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In vitro study of smooth muscle cells on polycaprolactone and collagen nanofibrous matrices

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Abstract

Biodegradable polycaprolactone and collagen nanofibers were produced by electrospinning, with fiber diameters of around 300–700 nm and features similar to the extracellular matrix of natural tissue. Human coronary artery smooth muscle cells (SMCs) seeded on nanofibrous matrices tend to maintain normal phenotypic shape and growth tends to be guided by the nanofiber orientation. The SMC and nanofibrous matrix interaction was observed by SEM, MTS assay, trypan blue exclusion method and laser scanning confocal microscopy. The results showed that the proliferation and growth rate of SMCs were not different on polycaprolactone (PCL) nanofibrous matrices coated with collagen or tissue culture plates. PCL nanofibrous matrices coated with collagen showed that the SMCs migrated towards inside the nanofibrous matrices and formed smooth muscle tissue. This approach may be useful for engineering a variety of tissues in various structures and shapes, and also to demonstrate the importance of matching both the initial mechanical properties and degradation rate of nanofibrous matrices to the specific tissue engineering.

Keywords: Polycaprolactone; Collagen; Smooth muscle cells; Nanofibers; Tissue engineering

1. Introduction

Vascular grafts are used to replace damaged or occluded blood vessels. The nanomatrix is designed and fabricated as three-dimensional scaffolds out of collagen and the cell secreting natural material can be used to seed the smooth muscle cells (SMCs) to mimic natural small diameter blood vessels. Studies suggest that muscle cells, once implanted in the scaffold, develop into the function, shape, morphology and cellular architecture of normal vessels (Bowlin, 2003). Synthetic polymer scaffold poly(L-lactid-*co*-ϵ-caprolactone) composed of aligned and random nanofibers mimics the native extracellular matrix suitable for SMCs and endothelial cells (ECs) interaction between the scaffolds. The electrospinning method used to fabricate the scaffold is simple for mass production and has good potential for blood vessel engineering (Mo et al., 2004; Xu et al., 2004). Human ECs, SMCs and fibroblasts were used in the inner, middle and outer layers, respectively, of the vascular constructs. A key challenge was to achieve the proper alignment, architecture, abundance of cell types and behavior in each cell layer. If successfully developed and approved for clinical use, the new technology could replace all other vascular grafts, reduce coronary bypass surgical costs by 10% and other hospital costs as well, and improve productivity and quality of life for people who undergo vascular graft procedures (Bowlin, 2003). The extracellular matrix surrounding vascular cells combines to provide the biomechanical properties of the tissue. The mechanical properties critical to blood vessel function include: the tensile stiffness, elasticity, compressibility and viscoelasticity (Ratcliffe, 2000). Nanotechnology

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produces natural human blood vessels grown around a scaffold made of collagen and polymers.

Biodegradable polymers such as poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA) and PGA coated with PLLA are being employed for cell transplantation and for in vivo regeneration of vascular tissue (Chu et al., 1999). Human ECs show little adhesion and no proliferation on the currently available vascular graft materials. These properties can be improved by coating grafts with fibronectin or other allogeneic human materials, such as fibrin glue, prior to seeding of the grafts (Li et al., 1992). Individually, collagen has less fiber strength to withstand longtime support for cell adhesion and degrades enzymatically within short periods. This study was aimed to modify the polycaprolactone with collagen, to increase the mechanical strength of nanofibers in tissue engineering and to allow the cells to secrete their own ECM to form natural blood vessels useful for the replacement of diseased vessels. In this study, we have examined the adhesion, growth and proliferation of SMCs on electrospun PCL, PCL nanofibrous matrices coated with collagen, and collagen nanofibrous matrices suitable for blood vessel engineering.

2. Materials and methods

2.1. Materials

Human coronary artery smooth muscle cells (SMCs) were obtained from American Type Culture Collection (ATCC, Arlington, VA, USA). Smooth muscle cell basal medium (SmBM), insulin, fetal bovine serum, human epidermal growth factor and gentamycin were purchased from Cambrex Bio Sciences (Walkersville, USA). Polycaprolactone (M_W 80,000) was obtained from Aldrich Chemical Company (USA) and methanol and chloroform from Merck (Germany). Calf skin collagen Type I, antibiotics and trypsin–EDTA were obtained from Sigma (USA). CellTiter 96® Aq_{ueous} One solution was purchased from Promega (USA).

2.2. Nanofiber fabrication

Polycaprolactone (7.5% w/w) was dissolved in methanol and chloroform (1:3) overnight with stirring. Collagen type I (75 mg/mL) was dissolved in 1,1,3,3,3-hexafluoro propanol. For electrospinning, polycaprolactone (PCL) and collagen solutions were placed in a 5 mL plastic syringe fitted with 27 gauge needle and the syringe pump was used to feed polymer solution into the needle tip and the feeding rate was fixed at 1 mL/h. The PCL and collagen nanofibers were fabricated by the electrospinning process and the applied voltage was 13 kV using a high voltage power supply (Gamma High Voltage Research). The ground collection plate of aluminum foil was located at a distance of 13 cm from the needle tip. A positive charged jet was formed from the Taylor cone and nanofibers sprayed on to the grounded aluminum foil target (Reneker and Chun, 1996). Coverslips of different sizes were spread on the aluminum foil target to collect nanofibers for the biocompatibility investigation, as well as to observe the structure and properties of nanofibers. Electrospun nanofibers were dried under vacuum at room temperature overnight. For characterization, electrospun nanofibers were sputter-coated with gold (JEOL, JFC-1200 fine coater). The morphology of the electrospun nanofibers was observed using SEM at an accelerating voltage of 15 kV and the fiber diameter was measured with the SEM images using the software Image J (National Institute of Health, USA).

2.3. Sterilization of nanofibers

Polycaprolactone and collagen nanofibers on coverslips were kept in 24 well culture plates. The nanofiber scaffolds on coverslips were sterilized and prewetted in decreasing concentrations of ethanol for 24 h. Thereafter, the scaffolds were soaked in PBS and in cell culture medium overnight prior to cell seeding, in order to facilitate protein absorption and cell attachment on nanofibers.

2.4. SMC cell culture

Human coronary artery smooth muscle cells were cultured in smooth muscle basal medium (SmBM) containing 5% FBS and growth factors in 75 cm^2 flasks. The cell culture was maintained at 37 °C in a humidified CO_2 incubator for 7 days and fed every 3 days and SMCs were harvested from 3rd passage cultures by trypsin–EDTA treatment and replated. Populations of cell lines used in this experiment were between passages 6 and 7.

2.5. Cell proliferation assay

Smooth muscle cells were seeded (2×10^4 cells/well) on control tissue culture plate (TCP), PCL, PCL nanofibrous matrices coated with collagen type I (10 mg/mL) and collagen nanofibrous matrices on 24 well plates. PCL nanofibrous matrices were soaked in collagen Type I solution overnight prior to cell seeding to facilitate cell attachment. The cell proliferation was monitored after 24, 48 and 72 h by MTS assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt). In order to monitor cell adhesion and proliferation on different substrates, the number of cells was determined using the colorimetric MTS assay (CellTiter 96® Aq_{ueous} Assay). The mechanism behind this assay is that metabolically active cells react with a tetrozolium salt in the MTS reagent to produce a soluble formazan dye that can be observed at 490 nm. The cellular constructs were rinsed with PBS followed by incubation