Plasma Processes for Innovative Vascular Prostheses Combining 3D Electrospun Nanofiber Matrices and Bioactive Coatings

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Abstract: We describe research designed to develop a new generation of synthetic vascular grafts (VGs) of small diameter (< 6 mm). Unlike current commercial VGs, these are based on electrospun PET scaffolds that are plasma treated in order (i) to optimize their mechanical properties; (ii) to allow colonization by the required cell types. Here, we emphasize adhesion of endothelial cells on the VG's innermost luminal (intima) layer.

Keywords: Vascular graft, electrospinning, plasma etching, plasma polymer, endothelium

1.General Introduction

Conventional large-diameter prosthetic vascular grafts (ePTFE, 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 thrombosis and to lack of patency [1]. 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 [2]. However, the efficacy of endothelial cell adhesion and growth on such PET scaffolds will likely be limited. Therefore, to 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 activities is to deposit a thin plasma-polymerized (PP) coating (for example, nitrogen-rich PP-ethylene, designated "L-PPE:N") on the surface. In this study, an innovative random 3D electrospun PET ("ePET") nanofiber scaffold for the lumen side of a graft was developed, one which is structurally and mechanically suitable for accommodating human umbilical vein endothelial cells (HUVECs) [3]. PP coating was then carried out and its effect on mechanical properties and HUVEC adhesion and growth was evaluated *in vitro* [3, 4]. We have also investigated scaffolds with aligned nanofibers that simulate the medial layer of natural blood vessels, as well as plasma-based etching designed to optimize scaffolds' mechanical compliance [5]. Finally, different PP coatings were compared in regard to HUVEC colonization efficacy [6].

2. Experimental Methodology

A schematic diagram of the electrospinning process is shown in Fig. 1, while Fig. 2 presents the radio-frequency (r.f.) low-pressure plasma reactor [7] used for etching and PP deposition experiments carried out here. Since both have been described in detail elsewhere [5, 7], no further details will be presented.

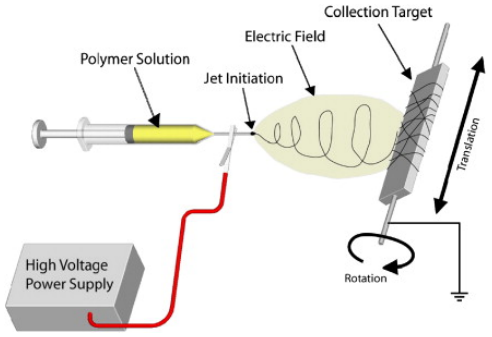

Fig. 1. Schematic diagram of the electrospinning process; from http://www.people.vcu.edu/~glbowlin.

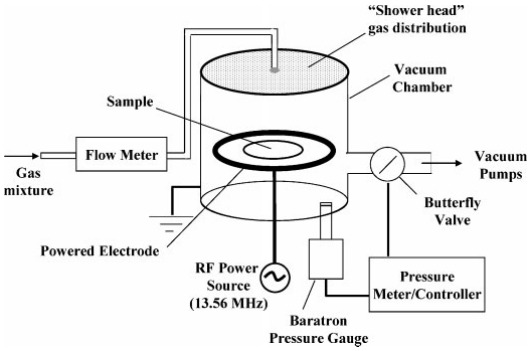

Fig. 2. Schematic diagram of the capacitively coupled plasma reactor [7].

Materials were characterized by several techniques: *Scanning electron microscopy (SEM).* The diameters of 100 randomly-selected fibers (at least two experiments with triplicate samples) were examined by SEM and analyzed using image analysis (NIH ImageJ software). *Mercury intrusion porosimetry.* Porosity and pore size of nano-fiber mats were determined by this method. *Tensile testing (dry and wet).* Mechanical properties of the mats were evaluated using a uniaxial tensile testing machine (Instron, ElectroPuls™ E10000). *Surface-chemical (XPS) analyses.* X-Ray photoelectron spectroscopy (XPS) analyses were performed in a VG

ESCALAB 3MkII instrument, using non-monochromatic Mg Kα radiation [7, 8]. For the case of N-rich mats, the surface-near concentrations of primary amine groups, [NH_2], were determined using the derivatization reaction with 4-(trifluoromethyl) benzaldehyde (TFBA) vapor and data acquired with XPS survey spectra [8]. *Biological Testing.* 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). The cell morphologies were also analyzed by SEM after fixation in 0.5% glutaraldehyde at 4 ºC and gold sputtering. The details have been described earlier [3].

3. Results and Discussion

Fig. 3 shows SE micrographs of three different types of electrospun PET mats (~ 100 µm thick), described in the caption, while Table 1 specifies typical mat characteristics.

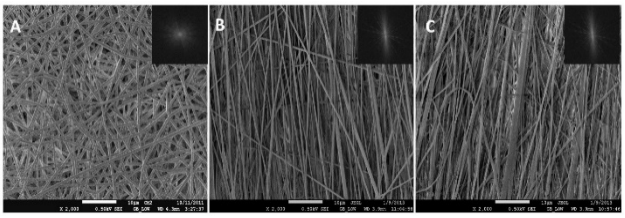

Fig. 3. SEM and 2D FFT images (insets) of (A) random (RL), (B) aligned (AL) mat for the luminal layer; and (C) aligned (AM) mat for the media layer (scale bar: 10 µm). Adapted from ref. [4].

Table 1- Porosity and pore properties of electrospun mats (bare and L-PPE:N-coated, n=4). Fibre diameter~550 nm.

Mat sample	Porosity (%)	Pore Diameter (µm)
Bare	87±1	3.2±0.5
PP-coated	86±1	2.5±0.9

It turned out that the mechanical compliance, inversely proportional to Young's modulus, was significantly higher than that of native blood vessels, for example human femoral artery. We therefore undertook a series of plasma-based etching experiments, both at low- (LP) and atmospheric pressure (HP), all using O_2 gas or O_2-based gas mixture, including in the apparatus of Fig. 2, but others as well [5]. Fig. 4 shows that all resulted in significantly reduced Young's moduli, close to that of femoral artery.

Next, a series of experiments was carried out which had the objective to study the effect of different surface compositions on the colonization by HUVECs. In order to render them feasible and simple, these were done not with ePET mats, but for the most part on smooth PET films, although two types of plasma-modified commercial cell-

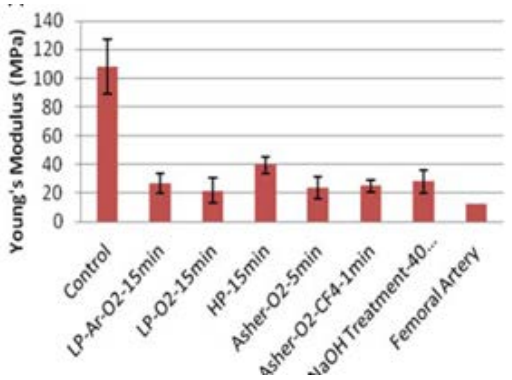

Fig. 4. Young's modulus of pristine and plasma-etched aligned ePET mats (three experiments, at least 12 samples in each experiment; adapted from ref. [5].

culture plates were also used as positive controls, namely tissue-culture polystyrene (O-rich "TCP"), and Primaria®, which contains both O- and N- surface functional groups [6]. This study, illustrated in Fig. 5, revealed that both O- and N-functionalized surfaces favour HUVEC adhesion to comparable extents: Both of these types of functionalizations displayed the ability to foster endothelialization, with results greatly superior than bare PET. The hoped-for synergistic effect of combined O- and N-bearing moieties observed on Primaria® could so far not be reproduced by plasma coating, although "L-PPE:O,N" does have potential for further improvement [6].

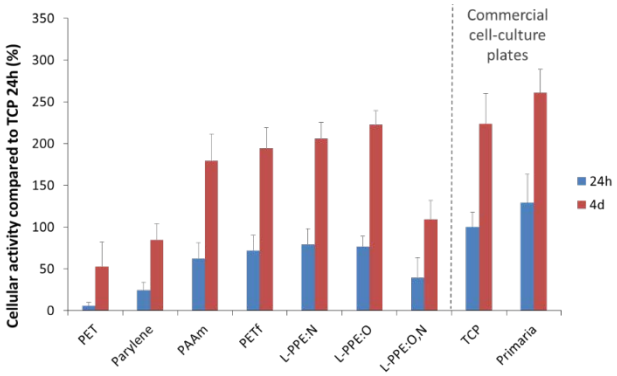

Fig. 5. Endothelial cell (HUVEC) culture results for the various bioactive materials examined (blue bars: after 24h; red bars: after 4d). All results are normalized with respect to those obtained with TCP plates after 24h (≡ 100%). Adapted from ref. [6].

Based on the investigations just described, one is faced with the choice between O- or N-rich PP coatings as the means to promote HUVEC adhesion on ePET mats. The main reason why we settled for L-PPE:N is that highly reactive primary amine (NH_2-) functional groups at the sample surface enable grafting of other bio-active groups or compounds, which turned out to be a major asset. The PP coating, by itself, favors not only colonization by HUVECs, but also by platelets in human blood, cells responsible for thrombosis (blood clot formation), of

course highly undesirable in this present context. Therefore, we have elected to covalently graft chondroitin sulfate (CS), a polysaccharide, to L-PPE:N-coated ePET surfaces. CS-containing coatings have already been shown to possess anti-apoptotic properties for vascular cells and to prevent platelet adhesion, while promoting HUVEC adhesion and growth [9-11]. Fig. 6 (top) compares the extent of HUVEC attachment after 21 days of incubation in media (a) on bare ePET; (b) on L-PPE:N-coated ePET, where the latter, unlike the former, clearly revealed near-complete coverage of the mat surface by a confluent monolayer of HUVECs. The two SEM images below, (c) and (d), respectively show corresponding (bare and L-PPE:N-coated) woven Dacron® surfaces: although (d) shows some HUVEC adhesion, it is clearly not possible to form the requisite confluent monolayer of HUVECs on such an irregularly profiled surface topography.

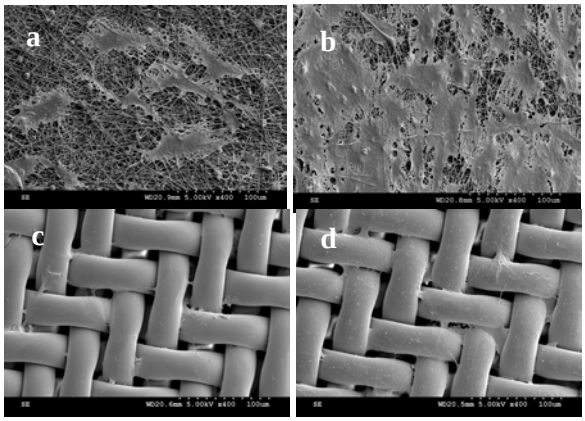

Fig. 6. SEM micrographs of electrospun nanofiber mats with HUVECs after 21 days of growth: a) bare mat (ePET); b) mat after L-PPE:N coating; c) bare woven PET (wPET, Dacron®); d) woven PET after L-PPE:N coating (scale bar: 100µm). Adapted from ref. [3].

Now, HUVEC coverage on a CS-grafted (L-PPE:N+CS, "LP+CS") ePET surface turned out to be comparable with (b), even slightly higher. The major difference, however, was noted when the cells' adhesion was tested in a special flow cell, designed to simulate shear stress on the HUVEC layer comparable to the 15 dynes.cm^{-2} caused by fluid flow in natural blood vessels. Fig. 7 illustrates the percentage of cell retention after 1 hour of exposure to shear, for the cases of (i) HUVECs on bare ePET; (ii) L-PPE:N (here "LP")-coated ePET; and (iii) "LP+CS"-coated ePET. Clearly, the latter shows statistically significantly improved performance over the former, both on random and aligned mats (see Fig. 3).

4. Conclusions

Clearly, the approach based on combining electrospun matrices with plasma processing has already led to much progress regarding the intima, emphasized here, but similar advances are now also being achieved for the case of the medial layer, populated by vascular smooth muscle cells.

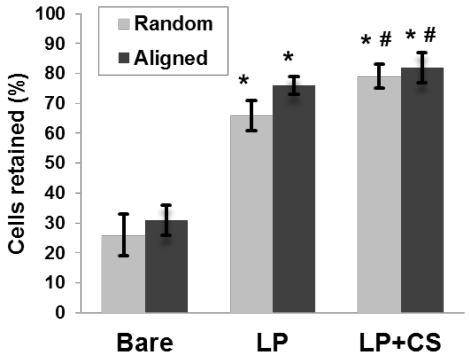

Fig. 7. HUVEC retention after laminar shear stress (15 dynes.cm^{-2},1h), evaluated by AlamarBlue assay (n=4). *, $^{\#}$ $p < 0.05$ with reference to bare and LP-coated surfaces under shear, respectively. Adapted from ref. [4].

One main advantage of these coatings is their versatility, because they can be deposited on any electrospun material and therefore enable one to optimize, in parallel, the compliance of the VG and its biocompatibility. The present bioactive coatings should help development of a novel generation of synthetic vascular prostheses with increased patency.

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