AIR-JET SPINNING OF BIOCOMPATIBLE POLYMERIC NANOFIBERS FOR WOUND DRESSINGS

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Abstract

Air-jet spinning (or solution blowing) is a method of nanofibrous production using high-velocity gas flow of air or gas to transform drops of a polymer solution into fibers. The technology is promising for relatively safe applying nanofibers directly to wounds, in-situ application. In this work, air-jet spinning of several polycaprolactone (PCL) and poly(L-lactic acid) (PLLA) solutions was performed utilizing a commercial airbrush for possible in situ application of skin wound dressings and for the production of flat samples of nanofibrous materials for further in-vitro testing. The average fiber diameters of air-jet spun materials were set 268 nm (6 wt% PCL in dichloromethane (DCM)), 305 nm (8 wt% PCL in DCM), 333 nm (10 wt% PCL in DCM) and 223 nm (6 wt% PLLA in chloroform (CF)). Cytotoxicity of fibrous mats and DNA quantification were investigated. The results showed that air-jet spun mats are nontoxic to mouse fibroblasts and the cell count significantly increased on the mats in comparison to the negative controls. Chemical changes in the biodegradable polymers structure and residual solvents in the fibrous mats were not found using Fourier-transform infrared spectroscopy. The degree of crystallinity was set for fibers from 8 wt% PCL in DCM – 58.9 %; 10 wt% PCL in DCM – 56.4 % and 6 wt% PLLA in CF – 22.0% via differential scanning calorimetry. The results of the tests performed show that air-jet spun PCL materials could be useful for in situ application of skin wound dressings.

Keywords: Air-jet spinning, airbrush, in situ deposition, nanofibrous scaffold, skin wound dressing

1. INTRODUCTION

Tissue engineering is a multidisciplinary field using applied science to provide tissue replacements or reconstruction [1]. There is significant interest in nanofibrous scaffolds mimicking the extracellular matrix. Nowadays, the most widely used way of nanofibrous scaffold fabrication is electrospinning. Nanofibers are produced with devices using DC or AC high voltage sources [2]. Besides this method, the air-jet spinning method (or solution blowing) is increasingly studied for nanofibrous material fabrication. Air-jet spinning uses air or gas flow to produce nanofibers [3]. The hydrodynamic force caused by air or gas friction on the surface of a solution droplet overcomes the surface tension of the polymeric droplet. This transforms the droplets into thin fibers.

It would be a remarkable achievement to fabricate nanofibrous scaffolds directly on a wound (in situ deposition) instead of surgical implantation. Portable electrospinning devices provide nanofibrous production in situ using high voltage, which can be dangerous for the human organism [4]. Electrospinning appears as an unsuitable

method for in situ deposition of nanofibrous scaffolds. Air-jet spinning seems relatively safe for applying nanofibers directly to wounds due to the use of gas flow instead of high voltage [4]. One of the devices which can be used for air-jet spinning is a commercial airbrush [3]. A suitable polymeric solution is placed into a reservoir of an airbrush. Then it flows from the reservoir onto a needle in an inner nozzle of the airbrush [5], see **Figure 1**. A solution droplet is formed on the top of the nozzle, and the gas flow elongates the droplet [6]. If the gas flow stretching force overcomes the surface tensions, the jets are made from the solution and taken by aerodynamic force out of the airbrush [6]. Fibers are made by solvent evaporating from the jets [6]. Important air-jet spinning parameters include solution parameters (viscosity, surface tension, molecular weight of the polymer, concentration of the solution, vapor pressure, etc.), process parameters (gas pressure, working distance, solution flow rate, nozzle diameter, and geometry, etc.), and others (temperature, relative air humidity, atmospheric pressure, etc.) [7].

Furthermore, the air-jet spinning method is more versatile than DC electrospinning as it does not require an electrically active collector. It typically has higher productivity as high values of solution flow through the nozzle can be used to form the fibers [4]. Air-jet spinning is useful for coating surfaces of various complex shapes [4]. The method can be easily implemented using a low-cost, user-friendly, portable device [4].

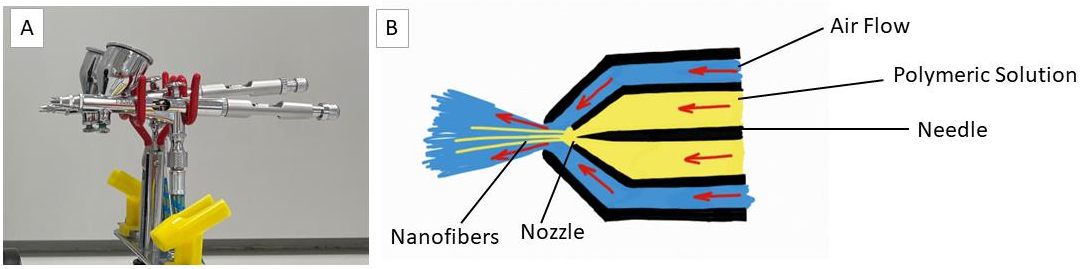

Figure 1 (A) Two airbrushes on a stand. (B) Scheme of air-jet spinning principle.

Air-jet spinning is not a brand-new technology of nanofibrous fabrication. Oliveira et al. fabricated biodegradable polymeric nanofibers using an airbrush and compared them to electrospun nanofibers [5]. Behrens et al. studied air-jet spinning of poly(lactic-co-glycolic acid) nanofibers for in situ deposition of a surgical sealant, hemostatic, and scaffolds [8]. Some water-soluble polymers, such as PVA, are very difficult to air-jet spin. The literature so far reports cases of PVA air-jet spinning with additional device for drying the fibers [9].

In this work, the biodegradable polymeric nanofibers were produced via air-jet spinning using a commercial airbrush. The optimal polymeric solutions for the technology were found. The morphology of the air-jet spun fibers were observed. A cytotoxicity test and DNA quantification were performed to prove suitability for potential medical use. Chemical changes in polymeric nanofibers were set by FTIR, and the degree of crystallinity was evaluated by DSC.

2. MATERIALS AND METHODS

2.1. Materials

The following polymers were used for the preparation of polymeric solutions: polycaprolactone (PCL, Mw 45 000 g·mol^{-1}, Merck, Germany) and poly(L-lactic acid) (PLLA, Mw 45 000–55 000 g·mol^{-1}, PolySciTech, USA). Dichloromethane (DCM, Penta, Czech Republic) and chloroform (CF, Penta, Czech Republic) were used for solvents. For further experiments, the following chemicals were utilized: Dulbecco's Modified Eagle's Medium (DMEM, Merck, Germany), fetal bovine serum (FBS, Biosera, France), Triton X-100 (Merck, Germany), Cell Counting Kit-8 (CCK, Dojindo, Japan), lysis buffer solution (prepared at the Technical University of Liberec, Department of Bioengineering), Quant-iT dsDNA Assay Kit, High Sensitivity (Invitrogen,

USA), phosphate buffer solution (PBS, prepared at the Technical University of Liberec, Department of Bioengineering).

2.2. Solution Preparation

For biocompatible polymeric nanofibers fabrication, the following solutions were prepared: 6 wt%, 8 wt%, and 10 wt% PCL in DCM; 6 wt% and 12 wt% PLLA in CF. The concentration of the solutions was set according to the literature and then other concentration values were adjusted [3], [5].

2.3. Air-jet Spinning and Electrospinning of Biocompatible Polymeric Fibers

Polymeric solutions were placed into a commercial airbrush Fengda BD-180 with 0.3 mm nozzle diameter and 1.8 bar air flow pressure. The air-jet spinning of biocompatible polymeric nanofibers was performed on different days when the temperature ranged between 21.1 and 23.3 °C, and the relative humidity ranged between 19.6 and 51.7%. Firstly, the solutions were air-jet spun as a nanofibrous layer on paper and then on a metal sieve as membranes, which can be taken down and further studied independently. Lastly, the solutions were air-jet spun on a hand inside a nitrile glove as evidence of possible in situ application (see **Figure 2**). Solutions of 10 wt% PCL in DCM and 16 wt% PCL in CF/EtOH (8:2) were fabricated with Nanospider™ (NS 1S500U, Elmarco, Czech Republic) to compare their properties with air-jet spun material of the same weight (50 gsm).

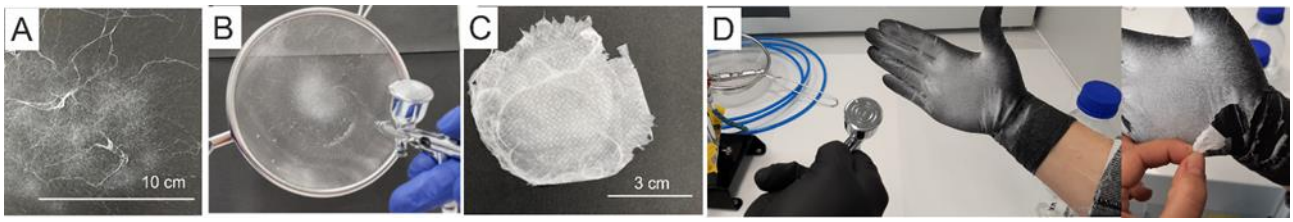

Figure 2 Air-jet spinning of PCL nanofibers onto a black paper (A); onto a metal sieve (B) to produce membrane (C) for further testing; and directly onto a hand inside a black nitrile glove (D).

2.4. Testing Methods of Prepared Fibers

The morphology of fibrous mats was observed with a scanning electron microscope (SEM, Tescan Vega S3B, Tescan Orsay Holding, Czech Republic). Fiber diameters were measured from SEM images using ImageJ software (National Institutes of Health, USA). Over 100 values of diameter were measured for each sample.

The in vitro cytotoxicity test was performed according to the ČSN EN ISO 10993-5 (855220) standard with the use of mouse fibroblasts 3T3-L1. Materials were tested using extracts from the materials. Nanofibrous mats from 8 wt%, 10 wt% PCL in DCM and 6 wt% PLLA in CF were chosen for testing cytotoxicity due to their optimal properties, such as compactness. Each material was air-jet spun for 1.5 minutes on a metal sieve. Immediately after nanofibrous material production, two samples were cut from each material into 1.3x1.3 cm squares and sterilized by UV light in a sterile flow box for 20 minutes from both sides directly following cytotoxicity testing. The cell viability was set from measured data. According to the ČSN EN ISO 10993-5 (855220) standard, the material is considered nontoxic if the value of cell viability is over 70 %. The rapid testing after nanofiber production was carried out to study the effect of any residual solvents in the air-jet spun nanofibrous materials.

After the cytotoxicity test, DNA quantification was studied using solution of Quant-iTTM dsDNA HS reagent and Quant-iTTM dsDNA HS buffer (in ratio 1:200). The excitation wavelength in the spectrophotometer was set to 485 nm, and the measured emitted wavelength was 523 nm.

Chemical changes in polymer chains and the remaining amount of the toxic solvents of air-jet spun mats from 8 wt%, 10 wt% PCL in DCM, and 6 wt% PLLA in CF were measured by Fourier transform infrared spectroscopy

(FTIR, Nicolet iZ10, Thermo Fisher Scientific, Czech Republic) in 4000–400 cm-1 range. The spectra of the nanofibrous mats were compared to the spectra of pure polymers from which the solutions were made. For comparison of air-jet spinning and electrospinning, a spectrum of the electrospun nanofibrous mat from 10 wt% PCL in DCM was measured by FTIR.

The degree of crystallinity of air-jet spun mats from 8 wt%, 10 wt% PCL in DCM, and 6 wt% PLLA in CF was investigated by differential scanning calorimetry (DSC, DSC 1 / 700, Mettler Toledo). Materials were weighted between 2–16 mg and dissolved in an aluminum cup. The temperature interval of heating was set at -20–200 °C in an inert nitrogen atmosphere with a gas flow of 50 ml/min and heating at 10 °C/min. The degree of crystallinity of the nanofibrous mats was compared to the degree of crystallinity of the granular form of polymers from which the solutions were prepared and 10 wt% PCL in DCM (electrospun).

3. RESULTS AND DISCUSSION

Successful nanofiber fabrication was possible from the following solutions: 6 wt%, 8 wt%, and 10 wt% PCL in DCM and 6 wt% PLLA in CF (see **Figure 3**). Average fiber diameter value from mats with confidence interval of 95% probability of: 6 wt% PCL in DCM is 268 ± 27 nm; 8 wt% PCL in DCM is 305 ± 24 nm; 10 wt% PCL in DCM is 333 ± 31 nm and 6 wt% PLLA in CF is 223 ± 19 nm. With a higher concentration of polymer in the solution, the entanglement of the polymer chains increases during air-jet spinning, thereby increasing the diameter of the produced fibers.

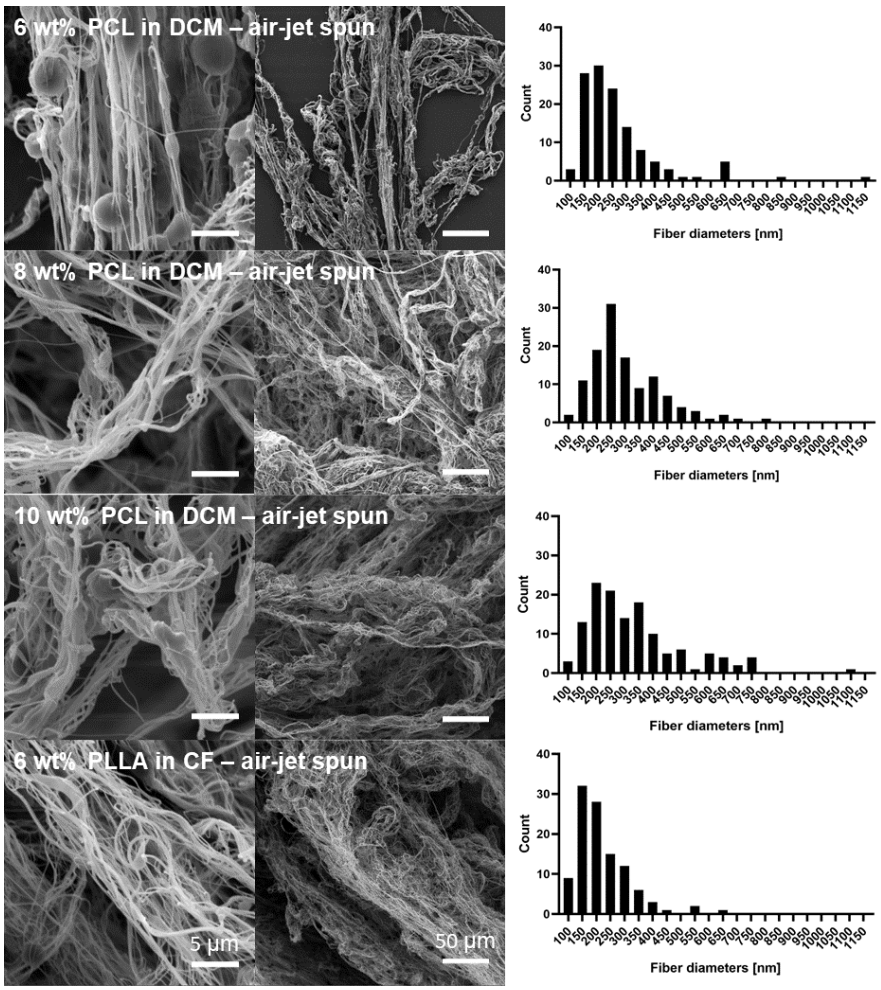

Figure 3 SEM images of selected air-jet spun nanofibers and histograms of their fiber diameters. Scale from left to right: 5 µm, 50 µm.

According to the ČSN EN ISO 10993-5 (855220) standard, the nanofibrous mats are considered nontoxic to the cells (see **Figure 4A**). Value of cell viability of materials from 8 wt% PCL in DCM was 124 ± 7 %, 10 wt% PCL in DCM was 110 ± 11 % and 6 wt% PLLA was 87 ± 12 %. Cell viability values over 100% could have been due to a higher metabolic rate of the cells caused by stress and not their higher cell amount. The viability value is less than 100% for PLLA material. This may be due to the effect of residual CF, which due to its higher boiling point evaporates more slowly during air-jet spinning than DCM. However, this must be a very small amount of residual solvent (CF from PLLA nanofibers) as FTIR showed no measurable residue there. Therefore, it seems that PCL solutions in DCM would be more suitable for in situ application of skin wound dressings by air-jet spinning. DNA quantification was performed to discover if the higher cell viability values are caused by their higher number (see **Figure 4B**). The DNA concentration was calculated from measured data with standard deviation for the wells with the cells from the material extract and negative control: 8 wt% PCL in DCM – 21 ± 4 ng/well, 10 wt% PCL in DCM – 19 ± 5 ng/well, 6 wt% PLLA in CF – 15 ± 4 ng/well and negative control – 13 ± 3 ng/well. For the materials with complete medium, the concentration values per well were higher than for the negative controls. Thus, there may be a higher number of cells per well in the materials than in the negative control wells. The results from cytotoxicity and DNA quantification show that the layers are not toxic and there could be an increase in cell count on these layers. This could be desirable in the use of nanofibrous layers as wound dressings.

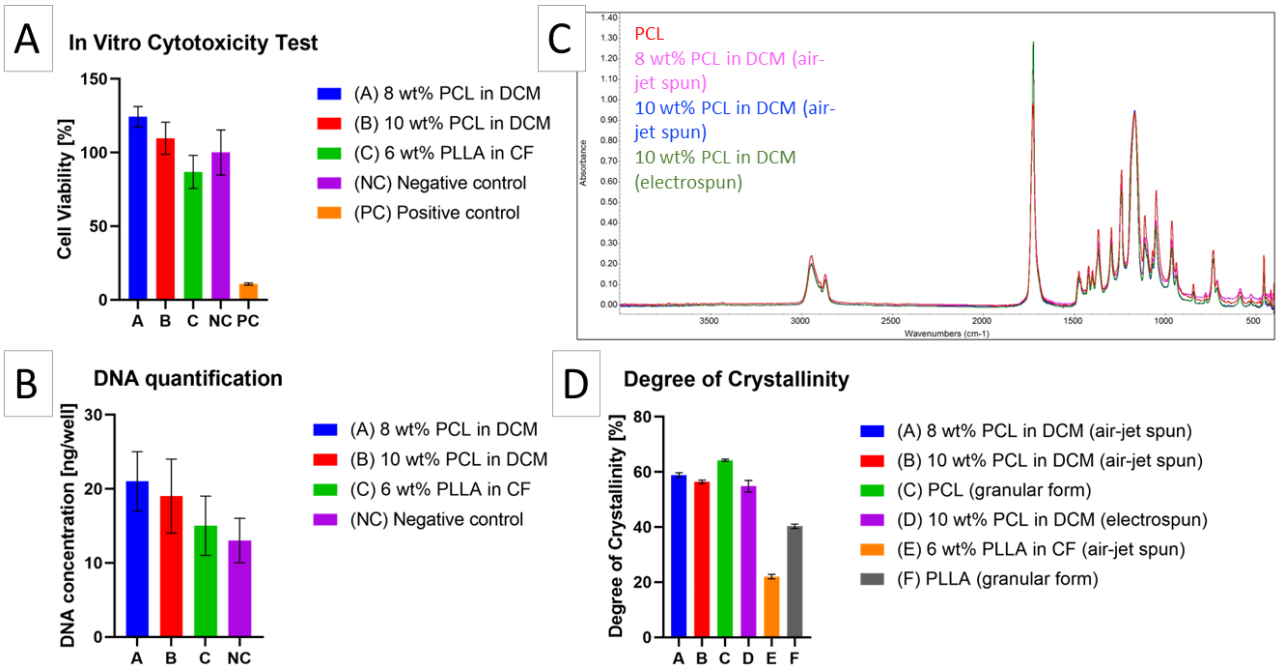

Figure 4 Results from biocompatibility testing (A, B); FTIR testing (C) and DSC testing (D)

No chemical changes were confirmed by FTIR (see **Figure 4C**). The remaining toxic solvents were in undetectable amounts by FTIR which is required. Residual solvents are undesirable in layers for skin wound dressings applied in situ.

The degree of crystallinity was set for following materials: 8 wt% PCL in DCM – 58.9 ± 0.8 %; 10 wt% PCL in DCM (air-jet spun) – 56.4 ± 0.6 %; 10 wt% PCL in DCM (electrospun) – 54.8 ± 2.1 %; PCL (granular form) – 64.2 ± 0.4 %; 6 wt% PLLA in CF – 22.0 ± 0.8 % and PLLA (granular form) – 40.2 ± 0.8 % (see **Figure 4D**). The degree of crystallinity values of the air-jet spun and electrospun materials were lower than those of the original granulates. Furthermore, the possible influence of the technology on the degree of crystallinity of the materials was shown by DSC. Although in electrospinning the fibers fly a longer path through the air and would have more time to form regular crystalline parts, they have a lower crystallinity value than fibers formed by

air-jet spinning. In both cases for PCL and PLLA, the granules have a higher degree of crystallinity than the air-jet spun materials. This is clearly an effect of technology i.e., the rate of solvent evaporation or solidification of the materials produced. In larger granules there is certainly more space for longer periods of time for the formation of crystalline regions, but for the relatively very fast liquid-to-solid transformations of the described fiber fabrication technologies there is probably less time for crystal formation. The values of the degree of crystallinity affect biodegradation which is an important parameter for nanofibrous scaffolds for wound dressings. Thus a different course (speed) of degradation can be expected, which was confirmed in further follow-up tests [10].

4. CONCLUSION

Air-jet spinning is being explored as an alternative to electrospinning. The nanofibers are formed by airflow instead of high voltage. This method of nanofiber fabrication allows safe, direct placement of the scaffold on the wound. In this paper, the biocompatible polymeric solutions from PCL and PLLA (6 wt%, 8 wt%, 10 wt% PCL in DCM and 6 wt% PLLA in CF) were successfully air-jet spun for possible in situ deposition of the fibrous scaffolds for wound dressings. The evaluated morphology of the fibers showed that the fiber diameters are according to the nanoscale. The cytotoxicity test proved that all produced nanofibrous materials are noncytotoxic (cytocompatible). The PCL materials showed higher viability compared to the PLLA materials and negative control. Via FTIR, chemical changes or remaining solvents were not present in the nanofibrous mats. The air-jet spun nanofibrous mats had higher degree of crystallinity than electrospun mats but lower than their original granular form according to DSC analysis. The air-jet spinning of 8 wt% PCL in DCM seems to be a great potential candidate for in situ deposition of nanofibrous scaffolds for wound dressings. However, it is necessary to continue studies imitating in situ application, to study possible changes in wound temperature during the application of nanofibers, and to address the issue of sterility of the spinning device.

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