ELECTROSPUN BIODEGRADABLE PCL, PEG AND PCL/PEG POLYURETHANE NANOFIBERS COATED BY AMINE-RICH PLASMA POLYMERS

KEDROŇOVÁ Eva[1,2,3]; KUPKA Vojtěch[4]; MANAKHOV Anton[1]; STOICA Adrian[1]; VOJTOVÁ Lucy[4], ZAJÍČKOVÁ Lenka[1,2]

[1] Plasma Technologies, CEITEC, Masaryk University, Brno, Czech Republic, EU
[2] Department of Physical Electronics, Faculty of Science, Masaryk University Brno, Czech Republic, EU
[3] Department of Chemistry, Faculty of Science, Masaryk University Brno, Czech Republic, EU
[4] Advanced Polymers and Composites, CEITEC, Brno University of Technology, Brno, Czech Republic, EU

Abstract

The electrospinning process was employed for the preparation of nanofiber substrates based on hydrophobic poly(ε-caprolactone) (PCL), hydrophilic poly(ethylene glycol) (PEG) and amphiphilic PCL/PEG linear polyurethane (PUR). The used polymer solution and electrospinning process influenced the structure and biodegradability of resulting nanofibers. While nanomaterials processed from PCL and PEG dissolved in acetic and formic acid mixture solvent showed well-structured nanofiber meshes, the electrospinning of PUR acidic solution resulted in electrosprayed film due to the insufficient molecular weight of PUR. In order to improve the biocompatibility of nanofibers a thin amine layer by means of the cyclopropylamine plasma polymerization in radio frequency capacitively coupled discharge was deposited on prepared nanofibrous meshes. The presence of the amine groups on the surface of nanofibrous substrate supposed to enhance the adhesion and proliferation of cells. The possibility for further functionalizing by grafting with chitosan biopolymer through carbodiimide-mediated coupling via citric acid has been tested on a model PCL/PEG PUR foil. Infrared spectroscopy confirmed successful grafting of chitosan onto the PUR surface. Optimizing PUR nanofibers processing can open possible use of the material in wound healing applications exploiting antibacterial properties of chitosan and excellent elasticity, controlled biodegradability and optimal stiffness of PUR.

Keywords: Electrospinning, polymer nanofibers, biodegradable polyurethanes, plasma amine coatings

1. INTRODUCTION

Studies have shown that naturally derived biomaterials such as decellularized matrices have superior functional attributes due to their extracellular matrix (ECM) composition. [1] In addition to biomechanical compatibility with the host, these implants also attract cells from the recipient and promote efficient scaffold remodeling. [2] However, these biologically derived substitutes have not found yet their way into clinical practice because of safety issues and the lack of regulatory approval. Electrospun nanofibers can mimic the desirable properties of decellularized matrix grafts. The ECM graft wall is a web of randomly orientated micro and/or nanofibers, which can be successfully imitated by appropriate nanofibers processing.

Many groups have spun a variety of biodegradable polymers. [3] Those consist of either natural (gelatin, elastin, cellulose derivatives etc.) or approved synthetic polymeric materials such as poly(lactic acid), poly(glycolic acid) or poly(ε-caprolactone) (PCL). However, those materials are very hydrophobic for e.g. wound dressings or tissue engineering grafts. Recently, electrospun polyurethanes (PURs) that have mechanical properties similar to the natural hydrogels such as collagen or epithelium in small diameter vessels have been evolved. [4] The PUR represent a diverse family of materials ranging from cast and thermoplastic

elastomers to flexible and rigid foams. Due to their toughness, durability, biocompatibility, and improved biostability, they have been incorporated in a wide variety of implantable biomedical devices. [5, 6].

Although a lot of publications have shown certain growing of the cells on the PUR, their biocompatibility is often low due to their artificial nature. The plasma deposition of functional coatings is the method of choice for the adjustment of surface composition and morphology of materials without affecting their bulk properties. [7] It was shown that stable amine-rich plasma films can be deposited by low pressure plasma polymerization of non-toxic cyclopropylamine (CPA) by using radio frequency capacitively coupled discharge plasma polymerization. [8 - 10] Choi et al. demonstrated that PCL or PCL/PEG electrospun nanofibers can be applied for in vivo wound healing of diabetic ulcers after grafting of amine groups and immobilization of recombinant human epidermal growth factor. [11] Furthermore, treated surface can be also functionalized by biologically active substances to provide certain role, e.g. in case of wound dressings, an antimicrobial substance, which can protect from the environment and facilitate the healing process. Chitosan is promising material for its antibacterial, antifungal and antitumor activity and has already been utilized in some applications in combination with PUR. [12, 13].

Our main aim is to provide biodegradable hydrogel-like (amphiphilic) nanofibrous layer with enhanced biological activity and also with certain biomechanical properties. For that purpose, we investigated the electrospinning of nanofibrous meshes and thereafter focused on grafting chitosan onto the surface to provide biologically active properties of the material. For our preliminary experiments, nanofibers from either PCL or PEG or PCL/PEG polyurethane were prepared and chitosan-grafting was applied on model amphiphilic PUR elastic foil (instead of nanofibers) based as well on PCL/PEG. The strategy for grafting of chitosan on PCL/PEG PUR is based on carbodiimide chemistry in combination with PUR's surface plasma treatment by cyclopropylamine (CPA) plasma. [14]

2. EXPERIMENTAL

Materials

Argon (99.998 %) was supplied by Messer. Acetic acid (99%, p.a. grade) and Formic acid (98%, p.a. grade) were purchased from Penta s.r.o. (Czech Republic). Cyclopropylamine (98%), poly(ε-caprolactone) flakes (M_n = 80 000 g·mol^{-1}) and poly(ethylene glycol) (M_n = 100 000 g·mol^{-1}) were purchased from Sigma-Aldrich (Germany). For PUR synthesis poly(ε-caprolactone) diol (M_n = 530 g·mol^{-1},) and poly(ethylene glycol) (M_n = 400 g·mol^{-1},) were degassed at 130 °C for 3 hours under the vacuum prior the synthesis. 1,6-diisocyanatohexane (HDI, 98%, Sigma-Aldrich) was degassed under the vacuum at the laboratory temperature for 3 hours. Tin(II) 2-ethylhexanoate (95%, Sigma-Aldrich) and gaseous nitrogen (99.999 %, SIAD Czech spol. s r.o.) were used as received. For coupling the chitosan onto the PUR chitosan from shrimp shells, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) (all three purchased from Sigma-Aldrich) and citric acid (99.8 %, Lach-Ner s.r.o.) were used. Ultrapure water (UPW) of type II (according to ISO 3696) was prepared on Elix 5 UV Water Purification System (Merck Millipore).

Preparation of polyurethane solution

The polyurethane (PUR) synthesis was carried out under nitrogen atmosphere. Macrodiols with the PCL/PEG = 75/25 weight ratio were degassed at 130 °C for 3 hours prior the synthesis. After cooling down to the laboratory temperature 0.03 mol.% of tin(II) 2-ethylhexanoate catalyst (related to moles of -OH groups in the mixture) were added followed by vigorous stirring for 1 hour to maximize dispersion. Finally, HDI in the molar ratio of NCO/OH = 1.0 was dosed into the diol mixture under nitrogen and the system was let to react for 15 minutes. Subsequently, the polyurethane polymer was dissolved in calculated amount of solvent (mixture of acetic and formic acid with the weight ratio 2:1) for 24 hours and used for electrospinning.

Electrospinning of nanofibers

The PCL and PEG electrospun nanofibers were prepared by electrospinning of the polymer solutions (9 wt.% and 11 wt.%) dissolved in a mixture of acetic acid and formic acid (2:1). Prepared solutions were stirred for 24 hours at room temperature followed by the electrospinning process using the Nanospider™ NSLAB 500 (ELMARCO). The polymer solutions were electrospun with a 20 cm long wired electrode by voltage from 40 up to 60 kV with interelectrode distance between 100-120 mm. The high-voltage electrode rotated at 5 rpm and the fabric collector at the grounded electrode moved at 12 $mm.min^{-1}$. Resulting polymer foils were compact and flexible with a thickness in a range of 30-40 μm. They were cut into 5×5 cm^2 pieces and modified in plasma discharge as described below.

Plasma Polymerization

The CPA plasma polymers were prepared in a stainless steel parallel plate reactor as depicted in **Figure 1**.[8] The bottom electrode, 420 mm in diameter, was capacitively coupled to a RF generator (13.56 MHz). The gases were fed into the chamber through a grounded upper showerhead electrode, 380 mm in diameter. The distance between the electrodes was 55 mm. The bottom electrode with substrates was negatively DC self-biased due to an asymmetric coupling. The CPA was polymerized in pulsed wave (PW) CPA/Ar plasmas at pressure of 50 Pa. The pulse duty cycle and repetition frequency were 33 % and 500 Hz, respectively. The flow rate of Ar was set to 20 sccm and regulated by an electronic flow controller Hastings whereas the flow rate of CPA vapors was set to 3 sccm by a needle valve. The deposition time was 30 min. The substrates were sputter-cleaned by pulsed Ar plasma for 10 minutes prior to the deposition.

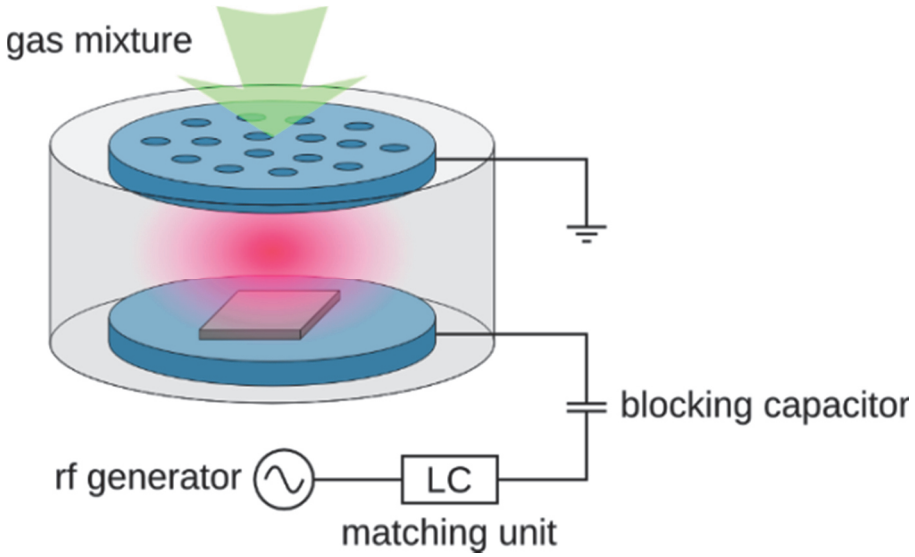

Figure 1 Schematic drawing of the plasma set-up [8]

Chitosan grafting onto the polyurethane foil

The PUR foils based on PCL/PEG (1:1) for further functionalization with chitosan were processed according to our previously evolved method. [14] Subsequently, the PUR foils were activated by CPA plasma via the procedure described in chapter 2.4 providing -NH_2 groups on the surface. The grafting was performed in two steps, both through carbodiimide-mediated coupling of the amine groups to carboxylic groups.[14] In the first step, citric acid (0.5 M solution) was grafted onto the PUR utilizing solution of 50 mM EDC and 20 mM NHS in ethanol at laboratory temperature. The PUR sample grafted by citric acid was transferred to the chitosan solution in 0.01 M hydrochloric acid with 50 mM EDC and 20 mM NHS and left there for 3 hours for coupling the chitosan to citric acid via amide bonding on PUR. Chitosan-grafted PUR samples were immersed in

ultrapure water (UPW) at room temperature for 1 hour and subsequently washed in new UPW for next 10 minutes to remove physically adsorbed chitosan on the surface. Finally, chitosan grafted PUR sample was dried *in vacuo* at 30 °C until the constant weight was reached.

Scanning Electron Microscopy

The surface of the samples was imaged by SEM Tescan LYRA3 in secondary emission mode (10 kV acceleration voltage, working distance 8-10 mm). The samples were coated with a 10 nm thick gold film deposited by RF magnetron sputtering prior to the imaging in order to avoid charging of surface.

Hydrolytic degradation tests

The stability in water was investigated by hydrolytic degradation tests carried out in an incubator at 37 °C in ultrapure water (UPW). Nanofibrous mats were cut into 1 × 1 cm pieces, immersed in UPW and removed at the given time from vials with UPW. Finally, the mats were dried at 30 °C *in vacuo* until the constant weight was reached. Mass loss was calculated according to formula (1):

$$\text{Mass loss } (\%) = (w_0 - w_t)/w_0 \times 100 \quad (1)$$

where w_0 is the weight of the sample and w_t is the weight of the dried sample after the given in from incubator. Each measurement was an average of 3 specimens and data were expressed as mean ± standard deviation.

Attenuated total reflection infrared spectroscopy

Attenuated total reflection infrared spectroscopy (ATR-IR) was performed on Fourier-transformed infrared spectrometer (Bruker Tensor 27, USA) equipped with a germanium crystal for ATR over the spectral range from 4000 to 600 cm^{-1} at the resolution of 4 cm^{-1} and 32 scans.

3. RESULTS AND DISCUSSION

The electrospinning process

The effect of electrospinning process on the resulting polymer nanofibers was studied in order to obtain suitable substrates for further plasmo-chemical depositions. The SEM micrographs (**Figure 2**) illustrate the effects of the main electrospinning parameters influencing the resulting structure and homogeneity of the fibers, which are the polymer concentration, the interelectrode distance and applied voltage and molecular weight of the used polymer.

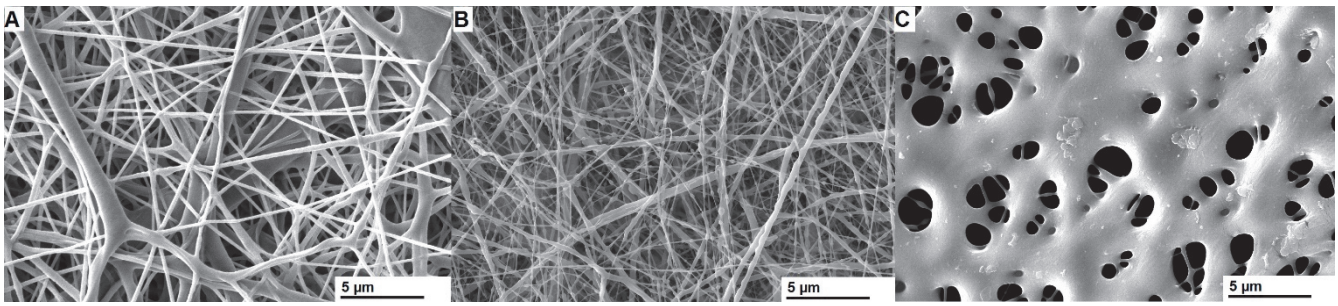

Figure 2 SEM micrographs of electrospun substrates at the magnification of 10 000 (A) PCL, polymer concentration of 11 wt.%, applied voltage of 60 kV (B) PEG, polymer concentration of 9 wt.%, applied voltage of 40 kV, (C) PUR, polymer concentration of 18 wt.%, applied voltage of 60 kV

Comparing SEM micrographs, it can be seen that a highly porous network of the fibers was observed for PCL and PEG polymers (**Figure 2A** and **2B**, respectively) and a porous substrate (**Figure 2C**) was observed for PUR polymer after the electrospinning process. The PCL fibers uniformity and higher regular morphology was likely affected by the electrospinning parameters, like high applied voltage, while the beaded fibers of PEG are more likely formed for less concentrated solutions, as viscosities are low and thus making the jet formation unstable. [16] In dependence on the combination of process parameters, it is possible to obtain PCL and PEG substrates with fine homogenous nanofibers. The preparation of PUR nanofibers was not very successful and the resulting substrate were formed by porous matrix, but not formed by separate fibers. This effect is likely caused by too low molecular weight of the prepared polymer solution. For each polymer the appropriate electrospinning condition have to be investigated with respect to desired resulting properties.

Hydrolytic degradation

The hydrolytic degradation tests of the prepared nanofibers were performed at 37 °C in UPW. The time dependence on the mass loss in the timescale of 14 days is presented in **Table 1**. Non-treated nanofibers behaved according to the nature of their composition consisted of hydrophilic PEG and hydrophobic PCL. The PEG nanofibers dissolved immediately in water, whereas nanofibers made of neat PCL lost only around 4 % of their initial weight during the given period of testing. Certain deviations from the weight representation of PCL and PEG polymers in nanofibers might be caused by slight inhomogeneities of samples. Treatment of nanofibers by CPA plasma had an impact on their hydrolytic stability. Neat PEG nanofibers did not completely dissolve after the first contact with water and a thin layer of the nanofibers remained stable for 4 days suggesting that the treatment by CPA increased the stability of the nanofiber mats in water.

Table 1 Hydrolytic stability of the nanofibers in the period of 14 days

	Weight loss non-modified (%)				**Weight loss CPA-modified (%)**			
	1 day	4 days	7 days	14 days	1 day	4 days	7 days	14 days
PCL	4.1 ± 2.0	3.6 ± 0.8	4.4 ± 1.7	3.2 ± 0.7	2.3 ± 1.9	2.3 ± 0.7	1.0 ± 1.7	1.7 ± 2.1
PEG	dissolved immediately in water				82.0 ± 2.2	90.7 ± 2.9	degraded	degraded

ATR-IR analysis of chitosan grafting onto PUR surface

The grafting of chitosan onto the PUR's surface was qualitatively evaluated using ATR-IR spectroscopy as showed in **Figure 3**. The spectrum of chitosan is characterized by broad band between 3650-2450 cm^{-1} attributed to stretching vibration of -OH groups, small peak localized on top of the broad -OH vibration band at 3368 cm^{-1} represents amine N-H symmetric stretching vibration. The absorption bands localized at 2880 cm^{-1} is attributed to -CH_2 stretching, absorption bands at 1377 cm^{-1} and 1152 cm^{-1} belong to furan C-H groups. The absorption bands at 1654 and 1594 cm^{-1} were assigned to C-C stretching of furan ring and the absorption bands with maxima at 1068 cm^{-1} and 1034 cm^{-1} are attributed to C-O stretching vibration. Characteristic absorption bands for the neat PUR are represented by amide II (urethane N-H stretching) vibration at 3322 cm^{-1}, the absorption of amide II (urethane N-H bending and C-N stretching) is located at 1536 cm^{-1} and strong absorption of C=O band at 1714 cm^{-1} was attributed to carbonyl of urethane together with carbonyl groups of PCL used as a feedstock for PUR. Other peaks presented in the spectra were assigned to asymmetric and symmetric CH_2 stretching located at 2935, 2863 cm^{-1} and further at 1463 cm^{-1}. The bands at 1250 and 1100 cm^{-1} were ascribed to C-O-C bond. The major difference between the spectra of neat PUR and PUR grafted with chitosan is manifested by broad band between 3660 cm^{-1} and 2390 cm^{-1} (red-dotted rectangle, **Figure 3**) attributed to stretching vibrations of free -OH groups of chitosan. Small differences can be seen as a shoulder between 1660-1610 cm^{-1} (red arrow in **Figure 3**) assigned to furan base of chitosan and also at 1033 cm^{-1} (inset in **Figure 3**) ascribed to C-O stretching of furan ring in chitosan.

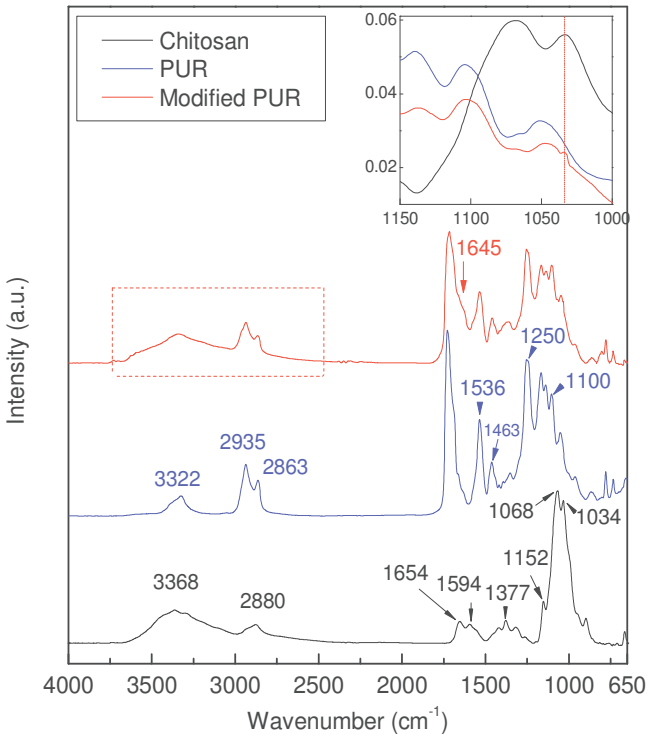

Figure 3 Infrared spectra of neat chitosan, polyurethane foil and polyurethane foil grafted with chitosan

CONCLUSION

Prepared electrospun PCL/PEG nanofibers were successfully plasma-coated in Ar/CPA mixtures by low pressure CCP discharge. Stability of nanofibers after immersion in water at 37°C is higher for amine-coated foils. Polyurethane foils were successfully activated by CPA providing -NH_2 layer for further coupling of biologically active substances (RGD, chitosan).

ACKNOWLEDGEMENTS

This research was carried out under the project CEITEC 2020 (LQ1601) supported by the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II and by COST Action MP1206 project n. LD15150 under the EU Framework Programme Horizon 2020.

REFERENCES

[1] SCHMIDT, C. E. & BAIER, J. M. Acellular vascular tissues: natural biomaterials for tissue repair and tissue engineering. Biomaterials 21, 2215-2231 (2000). doi: 10.1016/S0142-9612(00)00148-4.

[2] BERGMEISTER H, PLASENZOTTI R, WALTER I, PLASS C, BASTIAN F, RIEDER E, et al. Decellularized, xenogeneic small-diameter arteries: transition from a muscular to an elastic phenotype in vivo. J Biomed Mater Res B Appl Biomater 2008;87:95-104. doi: 10.1002/jbm.b.31074.

[3] HASAN, A., MEMIC, A., ANNABI, N., HOSSAIN, M., PAUL, A., DOKMECI, M. R., DEHGHANI, F. & KHADEMHOSSEINI, A. Electrospun scaffolds for tissue engineering of vascular grafts. Acta Biomaterialia 10, 11-25 (2014). doi: 10.1016/j.actbio.2013.08.022.

[4] BERGMEISTER, H., SEYIDOVA, N., SCHREIBER, C., STROBL, M., GRASL, C., WALTER, I., MESSNER, B., BAUDIS, S., FRÖHLICH, S., MARCHETTI-DESCHMANN, M., GRIESSER, M., DI FRANCO, M., KRSSAK, M., LISKA, R. & SCHIMA, H. Biodegradable, thermoplastic polyurethane grafts for small diameter vascular replacements. Acta Biomaterialia 11, 104-113 (2015). doi: 10.1016/j.actbio.2014.09.003.

[5] GUELCHER S. A.: Biodegradable polyurethanes: Synthesis and applications in regenerative medicine. Tissue Engineering Part B: Reviews, 14, 3-17 (2008). DOI: 10.1089/teb.2007.0133.

[6] SARTORI S., CHIONO V., TONDA-TURO C., MATTU C., GIANLUCA C.: Biomimetic polyurethanes in nano and regenerative medicine. Journal of Materials Chemistry B, 2, 5128-5144 (2014). DOI: 10.1039/C4TB00525B.

[7] KEDROŇOVÁ, E., ZAJÍČKOVÁ, L., HEGEMANN, D., KLÍMA, M., MICHLÍČEK, M. AND MANAKHOV, A. (2015), Plasma Enhanced CVD of Organosilicon Thin Films on Electrospun Polymer Nanofibers. Plasma Process. Polym., 12: 1231-1243.

[8] A. MANAKHOV, D. NEČAS, J. ČECHAL, D. PAVLIŇÁK, M. ELIÁŠ, L. ZAJÍČKOVÁ, Deposition of stable amine coating onto polycaprolactone nanofibers by low pressure cyclopropylamine plasma polymerization, Thin Solid Films. (2014). doi:10.1016/j.tsf.2014.09.015.

[9] A. MANAKHOV, P. SKLÁDAL, D. NEČAS, J. ČECHAL, J. POLČÁK, M. ELIÁŠ, et al., Cyclopropylamine plasma polymers deposited onto quartz crystal microbalance for biosensing application, Phys. Status Solidi. 211 (2014) 2801-2808. doi:10.1002/pssa.201431399.

[10] A. MANAKHOV, L. ZAJÍČKOVÁ, M. ELIÁŠ, J. ČECHAL, J. POLČÁK, J. HNILICA, et al., Optimization of Cyclopropylamine Plasma Polymerization toward Enhanced Layer Stability in Contact with Water, Plasma Process. Polym. 11 (2014) 532-544. doi:10.1002/ppap.201300177.

[11] CHOI JS, LEONG KW, YOO HS. In vivo wound healing of diabetic ulcers using electrospun nanofibers immobilized with human epidermal growth factor (EGF). Biomaterials 2008;29:587-96.

[12] YIFENG WANG, QUNFENG HONG, YANJUN CHEN, XINXIN LIAN, YANFEI XIONG, Surface properties of polyurethanes modified by bioactive polysaccharide-based polyelectrolyte multilayers, Colloids and Surfaces B: Biointerfaces, Volume 100, 1 December 2012, Pages 77-83, 10.1016/j.colsurfb.2012.05.030.

[13] AOYAGI, S., ONISHI, H., MACHIDA, Y. Novel chitosan wound dressing loaded with minocycline for the treatment of severe burn wounds (2007) International J. of Pharmaceutics, 330 (1-2), p. 138-145, 10.1016/j.ijpharm.2006.09.016.

[14] KUIJPERS, A. J., ENGBERS, G. H. M., KRIJGSVELD, J., et al, Cross-linking and characterisation of gelatin matrices for biomedical applications. J. of Biomaterials Science, Polym. 11, 225-243 (2000). doi: 10.1163/156856200743670

[15] KUPKA, V., VOJTOVA, L., FOHLEROVA, Z. & JANCAR, J. Solvent free synthesis and structural evaluation of polyurethane films based on poly(ethylene glycol) and poly(caprolactone). Express Polymer Letters 10, 479-492 (2016). doi: 10.3144/expresspolymlett.2016.46

[16] H. FONG, I. CHUN, AND D. RENEKER, “Beaded nanofibers formed during electrospinning,” Polymer (Guildf)., vol. 40, no. 16, pp. 4585-4592, 1999.