Structural, Mechanical and In Vitro Characterization of Plasma-Coated Electrospun Nanofiber Scaffolds for Vascular Graft Applications

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Statement of Purpose: Current off-the-shelf large-diameter prosthetic vascular grafts such as (expanded poly(tetrafluoroethylene), ePTFE; or woven Dacron® poly(ethylene terephthalate), PET) have shown poor endothelialization and compliance mismatch which lead to lack of patency and thrombosis when used for small-diameter vessels (below 6 mm) (Kannan R. Y. J. Biomed. Mater. Res., Part B. 2005; 74B: 570-581.). To overcome these issues, researchers have introduced electrospun nano/micro-fiber scaffolds which possess prominent structural and mechanical properties to imitate the ECM of the native blood vessels. These scaffolds contain interconnected open structure with high porosity and surface area. On the other hand, mechanical properties of the scaffolds can be optimized to fine-tune those of native blood vessels. Among different materials used, 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 (Hadjizadeh A. J Mech Behav Biomed 2011;4 (3): 340-351.). Most of the polymers possess inert surfaces which limits the endothelial cells adhesion and growth. Therefore, to promote the biocompatibility of the scaffolds, an appropriate surface modification technique is sought to improve cell-adhesion and growth. Plasma-polymerization is a versatile technique to promote protein adsorption and subsequent cell adhesion (Truica-Marasescu F. Plasma Processes and Polym. 2008; 5 (1): 44-57.). Here, we design and fabricate a novel 3D electrospun nanofiber PET scaffold modified with a nitrogen-rich thin coating to improve tensile properties and promote human umbilical vein endothelial cells (HUVECs) adhesion and growth.

Methods: Electrospinning was used to fabricate optimal 3D nano-fibrous PET mats to fine-tune the mat's structure and mechanical properties suitable for HUVECs. Thereafter, the porous substrates were plasma-coated in a plasma reactor; the tailored nitrogen-rich plasma-polymer coating being obtained from ammonia (NH_3) and ethylene (C_2H_4) mixture (ratio of $NH_3/C_2H_4 = 0.75$) in order to create a high concentration of primary amine groups [NH_2]. The stability of the functionalized coating was studied by immersing plasma-treated mats in an aqueous solution (milliQ water) for 7 days. Chemical surface analysis was conducted by X-ray Photoelectron Spectroscopy (XPS). The structural properties of the untreated and plasma-coated mats were conducted by Scanning Electron Microscopy (SEM) and Mercury Intrusion Porosimetry. Tensile properties of the mats were determined using a uniaxial tensile testing device in both dry and wet conditions, before and after plasma coating. To investigate HUVEC adhesion and growth, Alamar blue (resazurin; cell viability indicator) was used after different culture times (e.g. 1, 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 kit) 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.

Results: Surface chemical analysis of the mats showed high concentrations of the desired primary amine groups, [NH_2]/[N] selectivity >6%, on the substrates' top and bottom surfaces. This revealed that plasma species penetrate deep inside the porous structure. An increase in [O], accompanied by a slight decrease in [N] during immersion time in milliQ water was observed. Moreover, [NH_2] somewhat decreased ([NH_2]/[N]~5%) after 7 days, as expected. This indicated the presence of a stable functional coating on the mats. SEM images confirmed the random, smoothly-interconnected open structure, with an overall porosity of 87%, the average nano-fiber diameters being ca. 520 nm and 565 nm before and after coating, respectively. Tensile values such as young's modulus, tensile strength 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, while tensile stress was increased and the coated mats appeared somewhat stiffer. Tensile properties did not change significantly in wet conditions. In vitro cell-culture experiments showed that the plasma-coated mats did indeed promote the adhesion and growth of HUVECs. Cells were also shown to form a complete monolayer on the top surface of the scaffold.

Conclusions: Plasma-coated electrospun nanofiber PET scaffolds show finely controlled structural, mechanical and biocompatibility properties to mimic the luminal side of small-diameter vascular grafts.

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