Electrospinning Production of PLLA Fibrous Scaffolds for Tissue Engineering

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Nonwoven fibrous mats were produced in the process of solution electrospinning. Polymeric fibres generated in this process consist of poly(L-lactic) acid (PLLA), biodegradable and biocompatible polymer. Produced fibrous mats were examined by scanning electron microscopy and additionally degradation rate of fibrous material was investigated. Obtained fibres exhibit porous surface and fibre diameter varied from 200 nm to 1,2 m, depending on the process parameters. Low degradation rate of scaffold material was designed for long-term scaffold usage. The influence of solvent type and solution concentration as well as the solution flow rate, applied voltage and the setup geometry on the fibres morphology and diameter were examined and presented. The influence of polymer concentration on the solution viscosity was also evaluated. Further, the degradation rate of obtained fibres was investigated, as well as the influence of degradation process on surrounding environment. Materials produced in electrospinning process have potential application as long-term biodegradable scaffold for tissue engineering, especially in bone tissue, vascular tissue or cartilage tissue engineering.

Key words: nanofibre, electrospinning, poly(L-lactic acid), tissue engineering, biomaterial, degradation.

Introduction

One of the fields of biomedical engineering is tissue engineering, as defined by American National Institute of Biomedical Imaging and Bioengineering (NIBIB) (http://www.nibib.nih.gov/) as rapidly growing area that seeks to create, repair and/or replace tissues, organs by using combinations of cells, biomaterials, and/or biologically active molecules. For engineers, the main aim in this field is to create proper biomaterial, applicable as a scaffold for cells, and for tissues to grow. Ideal scaffold must have: (1) appropriate level and size of porosity in order to allow cells migration; (2) sufficient surface area and variety of surface properties to encourage cell adhesion, growth, and differentiation; (3) degradation rate that closely matches regeneration rate of the desired natural tissue [1]. One of the most widely used materials for scaffolding is nanofibrous or microfibrous non-woven mat manufactured by various methods, especially electrospinning.

Electrospinning as a method for producing fibres from solutions of polymers or polymeric melts is known since 1934, when first described by Formhals in his patent application [2]. Since then, the lack of equipment and knowledge were the main obstacle for applying electrospinning for practical use. However, in the last two decades we observed significant growth in numbers of publication concerning electrospinning as a method for producing non-woven materials containing fibres with diameter ranging from ten nanometers to few tenths of micrometers [3]. This significant growth is based on remarkable simplicity, versatility, and potential uses of electrospinning technique [4] and improvement in the state of electrospinning equipment. Indeed, all those electrospinning setup's features combined with unique properties of fibrous materials such as wide range of pore diameter distribution, high porosity, effective mechanical properties, and specific biochemical properties [5] make electrospinning one of the most popular technique for producing submicron diameter fibres and mats containing those fibres for biomedical applications. Also, processing parameters, solution parameters and setup parameters influence all attributes of non-woven materials. Therefore it is possible to design specific nanofibrous mats for desired application by finding the most suitable processing conditions.

Properties of nanofibres may vary in a wide range. Chosen parameters have a great influence on the electrospinning process, fibres morphology and alignment include polymer parameters (type, molecular weight), solvent parameters (types, vapour pressure, diffusitivity in air), additives (surfactants, salts), polymer concentration, solution properties (rheological behaviour, relax-

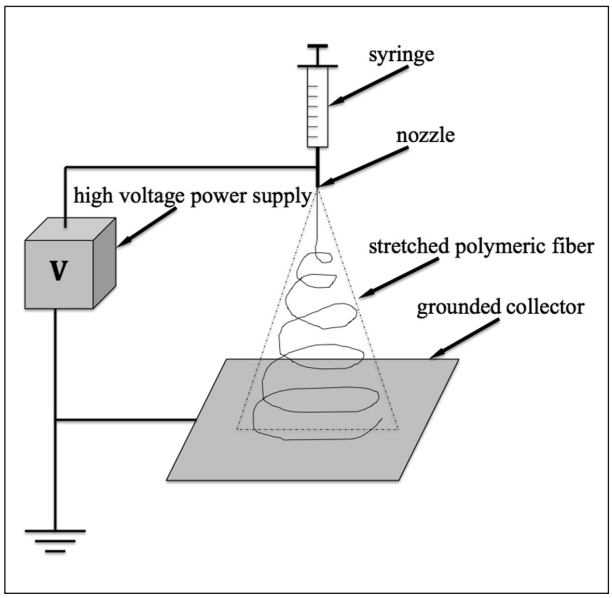

Fig. 1. Schematic of the electrospinning setup.

ation time, viscosity, surface tension, electric conductivity and dielectric permittivity), electric field (strength, geometry), solution feed rate, nozzle orifice diameter, distance from the nozzle to collector and ambient conditions (relative humidity, temperature, etc.) [6].

The elctrospinning setup and process mechanism is very simple (Fig. 1). The polymer solution is supplied through the nozzle connected to a high voltage power supply. Due to electrostatic forces, overcoming solution surface tension forces, Taylor's cone is created at the end of the nozzle [7] . The electrostatic field between the nozzle and grounded collector causes polymeric jet to stretch and bend, generating ultrafine polymeric fibres with diameter in the submicron range. Fibres produced this way are collected on a grounded collector as non-woven randomly aligned sheets or arrays.

The simplicity of the process, it versatility, and the ability to control generated material properties makes electrospinning technique extremely suitable for production of specifically designed scaffolds for tissue engineering and cell culturing [8–10]. Nature of electrospinning inspires many scientists to find the best conditions to produce desired nano and microfibrous polymeric materials for biomedical applications.

Background

The most commonly used polymer in biomedical applications is poly(lactic acid) (from here on abbreviated as PLA) which is basically a group of polymers consisting of three isomeric forms: poly(L-lactic acid) (PLLA), poly(D-lactic acid) (PDLA) and poly(D,L-lactic acid) (PDLLA). The most popular and widely used form of poly(lactic acid) is PLLA, and this isomeric form of PLA was used in this studies. In the last decade, the main use of poly(lactic acid) (PLA) was limited to medical applications such as implant devices, tissue scaffolds, and internal structures [11]. All of those biomedical applications are influenced by the properties of PLA such as biodegradability, biocompatibility, good mechanical properties and ability to be dissolved in common solvents for processing [12]. Also, PLA is one of the polymeric materials approved by FDA (US Food and Drug Administration) [13]. Due to its properties, PLA was chosen by many researchers as a basic material for manufacturing scaffolds for tissue engineering by electrospinning and other methods.

Nanofibrous electrospun mats from poly(lactic acid), more specifically L-isomer (PLLA), has been used by many groups of scientists in order for scaffolds preparation such as: culturing neural stem cells (NCS) or cardiomyocytes (CMs) to exam the influence of structure for cell proliferation and degradation rate of the scaffold [9, 10, 14]. Other application of this material is a wound dressing. Since the implementation of direct drugs encapsulation in nanofibres appeared, there has been a vast amount of research in the field of wound dressing and wound healing bandages. The influence of the presence of surfactants or drugs, for example benzalkonium chloride (BC), diclofenac sodium (DS) or lidocaine hydrochloride (LHC), was investigated by Zeng et al., and Toncheva et al. respectively [15, 16]. Additionally there is also an example of investigation of properties of single electrospun nanofibre made from poly(L-lactic acid) (PLLA) [17].

Furthermore, the next approach employing PLLA as a material for producing biomedical devices by electrospinning is blending PLLA with other polymers in order to provide better functionality and properties of resulting scaffold. Blends of PLLA with poly(-caprolactone) (PCL), poly(glycolic acid) (PGA) or poly(ethylene glycol) (PEG) were used to improve properties of the resulting material, such as ability to enhance structure of the scaffold, degradation rate or proliferation of seeded cells [18–20].

As it was mentioned before, one of the most important features of proper scaffold for tissue engineering is well-designed degradation rate, matched to the tissue regeneration rate. In order to design scaffold with suitable degradation rate, research on PLLA scaffolds were conducted [12, 21]. In fact, the degradation rate of PLLA is to low for certain application therefore poly(lactide-*co*-glycolide) is used for acceleration of degradation process [21, 22]. However, for long-term scaffold for tissue engineering, PLLA is the most suitable material.

In our project, we present a method of manufacturing nanofibrous scaffold by electrospinning. Described process is based on previously mentioned research conducted in this manner by groups of scientists and re-

searchers. Summary of some results of previous work in this field is also presented here. Nonwoven mat consist fibres made from poly(L-lactic acid) (PLLA) — an isomer L of poly(lactic acid) — solution, with diameter distribution between 200 and 1200 nanometers. The low degradation rate and the low influence on the surrounding pH level during degradation, crucial for cells proliferation and life in tissue engineering applications are achieved.

The resulting material might be used as a proper scaffold for tissue engineering, especially bone tissue, vascular tissue or cartilage tissue engineering, where long supporting time and low degradation rate is necessary. We evaluated changes of the viscosity of PLLA solution in order to find the most suitable polymer concentration for electrospinning process. In order to choose proper values of parameters we investigated how processing parameters, such as strength of electrostatic field, distance from the nozzle to collector and solution feed rate influence fibres morphology and diameter. Furthermore, we investigated the degradation rate of resulting fibrous material and influence of pH of the degradation medium on degradation of those mats.

Materials and Methods

Materials and solution preparation

Poly(L-lactic acid) (PLLA) with a molecular weight higher than 200 kDa called Biomer L9000 was used. Solvents used for preparing the solutions: dichloromethane (DCM) purchased from Chempur, Poland and N,N-dimethylformamide (DMF) purchased from Carlo Erba, France.

Solution was prepared from solid PLLA dissolved in mixture of solvents DCM and DMF with volume ratio 9:1. In the agreement with literature, this composition of solvents gives the best resulting fibres from electrospinning in our setup. Results of our experiments suggested that implementation of pure solvents evinced high instabilities and splashing, instead of jet formation. Furthermore, viscosity measurements were conducted. Solutions for viscosity measurements were prepared in a concentration (in %w/w) between 1% and 15%.

Viscosity measurements

The viscosity measurements were conducted with a Rotational Viscometer (Fungilab S.A., Spain). For solutions in the low viscosity range (from 1% to 7,5% PLLA concentration) spindle TL5 was used. Further, for solutions in the high viscosity range (from 7,5% to 15% PLLA concentration) spindle L1 was used. Both spindles were used with rotation speed between 6 rpm and 30 rpm. Spindles TL5 and L1 used in aforementioned range of rotation speed cover viscosity range for examined solution, and it is from 100 to 500 mPa·s for TL5 spindle, and from 200 to 1000 mPa·s for L1 spindle (maximum values of viscosity possible to measure with single spindle). Resulting plot (Fig. 2.) suggests that one of the most promising solution for electrospinning should be 5% w/w, due to it's level of viscosity allowing electrostatic forces to create polymeric jet, as well as maintaining stability. Also, the level of viscosity was low enough to develop polymeric jet at the end of the needle. The 5% PLLA solution was chosen for further experiments, as the highest possible concentration of polymer in the solution before a spike of viscosity.

We decided to choose the highest possible concentration, which is 5%w/w in order to provide good jet stability by surface tension forces based on the value of viscosity of the polymer solution.

For lower viscosities during the electrospinning process in our setup (Fig. 1) splashing of polymeric solution occurred causing defects on produced material. On the other hand, a PLLA solution with concentration over the 5% w/w had viscosity level too high, so the polymeric cloths occurred at the nozzle orifice and interrupted the process.

Electrospinning process

Electrospinning setup is shown in Fig. 1. The polymer solution was supplied to plastic syringe BD Discardit (Becton Dickinson S.A., Spain), then syringe was mounted on the syringe pump to supply polymer solution through the nozzle (needle EFD, USA; inner diameter 0,61 mm) with required feed rate. Nozzle was connected to high voltage power supply (self designed). The collector — aluminium plate — was grounded. The electrospinning

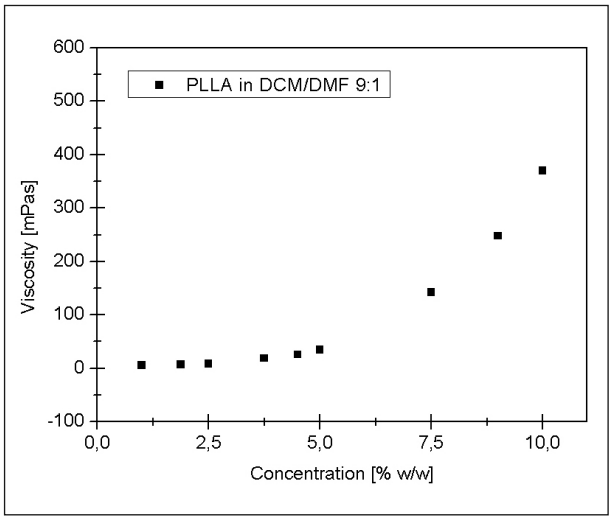

Fig. 2. Dependence of viscosity [mPa•s] on the concentration of the polymer solutions.

process was conducted in three different variants. When applied voltage was investigated, it was changed in range from 9,5 kV to 17,5 kV when other parameters were fixed: solution feed rate was 2 ml/h and distance between the nozzle and the collector was 15 cm. When distance nozzle/collector was investigated, it was changed in range from 9 cm to 19 cm when solution feed rate was set: first at 1 ml/h; and then at 2 ml/h; and applied voltage was fixed at 9,5 kV.

Degradation study

The electrospun nanofibrous mesh, after overnight drying in order to remove any residual solvents, was cut in 16 mm in diameter circle specimens, placed in bottles containing 20 ml of phosphate buffer solution (PBS, pH 7,41), and then incubated in vitro at 37°C for specified periods of time. After in-vitro degradation time, specimens were washed, and dried overnight in 40°C, and weighed. The weight loss percentage was calculated. Furthermore, the pH of residual PBS was measured (S40 SevenMulti pH meter, Metter Toledo, Switzerland) for all investigated specimens. All measurements were conducted three times for each degradation period, and average results were taken.

Morphology study

The morphology and diameter of single fibre in obtained submicron fibrous materials were investigated on images from scanning electron microscope (SEM) Phenom (Fei Company, USA) with commercial software Photoshop CS5. Samples were coated with 15 nm layer of gold before SEM examination. Further, statistical analysis was performed and fibrous mats were characterized by average fibre diameter and standard deviation.

Results and discussion

As mentioned above, 5% w/w PLLA solution in DCM/DMF volume ratio 9:1 were used. Solution in this concentration gives the best results in our electrospinning setup. Any instabilities of the jet caused splashing of the solution. Proper value of solution viscosity, and surface tension low enough to generate polymeric jet and sufficient enough to maintain it in stable condition, without ruptures occurred. In this mater, production of non-woven electrospun mats composed of ultrafine polymeric nanofibres is possible in stable way giving desired nanofabric product.

Further, experiments on the influence of changes of distance between the nozzle and the collector on the diameter of produced fibres for two different solution feed rates: 1 ml/h and 2 ml/h and fixed applied voltage 9,5 kV were conducted. Results (Fig. 3), in agreement with

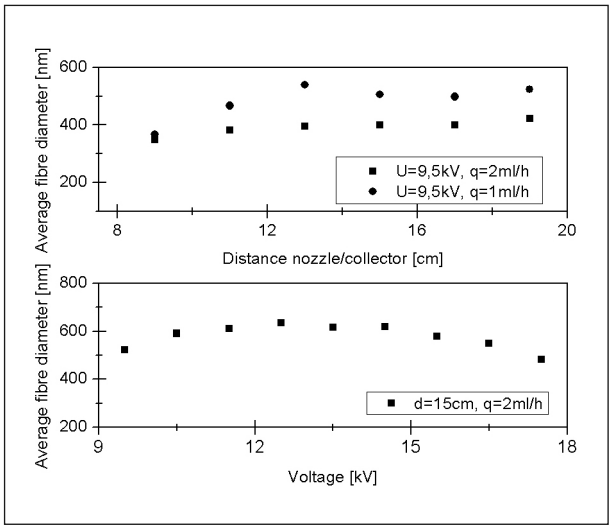

Fig. 3. Dependence of average fibre diameter on distance between the nozzle and the collector; applied voltage: U=9,5 kV, solution feed rates: 1 ml/h and 2 ml/h. Dependence of average fibre diameter on applied voltage; distance between the nozzle and the collector: 15 cm; solution feed rate: 2 ml/h.

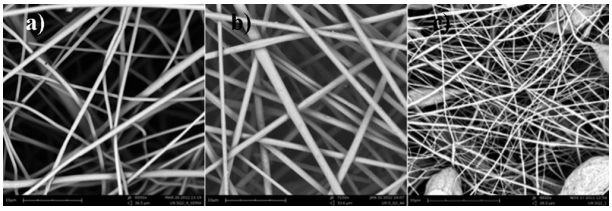

Fig. 4. SEM photographs of fibres obtained for distance between the nozzle and the collector: a) 9 cm, b) 13 cm, c) 19 cm; voltage: 9,5 kV; solution feed rate: 2 ml/h. Scale bar in all photographs represents 10 ≒m.

literature, show slight difference between those two values of solution feed rate in average fibre diameter on the level of about 100 nanometers. Also, we observe, for both solution feed rates, increase in average fibre diameter with increasing the distance between the nozzle and the collector. Morphology of resulting fibres can be observed on SEM photographs (Fig. 4). When the highest distance between the nozzle and the collector was set, resulting fibres possessed beaded structure. Moreover, when the lowest distance between the nozzle and the collector was set, resulting fibres became thinner, but diameter distribution in material obtained this way suggest, that there are a lot of thick fibres (Fig. 4, a)). Nanofibrous material with narrow diameter distribution and ultrafine fibres without beads occurred and was obtained since distance between the nozzle and the collector was set in range from 11 to 15 cm (Fig. 4, b)).

The results of examination on the influence of changes of applied voltage on average fibre diameter resulting in non-woven material shows, that there is a max-

Table 1. Parameters for producing nanofibrous biomaterial suitable for tissue engineering.

Parameter:	Voltage U	Distance nozzle/collector d	Solution feed rate q	Solution concentration of polymer c	Solution viscosity μ
Value:	9,5 kV	15 cm	2 ml/h	5 %w/w	34,6 mPa·s

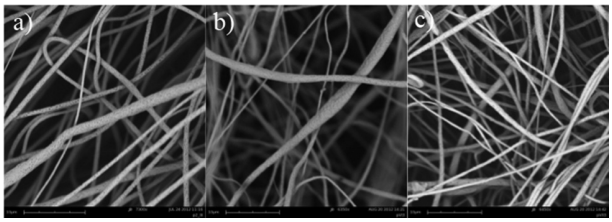

Fig. 5. Morphological changes in porous PLLA nanofibres after: a) 3 days, b) 21 days, c) 28 days of degradation in PBS, at 37°C.

imum average fibre diameter for 12,5 kV (Fig. 3). Thus, there are two possible voltage ranges, claimed as preferred in this process. Voltage below 12,5 kV allows conducting the process in a stable way, without any tearing and splashing of the electrospun polymeric solution during the process, also it is sufficient to create ultrafine fibres with low average diameter (Fig. 3). On the other hand, applied voltage above the 12,5 kV creates high instabilities in the stretched polymeric jet. This causes fibres to rupture and affects badly the material morphology.

Figure 5 shows changes in morphology for the obtained nanofibrous scaffold material in function of different periods of degradation time. There are no significant changes in morphology of nanofibres made from PLLA solution for degradation time of 28 days. Further, Fig. 6 shows weight loss and pH change as a function of time. It is known in literature, that PLLA has a low degradation rate. In this case, we found that nanofibrous material exhibits the same low degradation rate as described by others, independent of the fibrous structure. Thus, there is no significant influence of the fibrous morphology of the material on degradation rate. Moreover, it follows from the curve for pH of surrounding environment, that pH drop is low enough to be considered as a harmless for cell culture.

In fact, there is a weight increase at 7th day of degradation. It is caused by salts or any other residues from buffer solution (PBS), which might were not be washed properly from tested material or rested in pores of this highly porous material. Also, weight increase might be caused by using the average value of degradation percentage from three different samples. Regardless, discarding this value, during all degradation time weight loss is nearly linear for each specimen, with scope suggesting long degradation time, which is highly preferable for material designed as a long-term scaffold for tissue engineering — the goal of this work.

It is well established, that applied voltage, distance between the nozzle and the collector and solution feed rate have great influence on resulting electrospun polymeric nanofibres morphology and diameter [23]. In order to obtain desired, applicable in tissue engineering, nanofibrous polymeric non-woven material, which is the goal of this work, it is necessary to adjust at least before mentioned processing parameters of electrospinning. Indeed, the most significant influence on fibres diameter displays applied voltage and needs to be carefully picked. Nevertheless, solution feed rate and distance between the nozzle and collector influence fibre morphology and dimensions as well, which was also established earlier by many other researchers groups. In order to produce non-woven material from poly(L-lactic acid) (PLLA), suitable as a scaffold for tissue engineering, having desired fibres diameters in range from about 200 nm to about 1200 nm, desired low degradation rate and causing low acidosis in the surrounding environment, parameters showed in Table 1 should be applied for electrospinning setup shown in Fig. 1, used by our group. Applied voltage and solution feed rate values used in this work give possibility to obtain fabric material with fibres possessing desired diameter distribution and average diameter. Distance between the nozzle and the collector gives an opportunity to produce well-connected multilayer material without any imperfections, such as beads. Nanofibrous polymeric material, suitable for tissue engineering of bones, blood vessels or cartilages, being a result of this work, with diameter distribu-

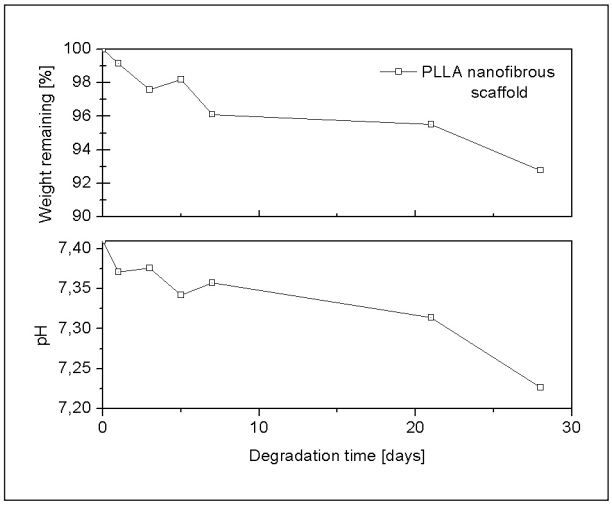

Fig. 6. Weight loss and pH changes during in-vitro degradation of PLLA nanofibres.

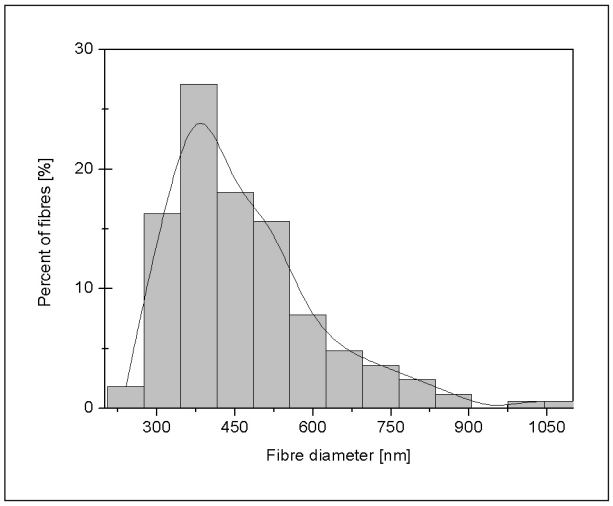

Fig. 7. Fibres diameter distribution for material produced by electrospinning; voltage: 15 kV, distance nozzle/collector: 15 cm; solution feed rate: 2 ml/h; polymer concentration in the solution: 5 %w/w.

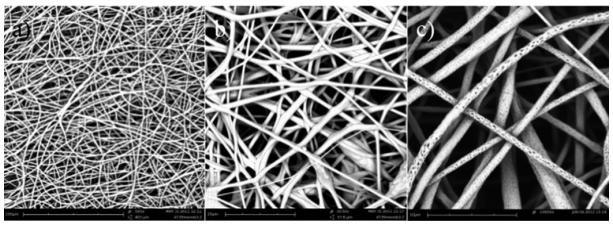

Fig. 8. SEM photographs of resulting fibrous material, scale bar represents: a) 200 ≒m; b) 20 ≒m; c) 8 ≒m.

tion shown in Fig. 7, the average diameter 500 ± 110 nm, and morphology depictured in Fig. 8, having long in-vitro degradation time, was obtained by electrospinning.

This paper shows how to create specially designed nanofibrous scaffold material for tissue engineered human blood vessels, cartilage and bones by seeding cells on it, by using simple electrospinning setup. The PLLA scaffold was made by tailoring strength of the applied electric field, distance from the nozzle to collector and solution feed rate in order to obtain designed fibre diameter distribution and morphology, suitable for cell culturing.

Electrospun poly(L-lactic acid) (PLLA) solutions into nanofibrous materials, well known as biodegradable and biocompatible polymeric scaffold, are now commonly used in bioengineering. To produce proper scaffold for cell culturing or tissue engineering, it is necessary to understand production method and all parameters affecting resulting biomaterial. However, it is necessary to establish if the resulting material have a proper biocompatibility and are non-toxic for any kind of cultured cells. Evaluating cytotoxicity and biocompatibility of materials obtained in this work will be conducted in the future.

Conclusions

Electrospinning is a versatile method to produce fibrous mats from PLLA. Obtained fibrous material is porous and exhibits diameter ranging from a two hundred nanometers to one micrometer (Fig. 7), possessing desired biodegradation time and rate. The porous structure and application of biocompatible and biodegradable polymer allows the application of obtained mats in tissue engineering, what will be the goal of future experiments.

Acknowledgements

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