 DOI: 10.1002/masy.201100037

Charged Poly(D,L-lactide) Nanofibers: Towards Customized Surface Properties

Florence Croisier,[1] *Abdelhafid Aqil,*[1] *Cédric Malherbe,*[2] *Bernard Gilbert,*[2] *Christophe Detrembleur,*[1] *Christine Jérôme**[1]

Summary: Surface-charged nanofibers were prepared by electrospinning technique (ESP). For this purpose, a copolymer bearing carboxylic acid functions was added to a poly(D,L-lactide) solution just before ESP process. In a basic medium, negative charges were therefore revealed on fiber surface. By deposition of positively charged particles or polyelectrolytes, surface properties of the fibers could be tailor-made for a specific application. This versatile method can, for example, be applied to the preparation of new biomedical scaffolds.

Keywords: charged nanofibers; electrospinning; surface modification

Introduction

Poly(lactide) (PLA) belongs to the aliphatic polyester family. These polymers possess interesting features of biodegradability, due to the presence of ester bonds that can be hydrolytically and enzymatically cleaved to form non-toxic residues.[1,2] The rate of degradation depends mainly on the initial molecular weight,[3] and is generally set around a few months to a year for PLA.[4] This offers promising prospects for short and medium terms applications. Moreover, PLA can be derived from renewable resources[5] and has shown notable biocompatibility, which makes poly(lactide) an outstanding candidate for pharmaceutical and biomedical applications.[6,7] In this respect, PLA has already been applied as medical sutures, bone implants, tissue repair and controlled drug-delivery devices.[3,8] In the field of tissue engineering, polymer nanofiber mats are particularly relevant, as they can form scaffolds for cellular growth.[9,10] To produce such nanofiber structure, electrospinning (ESP)[11] has revealed itself as a powerful technique: from polymer solution or melt, it allows the preparation of fibers with diameters ranging from a few nanometers to microns.[10] The protocol of ESP is quite straightforward. In brief, a polymer solution is conducted through a syringe needle and a voltage is applied between this needle and a grounded collector. At a critical voltage value, the surface tension of the polymer solution droplet is overcome and an electrically charged jet of polymer is formed. Fibers are then collected on the grounded collector.[12] The produced fiber mats exhibit high surface area and porosity, while the nonwoven assembly of the fibers mimics extracellular matrix.[10] Such scaffolds can thus be used as a structural support for cells attachment and development. In the view of tissue engineering application, factors for promoting cellular growth could furthermore be added to the fibers, before or after electrospinning process.

In this prospect, we report in this paper a method for modifying PLA nanofibers surface, after ESP operation. It is based on the preparation of charged poly(D,L-lactide) nanofibers by electrospinning,

[1] Center for Education and Research on Macromolecules (C.E.R.M.), Department of Chemistry, University of Liège, Sart Tilman, B6a, 4000 Liège, Belgium
Fax: (+32) 4 366 34 97;
E-mail: C.Jerome@ulg.ac.be
[2] Analytical Chemistry and Electrochemistry, Department of Chemistry, University of Liège, Sart Tilman, B6c, 4000 Liège, Belgium

followed by electrostatic deposition[13] of charged particles or polyelectrolytes on the fiber surface. A schematic representation of this approach is shown on Figure 1. With this versatile method, coated nanofibers could have tailor-made surface properties for a specific application (e.g. immunostimulant, antibacterial or adhesive coating), with a fiber core that preserves good mechanical resistance. This method could thus be applied to the preparation of a wide diversity of new biomedical scaffolds.

Materials and Methods

Preparation of Charged Poly(D,L-lactide) Fibers by Electrospinning

A poly(methyl methacrylate-*b*-methacrylic acid) block copolymer was synthesized by atom transfer radical polymerization (ATRP) of methyl methacrylate and trimethylsilyl methacrylate, initiated by 2-bromo-ethylisobutyrate and catalyzed by dibromobis(triphenylphosphine) nickel.[14] The pendant silylated groups were hydrolyzed as reported elsewhere.[15,16] The resulting copolymer, poly(MMA_{48}-*b*-MA_{43}), presents a polymerization degree of 48 in methyl methacrylate (MMA) and 43 in methacrylic acid (MA), respectively. Poly(D,L-lactide) (PD,LLA) was purchased from Boehringer Ingelheim (Resomer® R208) (Mn∼70,000 g/mol, PDI: 2.8). PD,LLA and poly(MMA-*b*-MA) were dissolved in *N,N*-dimethylformamide (DMF) at a concentration of 22% by weight and with a PD,LLA to poly(MMA-*b*-MA) ratio of 9:1. Then, the electrospinning process

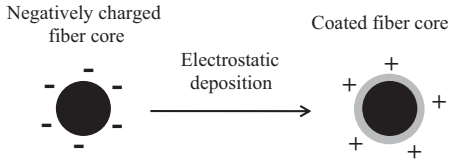

Figure 1.
Schematic representation of coated fiber preparation (cross-sectional view of a fiber). Fiber is depicted in black and fiber coating in grey. For convenience, counter-ions were not depicted.

was conducted as follows: the polymer mixture solution was transferred into a glass syringe with an orthogonally cut-ended needle (0.8 mm in external diameter). A syringe driver was used to regulate the flow rate of solution to 0.8 mL/h and a grounded aluminum collector plate was placed at 18 cm of the needle-end. To produce charged poly(D,L-lactide) based nanofibers, a voltage of 10 kV was applied between the collector and the syringe needle. Fibers were collected on an aluminum foil of 20×20 cm placed on the grounded collector. Platinum coating was applied to the fibers for Scanning Electron Microscopy analysis (SEM, Jeol JSM-840A). For convenience, the fibers produced hereinbefore will be called ''PD,LLA blended fibers'' in the following paragraphs.

For the sake of comparison, reference fibers of PD,LLA without adding the copolymer were prepared in the same electrospinning conditions starting from a PD,LLA (Resomer® R208, Boehringer Ingelheim) solution in *N,N*-dimethylformamide (DMF) at a concentration of 22% by weight.

Deposition of Magnetite (Fe_3O_4) Nanoparticles on PD,LLA Blended Fibers

To confirm the presence of negative charges on the PD,LLA blended fibers surface, magnetite nanoparticles (Fe_3O_4), known to be positively charged in acidic medium, were used. The preparation of these particles is described elsewhere.[17] PD,LLA blended fibers were directly electrospun on copper Transmission Electron Microscopy (TEM) grids for this experiment. The grids were then first immersed for 1 minute in a phosphate buffer solution (pH = 8) – in order to reveal negative charges on fiber surface –, next dipped for 1 minute in an acidic aqueous magnetite nanoparticles solution (pH = 3.5) and finally rinsed 1 minute with milliQ water. The samples were let to dry under air and at ambient temperature. They were then analyzed with a Philips CM100 Transmission Electron Microscope

equipped with an Olympus camera and the images were transferred to a computer under Megaview system.

The obtained images were compared to reference images, taken on PD,LLA fibers which do not contain poly(MMA-*b*-MA) copolymer.

Deposition of Poly(allylamine hydrochloride) on PD,LLA Blended Fibers

Poly(allylamine hydrochloride) (PAH) with a molecular weight of 15,000 g/mol was purchased from Sigma Aldrich. Aqueous saline[18] solution (0.1 M in NaCl) of PAH with a concentration of 5 g/L was prepared for the deposition experiment.

The deposition of PAH on the surface of PD,LLA blended fibers was conducted as described hereafter: fiber mats were first immersed for 1 minute in a phosphate buffer solution (pH = 8) in order to deprotonate carboxylic acid functions. Afterwards, fiber mats were dipped in the PAH solution for 30 minutes, and then rinsed in milliQ water for 1 minute. The samples were then dried under reduced pressure at ambient temperature for 2 days. For SEM analysis, fibers were firstly covered with platinum coating (Jeol JSM-840A).

For TEM analysis (Philips CM100, Olympus camera and Megaview system), PD,LLA blended fibers were directly electrospun on copper grids. The grids were then first immersed for 1 minute in a phosphate buffer solution (pH = 8), next dipped for 1 minute in PAH solution and finally rinsed 1 minute with milliQ water. The samples were let to dry under air and at ambient temperature before TEM analysis.

Indirect Zeta Potential measurements on fiber surfaces were performed using the flat surface cell of a Beckman Coulter Delsa Nano C analyzer. The data were processed via the Delsa Nano UI 2.21 software. All the measurements were carried out at 25 °C at a measuring angle of 15°, with standard particles for solid samples (Otsuka Electronics co., LTD, A54496). This technique allowed to estimate the charges of the fiber surfaces, before and after deposition of PAH. For the analysis, fiber mats electrospun during 30 minutes were peeled off the aluminum foil and cut with scissors to fit into the analyzing flat surface cell. Measurement of the Zeta Potential was repeated on four different samples coming from the same batch to check the reproducibility of the obtained results.

Raman spectroscopy was also used to confirm the deposition of PAH on PD,LLA blended fibers. The Raman spectra were recorded with a Dilor Labram spectrometer using the 514.5-nm line of a Spectra Physics 164 argon-ion laser, with a power of 60 mW. Five types of samples were analyzed: PD,LLA polymer alone, poly(MMA-*b*-MA) copolymer alone, PAH polymer alone, PD,LLA blended fibers, and PD,LLA blended fibers after deposition of PAH.

Results and Discussion

Preparation of Charged Poly(D,L-lactide) Fibers by Electrospinning

Electrospinning of poly(D,L-lactide) in presence of a poly(methyl methacrylate-*b*-methacrylic acid) copolymer was conducted as described in the Materials and Method section. Representative Scanning Electron Microscopy (SEM) images of electrospun PD,LLA blended fibers are presented in Figure 2 (a) and (b). Homogeneous fiber mats were obtained, without any beaded defect. The average diameter of the collected fibers is around 500 nm. For the sake of comparison, SEM images of PD,LLA fibers electrospun without the methacrylic acid-based copolymer are shown in Figure 2 (c) and (d). No significant difference is observed in terms of fiber morphology or diameter.

Deposition of Magnetite (Fe_3O_4) Nanoparticles on PD,LLA Blended Fibers

Due to the presence of carboxylic acid functions along the poly(MMA-*b*-MA) copolymer backbone, negative charges are present on this copolymer in basic

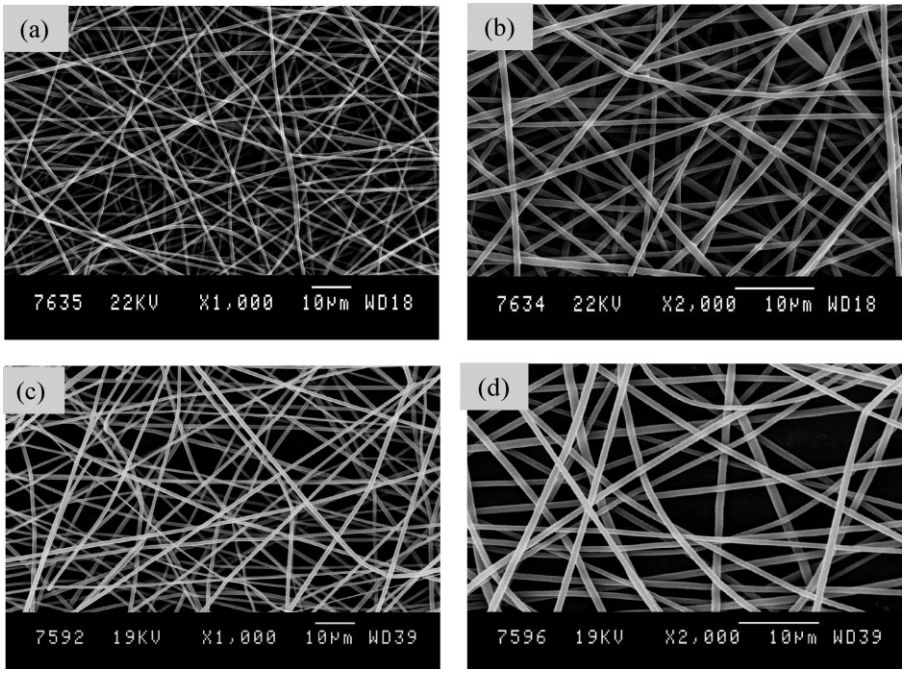

Figure 2.
SEM images of blended PD,LLA electrospun fibers at two different magnifications (a) and (b) of pure PD,LLA electrospun fibers at two different magnifications (c) and (d). All fibers were collected on aluminum foil and metal coated with Pt before observation.

medium. PD,LLA blended fibers were thus immersed in a pH = 8 buffer solution to disclose negative charges on their surface. In order to confirm the presence of negative charges on fiber surface, magnetite nanoparticles (Fe_3O_4), which are positively charged in acidic medium, were brought into contact with the fibers. Transmission Electron Microscopy (TEM) analysis was then performed on two different samples: the first one was composed of PD,LLA electrospun fibers, without the methacrylic acid copolymer. These fibers were used as a reference. They were also immersed in a pH = 8 buffer solution, then in an acidic solution containing Fe_3O_4 particles and finally rinsed with water. The second sample was composed of PD,LLA blended fibers (containing the poly(MMA-*b*-MA) copolymer). These were subjected to the same treatment (immersed in buffer, then in Fe_3O_4 particles solution, then in water). Figure 3 compares the TEM images of these two samples.

Clearly, these images are significantly different; while on PD,LLA electrospun fibers (Figure 3 (a)), only very few particles are attached on the fiber surface, a massive deposition of magnetite particles is observed on the fibers containing methacrylic acid copolymer (Figure 3(b)). This corroborates the presence of negative charges at the surface of the PD,LLA blended fibers that trigger the deposition of oppositely charged particles. In the case of PD,LLA fibers not containing the methacrylic acid-based copolymer, the deposition of a few particles is most likely due to the presence of a couple of carboxylic acid functions, resulting from a minor degradation of the PD,LLA polymer.

In view of these results, the electrostatic deposition of a polyelectrolyte was then attempted on the PD,LLA blended fibers.

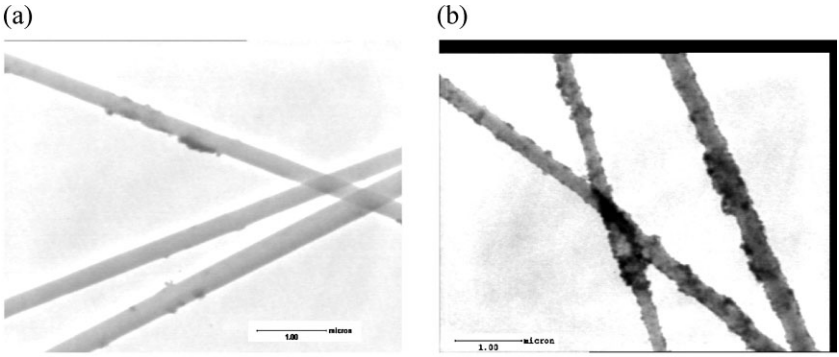

Figure 3.
(a) TEM images of PD,LLA electrospun fibers collected on copper grid, after immersion in acidic magnetite particles solution. (b) PD,LLA blended electrospun fibers (containing poly(MMA-*b*-MA) copolymer) collected on copper grid, after immersion in acidic magnetite particles solution.

Deposition of Poly(allylamine hydrochloride) on PD,LLA Blended Fibers

The electrostatic deposition of poly(allylamine hydrochloride) was tried on PD,LLA blended fibers. As in the case of the deposition of magnetite nanoparticles, the fibers were firstly immersed in a pH = 8 buffer solution, in order to disclose negative charges on the fiber surface. The fibers were then dipped in a saline PAH solution and finally rinsed with water. For Scanning Electron Microscopy analysis, the experiment was performed on fibers deposited on an aluminum foil, while for Transmission Electron Microscopy analysis, the fibers were directly electrospun on copper TEM grids.

The Figure 4 shows a representative SEM image of PD,LLA blended fibers after deposition of PAH. From this SEM image, the deposition of PAH cannot be ascertained: the resolution of the microscope used may not be sufficient, the layer deposited on fiber surface being really thin (a few nanometers thick, as reported in the literature on film surfaces[13,19]). However, SEM at least confirms the integrity of the fibers after their immersion in PAH solution. The morphology of the fibers remains in fact identical to that before the deposition treatment (referring to Figure 2(b)).

Transmission Electron Microscopy was then used to highlight the presence of PAH on PD,LLA blended fiber surface. Figure 5 shows representative TEM images of PD,LLA blended fibers after immersion in phosphate buffer only (Figure 5(a)) and of PD,LLA blended fibers after immersion in PAH solution (Figure 5(b)). A clear much more granular surface morphology can be observed on Figure 5(b), most probably indicating the presence of some PAH deposition on the fibers. This granular rather than smooth aspect mainly arises from the fact that fibers were immersed in PAH solution for only 1 minute (and not 30 minutes as for the SEM sample), to avoid blocking the TEM grid. According to the literature, electrostatic deposition should

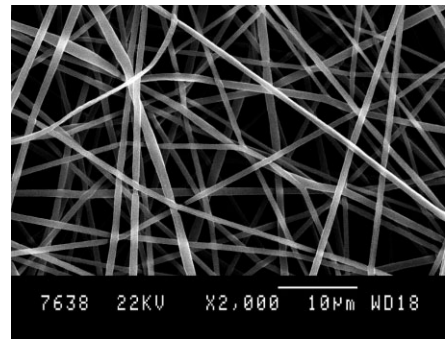

Figure 4.
SEM image of PD,LLA blended electrospun fibers after immersion in PAH solution. Fibers were metal coated with Pt before analysis.

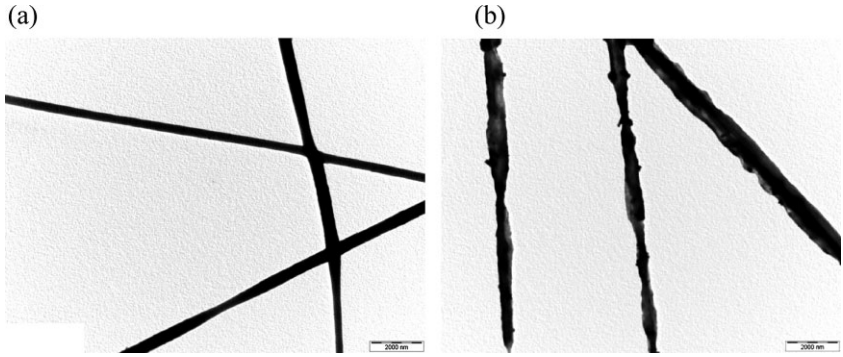

Figure 5.
(a) TEM images of PD,LLA blended electrospun fibers collected on copper grid, after immersion in pH = 8 buffer solution. (b) PD,LLA blended electrospun fibers collected on copper grid, after immersion in poly(allylamine hydrochloride) solution.

be conducted for at least 20 minutes for the deposition process to equilibrate and the deposit to become smooth.[20] The TEM analysis yet allowed to disclose the presence of PAH on PD,LLA blended fiber surface.

Indirect Zeta Potential measurements were also developed to estimate the charge of the surface constituted by the electrospun fiber mat. In this technique, the surface to be analyzed is brought into contact with standard particles solution whereof the charge is accurately known. In an electric field, the standard particles will migrate, and this migration will be influenced, not only by their own charges, but also by the charges of the surface in contact. This indirect technique allows to determine the Zeta Potential of the fiber surface. Three batches of samples were tested: the first batch contained PD,LLA fibers and the second, PD,LLA blended

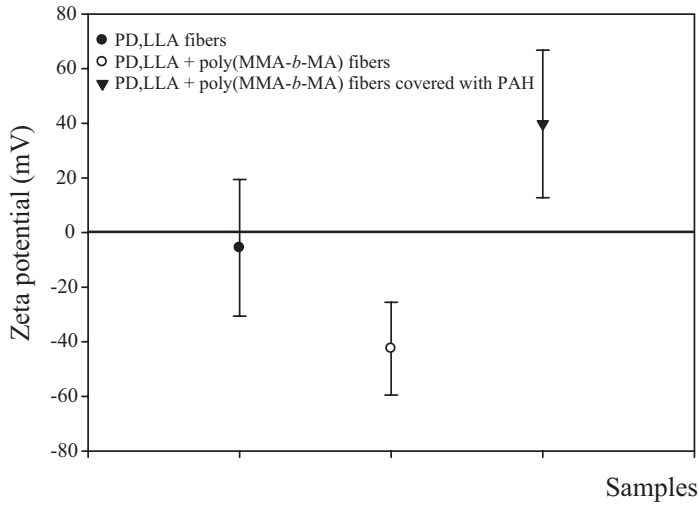

Figure 6.
Graphic representation of indirect Zeta Potential measurements on PD,LLA electrospun fibers (black dot), PD,LLA blended electrospun fibers after immersion in buffer solution (pH = 8) (white dot) and the same fibers after immersion in PAH solution (black triangle). The error bars in the figure represent the standard deviation derived from multiple measurements.

fibers immersed in pH = 8 buffer and then dried at ambient temperature. The third batch was composed of the same fibers, but immersed in PAH solution, rinsed with water and finally dried at ambient temperature. The results are graphically presented in Figure 6.

The results corresponding to PD,LLA fibers are represented by a black dot, the results of PD,LLA blended fibers before deposition of PAH are delineated by a white dot, while a black triangle depicts the results of the PD,LLA blended fibers after deposition of PAH. The standard deviation calculated on each batch is also shown on the graph of the Figure 6. The first batch, composed of PD,LLA fibers, gives a slightly negative Zeta Potential. This is probably due to the presence of a couple of carboxylic acid functions along the fibers, resulting from a minor degradation of the PD,LLA polymer. The indirect Zeta Potential of the second batch (without PAH) is clearly negative, which indicates that negative charges are present on fiber surface as expected for neutralized carboxylic acid blends. For the third batch (same fibers, with PAH), positive Zeta is obtained; this is consistent with the deposition of PAH, which is a positively charged polyelectrolyte in water. Indirect Zeta potential measurement technique turned out to be a powerful tool for disclosing an electrostatic deposition on a solid surface – in that case composed of fibers.

Finally, Raman spectroscopy was used, first to assess the composition of the initial PD,LLA blended fibers and confirm the presence of the poly(MMA-*b*-MA) copolymer, and in a second time, to highlight the existence of a PAH deposition. Raman spectra are presented in Figure 7. Five types of samples were analyzed: PD,LLA polymer alone (Figure 7(a)), poly(MMA-*b*-MA) copolymer alone (Figure 7(b)), PD,LLA blended fibers (Figure 7(c)), PD,LLA blended fibers after deposition of PAH (Figure 7(d)), and PAH polymer alone (Figure 7(e)). Dashed lines in Figure 7 refer to peaks that are common

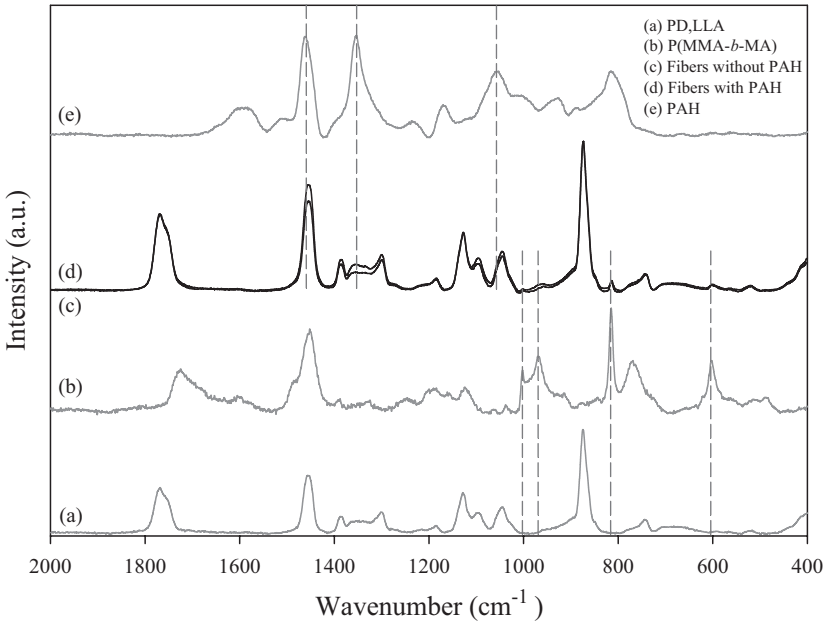

Figure 7.
Comparison of Raman spectra obtained for 5 types of samples – (a) PD,LLA polymer alone, (b) P(MMA-*b*-MA) copolymer alone, (c) PD,LLA blended fibers, (d) PD,LLA blended fibers after PAH deposition, (e) PAH polymer alone. Vertical dashed lines refer to peaks that are shared by two or more samples.

to two or more samples. In the spectrum of the copolymer (Figure 7(b)), representative peaks are present at 1002, 968, 815 and 602 cm^{-1}. These peaks are also present in the spectrum of the PD,LLA blended fibers (Figure 7(c)) and in the spectrum of the same fibers after PAH deposition (Figure 7(d)), which confirms the presence of the poly(MMA-*b*-MA) in the PD,LLA blended fibers. As to our second concern, representative peaks of the PAH polymer (Figure 7(e)), at 1461, 1354 and 1055 cm^{-1}, can be found in the spectrum of the PD,LLA blended fibers after PAH deposition (Figure 7(d)). For that matter, spectra (c) and (d) were superimposed to highlight those peaks. We can thus assume that PD,LLA blended fibers were indeed covered with PAH (Figure 7(d)).

The combination of the results obtained by Transmission Electron Microscopy, indirect Zeta Potential measurement and Raman spectroscopy, confirms the formation of a PAH coating on PD,LLA blended with poly(MMA-*b*-MA) fibers.

Conclusion

A method for customizing PD,LLA fiber surface, based on electrostatic coating, was developed in this paper. This technique could also be applied to deposit other positively charged particles or polyelectrolytes, in order to modify the surface properties of the fibers, in view of their future application. For example, in the field of tissue engineering and biomedical application, the surface of the fibers could be coated with immunostimulant or antibacterial coating. Moreover, a system with positively charged fibers and a negative coating could also be envisaged (with the addition of a copolymer bearing tertiary amine to the electrospinning solution). This method turned out to be really versatile to tailor surface properties of the fibers while keeping their morphology and mechanical properties.

Acknowledgements: F.C. is grateful to the Belgian « Fonds pour la Formation à la Recherche dans l'Industrie et dans l'Agriculture » (FRIA) for financial support. C.D. is « Maître de Recherche » by the FRS-FNRS. The present work has been supported by the Science Policy Office of the Belgian Federal Government (IAP 6/27). CERM thanks the Walloon Region for supporting this research in the frame of GO-CELL project.

[1] A.-C. Albertsson, I. K. Varma, *Advances in Polymer Science* **2002**, *157*, 1.
[2] R. Smith, "Biodegradable Polymers for Industrial Applications", Woodhead Publishing Ltd, **2005**, 531 pp.
[3] B. Gupta, N. Revagade, J. Hilborn, *Progress in Polymer Science* **2007**, *32*, 455.
[4] R. G. Sinclair, *Journal of Macromolecular Science, Pure and Applied Chemistry* **1996**, *A33*, 585.
[5] O. Avinc, A. Khoddami, *Fibre Chemistry* **2009**, *41*, 391.
[6] C. Jerome, P. Lecomte, *Advanced Drug Delivery Reviews* **2008**, *60*, 1056.
[7] M. Vert, S. M. Li, G. Spenlehauer, P. Guerin, *Journal of Materials Science: Materials in Medicine* **1992**, *3*, 432.
[8] H. Sawalha, K. Schroen, R. Boom, *Journal of Applied Polymer Science* **2008**, *107*, 82.
[9] K. Kim, M. Yu, X. Zong, J. Chiu, D. Fang, Y.-S. Seo, B. S. Hsiao, B. Chu, M. Hadjiargyrou, *Biomaterials* **2003**, *24*, 4977.
[10] S. Ramakrishna, K. Fujihara, W. E. Teo, C. T. Lim, Z. Ma, "An introduction to electrospinning and nanofibers", World Scientific Publishing Co. Pte. Ltd, Singapore **2005**, 396 pp.
[11] US patent 2187306 **1940**, Schreiber-Gastell, Richard, inv. A. Formhals.
[12] M. K. Leach, Z.-Q. Feng, S. J. Tuck, J. M. Corey, *Journal of Visualized Experiments* **2011**, 2494/1.
[13] G. Decher, *Science (Washington, D.C.)* **1997**, *277*, 1232.
[14] J. Rieger, C. Passirani, J.-P. Benoit, K. Van Butsele, R. Jerome, C. Jerome, *Advanced Functional Materials* **2006**, *16*, 1506.
[15] S. Gautier, N. Grudzielski, G. Goffinet, S. H. de Hassonville, L. Delattre, R. Jerome, *Journal of Biomaterials Science, Polymer Edition* **2001**, *12*, 429.
[16] G. Moineau, M. Minet, P. Dubois, P. Teyssie, T. Senninger, R. Jerome, *Macromolecules* **1999**, *32*, 27.
[17] F. Stoffelbach, A. Aqil, C. Jerome, R. Jerome, C. Detrembleur, *Chemical Communications (Cambridge, United Kingdom)* **2005**, 4532.
[18] S. Boudard, B. Seantier, C. Breffa, G. Decher, O. Felix, *Thin Solid Films* **2005**, *495*, 246.
[19] T. Cassier, K. Lowack, G. Decher, *Supramolecular Science* **1998**, *5*, 309.
[20] V. V. Tsukruk, V. N. Bliznyuk, D. Visser, A. L. Campbell, T. J. Bunning, W. W. Adams, *Macromolecules* **1997**, *30*, 6615.

