation of fibers with lower mean diameters can be attributed to the observed more rapid fiber formation onto the collector in the presence of B70 as compared to the electrospinning of B60-containing solutions. The studied copolymers contain polycationic PDMAEMA-block. The configuration for generation of the electric field for the electrospinning process was +/− (the anode was connected to the feeding solution, and the cathode – to the collector). It can be assumed that the observed faster fiber formation using the copolymer B70 is due to the much higher amount of DMAEMA-units and is a result of the stronger attraction between B70 and the negatively charged collector, than in the case of B60 copolymer, similarly to the recently reported case of electrospinning of oligopeptide copolymers [6]. Moreover, during the jet flow the organic solvent evaporates. At the jet-air interface hydrophilicity-driven orientation of the hydrophilic PDMAEMA-segments can take place. The hydrophilicity is increased by the electrical charges induced by the high-voltage field. A change in the surface tension may also contribute to the segregation. On a next stage studies on the surface chemical composition of the fibers were performed.

3.2. Effect of the PCL-b-PDMAEMA content in PCL-based fibers on the surface chemistry and mechanical behavior of the fibers as well as on their thermal behavior

As mentioned above the application of the approach of electrospinning of mixed solutions of PCL homo- and copolymers is a suitable way for the preparation of fibers with modified chemical composition. The presence of tertiary amino groups from PDMAEMA in the fibers obtained in the present study is easily demonstrated by their coloring by immersion of the fibrous mats into aqueous solution of the negatively charged dye fluorescein [8,10]. Fluorescein has a carboxylic group that can interact

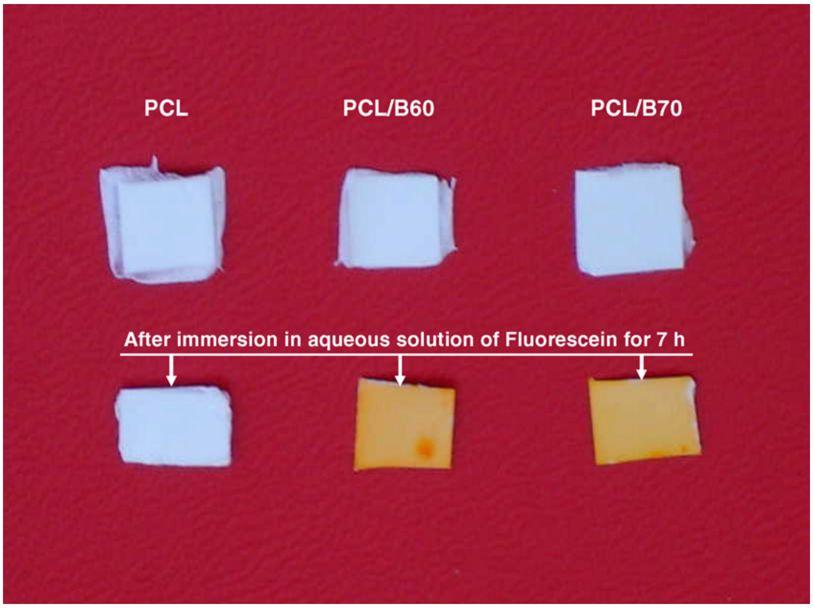


Fig. 4. Photograph of fibrous mats of PCL (PCL concentration– 7.5 wt.%), PCL/B60 [(PCL^{homo} + PCL^{block})/PDMAEMA^{block} = 2.5/1 (w/w)] and PCL/B70 [(PCL^{homo} + PCL^{block})/PDMAEMA^{block} = 2/1 (w/w)] before and after 7 h stay in aqueous solution of fluorescein.

ionically with the tertiary amino groups of PDMAEMA while in the case of PCL due to the lack of appropriate functional groups such interaction cannot occur. A photograph of PCL, PCL/B60 and PCL/B70 mats (non)interacted with fluorescein is shown in Fig. 4.

As expected, after 7 h stay in aqueous solution of the dye the PCL mats did not change their color. On the contrary, the mats containing PCL-*b*-PDMAEMA-block copolymers became intensively colored in orange thus indicating that fluorescein had interacted ionically with the positively charged tertiary amino groups of DMAEMA-units. Noteworthy, all of the mats preserved their integrity after their 7 h stay in the aqueous solution of the dye.

Studies on the surface chemical composition of the prepared fibers were performed by X-ray photoelectron spectroscopy (XPS). The analyses were carried out at detection limit of ca. 0.1 at.% (according to the manufacturer) and probe depth (ca. 1–3 nm). Only the expected elements were detected in the samples. In the spectrum (XPS spectra not presented here) of the PCL fibers peaks for C_{1s} at 280.7 eV and for O_{1s} at 527.9 eV were observed, while in the spectra of the PCL/PCL-*b*-PDMAEMA fibers besides the peaks of the above-mentioned elements a peak for N_{1s} at 395.1 eV was observed. The latter was attributed to the tertiary amino groups of PDMAEMA-block, and it was used for quantitative analysis. The theoretically calculated and the experimentally determined values of the ratio between the percent content of N and C ([N]/[C]) on the surface of PCL, PCL/B60 and PCL/B70 fibers at different weight ratios PCL/copolymer are listed in Table 3.

The experimentally determined values of the [N]/[C] ratio were much higher than the theoretically calculated ones. This is a proof that the surface of the PCL/copolymer fibers is enriched in DMAEMA-units. This result is in agreement with those obtained by electrospinning of polyoxyethylene and polyoxyethylene-peptide conjugate [6]. The N-amount on the fiber surface increased on increasing the copolymer content in the spinning solution. For the fibers of PCL/B70 the experimentally determined values of the [N]/[C] ratio at (PCL^{homo} + PCL^{block})/PDMAEMA^{block} = 2/1 (w/w) and 1/1 (w/w) are 2-fold and 1.5-fold higher than the theoretically calculated one, respectively. Similarly, in the case of fibers of PCL/B60 at (PCL^{homo} + PCL^{block})/PDMAEMA^{block} = 2.5/1 (w/w) and 2/1 (w/w) the experimentally determined values of the [N]/[C] ratio are 2-fold higher than the theoretically calculated one. The experimentally determined value of the ratio [N]/[C] on the surface of the fibers prepared by electrospinning of PCL/B60 at (PCL^{homo} + PCL^{block})/PDMAEMA^{block} = 1/1 (w/w) is only 1.2-fold higher than the theoretically calculated

Table 3
Ratio between the atomic percent content of N- and C-atoms on the surface of PCL and PCL/copolymer fibers depending on the fibers composition

Fibers composition	[N]/[C]$^{\mathrm{Theor},a}$ [%]	[N]/[C]$^{\mathrm{Exp},b}$ [%]
PCL (PCL concentration – 7.5 wt.%)	–	–
PCL/B70 (PCL$^{\mathrm{homo}}$ + PCL$^{\mathrm{block}}$)/PDMAEMA$^{\mathrm{block}}$ = 2/1 (w/w)	4.2	8.3
PCL/B70 (PCL$^{\mathrm{homo}}$ + PCL$^{\mathrm{block}}$)/PDMAEMA$^{\mathrm{block}}$ = 1/1 (w/w)	6.4	9.7
PCL/B60 (PCL$^{\mathrm{homo}}$ + PCL$^{\mathrm{block}}$)/PDMAEMA$^{\mathrm{block}}$ = 2.5/1 (w/w)	3.6	7.1
PCL/B60 (PCL$^{\mathrm{homo}}$ + PCL$^{\mathrm{block}}$)/PDMAEMA$^{\mathrm{block}}$ = 2/1 (w/w)	4.2	8.2
PCL/B60 (PCL$^{\mathrm{homo}}$ + PCL$^{\mathrm{block}}$)/PDMAEMA$^{\mathrm{block}}$ = 1/1 (w/w)	6.4	7.9

[a] Theoretically calculated value as determined by the content of PCL and copolymers in the spinning solution used for preparation of the fibers by electrospinning.

[b] Experimentally determined value by XPS from the ratio between the corresponding chemical elements.

one. It may be assumed that this difference is related to the difference in the mean fiber diameter similarly to the reported data for fibers prepared by electrospinning of amino groups-containing natural polyelectrolytes [14]. While the mean diameters of the fibers of PCL/B60 at weight ratio (PCL$^{\mathrm{homo}}$ + PCL$^{\mathrm{block}}$)/PDMAEMA$^{\mathrm{block}}$ = 1/1 are ca. 900 nm, those of PCL/B70 at the same weight ratio are ca. 640 nm (Table 2). As was already mentioned, the formation of mats on the collector was slower in the case of PCL/B60. Thus, in this specific case it can be assumed that more significant chain rearrangements may take place during the longer time of the jet flight.

It is expected that the differences in the surface chemistry of the PCL, PCL/B70 and PCL/B60 fibers could affect their surface stiffness. It is known that AFM in tapping mode can be used for characterization of soft materials without inducing severe damage. Recently, the possibility for evaluation of the changes in the mechanical and topographic behavior of materials consisted of hard and soft segments using approach curves by AFM in tapping mode has been shown [21]. In the present study this approach has been applied to evaluate the dependence of the surface stiffness of PCL and PCL/PCL-*b*-PDMAEMA fibers on their composition. The approach curves of PCL and PCL/B60 fibers at different PCL/copolymer weight ratios recorded by AFM in tapping mode are presented in Fig. 5.

A relationship between the surface stiffness of a certain polymer material and the slope value of its

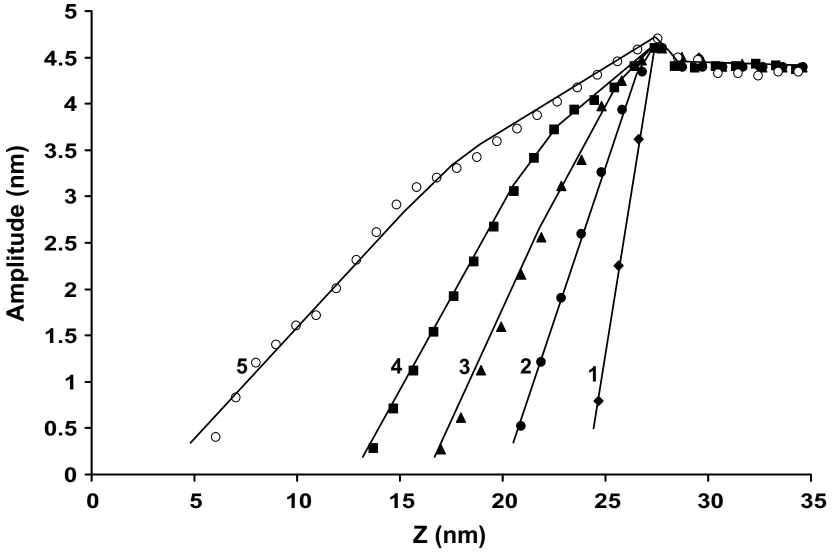


Fig. 5. Experimental approach curves recorded by AFM on fibers of PCL (PCL concentration – 7.5 wt.%) (2) and PCL/B60 at weight ratios (PCL$^{\mathrm{homo}}$ + PCL$^{\mathrm{block}}$)/PDMAEMA$^{\mathrm{block}}$ = 2.5/1 (3); 2/1 (4) and 1/1 (5). The mica approach curve (1) was used as a reference one with a slope value of 1.0.

approach curve exists. The higher the slope value the more pronounced is the surface stiffness. The approach curve of the mica support is shown in the figure as well and it was used in the present study as a reference curve, the slope of which was normalized to be equal to 1. Slope value of 1 means that the oscillator recognizes the surface as a hard one. As seen from Fig. 5, the surface of PCL fibers has a significant stiffness close to that of mica. Introduction of 29 wt.% of PDMAEMA [$(\mathrm{PCL}^{\mathrm{homo}} + \mathrm{PCL}^{\mathrm{block}})/\mathrm{PDMAEMA}^{\mathrm{block}} = 2.5/1$ (w/w)] from the block copolymer B60 leads to some decrease of the slope. This is an indication that the surface of these fibers has reduced stiffness compared to the PCL ones. Increasing the PDMAEMA content triggers a decrease of the slope of the curves, i.e. the surface stiffness decreases. The dependence of the slopes of the linear section of the approach curves on the fibers composition is presented in Fig. 6.

The mean slope value was determined by five measurements using an image of 100 nm^2, and the standard deviation was 0.1. The images were focused on the fiber center to remove the curvature effect of the object. For sake of comparison, the slope value of the approach curve of a PDMAEMA thin film is presented as well. As observed, the approach curve of PCL fibers has the highest slope of ca. 0.75. This is an indication that the fibers surface has a significant stiffness. The presence of 29 wt.% PDMAEMA in the PCL/B60 fibers [$(\mathrm{PCL}^{\mathrm{homo}} + \mathrm{PCL}^{\mathrm{block}})/\mathrm{PDMAEMA}^{\mathrm{block}} = 2.5/1$ (w/w)] decreases the slope value by ca. 0.2. Further increase of the PDMAEMA content in the fibers provokes additional decrease of the slope value, and the slope of the curve for fibers of $(\mathrm{PCL}^{\mathrm{homo}} + \mathrm{PCL}^{\mathrm{block}})/\mathrm{PDMAEMA}^{\mathrm{block}} = 1/1$ (w/w) is close to that determined for the PDMAEMA thin film. These results for the surface stiffness depending on the fibers composition are in good agreement with those obtained by XPS for the surface chemistry of the fibers.

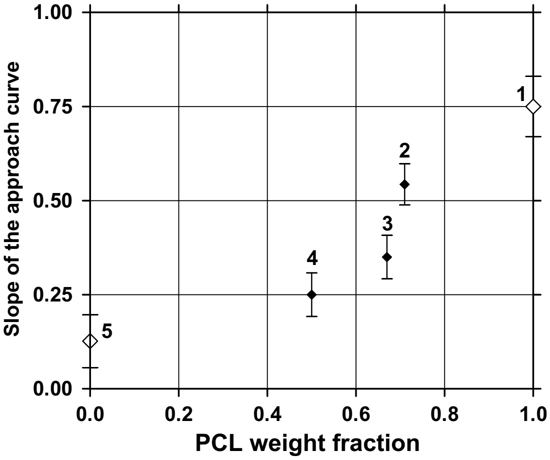


Fig. 6. Dependence of the slope of the linear sections of the approach curves presented in Fig. 5 on the PCL weight fraction in fibers of: PCL alone (1) and PCL/B60 at weight ratios $(\mathrm{PCL}^{\mathrm{homo}} + \mathrm{PCL}^{\mathrm{block}})/\mathrm{PDMAEMA}^{\mathrm{block}} = 2.5/1$ (2); 2/1 (3) and 1/1 (4). The slope value of PDMAEMA alone (5) was determined using a thin film prepared by spin casting.

The performed XPS and AFM analyses for, respectively, evaluating the chemical composition and mechanical behavior of the fibers surface demonstrate that the fibers prepared by electrospinning of mixed solutions of PCL and its copolymers with PDMAEMA have surface enriched in DMAEMA-units. Interesting evidence that this enrichment is more likely due to the electrospinning process is the comparison between the slopes of the approach curves of fibers and thin films with one and the same PCL and copolymer content. In the case of PCL (in absence of copolymer) the curve slope did not depend on the material structure and preparation mode (film or fibers). In contrast, significant differences were observed comparing the slopes of the curves obtained for the fibers and films of PCL/copolymer. The films of PCL/copolymer have surface stiffness close to the PCL one. Contrariwise, as seen from Figs. 5 and 6 the fibers with the same composition had lower surface stiffness tending to reach the slope value of the surface of PDMAEMA film. This may be attributed to the preferable orientation of the PDMAEMA blocks to the surface of the fibers. The difference between the surface stiffness of thin films and fibers indicates that PDMAEMA-soft segments orientate during the electrospinning. These findings are in agreement with the results obtained by XPS and show that the DMAEMA-units segregate preferably on the fiber surface. They are also in agreement with similar data on the enrichment of fibers in polyanionic peptide blocks [6].

The thermal behavior of PCL, PCL/B60 and PCL/B70 fibers depending on their composition was studied by differential scanning calorimetry (DSC). The DSC thermograms of PCL and PCL/B60 fibers are shown in Fig. 7 for the first heating runs.

The melting temperature of PCL is ca. 60 °C [22]. In previous studies some of us showed that the copolymers B60 and B70 have a lower melting

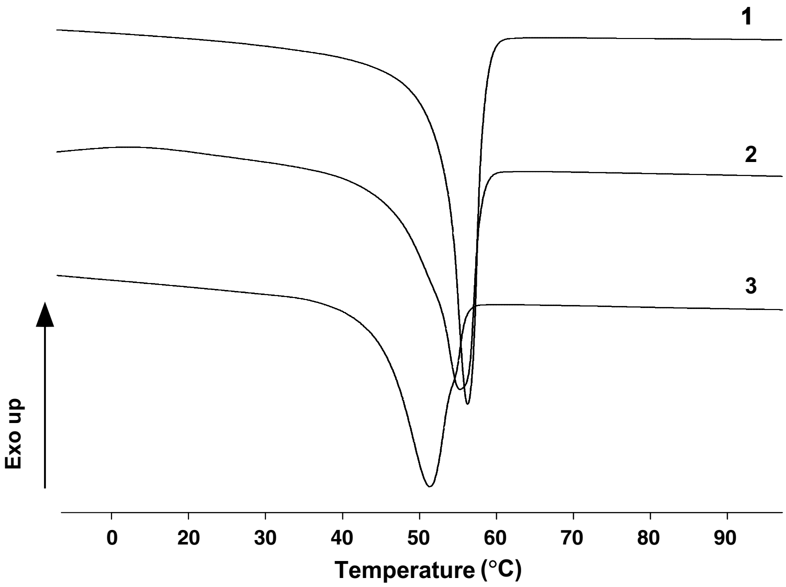


Fig. 7. DSC thermograms of fibers of PCL (PCL concentration in the spinning solution – 7.5 wt.%) (1) and PCL/B60 at different weight ratios ($(PCL^{homo} + PCL^{block})/PDMAEMA^{block} = 2/1$ (2) and 1/1 (3); first heating.

temperature (ca. 52 °C) [18]. As seen from Fig. 7, the introduction of the copolymer into the fiber composition leads to some decrease of the melting temperature in comparison with the fibers prepared by electrospinning of PCL alone. The melting temperature (T_m), melting enthalpy (ΔH_m) and degree of crystallinity (X_c) values of the starting (co)polymers and the fibrous mats prepared by their electrospinning are listed in Table 4.

The electrospinning of PCL decreases the melting temperature (from 60 to 57 °C) and the degree of crystallinity (from 56 to 47%) as compared to the starting polymer. This result is in agreement with the finding that PCL fibers prepared by electrospinning have a lower degree of crystallinity compared to the starting polymer due to rapid solvent evaporation, i.e. not enough time for rearrangement of the polymer chains [30]. The introduction of PDMAEMA into the fibers structure leads to some further decrease of the melting temperature as well as of the degree of crystallinity with respect to the starting PCL. This result is expected since the amorphous domains of PDMAEMA additionally hinder the crystallization of PCL in the fibers. As seen from Table 4 increasing the copolymer content into the fibers allows for reducing the degree of crystallinity,

Table 4
Melting temperature (T_m), melting enthalpy (ΔH_m) and degree of crystallinity (X_c) of the PCL in the starting (co)polymers and in the electrospun fibrous mats

Sample	T_m [°C][a]	ΔH_m [J/g][a]	X_c [%][b]
Starting (co)polymers			
PCL	60	76.6	56
B70	52	30.0	22
B60	52	33.1	24
Electrospun fibrous mats			
PCL (PCL concentration – 7.5 wt.%)	57	66.0	47
PCL/B70 $(PCL^{homo} + PCL^{block})/PDMAEMA^{block} = 2/1$ (w/w)	56	54.0	39
PCL/B60 $(PCL^{homo} + PCL^{block})/PDMAEMA^{block} = 2/1$ (w/w)	55	60.0	43
PCL/B60 $(PCL^{homo} + PCL^{block})/PDMAEMA^{block} = 1/1$ (w/w)	52	49.0	35

[a] Melting temperature and melting enthalpy as determined by DSC.

[b] Degree of crystallinity as calculated form the ratio between the melting enthalpy of a given sample and the melting enthalpy of PCL with 100% degree of crystallinity ($\Delta H_m^0 = 139.5$ J/g [29]).

and at $(\text{PCL}^{\text{homo}} + \text{PCL}^{\text{block}})/\text{PDMAEMA}^{\text{block}} = 1/1$ (w/w) (for the PCL/B60 pair) the degree of crystallinity is ca. 1.6-fold higher than that of the starting B60 and ca. 1.6-fold lower than that of the starting PCL. It is worth to be noted that the fibers containing the copolymer B70 have slightly lower degree of crystallinity as compared to the PCL/B60 fibers (Table 4). This might be attributed to the 2-fold higher PDMAEMA-block length in B70 compared to B60.

4. Conclusions

In the present work one-step preparation of PCL-based fibers having surface enriched in positively ionizable in aqueous medium amino groups was shown. This was achieved by electrospinning of mixed solutions of PCL and block copolymers consisted of PCL and PDMAEMA blocks. It was found out that fibers with targeted amount of tertiary amino groups on their surface can be prepared in a controlled manner tuning up the ratio PCL/copolymer and the PDMAEMA-block length in the copolymer. Such materials are of great interest as potential candidates for biomedical applications. Their macroscopic physico-chemical as well as biological behavior will be subject of a forthcoming publication.

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References

[1] Huang ZM, Zhang YZ, Kotaki M, Ramakrishna S. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. Compos Sci Technol 2003;63:2223–53.

[2] Li D, Xia Y. Electrospinning of nanofibers: reinventing the wheel? Adv Mater 2004;16:1151–70.

[3] Greiner A, Wendorff JH. Electrospinning: a fascinating method for the preparation of ultrathin fibers. Angew Chem Int Ed 2007;46:5670–703.

[4] Nair LS, Laurencin CT. Biodegradable polymers as biomaterials. Prog Polym Sci 2007;32:762–98.

[5] Deitzel M, Kosik W, McKnight SH, Tan NCB, DeSimone JM, Crette S. Electrospinning of polymer nanofibers with specific surface chemistry. Polymer 2002;43:1025–9.

[6] Sun XY, Shankar R, Börner HG, Ghosh TK, Spontak RJ. Field-driven biofunctionalization of polymer fiber surfaces during electrospinning. Adv Mater 2007;19:87–91.

[7] Harris LG, Mead L, Müller-Oberlander E, Richards RG. Cytocompatibility of coated titanium surfaces impregnated with an antiseptic to staphylococci and fibroblasts. Eur Cells Mater 2005;10:24.

[8] Spasova M, Paneva D, Manolova N, Radenkov Ph, Rashkov I. Electrospun chitosan-coated fibers of poly(L-lactide) and poly(L-lactide)/poly(ethylene glycol): preparation and characterization. Macromol Biosci 2007. doi:10.1002/mabi.200700129.

[9] Yancheva E, Paneva D, Maximova V, Mespouille L, Dubois Ph, Manolova N, et al. Polyelectrolyte complexes between (crosslinked) *N*-carboxyethylchitosan and (quaternized) poly[2-(dimethylamino)ethyl methacrylate]: preparation, characterization and antibacterial properties. Biomacromolecules 2007;8:976–84.

[10] Huang J, Murata H, Koepsel RR, Russel AJ, Matjyaszewski K. Antibacterial polypropylene via surface-initiated atom transfer polymerization. Biomacromolecules 2007;8:1396–9.

[11] McKee MG, Hunley MT, Layman JM, Long TE. Solution rheological behaviour and electrospinning of cationic polyelectrolytes. Macromolecules 2006;39:575–83.

[12] Pornsopone V, Supaphol P, Rangkupan R, Tantayanon S. Electrospinning of methacrylate-based copolymers: effects of solution concentration and applied electrical potential on morphological appearance of as-spun fibers. Polym Eng Sci 2005;45:1073–80.

[13] Spasova M, Manolova N, Paneva D, Rashkov I. Preparation of chitosan containing nanofibres by electrospinning chitosan/poly(ethylene oxide) blend solutions. e-Polymers 2004. no. 056.

[14] Duan B, Dong C, Yuan X, Yao K. Electrospinning of chitosan solutions in acetic acid with poly(ethylene oxide). J Biomater Sci Polym Ed 2004;15:797–811.

[15] Mincheva R, Manolova N, Paneva D, Rashkov I. Preparation of polyelectrolyte-containing nanofibers by electrospinning in the presence of a non-ionogenic water-soluble polymer. J Bioact Compat Polym 2005;20:419–35.

[16] Ignatova M, Starbova K, Markova N, Manolova N, Rashkov I. Electrospun nano-fibre mats with antibacterial properties from quaternized chitosan and poly(vinyl alcohol). Carbohydr Res 2006;341:2098–107.

[17] Ignatova M, Manolova N, Rashkov I. Novel antibacterial fibers of quaternized chitosan and poly(vinyl pyrrolidone) prepared by electrospinning. Euro Polym J 2007;43:1112–22.

[18] Bougard F, Jeusette M, Mespouille L, Dubois Ph, Lazzaroni R. Synthesis and supramolecular organization of amphiphilic diblock copolymers combining poly(*N*,*N*-dimethylamino-2-ethyl methacrylate) and poly(ϵ-caprolactone). Langmuir 2007;23:2339–45.

[19] Spasova M, Mincheva R, Paneva D, Manolova N, Rashkov I. Criteria for complex evaluation of the morphology and alignment of electrospun polymer nanofibers. J Bioact Compat Polym 2006;21:465–79.

[20] Rasband WS. ImageJ, U.S. National Institute of Health, Bethesda, Maryland, USA; 1997–2006. http://rsb.info.nih.gov/ij/.

[21] Kopp-Marsaudon S, Leclère Ph, Dubourg F, Lazzaroni R, Aimé JP. Quantitative measurements of the mechanical

contribution to tapping-mode atomic force microscopy images of soft materials. Langmuir 2000;16:8432–7.

[22] www.solvaycaprolactone.com.

[23] McKee MG, Wilkes GL, Colby RH, Long TE. Correlations of solution rheology with electrospun fiber formation of linear and branched polyesters. Macromolecules 2004;37: 1760–7.

[24] Shenoy SL, Bates WD, Frisch HL, Wnek GE. Role of chain entanglement on fiber formation during electrospinning of polymer solutions: good solvents, non-specific polymer–polymer interaction limit. Polymer 2005;46: 3372–84.

[25] Gimenez J, Cassagnau Ph, Michel A. Bulk polymerization of (ϵ-caprolactone: rheological predictive laws. J Rheol 2000;44:527–47.

[26] Stoilova O, Jérôme C, Detrembleur C, Mouithys-Mickalad A, Manolova N, Rashkov I, et al. New nanostructured materials based on fullerene and biodegradable polyesters. Chem Mater 2006;18:4917–23.

[27] Stoilova O, Jérôme C, Detrembleur C, Mouithys-Mickalad A, Manolova N, Rashkov I, et al. C_{60}-containing nanostructured polymeric materials with potential biomedical applications. Polymer 2007;48:1835–43.

[28] Kwon IK, Kidoaki S, Matsuda T. Electrospun nano- to microfiber fabrics made of biodegradable copolyesters: structural characteristics, mechanical properties and cell adhesion potential. Biomaterials 2005;26:3929–39.

[29] Skoglund P, Fransson Å. Continuous cooling and isothermal crystallization of polycaprolactone. J Appl Polym Sci 1996;61:2455–65.

[30] Thomas V, Jose MV, Chowdhury S, Sullivan JF, Dean DR, Vohra YK. Mechano-morphological studies of aligned nanofibrous scaffold of polycaprolactone fabricated by electrospinning. J Biomater Sci Polym Ed 2006;17:969–84.