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Short communication

Poly (lactic acid)–chitosan–collagen composite nanofibers as substrates for blood outgrowth endothelial cells

B. Swarnalatha[a,1], S.L. Nair[b,c,1], K.T. Shalumon[a], L.C. Milbauer[c], R. Jayakumar[a], B. Paul-Prasanth[a], K.K. Menon[a], R.P. Hebbel[c], A. Somani[b,c,d,*], Shantikumar V. Nair[a,**]

[a] Amrita Centre for Nanosciences and Molecular Medicine, Amrita Institute of Medical Sciences and Research Centre, Amrita Vishwa Vidyapeetham, Kochi, 682041, India
[b] Pediatric Critical Care Medicine, University of Minnesota, Minneapolis, USA
[c] Vascular Biology Centre, Department of Medicine, University of Minnesota, Minneapolis, USA
[d] Pediatric Critical Care, McMaster University, Hamilton, Canada

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ABSTRACT

In this work, the attachment, viability and functionality of rat Blood Outgrowth Endothelial Cells (rBOEC) and genetically modified rBOEC (rBOEC/eNOS-GFP), which over express endothelial nitric oxide synthase (eNOS), were investigated on Poly(lactic acid) (PLA)–chitosan and PLA–chitosan–collagen nanofibrous scaffolds. Both the cell types displayed good attachment, remained viable and functional on both scaffolds. Moreover, incorporation of collagen in the scaffold helped in sustaining the rBOEC for upto one week, although collagen was not found necessary for rBOEC/eNOS-GFP. We conclude that PLA–chitosan based nanofibrous scaffolds can be a potential candidate for BOEC based wound healing applications.

1. Introduction

Blood outgrowth endothelial cells (BOEC) are a robust endothelial cell lineage isolated from peripheral blood with very high proliferative capacity [1]. These endothelial cells, genetically engineered to overexpress eNOS (rBOEC/eNOS-GFP), hold promise for vascular and wound healing applications [2]. In order to use endothelial cells for wound healing, it is necessary to support these cells on a suitable substrate. Materials such as silk fibroin [3], poly (urethane) coated with cholesterol [4], and combinations of poly (lactic acid) (PLA) and collagen [5] have been studied for BOEC compatibility for vascular tissue engineering. A collagenous support is considered to be an ideal matrix for BOEC support [1,2]. However, collagen is not cost effective. On the other hand, chitosan is a biological macromolecule with components similar to ECM and is a well-known cost-effective, haemostatic, biocompatible, bioadhesive agent, that has been used extensively as a wound healing material [6,7]. Further, nanofibrous scaffolds enhance cell–material interaction [7] and protein adsorption [8]. Therefore, this study explores the utility of chitosan in nanofibrous form as a host material for BOEC. Since chitosan alone cannot be electrospun, a synthetic polymer, PLA whose biocompatibility to BOEC has been established [5], was chosen as a binder for electrospining [7]. PLA–chitosan-based nanofibrous support was investigated for the first time as a potential matrix for BOEC support with and without the addition of collagen.

2. Materials and methods

2.1. Materials

PLA (M_W 350,000 kDa) was purchased from Good Fellow Cambridge Ltd. (Huntingdon, UK). Chitosan (87% degree of

* Corresponding author at: Pediatric Critical Care, University of Minnesota, 5th Floor East Building, MB538, 2450 Riverside Ave, Minneapolis, MN 55454, USA. Tel.: +1 612 624 5649; fax: +1 612 626 0413/McMaster University, Faculty of Health Sciences, 1280 Main Street West, HSC 3Y, Hamilton, ON, L8S 4K1, Canada. Tel: +1 905 521 2100x75823.
** Corresponding author at: Amrita Vishwa Vidyapeetham, Amrita Institute of Medical Sciences and Research Centre, Amrita Centre for Nanosciences and Molecular Medicine, Ponekkara, Kochi, Kerala 682041, India. Tel.: +91 484 4008750; fax: +91 484 2802030.
E-mail addresses: soman007@umn.edu, somani@mcmaster.com (A. Somani), nairshanti@gmail.com (S.V. Nair).
[1] The authors hold equal contribution towards the work.

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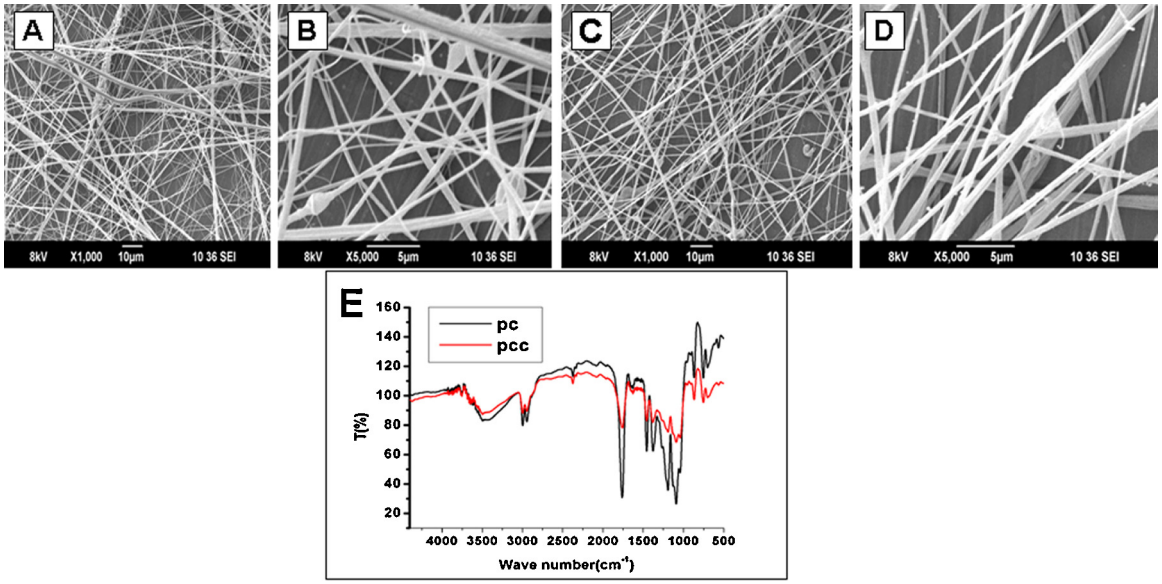

Fig. 1. SEM images of (A, B) PC nanofibrous scaffold; (C, D) PCC nanofibrous scaffold. (E) FTIR spectra of PC and PCC nanofibrous scaffolds.

deacetylation) (M_W 100–150 kDa) was obtained from Koyo Chemicals Co Ltd. (Japan) and Collagen type 1 from BD Biosciences (California, USA). Acetylated low density lipoprotein (Ac-LDL), 4', 6-diamidino-2-phenylindole (DAPI) and Alamar Blue were purchased from Invitrogen Inc. (Grand Island, USA). Gluteraldehyde was procured from Fluka chemical corp. (Milwaukee, USA).

2.2. Preparation of PLA–chitosan and PLA–chitosan–collagen scaffold

The PLA–chitosan (PC) solution for electrospinning was prepared by dissolving PLA and chitosan in formic acid/chloroform/acetone (60:20:20) solvent mixture. 12% (W/V) PLA and 4% (W/W) Chitosan (with respect to the weight of PLA) were dissolved in the solvent mixture by stirring [7]. For making PLA–chitosan–collagen (PCC) solution, 100 μl of 0.05% collagen solution was added drop wise to the PLA–chitosan solution while stirring. After 2 h of mixing, a clear and homogenous solution was loaded in a 10 ml syringe needle (internal diameter-0.5 mm) fitted in an infusion syringe pump and delivered at a constant flow rate of 1 ml/h, tip-target distance of 11 cm and a positive voltage of 10 kV from a high voltage power source. Random nanofibers were collected on grounded aluminum foil.

2.3. Characterization of nanofibrous PC and PCC scaffolds

Microscopic characterization of the fibers was carried out using a JEOL analytical Scanning Electron Microscope [JSM-6490 LA,

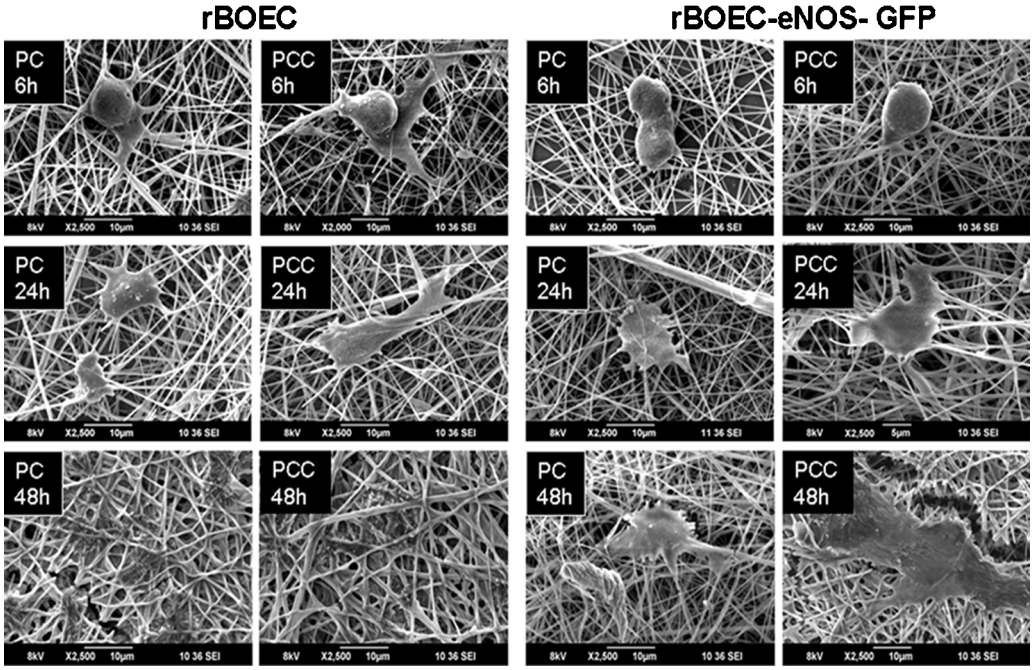

Fig. 2. Cell morphology studies. SEM images representing the cell attachment by rBOEC seeded on PC and PCC respectively at 6, 24 & 48 h and rBOEC/eNOS-GFP on PC and PCC respectively at 6, 24 & 48 h.