From 3D Electrospun Nanofiber Scaffolds to Small-Diameter Vascular Grafts

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Mr. Savoji obtained his B.Sc. (2001) and M.Sc. (2004) in Chemical Engineering from two well-distinguished Engineering Universities in Iran. He led projects as a Senior Engineer and R&D Researcher in several Consulting Engineering Companies before moving to Canada. He was a Research Associate in Industrial Membrane Institute of University of Ottawa. He is now a 3rd year Ph.D. Candidate at the Institute of Biomedical Engineering at Ecole Polytechnique de Montreal, studying the "fabrication of innovative vascular prostheses comprising electrospun nanofiber matrices and bioactive coatings prepared by plasma polymerization". He has a number of publications in peer-reviewed journals, as well as several technical reports. He has presented more than 30 oral and poster presentations in international and national conferences. He is a recipient of several scholarships and awards including FQRNT Merit Doctoral Research Scholarships. He has initiated the first Montreal Student Chapter of the Canadian Biomaterials Society where he is currently the president. He enjoys hiking in beautiful trails of Quebec with his wife.

Abstract

Conventional large-diameter prosthetic vascular grafts (ePTFE, PET), have proven unsatisfactory for small-diameter vessels (below 6 mm) due to poor endothelialization and compliance mismatch, which lead to the lack of patency and to thrombogenesis. To address these vital issues, scaffolds that simulate the extracellular matrix (ECM) of native blood vessels and that possess similar 3D nano-fibrous structure, can be produced by electrospinning, which is a "re-discovered" technique from textile industry. However, the efficacy of endothelial cell adhesion and growth on such PET scaffolds may be limited. Therefore, to further improve biocompatibility of a polymeric scaffold, a suitable surface treatment is needed to enable strong cell-adhesion and -growth. A particularly powerful method to promote cell adhesion is to deposit a plasma-polymerized nitrogen-rich coating

(so-called L-PPE:N) on the surface. In this study, we developed an innovative random 3D electrospun nanofiber scaffold for the lumen side of a graft, one which is structurally and mechanically suitable for accommodating human umbilical vein endothelial cells (HUVECs). Coating with L-PPE:N was then carried out and its effect on mechanical properties and HUVEC adhesion and growth was evaluated *in vitro*.

To this aim, 3D nano-fibrous PET mats were prepared by electrospinning, to fine-tune the mat's morphology and mechanical properties suitable for culture of HUVECs. Thereafter, the substrates were L-PPE:N-coated in a capacitively coupled radio-frequency (r.f.) glow discharge plasma reactor; the tailored plasma-polymer coating were obtained from ammonia (NH_3) / ethylene (C_2H_4) mixture, in order to create a high concentration of primary amine groups [NH_2] in these porous structures. [NH_2] was determined by X-ray Photoelectron Spectroscopy (XPS), before and after chemical derivatization. The morphology of the untreated and plasma-coated mats was studied by Scanning Electron Microscopy (SEM) and Mercury Intrusion Porosimetry. Mechanical properties of the mats were determined using tensile testing (Instron), under both dry and wet conditions. To investigate HUVEC adhesion, growth and distribution on the mats, live/dead assay was used and followed by Laser Scanning Confocal Microscopy. The morphology of the cells was also analyzed by SEM.

Chemical derivatisation / XPS showed the desired high [NH_2] values (6%) on the mats' outer and inner surfaces, clearly illustrating the plasma species' ability to penetrate through the fiber mats. SEM confirmed smoothly-interconnected open structure, with an overall porosity of 87% and average nano-fiber diameters of ca. 551 nm and 567 nm before and after coating, respectively (Fig. 1). Tensile strain was found to be reduced after coating, as expected, while tensile stress was increased and the coated mats appeared somewhat stiffer, under both dry and wet conditions. *In vitro* cell-culture showed a significant increase of cell number on the plasma-coated mats, confirming L-PPE:N as an excellent pre-treatment to promote the requisite HUVEC adhesion and growth (Fig. 2). Cells were also shown to form a complete monolayer on the scaffold (Fig. 3).

To conclude, the methodology proposed in this research provides adequate scaffolds for the luminal side of small-diameter vascular prostheses, with finely-controlled structural, mechanical and surface properties required for complete and stable endothelialisation of small vascular grafts.