Materials Science and Engineering C 35 (2014) 347–353

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Evaluation of an air spinning process to produce tailored biosynthetic nanofibre scaffolds

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ARTICLE INFO

Article history:
Received 25 June 2013
Received in revised form 5 October 2013
Accepted 2 November 2013
Available online 14 November 2013

Keywords:
Nanofibre scaffold
Spinning
Poly(lactic acid)
Multilinear regression
Vascular regenerative medicine

ABSTRACT

We optimised the working parameters of an innovative air spinning device to produce nanofibrous polymer scaffolds for tissue engineering applications. Scanning electron microscopy was performed on the fibre scaffolds which were then used to identify various scaffold morphologies based on the ratio of surface occupied by the polymer fibres on that covered by the entire polymer scaffold assembly. Scaffolds were then produced with the spinning experimental parameters, resulting in 90% of fibres in the overall polymer construct, and were subsequently used to perform a multiple linear regression analysis to highlight the relationship between nanofibre diameter and the air spinning parameters. Polymer solution concentration was deemed as the most significant parameter to control fibre diameter during the spinning process, despite interactions between experimental parameters. Based on these findings, viscosity measurements were performed to clarify the effect of the polymer solution property on scaffold morphology.

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1. Introduction

In the most serious cases of vascular disease, surgeons have no choice but to replace the arteries with vascular prostheses [1,2] which are manufactured in either woven or knitted polyethylene terephthalate (PET) or microporous expanded polytetrafluoroethylene (ePTFE) and are used to replace arteries with a diameter greater than 6 mm. For smaller diameters, no long-term solution exists for artery replacement [3], primarily because these materials are highly thrombogenic. Indeed, when the surface of the material is in contact with blood, coagulation factors are activated which leads to blood clot formation [4]. This foreign-body reaction [5] can be avoided that the prosthesis is able to accommodate an endothelial cell monolayer that resembles the inner surface of the artery.

Our previous studies have demonstrated the ineffectiveness of endothelial cell monolayer growth on the textile structure of prostheses due to a dimensional mismatch between prosthesis structure scale and cell size [6]. Our strategy is to bridge this mismatch by coating the internal surface of the prosthetic tube with a polymeric nanofibre scaffold to produce a temporary synthetic extracellular matrix [7]. In addition to making it possible for endothelial cells to adhere and proliferate, this coating helps preserve the mechanical properties of the prosthesis.

Choosing the right polymer for this coating is crucial [8]. The polymer must be biocompatible and easy to produce. In this regard, high molecular weight poly(lactic acid) (PLA) is an ideal candidate, as medical devices made with this polymer are approved by the Food and Drug Administration [9] and is commonly deployed in medical and paramedical applications. Used as suture thread for many years [10], PLA was recently shown to provide an alternative to permanent stents [11,12], and it is also a suitable polymer for drug delivery applications [13] due to its biodegradation features. This aliphatic polyester consists of a linear chain synthesised by the ring opening polymerisation and polycondensation of the lactide dimer. Thanks to its ester-containing structure, this polymer can be degraded through hydrolysis and its degradation rate can be tailored to meet the requirements of each application [14,15].

The most common method to produce nanofibres is the electrospinning process [16]. In this technology, a high voltage is applied between a needle and a collector to draw fibres from a solution. This easy process has been widely studied and can produce either classical or porous [17] synthetic and natural fibres. More complex morphologies have been spun, such as composite fibres [18], core-shell fibres [19], and ceramics, as well as carbon nanotubes and nanofibres [20]. The shape morphology can also be controlled to produce unidirectional and multidirectional patterns [21]. Finally, the variety of yarns and shapes provide a wide range of applications in numerous scientific fields.

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0928-4931/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.msec.2013.11.004

Despite the definite advantages of electrospinning, this method cannot readily be used to coat the luminal surface of a tubular textile vascular prosthesis [7] because of the small distances between the needle and the surface. To address this issue, we developed an alternative device, called air spinning which involves the stretching of a polymer solution under high-speed air flow. This technique was introduced by our group in 2008 [7] and has been continuously upgraded for the coating of tubular shapes [22].

Nanofibre technology is extensively used in biomedical and tissue engineering applications [23], as well as in bone and cartilage applications [24,25], in skin regenerative medicine [26] or cardiac tissue repair applications [27]. Nanofibres are now being formed as scaffolds to promote cell adhesion and proliferation and also differentiation [28] and guided migration [29], and their surface and physical properties are tuneable to target protein adsorption [30]. In addition, natural or synthetic scaffolds can be functionalised to add biomimicry and improve bioactivity [31].

François et al. [7] showed that cell behaviour depends on the quality of the air-spun nanofibre scaffold which can be determined by the number of fibre fractures, fibre diameter, and scaffold morphology. In a previous study [7], Fibre ruptures were quantified and correlated with spinning parameters, yet no information was provided as to fibre diameter and scaffold morphology with the air-spinning device. Oliveira et al. [32] characterised solution blow-spinning using a system similar to the one previously developed in our laboratory. These authors correlated blow-spinning parameters with fibre diameter but with a different set of parameter ranges. Moreover, some important parameters, such as needle diameter and substrate/needle distance were not considered.

The aim of this study was thus to further the state of knowledge regarding the air spinning process to better understand fibre formation, optimise spinning parameters for a more uniform fibre shape, and develop methods and tools to predict optimal spinning conditions.

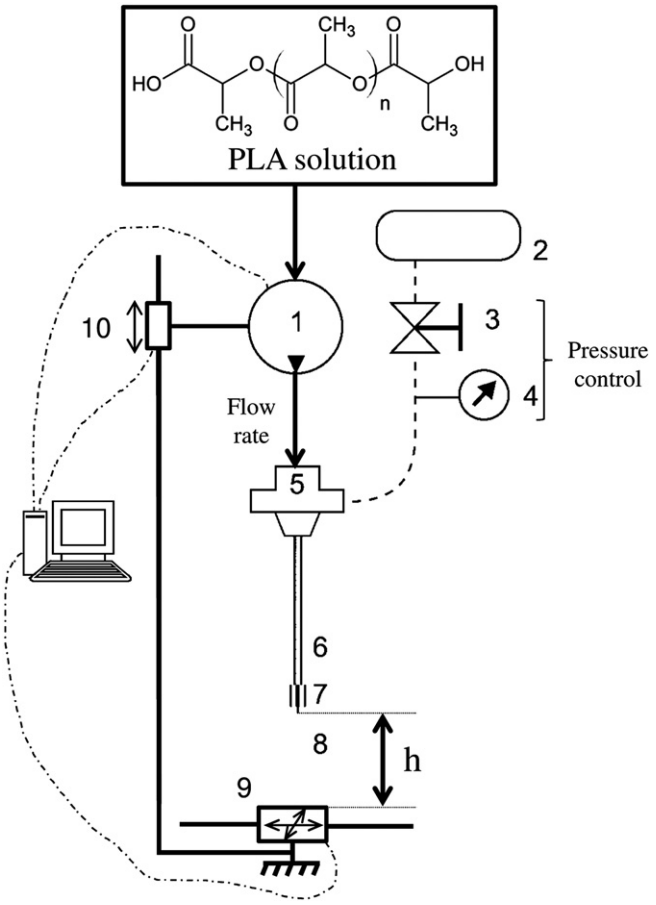

Fig. 1. Air spinning system set-up. (1) syringe pump; (2) compressed air tank; (3) air valve; (4) manometer; (5) atomiser; (6) injector with small needle; (7) nozzle; (8) pulverisation cone; (9) XY translation stage and collector; and (10) Z-stage.

2. Materials and methods

2.1. Air spinning process and parameters

PLA (Mw = 150,000, Ip = 2.45, D/L = 4.5%, Tg = 61 °C, Tm = 144 °C, CML poly(lactic acid), Hycail Finland Oy, Turku, Finland) was totally solubilised in chloroform (99.8%, Laboratoire Mat, Québec, QC, Canada) and injected into an air spinning device (Fig. 1 [7]). The PLA solutions were then stretched under high-speed air flow and pulverised on PET films that were fastened onto the collector (Goodfellow, Oakdale, PA, USA). The polymer solution residence in the air flow enabled the nanofibres to form upon solvent evaporation.

The air spinning apparatus was equipped with a flow control syringe pump (NE-1010, New Era Pump System, Farmingdale, NY, USA) and a 20 mL luer lock glass syringe containing the polymer solutions. The syringe was first mounted onto an injector equipped with a small needle (1/2" Straight Cannula Crimp Sealed, I&J Fisnar, Wayne, NJ, USA), then attached to a homemade atomiser fed with compressed medical-grade air. The nanofibre coating was homogeneously settled on the collector with X and Y computerised translation stages (Velmex, Bloomfield, NY, USA).

The effect of polymer solution concentration, needle diameter, flow rate, pressure, and nozzle-to-sample distance was investigated in terms of its influence on fibre diameter, with the ranges determined either from previous studies [7] or on the basis of physical constraints. Flow rates and pressure values were set accordingly between 10 mL/h and 50 mL/h and between 5 MPa and 10 MPa, respectively, while polymer solutions were investigated at concentrations ranging between 1% and 15% to enable flow rate control in the lower diameter needle (0.15 mm) while preventing clotting in the higher diameter one. The nozzle-to-sample distance values were set at between 200 mm and 300 mm, as a previous study by our group demonstrated that these lengths were sufficient to produce solvent-free polymer fibres.

2.2. Morphology study and fibre diameter measurements

Thirteen solutions of varying PLA concentrations (1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 15% w/v) were randomly air-spun with four needles of different diameters (0.6, 0.41, 0.25, and 0.15 mm). For this section of the experiment, flow rate, air pressure, and nozzle-to-sample distance (h) were kept constant at 10 mL/h, 5 MPa, and 200 mm, respectively. Each sample was duplicated, gold-coated, and subsequently observed under a scanning electron microscope (JSM840A, JEOL, Tokyo, Japan). Three images per sample were randomly taken at a magnitude of 1000 for the morphology study and either 3000 or 10,000 for the fibre diameter measurements. Each image was then analysed with image treatment software (Image J, National Institutes of Health, Bethesda, MD, USA). The ratio between the surface covered by the fibres (S_{fibres}) and the surface covered after spinning ($S_{covered}$) was then calculated.

Finally, approximately 25 fibre diameters were evaluated per image for an approximate total of 150 diameter measurements for each experiment. The measurements were compared with a Gaussian profile to determine the relevance of using the diameter average for the rest of the study, which was a condition to performing a statistical analysis.

2.3. Experimental design and statistical analysis

A least squares multiple linear regression was performed to determine the relationship between the spinning experimental parameters and the fibre diameter. This method consists in fitting an experimental parametric model with a linear mathematical model. The calculation

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