Design and testing of plasma-coated electrospun nanofiber scaffolds for vascular grafts

+ [1,2]Savoji, H; [3]Hadjizadeh, A; [2]Maire, M; [2,4]Lerouge, S; [3]Ajji, A; [1,5]Wertheimer, MR

École Polytechnique de Montréal, Montreal, QC, Canada ([1]Institute of Biomedical Engineering, [3]Department of Chemical Engineering, [5]Department of Engineering Physics)
[2] Laboratory of Endovascular Biomaterials (LBeV), Research Centre, Centre Hospitalier de l'Université de Montreal (CRCHUM), Montreal, QC, Canada
[4] Department of Mechanical Engineering, École de Technologie Supérieure, Montreal, QC, Canada

Introduction:

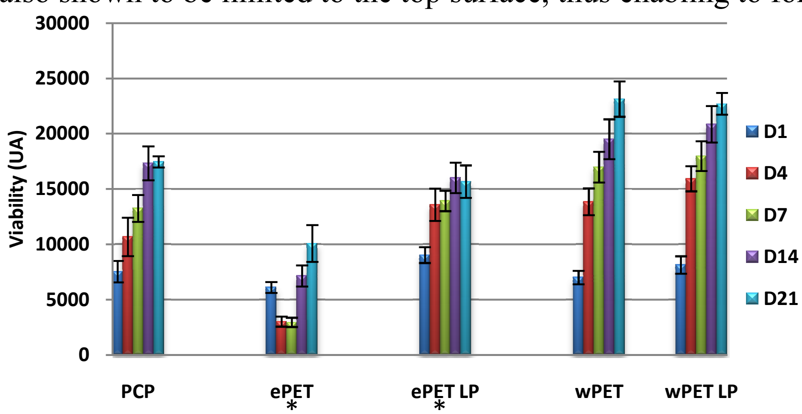
Development of prosthetic vascular grafts has been a rapidly growing area of research, designed to alleviate insufficient availability of autologous grafts. Conventional large-diameter prosthetic materials such as expanded poly(tetrafluoroethylene), ePTFE; or woven Dacron® poly(ethylene terephthalate), 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[1]. To address these vital issues, scaffolds that simulate mechanical and morphological properties of the extracellular matrix (ECM) of native blood vessels and that possess similar 3D nano-fibrous structure and porosity, can be produced by electrospinning. Many materials can be used to create such electrospun scaffolds. Among them, PET offers distinct advantages as the polymer material for small-diameter grafts, including its mechanical properties, stability, biocompatibility, cost effectiveness, but foremost the fact that it is already FDA approved for this application[2]. However, literature data suggest that 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 -proliferation. A particularly powerful method to promote protein adsorption and subsequent cell adhesion, developed by our team, is to deposit a plasma-polymerized nitrogen-rich coating (so-called L-PPE:N) on the surface[3]. 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 LPPE:N was then carried out and its effect on mechanical properties and HUVEC adhesion and growth was evaluated *in vitro*.

Materials and Methods:

3D nano-fibrous PET substrates were prepared by electrospinning using a rotating mandrel under optimized conditions, 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 with a ratio of $NH_3/C_2H_4 = 0.75$, in order to create a high concentration of primary amine groups [NH_2] in these porous structures[3]. [NH_2] was determined by X-ray Photoelectron Spectroscopy (XPS), before and after chemical derivatization with 4-(trifluoromethyl) benzaldehyde (TFBA). The morphology of the untreated and plasma-coated mats was studied by Scanning Electron Microscopy (SEM) and Mercury Intrusion Porosimetry, to determine fiber diameter, average pore size and overall porosity. Mechanical properties of the mats were determined using tensile testing, under both dry and wet conditions. To investigate HUVEC adhesion and growth, Alamar blue (resazurin; cell viability indicator) was used after different culture times (e.g. 1, 4, 7, 14 and 21 days). In addition, cell survival and distribution on the mats was observed using calcein-ethidium homodimer-1 staining (LIVE/DEAD viability/cytoxicity kit) and followed by Laser Scanning Confocal Microscopy. The morphology of the cells was also analyzed by SEM after fixation in 0.5% glutaraldehyde at 4 ºC and gold sputtering. Statistical analysis of the data was performed to report the significance in differences at the 0.05 level (e.g. via analysis of variance, ANOVA).

Results and Discussion:

Chemical derivatization / 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. 521 nm and 565 nm before and after coating, respectively. Mechanical properties such as tensile strength, Young's modulus and elongation at break were found to be improved significantly compared to those of commercial prosthetic vascular grafts (e.g. Dacron® and ePTFE). Tensile strain was found to be somewhat reduced after coating, as expected, while tensile stress was increased and the coated mats appeared somewhat stiffer. Tensile properties did not change significantly under wet conditions. *In vitro* cell-culture showed a significant increase of cell number on the plasma-coated mats (Fig.1), confirming L-PPE:N as an excellent pre-treatment to promote the requisite HUVEC adhesion and proliferation[4]. In contrast to woven PET, cells were also shown to be limited to the top surface, thus enabling to form a complete monolayer (Fig.2).



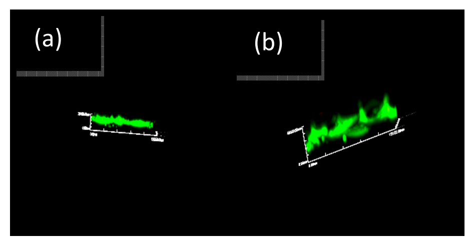


Fig.2: Z-stack confocal micrograph of ePET (a) and wPET (b)

Fig.1: Adhesion and growth of HUVEC on untreated (ePET) and plasma-treated (ePET-LP) electrospun PET and untreated (wPET) and plasma-treated (wPET-LP) woven PET (N=3, (*$p < 0.05$))

Conclusion:

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; such innovative devices may help prevent clinical complications encountered when using current off-the-shelf prosthetic vascular grafts.

Acknowledgments: Financial support from FQRNT, NSERC and CIHR is gratefully acknowledged.

References:

(1) Sarkar, S. et al., Medical & Biological Engineering & Computing 2007, 45 (4), 327-336.
(2) Ma, Z. et al., Biomaterials 2005, 26 (15), 2527-2536.
(3) Truica-Marasescu, F. et al., Plasma Processes and Polymers 2008, 5 (1), 44-57.
(4) Gigout, A. et al., Macromolecular Bioscience 2011, 11 (8), 1110-1119.