

Electro-spinning of PLGA/PCL blends for tissue engineering and their biocompatibility

Nguyen Thi Hiep · Byong-Taek Lee

Received: 30 November 2009 / Accepted: 1 March 2010 / Published online: 16 March 2010
© Springer Science+Business Media, LLC 2010

Abstract In this study, an electro-spun co-polymer PLGA/PCL blend was fabricated using various percentages of PLGA in the blend PLGA/PCL solutions. The PLGA/PCL ratios used to fabricate the electrospun fibrous mats were reflected in the FT-IR (Fourier Transform Infrared Spectroscopy) data. Experimental results from the MTT assay showed that the biocompatibility of the electro-spun co-polymer increased at increasing percentages of PLGA. In vitro cells adhesion and proliferation of fibroblast cells on electro-spun mats were characterized by SEM morphology. In addition, we found that increasing PLGA concentrations affected the mechanical properties of electro-spun membranes and increased the biocompatibility of PLGA/PCL electro-spun fibrous mats.

1 Introduction

Tissue engineering is an emerging multidisciplinary field involving biology, medicine, and engineering and this field is likely to revolutionize the ways we improve health by restoring, maintaining, or enhancing tissue and organ function [1]. The foundation of tissue engineering for diagnostic applications is based on the ability to exploit living cells in a variety of ways. Tissue engineering research includes the following areas: biomaterials, cells, biomolecules, engineering design aspects, biomechanical aspects of design, informatics to support tissue engineering, stem cell research, etc. [2]. In terms of biomaterials, novel

biomaterials have been designed to direct the organization, growth, and differentiation of cells in the process of forming functional tissue by providing both physical and chemical cues [3]; The involvement of cells in the tissue engineering field include developing novel methods for the proliferation and differentiation of cells. The biomechanical aspects of tissue engineering design includes properties of native tissues, identification of minimum properties required for engineered tissues, mechanical signals regulating engineered tissues, and efficacy and safety of engineered tissues [4].

The electro-spinning method is one of the simplest among all methods for the preparation of fibrous mats as extra-cellular matrices. Electro-spinning offers a new direction for biopolymers since this approach allows one to decrease the diameter of the pore size from micrometers to nanometers; thereby, increasing the surface area to volume or mass ratio and the mechanical properties of the polymer [5]. The advantages of the electro-spinning technique are the production of very thin fibers, on the order of few nanometers or micrometers, with large specific surface areas, which allow for easy functionalization for various applications, superior mechanical properties and ease of processing, as suggested by many experts in the tissue engineering field [6]. Electro-spun scaffolds allow cells to grow while providing sufficient mechanical support. Tan et al. reported that the mechanical properties of fibers increased when the inner diameter of the fiber was decreased [7]. The scaffolds currently used for arteries, however, are still far from optimal in terms of mechanical endurance and biocompatibility. The structure and properties of electro-spun biopolymer scaffolds are of critical importance for their capable application in tissue engineering. It has been generally accepted that the electrospun mats need to be biocompatible, biodegradable with

N. T. Hiep · B.-T. Lee (✉)
Department of Biomedical Engineering and Materials,
College of Medicine, Soonchunhyang University,
Cheonan 330-090, Korea
e-mail: lbt@sch.ac.kr

degradation or resorbable rates that match the tissue replacement and possess good mechanical properties to match those of the tissues at the site of implantation. The characteristics of electrospun mats include an appropriate extracellular matrix structure to facilitate cell adhesion, proliferation and differentiation, architecture similar to a real tissue made from biodegradable or biocompatible material, an internal architecture with fully interconnected pores and mechanical strength that satisfies physiological requirements. In recent years, many polymers have undergone electro-spinning to fabricate nano-fibers for different applications such as artificial skin [8], artificial blood vessels [9], bone tissue engineering [10], bladder, etc. The polymer combinations used in these processes have included the use of a single natural polymer [11], synthetic polymer [12], copolymer consisting of a natural and synthetic polymer [13], etc. However, to overcome the limitation associated with a single polymer, a new method was developed that involves the simultaneous electro-spinning of a blend of two polymer solutions delivered by mixing the two polymer then electro-spinning. The blend electro-spun method not only overcomes the limitations associated with a single polymer, but also creates a new component without the need for synthesizing a new co-polymer, which is difficult and complex to achieve using co electro-spinning of two single biopolymers. In the blend electro-spun method, one can blend the two polymers using an electro-spun 2-eject system, which contains a mix of the polymers and then electro-spin the mix. For example, Duan et al. [8] investigated electro-spun PLGA–PVA/chitosan blends for skin applications, where they mixed PVA and chitosan during the first electro-spinning injection and PLGA was added during the second electro-spinning injection. Using this approach, they obtained PLGA-PVA/chitosan mats that had the strong mechanical properties of PLGA, absorptive properties of PVA and antibacterial activity of chitosan.

In this research, electro-spun PLGA/PCL mats were fabricated from PLGA (85:15), which is an expensive synthesis polymer. Therefore, PCL was used as the main component because it is relatively inexpensive and stable in material handling and storage [14]. PCL is a flexible biopolymer that has been used to overcome the brittle and low elongate properties of PLGA [15]. However, PLGA is better than PCL in respect to cell adhesion and proliferation due to its more hydrophilic property [15]. Polycaprolactone (PCL) is hydrophobic and, thus, does not have any physiological active sites, which makes PCL unfavorable for cell growth when it comes into contact with the living body. Although, when the behavior of epithelial cells was evaluated on a PCL membrane, the PCL membrane was shown to enhance epithelium growth. However, cell affinity towards PCL is generally poor due to their low

hydrophilicity and lack of surface cell recognition sites [16]. Even though the electrospun PCL mats mimic the identity of ECM in living tissues, its poor hydrophilicity caused a reduction in cell adhesion, migration, proliferation, and differentiation [17, 18]. The advantage of using PCL in this composite is PCL has high mechanical strength, which is a high valuable property for tissue engineering applications. PLGA was added to the composite to support cells attachment, adhesion and cells proliferation on the PLGA/PCL electro-spun mats, which was previously demonstrated for human mesenchymal stem cells and osteoblast cells [19, 20]. The morphology of the fine PLGA/PCL electro-spun fibrous mats was imaged by SEM. The composition and mechanical properties of the blend electrospun fibrous mats were characterized by FT-IR and tensile strength tests, respectively. In vitro cell adhesion and proliferation were tested by seeding cells for 60 min, 1 day and 3 days. The MTT assay showed that the PLGA/PCL electrospun mats were highly biocompatibility.

2 Materials and methods

2.1 Materials

PLGA (85:15), polycaprolactone (M_n 80 000), tetrahydrofuran (THF, minimum 99%), Dimethylformamide (DMF, 99%) were purchased from Sigma-Aldrich, USA. Methyl Chloride (MC, SK Chemicals Co., Korea). DMSO (Dimethylsulfoxide 99, 0%, Samchun Pure Chemical Co., LTD, Korea), Ethanol (Merck, Germany). Fetal bovine serum (FBS), P.S. (penicillin/streptomycin [antibiotics]), Dulbecco's phosphate buffered saline (D-PBS) without calcium or magnesium, MTT solution and trypsin–EDTA were purchased from GIBCO (Carlsbad, CA). The L-929 cells line was obtained from the ATCC Cell Line (CCL-1TM, NCTC clone 929 [L cell, L-929, derivative of Strain L], Korea). HDMS (Hexamethyldisilazane, Sigma, USA).

2.2 Preparation of polymer solutions

10 wt% PLGA was dissolved in THF: DMF (50:50) [8] and 12 wt% PCL was dissolved in MC: DMF (20:80) [21]. Both solutions were prepared overnight until the solution became homogeneous. Then, several PLGA/PCL azeotrope solutions were prepared by adding a homogeneous PLGA solution to a homogeneous PCL solution.

2.3 Electro-spinning setting

PLGA/PCL blend solutions were placed in a plastic syringe (lure-lock type, 12 ml) and connected through a metal

syringe needle, which was already connected to a nozzle gauge (25 gauge, inner diameter 0.25 mm) on the pump. The parameters of electrospinning were as follows: the solution flow rate was 0.5 ml/h, a high voltage of 25 kV was supplied directly from a high DC voltage power supply (NNC-30 kilovolts-2 mA portable type, NanoNC Korea) and the samples were collected through a steel plate 20 cm away from the tip of the syringe needle.

2.4 Characterization

2.4.1 Morphology

The detailed structure of each polymer fibrous mat was imaged by scanning electron microscopy (SEM) (SM-65F, JEOL, and Japan) at an acceleration voltage of 25 kV. Before SEM observation, all of the samples cut from the electrospun fiber mats (1 cm × 1 cm) were sputter coated with gold under a JEOL JFC-1200 fine coater for 60 s. The average fiber diameter of the electrospun fibers was measured from the SEM images using Adobe Photoshop 5.0 software.

2.4.2 FT-IR analyze

The electro-spun PCL, PLGA and blend PLGA/PCL nanofibrous mats were characterized by attenuated reflectance Fourier transform spectroscopy (Spectrum GX, PerkinElmer, USA). The infrared spectra of the samples were measured over a wavelength range of 4,000–500 cm⁻¹. All spectra were taken in the spectral range by the accumulation of 64 scans with a resolution of 4 cm⁻¹.

2.4.3 Mechanical properties

The dimensions of the samples were measured by SEM before measuring the tensile strength (thickness 100 μm, width 1 mm and length 27 mm). Mechanical characterization was achieved by applying tensile test loads to specimens prepared from the electro-spun ultra fine non-woven fiber mats. Since a single polymer nano-fiber is very weak, the resulting non-woven mat was so delicate that any direct contact with the mat surface during manipulation could damage the fibers. Thus, sufficient care was taken in preparing and handling the tensile specimens in order to avoid severe damage. The specimens were prepared using the following method. First, a white paper frame was cut as shown in Fig. 4, and sample (1) was glued (2) onto the top and bottom areas of one side. The double-sided tape (3) was 2 mm and the hold (4) was used to remove the frame from the load-cell. The frame was then glued onto the topside of the fiber mat, and was cut into rectangular pieces along the vertical lines.

2.5 In vitro study

2.5.1 Sterilization approach and preparation sample for in vitro

All electro-spun mats were placed in a vacuum oven for 48 h at room temperature and sterilized using 70% EtOH for 30 min before conducting in vitro testing.

2.5.2 Cell line and maintenance

Cell culture studies were carried out with the L929 cell line obtained from ATCC Cell Line (CCL-1TM, NCTC clone 929 [L cell, L-929, derivative of Strain L], Korea). The L-929 cells were subcultured in flasks using RPMI media, supplemented with 10% (v/v) FBS (fetal bovine serum) and 1% PS (penicillin/streptomycin (antibiotics)), 50 μg/ml ascorbic acid (Sigma) and 10 mM β-glycerophosphate (Sigma) at 37°C, 95% humidity and 5% CO₂ (Incubator, ASTEC, Japan). Cells were dissociated with trypsin-EDTA (GIBCO) centrifuged and resuspended in medium. The culture medium was replaced every 2 days. Prior to cell culture experiments, 24-well or 96-well tissue culture test plates gamma sterilized (SPL Life Science, Becton-Dickinson Labware) were prepared. The culture plates were then sprayed with 70% ethanol before being placed under UV light for 30 min to sterilize them.

2.5.3 MTT assay/cytotoxicity assay

The cell samples were washed in PBS 1 time and medium 2 times before the cells were seeded. The cellular viability % of fibroblast cells on each polymer fibrous mat was determined using the Cytotoxicity or MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assays. The L-929 mouse fibroblasts cells were seeded in 96-well tissue culture plates at 1,000 cells/well in 100 μl RPMI. All media contained 10% FBS and all cell lines were incubated overnight. Diluted extract solutions (100 μl) of every fibrous mat at various concentrations (100, 75, 50, 25 and 0%) were then added. The L 929 fibroblast cells were treated for 1, 2, and 3 days and then 20 μl of filtered MTT solution (5 mg/mL in PBS) was added. After incubation at 37°C for 3.5 h, the medium was removed from the well and 150 μl of DMSO was added to dissolve any insoluble formazan crystals. The absorbance was measured at 560 nm using an ELISA reader (Turner Biosystems CE, Promega Corporation, USA) and the cell viability was calculated as the percentage relative to the untreated control cells.

2.5.4 Adhesion, growth behavior of cells

Sample membranes 20 mm in diameter were sterilized with 70% EtOH (30 min), then washed with PBS and suspended in conditioning medium (15 min). The fibroblast cells (L-929) were cultured on TCPs (Tissue Culture Polystyrene), control-like and samples. The cells were seeded at a cell density of 10^4 cells/cm² in RPMI and cultured for 1 and 3 days at 37°C in a humidified air atmosphere with 5% CO₂. After 1 and 3 days of culture, cellular constructs were harvested, rinsed twice with PBS to remove non-adherent cells and subsequently fixed with 2% glutaraldehyde for 15 min and washed with DPBS two times (15 min/time). The samples were then dehydrated through a series of graded ethanol (EtOH) solutions (50, 75, 90, 95 and 100%) and 5 min was used for every washing time. The cells were then washed three times in 100% EtOH. Finally, the samples were washed two times with HDMS. All samples were air-dried overnight. Dry cellular constructs were sputtered with gold and imaged by SEM.

3 Results

3.1 SEM morphology of co-polymer PLGA/PCL electro-spun

The morphology of electro-spun ultra-fine fibers is influenced by various parameters such as the applied voltage, solution flow rate, distance between capillary and collector, and especially the properties of the polymer solutions

including concentration, surface tension and the nature of the solvents [22]. However, the solubility of the polymer should be examined in more detail when the electro-spun material is a co-polymer because the uniform electro-spun material just appears as a homogenous dissolution. In this study, we mixed several ratios of PLGA and PCL (10, 20 and 30% PLGA) that were from separate homogenous solutions. The mixed polymers were incubated together for 6 h at room temperature to create a homogenous azeotrope. The electro-spun PLGA/PCL mats were then fabricated using an electro-spinning machine (NNC-30 kV-2 mA portable type, Nano NC. Korea.). The parameters used for the fabrication of each polymer fibrous mats were as follows: voltage 25 kV, distance 20 cm, flow rate 0.5 ml/h and nozzle 25 G. SEM images and the diameter distributions of each different type of fiber are shown in Fig. 1a, b and c. Based on the analysis of the SEM images, the electro-spun mats were homologous and uniform over a large area. However, morphology of each sample showed differences in fibers; for example, in cases of PLGA/PCL (10/90) (Fig. 1a), fibers were not cylindrical and were attached to each other. Contrary to Fig. 1a, the case of PLGA/PCL (20/80) (Fig. 1b) showed that electro-spun fibers were cylindrical and separated with continuous fibers. On the other hand, when the concentration of PLGA was increased to 30, PLGA/PCL (30/70) (Fig. 1c) showed that the fibers were attached and had melted into each other. Variation in fibers for each sample also changed in cases of PLGA/PCL (20/80), the diameter for over 60% of the fibers was around 500 ± 100 nm. For the PLGA/PCL (10/90) electro-spun fibers, the diameter for over 60% of the fibers was around $1,000 \pm 200$ nm (Fig. 1a). Finally,

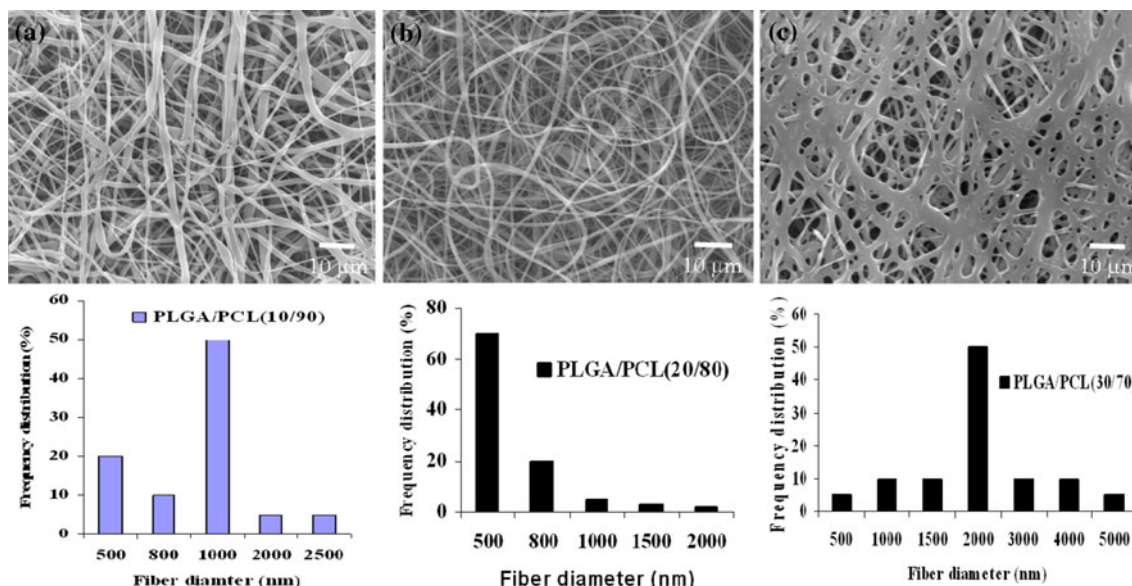


Fig. 1 SEM morphology of the PLGA/PCL blend (10/90) (a), (20/80) (b), (30/70) (c) electrospun nano-fibrous mats

the PLGA/PCL (30/70) electro-spun fibers were attached to each other and the diameter for over 50% of the fibers were around $2,000 \pm 200$ nm and the remaining fibers were very large.

3.2 FT-IR

To determine if the blended PLGA/PCL electro-spun fibers were successfully fabricated, we analyzed the FT-IR spectrum of PCL, PLGA, and PLGA/PCL at different PLGA/PCL ratios (Fig. 2). The FT-IR spectrum of the blend PLGA/PCL electro-spun fibrous mats showed that the structure of blend electro-spun mats contained all the peaks corresponding to PCL and PLGA. More specifically, all of the PCL peaks in the PLGA/PCL composite overlapped with all of the PLGA peaks without the peak at 865 cm^{-1} , which appeared separate from the PCL peaks. In this case, the PLGA peaks had large widths but not depths while the PCL peaks had large depths but not widths. In addition, the amount of PLGA was smaller than the amount of PCL. Therefore, a larger PCL width in the blend electro-spun PLGA/PCL fibers was expected. Figure 2 also demonstrates that no reaction between PCL and PLGA occurred; these fibers were just blended composites. Almost peaks of the blend PLGA/PCL electro-spun fibrous mats overlapped PCL peaks, it is easy to confuse but the peaks at 865 cm^{-1} which asserted PLGA present. However, the intensity of this peak increased with increasing amounts of PLGA. The combined results demonstrate that the blend PLGA/PCL electro-spun fibrous mats were successfully fabricated.

3.3 Mechanical property

Since a single polymer nano-fiber is very weak, the resulting non-woven mat was so delicate that any direct

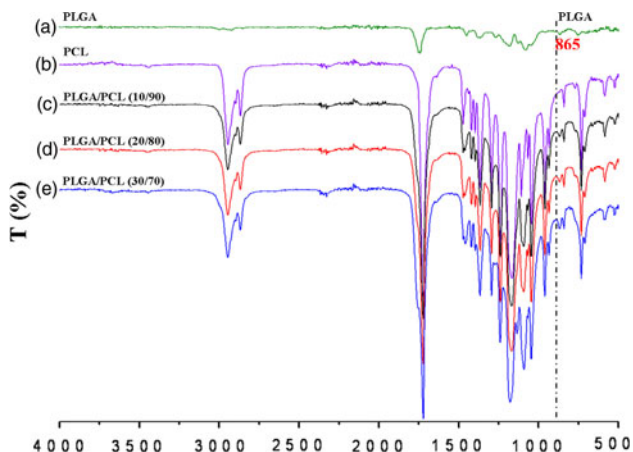


Fig. 2 FT-IR curves of electrospun PLGA (a), PCL (b), PLGA/PCL (10/90) (c), (20/80) (d), (30/70) (e)

contact with the mat surface during manipulation could damage the fibers. Therefore, the samples were collected using alumina foil at a similar polymer concentration, and then the thickness of each sample was measured by SEM. These samples were measured, cut carefully and glued on the frame (Fig. 3), and then the aluminum foil was removed. The paper frame was cut before initiating the tensile strength measurements. The tensile strengths of the electro-spun PLGA/PCL fiber mat at different PLGA concentrations were measured using a tensile tester (Universal Testing Machine, R&D Co., Ltd., Korea). Figure 4A shows the curves of PCL and the PLGA/PCL composites at different PLGA concentrations. In these experiments, we found that the stress in the electro-spun PLGA/PCL electro-spun mats increased as the amount of PLGA increased. However, the strain did not always increase following an increase in the amount of PLGA. In the case of PCL (Fig. 4Aa) and PLGA/PCL (10/90) (Fig. 4Ab), the stress increased from 1.8 ± 0.05 to 2.5 ± 0.05 MPa and the strain increased from 130 ± 5 to 134 ± 5 (%). However, this same trend was not observed when the PLGA concentration was increased from 10% [PLGA/PCL (10/90)] (Fig. 4Ab) to 20% [PLGA/PCL (20/80)] (Fig. 4Ac). In this scenario, the stress increased from 2.5 ± 0.5 MPa to 6.1 ± 0.5 MPa but the strain decreased from 134 ± 5 to 88 ± 5 (%). When the PLGA concentration was increased

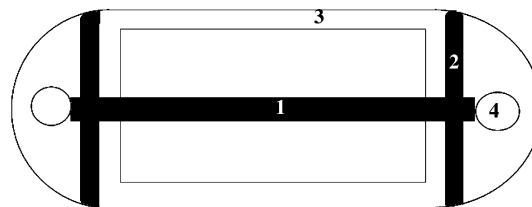


Fig. 3 A schematic of the paper frame used to prepare the tensile specimens from the electro-spun non-woven mats

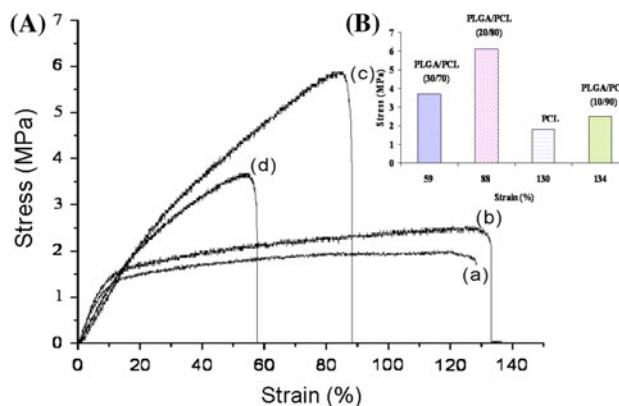


Fig. 4 A Stress–strain curves of electrospun PCL (a), PLGA/PCL (10/90) (b), PLGA/PCL (20/80) (c), PLGA/PCL (30/70) (d). B Variation of tensile strength of PCL and PLGA/PCL blend

from 20% [PLGA/PCL (20/80)] (Fig. 4Ac) to 30% [PLGA/PCL (30/70)] (Fig. 4Ad) the strain and stress both suddenly decreased from 6.1 ± 0.5 to 3.8 ± 0.5 MPa and 88 ± 5 (%) (strain) to 59 ± 5 (%), respectively. The variations in tensile strength, which contains more detail on the relationship between the stress and strain of each mat, is shown in Fig. 4B. As shown in Fig. 4Ad, the stress and strain were not high even though the amount of PLGA was high. These results most likely occurred because the electro-spun fibers containing 30% PLGA were not fine as the PLGA/PCL (20/80) and PLGA/PCL (10/90) fibers. In addition, PLGA is a very brittle material [15], which can easily break if the stress increases. Duan et al. [8]. reported a stress of 9 MPa and strain of 30% for only PLGA electrospun mats. For this reason, the mechanical properties of the electrospun PLGA/PCL mats increased at higher amounts of PLGA. Based on the combined results presented above, we concluded that the stress of the PLGA/PCL fibers was dependent on both the amount of PLGA and the uniformity of the electro-spun fibers on the mats. Because the fibers of the electro-spun PLGA/PCL (30/90) aggregated with each other and the composite was not uniform, the stress was lower than the PLGA/PCL (20/80) mats. The co-polymer containing 10 wt% PLGA had a strain value that was higher than the composites not containing PLGA. The stress–strain data showed that PCL was a flexible polymer and if it was added to brittle PLGA materials (non-flexible polymer) in small amounts, the mechanical strength under fine fibrous mats conditions was increased.

3.4 Cytotoxicity results

The cytotoxicity of PLGA/PCL nanofiber mats was tested by quantitative analysis using the MTT test. After allowing the cells to spread for 3 days at the various extract dilutions (0, 25, 50, 75 and 100%), the proliferation of L-929 cells was measured by the MTT assay. In the case of the control, the extract solution containing cultured cells was not added. Absorbance values were used to measure cellular proliferation. The extracts containing the blend electrospun PLGA/PCL nanofiber mats did not inhibit cell metabolism relative to the control group. We found that cell metabolism of the blend electro-spun PLGA/PCL nano-fibrous increased with an increase in the PLGA concentration. Based on the quantitative scores, it was concluded that the extracts of the nanofibers mats displayed no cytotoxic reactivity in this test, as shown in Fig. 5. In addition, neither cell death nor morphological disorder was observed throughout the incubation period. Thus, it was concluded that PLGA/PCL nano-fibrous mats produced from this process displayed no cytotoxic effect due to the presence of remnant solvents.

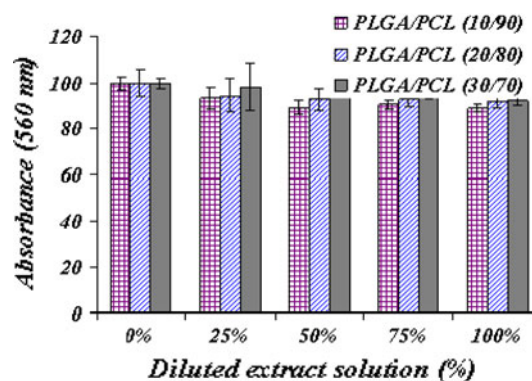


Fig. 5 MTT cytotoxicity of the blend electro-spun PLGA/PCL mats at different PLGA/PCL ratio

3.5 Cellular proliferation

The proliferation of fibroblast cells on the blend electro-spun PLGA/PCL fibrous mats was evaluated by the reduction of MTT to formazan by mitochondrial succinate dehydrogenase in complex II (succinate–ubiquinone oxidoreductase complex) [23]. The level of reduction of MTT into formazan reflects the level of cell metabolism. Figure 6 compares the time course of formazan absorbance of blend PLGA/PCL nanofiber mats fabricated with different concentrations of PLGA. Biomimetic tissue formed on the blend PLGA/PCL nanofibrous mats, which affected the viability and proliferation of cells. Cell attachment and proliferation on the electro-spun PLGA/PCL (10/90) co-polymer doubled between day 1 and day 3. Moreover, the absorbance values showed that cell attachment and proliferation increased with an increase of PLGA concentration in the PLGA/PCL composite; however, between PLGA/PCL (20/80) and PLGA/PCL (30/70), a slight increase was observed. According to the results, we concluded that electro-spun PLGA/PCL (20/80) had the greatest effect on ECM production when compared to PLGA/PCL (30/70) and PLGA/PCL (10/90) (Fig. 6).

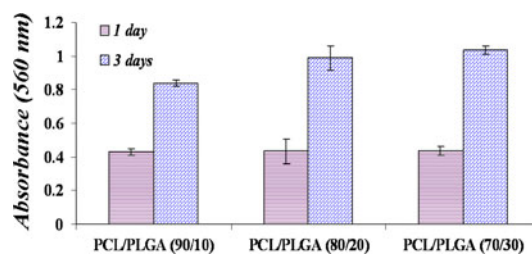


Fig. 6 Cellular proliferation of the blend electro-spun PLGA/PCL of fibroblast cells L-929 after seeding the cells for 1 day and 3 days

3.6 Cell attachment and cell proliferation

3.6.1 One cell morphology-cell attachment

The cell morphology was examined to continually check the biocompatibility of all PLGA/PCL blend membranes. Figure 7 shows the cell morphology of one fibroblast L-929 after seeding the cell for 1 h on a tissue culture plate (TCPs) (Fig. 7a1–a3). Figure 7a2, a3 shows an image of cell attachment on the PLGA/PCL (10/90) electro-spun mat, while Fig. 7b2, 3 shows an image of cell attachment on the PLGA/PCL (20/80) electro-spun mat. Finally, Fig. 7c2, c3 shows an image of cell attachment on the PLGA/PCL (30/70) electro-spun mat. By comparing the filopodium and lamellipodium in the control sample with each condition, we found that filopodium and lamellipodium growth was as good as the control. However, by comparing (Fig. 7a2, b2, c2), we found that filopodium and lamellipodium growth increased as the content of PLGA increased. On the other hand, in the enlarged image

(Fig. 7a3, b3, c3), which shows more detail, the L-929 cells were shown to be well attached to the PLGA/PCL fiber. Therefore, the PLGA/PCL electro-spun fibrous has high biocompatibility that is suitable for biomaterial applications.

3.6.2 Cell proliferation

To test cell proliferation on the PLGA/PCL electro-spun fibrous mats, we seeded the cells at the same density and incubated the samples for different time periods (short-time). Figure 8 shows the cell proliferation after seeding the cells for 1 day, while Fig. 9 shows the cell proliferation after seeding the cells for 3 days. After 1 and 3 days of cell seeding the cells were spread on all scaffolds. In Fig. 8, the images showed that the fibroblast cells were spread on the TCP and PLGA/PCL fibrous mats after seeding the cells for 1 day. These results demonstrated cellular attachment and proliferation of mouse fibroblast L-929 cells under static seeding on all PLGA/PCL electrospun mats.

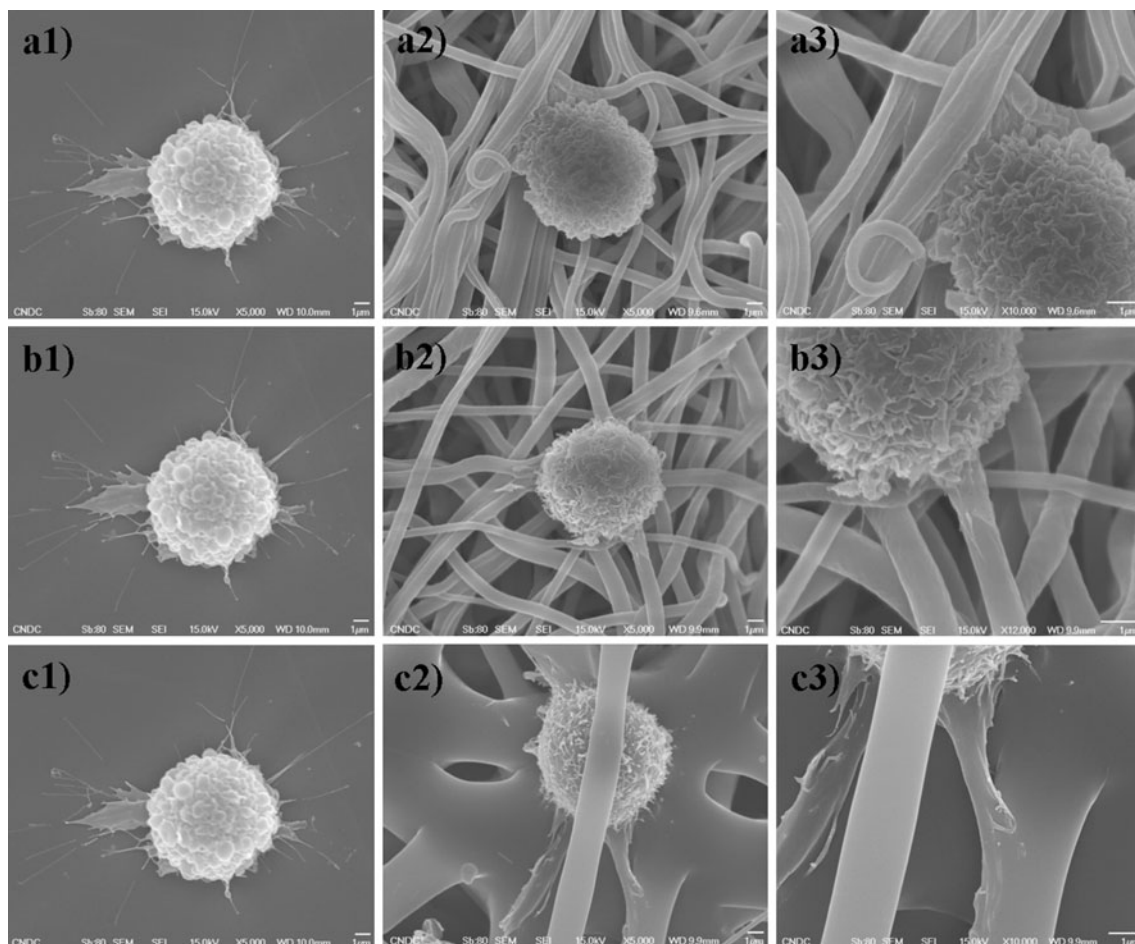


Fig. 7 Cell morphology of fibroblast cell growth on control (**a1**, **b1**, **c1**), blend PLGA/PCL (10/90) (**a2**, **a3**), PLGA/PCL (20/80) (**b2**, **b3**) and PLGA/PCL (30/70) (**c2**, **c3**) electro-spun after seeding cell 1 h

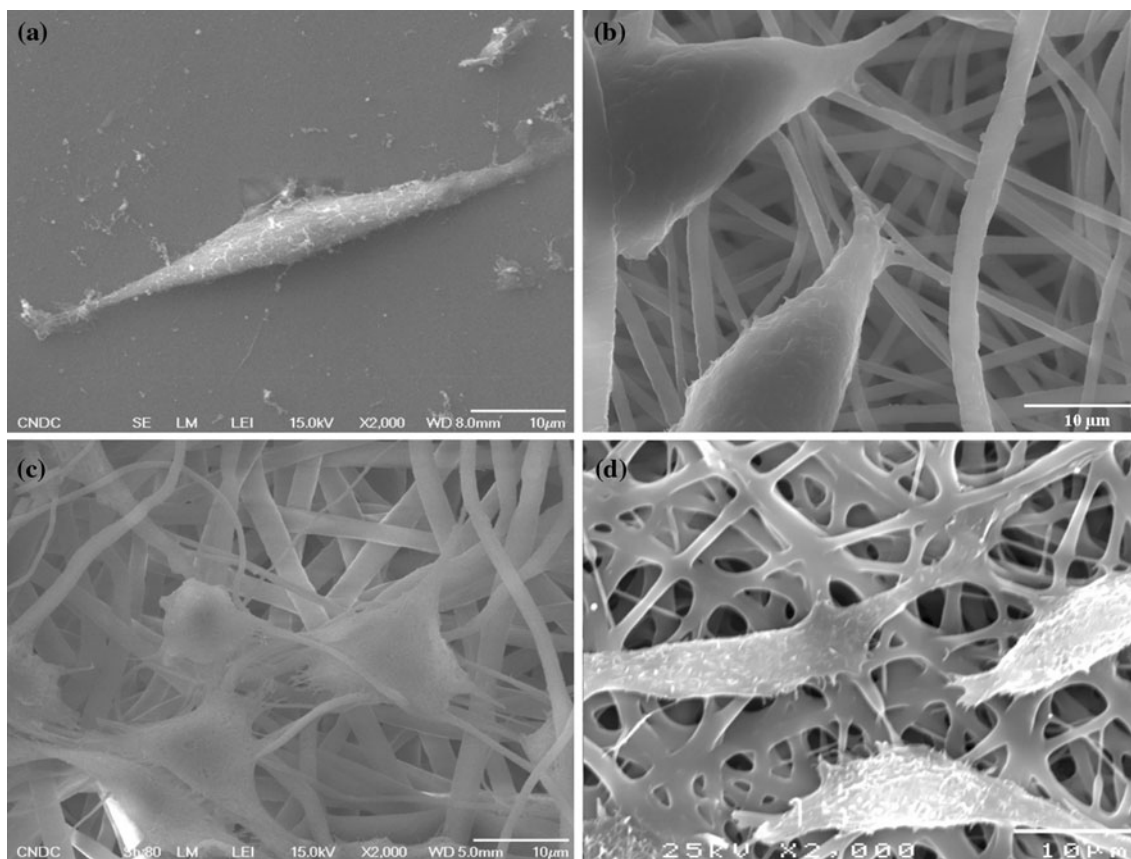


Fig. 8 SEM morphology of fibroblast cells grown on the control (a), blend PLGA/PCL (10/90) (b), PLGA/PCL (20/80) (c) and PLGA/PCL (30/70) (d) electro-spun after seeding cell 1 day

However, cell proliferation on the samples [Fig. 8b–d] was clearly better than the TCP surface [Fig. 8a] as evidenced by the development of lamellopodia, which is an indicator of cell attachment to the surface. In addition, after 1 day of seeding, cells were well grown and had spread uniformly and extensively, covering the surfaces of the biomaterials, which was similar to the control sample. The presence of lamellapodia and an interlaced fibrous network demonstrated that cell adhesion was very good. Cell spreading increased on the PLGA/PCL electrospun mats when the PLGA concentration was increased (Figs. 8b and 8 [c, d]).

After 3 days (Fig. 9), cells completely covered the electro-spun mats. To assess cell proliferation and cells attachment on the surface, we took images of each sample at different magnifications. At the highest magnification (Fig. 9a3, b3, c3), we found that cell proliferation increased at higher PLGA concentrations. In addition, cell attachment was more clearly observed on the PLGA/PCL (30/70) electro-spun fibrous mat. Even though the incubation time was just 3 days, the shape of the fibroblast cells changed from round to elongated (Fig. 9a3, b3, and c3). Based on these results, we can conclude that the electro-spun PLGA/

PCL co-polymer is a good composite for tissue engineering applications.

4 Discussion

In this study, we investigated a new component for biomaterials and tissue engineering applications; the electro-spun PLGA/PCL blend. We fabricated PLGA/PCL electro-spun mats by combining different PLGA and PCL ratios into a single PLGA/PCL component. Calvert et al. [24] and Lee et al. [25] reported that PCL should not be used at high percentages in PLGA/PCL composites due to its high cost and stability during handling and storage [14]. Therefore, we combined PCL with different ratios of PLGA to fabricate PLGA/PCL electro-spun fibrous mats. On the other hand, Calvert et al. [24] also reported that a high percentage of PLGA was not always as good as a low percentage; therefore, we only used a maximum PLGA concentration of 30% in the PLGA/PCL blend. The SEM morphology shown in Fig. 1 clearly demonstrates that PLGA concentration was very important in obtaining fine PLGA/PCL fibers mats. The PLGA/PCL electro-spun fiber

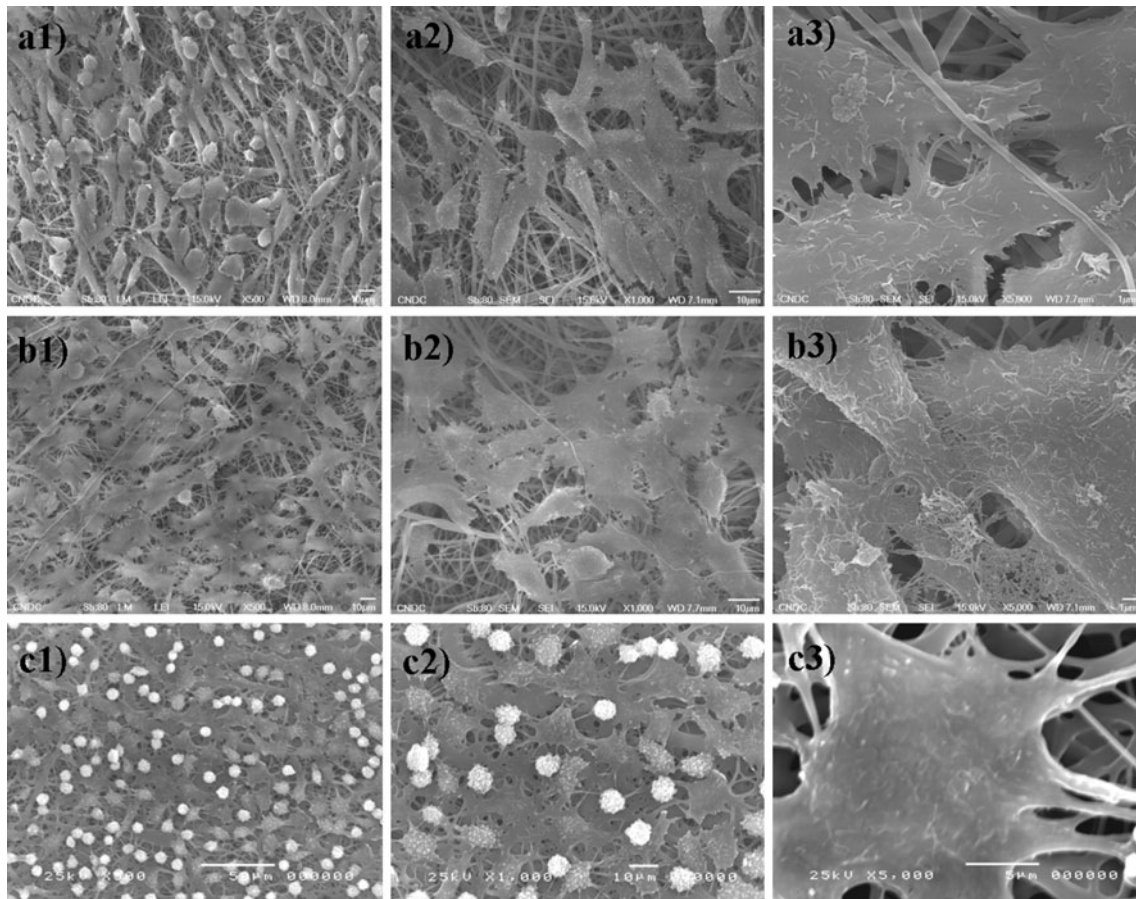


Fig. 9 SEM morphology of fibroblast cells grown on blend PLGA/PCL (10/90) (**a1**, **a2**, **a3**), PLGA/PCL (20/80) (**b1**, **b2**, **b3**) and PLGA/PCL (30/70) (**c1**, **c2**, **c3**) electro-spun after seeding the cells for 3 days

for PLGA/PCL (10:90) was very large and its mechanical strength was much higher than the composite not containing PLGA. However, at 20% PLGA, the PLGA/PCL electro-spun blend contained very fine fibers with small, smooth, cylindrical, and humongous fibers, at this composition the stress of the electro-spun mat was very high but the strain was lower relative to the PLGA/PCL (10/90) electro-spun mat. As for the PLGA/PCL (30/70) composite, the high PLGA concentration changed the heat of fusion, which affected the blend solution, resulting in a morphology that showed a blend of PLGA/PCL (30/70) electro-spun fibers were larger. The morphology of PLGA/PCL (30/70) electro-spun mat is a key to make the mechanical strength the PLGA/PCL (30/70) electro-spun mats were lower than the PLGA/PCL (20/80) electro-spun mat. Lee et al. [25] fabricated PCL electro-spun nonwoven mats from PCL in a DMF: MC (25/75) solution, which displayed a strain of over 200% and stress of approximately 1.7 MPa. In this study, the PCL electro-spun mat was fabricated from PCL in a DMF: MC (80/20) solution and had a similar stress but lower strain (130%). The PLGA/PCL (20/80) electro-spun mat showed the best mechanical strength and modulus

strength because this fibrous mat was uniform and small in diameter, both of which are important factors dictating the mechanical properties of electrospinning [7, 26]. The FT-IR curves showed that PLGA/PCL electro-spun mats were successfully fabricated using the PLGA/PCL blend due to the presence of the 865 cm^{-1} peak. The PLGA/PCL electro-spun mat was shown to not be toxic at all compositions (Fig. 5). In addition, cell proliferation of fibroblast doubled between day 1 and day 3 day (Fig. 6) through MTT results. On the other hand, the cells became attached to the PLGA/PCL electro-spun mats within 60 min (Fig. 7). The SEM images showed that filopodium and lamellipodium growth on the electro-spun mats was as good as the control. But, the cell proliferation and attachment was better as the PLGA concentration in the PLGA/PCL electro-spun mats was increased after seeding the cells for 1 day (Fig. 8) and 3 days (Fig. 9). However, in the case of PLGA/PCL (30/70), the cells did plate on the electro-spun mat but the cells shrunk. These combined results clearly indicate that the presence of the PLGA support in the PLGA/PCL electro-spun mats improved biocompatibility, cell attachment and cell proliferation.

5 Conclusion

In this study, we successfully fabricated electro-spun PLGA/PCL mats containing different concentrations of PLGA. PCL was used as the major material because it is inexpensive and stable during handling and storage. PCL is a flexible material and PLGA is a brittle material; thus, the PLGA/PCL electro-spun mats had a stronger mechanical strength than PCL. The addition of PLGA as a doping agent in the PLGA/PCL electro-spun mats increased the value of this composite for biomaterial and tissue engineering applications because it increased biocompatibility, cell attachment and cell proliferation. We investigated a new electro-spun component and demonstrated that it holds promise for use as a biomaterial for tissue engineering and biomedical applications.

References

- Vunjak-Novakovic G, Freshney R. Culture of cells for tissue engineering. New York: Wiley; 2006.
- Rickne A. Swedish possibilities within tissue engineering and regenerative medicine. VINNOVA—Swedish Governmental Agency for innovation systems/Verket för Innovations system; 2009.
- Segvich S, Kohn DH. Biological interactions on materials surfaces understanding and controlling protein, cell, and tissue responses. New York: Springer; 2009.
- Dawson E, Mapili G, Erickson K, Taqvi S, Roy K. Biomaterials for stem cell differentiation. *Adv Drug Deliv Rev.* 2008;60:215–28.
- Supaphol P, Chuangchote S. On the electrospinning of poly(vinyl alcohol) nanofiber mats: a revisit. *J Appl Polym Sci.* 2008;108:969–78.
- Agarwal S, Wendorff JH, Greiner A. Use of electro-spinning technique for biomedical applications. *Polymer.* 2008;49:5603–21.
- Tan EPS, Ng SY, Lim CT. Tensile testing of a single ultrafine polymeric fiber. *Biomaterials.* 2005;26:1453–6.
- Duan B, Yuan X, Zhu Y, Zhang Y, Li X, Zhang Y, et al. A nanofibrous composite membrane of PLGA-chitosan/PVA prepared by electrospinning. *J Eur Polym.* 2006;42:2013–22.
- Vaz CM, Tuijil SV, Bouten CVC, Baaijens FPT. Design of scaffolds for blood vessel tissue engineering using a multi-layering electrospinning technique. *Acta Biomater.* 2005;1:575–82.
- Zhang Y, Venugopal JR, El-Turki A, Ramakrishna S, Su B, Lim C. Electrospun biomimetic nanocomposite nanofibers of hydroxyapatite/chitosan for bone tissue engineering. *Biomaterials.* 2008;29:4314–22.
- Lee KY, Jeong L, Kang YO, Lee SJ, Park WH. Electro-spinning of polysaccharides for regenerative medicine. *Adv Drug Deliv Rev.* 2009;61:1020–32.
- Bini TB, Gao S, Tan TC, Wang S, Lim A, Hai LB, et al. Electrospun poly(L-lactide-co-glycolide) biodegradable polymer nanofibre tubes for peripheral nerve regeneration. *Nanotechnology.* 2004;15:1459–64.
- Bajgai MP, Aryal S, Bhattarai SR, Bahadur KCR, Kim KW, Kim HY. Polycaprolactone grated dextran biodegradable electrospun matrix: a novel for tissue engineering. *J Appl Polym Sci.* 2008;108:1447–54.
- Tang ZG, Callaghan JT, Hunt JA. The physical properties and response of osteoblasts to solution cast films of PLGA doped polycaprolactone. *Biomaterials.* 2005;26:6618–24.
- Kim JY, Cho DW. Blended PCL/PLGA scaffold fabrication using multi-head deposition system. *Microelectron Eng.* 2009;86:1447–50.
- Ciardelli G, Chiono V, Vozzi G, Pracella M, Ahluwalia A, Barbani N, et al. Blends of poly(3-caprolactone) and polysaccharides in tissue engineering applications. *Biomacromolecules.* 2005;6:1961–76.
- Kim CH, Khil MS, Kim HY, Lee HU, Jahng KY. An improved hydrophilicity via electrospinning for enhanced cell attachment and proliferation. *J Biomed Mater Res B.* 2006;78B:283–90.
- Li WJ, Cooper JA, Mauck RL, Tuan RS. Fabrication and characterization of six electrospun poly(α -hydroxyester)-based nanofibrous scaffolds for tissue engineering applications. *Acta Biomater.* 2006;2:377–85.
- Curran JM, Tang Z, Hunt JA. PLGA doping of PCL affects the plastic potential of human mesenchymal stem cells, both in the presence and absence of biological stimuli. *J Biomed Mater Res A.* 2008. doi:10.1002/jbm.a.31966.
- Tang ZG, Hunt JA. The effect of PLGA doping of polycaprolactone films on the control of osteoblast adhesion and proliferation in vitro. *Biomaterials.* 2006;27:4409–18.
- Khil MS, Shanta RB, Kim HY, Kim SZ, Lee KH. Novel fabricated matrix via electrospinning for tissue engineering. *J Biomed Mater Res.* 2005;72B:117–24.
- Deitzel JM, Kleinmeyer J, Harris D, Tan NCB. The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer.* 2001;42:261–72.
- Karakecili AG, Satriano C, Gümüşderelioğlu M, Marletta G. Enhancement of fibroblastic proliferation on chitosan surfaces by immobilized epidermal growth factor. *Acta Biomater.* 2008;4:989–96.
- Jay W, Calvert KGM, Lisa C, Prashant NK, Paul AD, Lee EW. Characterization of osteoblast-like behavior of cultured bone marrow stromal cells on various polymer surfaces. *J Biomed Mater Res.* 2000;52A:279–84.
- Lee KH, Kim HY, Khil MS, Ra YM, Lee DR. Characterization of nano-structured poly(caprolactone) nonwoven mats via electrospinning. *Polymer.* 2003;44:1287–94.
- Li Y, Huang Z, Lu Y. Electro-spinning of nylon-6,6,1010 terpolymer. *Eur Polym J.* 2006;42:1696–704.