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A poly(L-lactic acid) nanofibre mesh scaffold for endothelial cells on vascular prostheses

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Abstract

The absence of neoendothelium covering the intimal surface of small-diameter PET vascular prostheses is known to be one cause of failure following implantation in humans. Protein coatings currently used to seal porous textile structures have not shown evidence of in vivo neoendothelium formation. In this study, we covered the inner wall of textile prostheses with a biodegradable synthetic scaffold made of poly(L-lactic) acid (PLLA) nanofibres obtained by an air-spinning process we developed that produces nanofibres by stretching a solution of polymer with a high-speed compressed air jet. The air spinning was designed to process a scaffold that would support good endothelial cell proliferation. Our innovative process enabled us to very rapidly cover textile samples with PLLA nanofibres to determine the influence of air pressure, polymer solution flow rate and polymer concentration on fibre quality. High air pressure was shown to induce a significant number of ruptures. High polymer flow rate stimulated the formation of polymer droplets, and the fibre diameter mean increased for the 4% and 7% polymer concentrations. The adherence and proliferation of bovine aortic endothelial cells was assessed to compare prosthesis samples with or without the PLLA nanofibre scaffold and PET film. The PLLA nanofibres displayed a significantly better proliferation rate, and enabled endothelial cells to proliferate in the monolayer. Our novel approach therefore opens the door to the development of partially degradable textile prostheses with a blood/textile interface that supports endothelial cell proliferation.

Keywords: PLLA; Nanofibres; Air spinning; Endothelial cells; Vascular prostheses

1. Introduction

The gold standard for small-diameter vessel replacement is autologous vessels; however, these are often unavailable or are of poor quality, thereby forcing surgeons to use synthetic vascular prostheses. Expanded polytretrafluoroethylene (ePTFE) grafts are currently the conduits of choice in small-diameter applications [1]. Currently available textile vascular prostheses are made of polyethylene terephthalate (Dacron™ or PET) and are used clinically to replace thoracic, abdominal or lower limb arteries [1]. These protheses are usually coated with gelatine or collagen to prevent blood loss through the prosthesis wall at the time of implantation. Although their patency rate is acceptable for large-diameter prostheses, it decreases dramatically with smaller diameters [2,3], which explains why PET textile prostheses are not used for diameters of

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less than 8 mm, despite the enormous clinical need for such small arterial substitutes.

The absence of neoendothelialization with luminal textile prostheses is known as one cause of failure. Good results (partial to total endothelialization) have been observed with coated prostheses in animal models; unfortunately, the same cannot be said with regard to implantation in humans [4]. Moreover, with the increasing longevity of populations in industrialized countries, vascular prostheses will have to last longer and show better patency rates accordingly. In light of this context, we focused on developing a new approach to optimize textile vascular prostheses and to promote neoendothelium development.

Textile vascular prostheses display good mechanical properties: they are able to resist cyclic stresses over a long period, and can still be found working after a lengthy implantation [5]. Their textile structure is porous, and therefore without the proper coating (collagen, albumin, gelatin or even pre-clotting), implantation is followed by dramatic blood loss [6] through the prosthesis wall. Moreover, previous investigations by our laboratory have demonstrated that bare textiles do not provide adequate support for endothelial cells to proliferate due to oversized threads [7]. Currently available coating solutions have shown interesting results by helping to prevent blood loss through the prosthesis wall; however, they are not designed to promote in vivo endothelium formation [8]. Studies on explanted prostheses have shown that endothelialization is limited to perianastomotic areas [4]. This may be due, in part, to the fact that collagen or gelatine is composed of fragmented biological tissue that is not specifically created to optimize endothelial cell migration and proliferation.

The approach presented in this study centres on designing a partially biodegradable vascular substitute to increase the patency rate of small-diameter PET textile prostheses. To achieve this goal, we decided to cover the intimal surface of textile vascular prostheses with a biodegradable scaffold that could be successfully processed through the tubular shape of the prostheses, adhere to the textile and move with it, and be flat enough to enable surface endothelialization. We therefore applied a nanofibre scaffold made of poly(L-lactic) acid (PLLA) onto the PET textile surface. PLLA is a biodegradable polymer that is widely used for biomaterial applications [9–12]. Its in vivo degradation is related to PLLA ester link hydrolysis, thereby creating metabolizable lactic acid by-products. This FDA-approved polymer can be dissolved and is thus easily processed.

Electrospinning is a common technique used in laboratories to produce high-quality nanofibres [10,11]. Although well adapted to the research setting, this approach is both time-consuming and hardly acceptable for industrial scale-up. Moreover, due to the high electric tension involved in the process, electrospinning cannot be used to treat the inner wall of tubular shapes (e.g. vascular prostheses) because of the small space between needle and surface. An alternative method consists in obtaining PLLA nanofibre meshes through air spinning. Known also as air spinning or blow spinning, this technique enables surfaces to be covered very rapidly with a non-woven polymer fibre matrix. It basically uses an air jet to stretch a solution of polymer into fibres with a simultaneous vaporization of solvent.

The goal was to examine the potential of air spinning as a method of producing biomaterial scaffolds. To our knowledge, no other research has addressed this application. We focused our investigation on an air-spinning technique, with emphasis on its capability to process solubilized PLLA into nanofibres and its potential to produce a scaffold that would enable good endothelial cell proliferation. Our hypothesis was that endothelial cells would proliferate in a monolayer when encountering a structure whose dimensions were at the proper scale to retain cells at the surface of the graft. If composed of biodegradable material, this scaffold would slowly yield to the developing biological tissue produced by cells stimulated by the resorption of their scaffold. Moreover, this process would subsequently make room for capillary ingrowth, which would then stimulate transmural neoendothelialization. Finally, the mechanical properties of the graft are preserved by the remaining PET textile structure within our partially biodegradable vascular graft.

2. Materials and methods

2.1. Materials

PLLA was obtained from Hycail-Finland Oy (Turku, Finland, Mn = 126239, Mn/Mw = 1.78) and was solubilized in chloroform (Laboratoire MAT Inc., Montréal, QC, Canada) at concentrations of 1%, 4% and 7% (w/v). Following total dissolution of the polymer, the solution was injected into a compressed air spray apparatus designed in our laboratory (Fig. 1). Basically, this apparatus consists of a customized atomizer plugged into compressed medical-grade air and filled with PLLA solution through a glass syringe (Becton-Dickson, Franklin Lakes, NJ, USA) equipped with a stainless steel needle. The air spray apparatus enabled the atomizer to translate vertically for air-spinning distance adjustments. A computer-controlled carriage moved the sample under the spray. PLLA was injected through the atomizer (Spraying Systems, Wheaton, IL, USA) with a computerized syringe pump (74900-00, Cole Parmer, Vernon Hills, IL, USA). Polymer solution was ejected through the needle and stretched by high-speed air flow. During this stretching process, solvent was vaporized (with a simultaneous increase in the viscosity of the PLLA solution) and polymer droplets were elongated to form fibres under air flow stress. Air pressure and polymer flow were precisely monitored to avoid fibre fragmentation and uncontrolled droplet production.

Samples were taken from non-crimped PET textile tubes (semi-finished Dialine II prostheses, C.R. BARD/Cardial, Saint-Etienne, France), hereafter referred to as Cardial

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