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Human coronary artery smooth muscle cell response to a novel PLA textile/fibrin gel composite scaffold

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Abstract

Previous studies have demonstrated the potential of fibrin as a cell carrier for cardiovascular tissue engineering applications. Unfortunately, fibrin exhibits poor mechanical properties. One method of addressing this issue is to incorporate a textile in fibrin to provide structural support. However, it is first necessary to develop a deeper understanding of the effect of the textile on cell response. In this study, the cytotoxicity of a polylactic acid (PLA) warp-knit textile was assessed with human coronary artery smooth muscle cells (HCASMC). Subsequently, quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was employed to examine the gene expression of HCASMC embedded in fibrin with and without the textile. Five genes were examined over a 3-week period: smooth muscle α-actin (SMαA), myosin heavy chain 11 smooth muscle (SM1/SM2), calponin, myosin heavy chain 10 non-muscle (SMemb) and collagen. Additionally, a microarray analysis was performed to examine a wider range of genes. The knitting process did not adversely affect the cell response; there was no dramatic change in cell number or metabolic rate compared to the negative control. After 3 weeks, there was no significant difference in gene expression, except for a slight decrease of 10% in SMemb in the fibrin with textile. After 3 weeks, there were no obvious cytotoxic effects observed as a result of the knitting process and the gene expression profile did not appear to be altered in the presence of the mesh in the fibrin gel.

Keywords: Fibrin; Polylactic acid; Smooth muscle cell; Composite scaffold; Warp-knit

1. Introduction

Coronary artery disease (CAD) is a disease with one of the greatest incidences of death in the western world [1]. With the rising numbers of obesity and poor nutritional habits, CAD is set to remain a serious issue for years to come. One common treatment for CAD is a coronary bypass using an autologus graft from a patient's vessel. Unfortunately, a patient may not always have a suitable vessel for implantation because of disease or age. Successful synthetic grafts have been designed with diameters >6 mm using polyethylene terephthalate (PET) and expanded polytetraflouroethylene (ePTFE). Currently, problems still exist with small-diameter (<6 mm) artificial cardiovascular grafts, such as thrombogenicity and compliance mismatch, despite many years of effort and various approaches [2]. Tissue engineering techniques are being used to overcome these limitations.

Fibrin gel has been studied as a scaffold for tissue-engineered vascular grafts (TEVG) [3–6]. An advantage of fibrin is that it can be produced from a patient's own blood and used as an autologous scaffold without the risk of a foreign body reaction. Conversely, fibrin does not possess the mechanical integrity to maintain the desired shape

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and structure after implantation. Since the mechanical strength of fibrin gel is not comparable to that of a native coronary artery [7], we propose the addition of a PLA textile to reinforce the gel. This would create a two-phase structure with the fibrin gel acting as a cell carrier, and the textile providing the necessary mechanical support. Warp-knit textiles are especially interesting, and previous research has shown positive results for cartilage repair using a composite hydrogel/warp-knit textile construct [8] and heart valve conduits composed of a fibrin gel and a warp-knit textile [9,10].

The overall aim of this research study is to produce a TEVG in vitro, whereby a PLA mesh is embedded in a smooth muscle cell seeded fibrin gel and mechanically conditioned in a bioreactor in the hope that the PLA mesh will degrade and extracellular matrix will be formed in vitro. Thereby reducing any negative side effects that have been seen to occur in vivo when PLA degrades. In order to achieve this goal, it was first necessary to examine the degradation profile of the PLA fibres and assess the affect of the mesh production methods on HCASMC response in vitro. Initially, changes in molecular weight, tensile strength and pH were monitored for PLA fibres stored in phosphate buffer solution (PBS) over a 36-week period. Direct contact cytotoxicity studies were performed whereby cell viability and cell number were investigated for HCASMC seeded in the fibrin gels with and without the textile. In addition gene expression was monitored over a 3-week period for a range of HCASMC and extracellular matrix (ECM) markers using quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) and microarray technology.

2. Materials and methods

2.1. Degradation profile of the PLA fibres

Medical grade polymer poly(L/D)lactide 96/4 with an intrinsic viscosity of 2.18 dl g^{-1} (Purac Biochem, Goringhem, The Netherlands) was used for fibre production. The polymer was melt-spun into multi-filament fibres (12-ply, single orifice Ø 0.2 mm), using a Gimac microextruder (Gimac, Gastronno, Italy) with a screw diameter of 12 mm. The fibres were drawn using caterpillars and ovens at 90 and 140 °C to an average diameter of 40 ± 3 μm.

Unsterilized fibres were incubated from 0 to 36 weeks at 37 °C in PBS (3.48 g dm^{-3} Na_2HPO_4/0.755 g dm^{-3} NaH_2PO_4/5.9 g dm^{-3} NaCl-buffered saline) at pH 7.4. The average ratio of fibre weight to buffer volume was 0.045 g per 30 ml. The buffer solution was changed and the pH measured fortnightly. In parallel, the tensile properties of specimens 50 mm in length and 40 μm in diameter were tested using an Instron 4411 Materials Testing Machine (Instron Ltd., High Wycombe, England) at a crosshead speed of 30 mm min^{-1} ($n = 5$).

The molecular weight (M_w) and polydispersity (PD) were measured by gel permeation chromatography (GPC) relative to narrow polystyrene standards. GPC consisted of a Waters 410 RI differential refractometer detector and a Waters 515 HPLC pump (Waters, Milford, MA, USA). The GPC columns were PL gel 5 μm Guard and 2 PL gel 5 μm mixed-C. The injection volume was 150 μl and the flow rate of eluent was 1 ml min^{-1}. Calibration was performed using monodisperse polystyrene standards applying Mark–Houwink parameters for PS ($K = 1.12 \times 10^{-4}$ and $a = 0.73$). The samples were dissolved in 0.1% w/v solutions in chloroform at room temperature.

Upon completion of the degradation study of the fibres, a warp-knit structure was formed from the melt-spun fibres using a custom-designed double warp-knitting machine, type DR 16 EEC/EAC (Karl Mayer Textilmaschinenfabrik GmbH, Germany). A fineness of E30 was applied and the fibres were pulled singly from the creel. A tricot pattern was used with a single layer setting. A cylindrical warp-knit structure was produced with an inner diameter of 6 mm, a pore size of 1 mm, a stress at failure of 33 MPa and an elongation at break of 52%.

2.2. Assessment of mesh production methods on HCASMC response

The cytotoxicity of the warp-knit mesh after the knitting process was examined using direct contact methods according to the International Standard 10993-5: Biological Evaluation of Medical Devices – Part 5: Tests for Cytotoxicity: In Vitro Methods [11]. High-density polyethylene (US Pharmacopeia, USA) served as the negative control and organo-tin-stabilized poly(vinylchloride) (Portex Ltd., UK) as the positive control, both of which were chosen as controls based on the ISO reference standards. The textile was soaked in 70% industrial methylated spirits (IMS) for 15 min followed by a sterile PBS rinse. The textile was plasma sterilized using a Sterrad 100S Sterilizer (Johnson & Johnson, USA). The controls were sterilized by soaking in 70% IMS for 30 min, followed by a sterile PBS rinse. Human coronary artery smooth muscle cells (HCASMC, Cambrex, UK) were seeded onto 24-well plates at a density of 9000 cells cm^{-2} and incubated for 24 h. The textile and controls were added to the subconfluent monolayers so that the cells were in direct contact with the materials ($n = 6$). After 24 h incubation, the cell metabolic activity was examined using an alamarBlue™ assay (Biosciences, USA), while cell number was determined using a PicoGreen® assay (Molecular Probes, USA).

Briefly, the media was removed from the wells and cells were rinsed with sterile PBS. A 10% alamarBlue® and Hanks' balanced salt solution (HBSS) mixture was added to each well and the plates were incubated for 1 h. Aliquots from each well were transferred to a black 96-well plate and the fluorescence was measured using an excitation wavelength of 530 nm and an emission wavelength of 590 nm with an FLX800 Microplate Fluroescence Reader (Biotek Instruments, Inc., Ireland). The remaining 10% alamar-

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