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## Endothelial/Smooth Muscle Cells Growth into Nonwoven Fiber Vascular Grafts

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**Introduction:** Coronary artery and peripheral vascular diseases is the leading cause of death in Canada (1 in 3), with 80% of Canadians having at least 1 risk factor for cardiovascular diseases and 10% more than 3 risks or more<sup>1</sup>. These figures stand for all Western countries. The standard surgical intervention for small diameter vessels is currently autologous vein (saphenous) bypass grafting when available and, for larger diameter (6 mm and more), synthetic grafts made of woven polyethylene terephthalate (PET) fibers (Dacron<sup>TM</sup>) or expanded polytetrafluoroethylene (ePTFE) characterized by a low compliance. The compliance mismatch between the graft and artery contributed to myointimal hyperplasia, particularly at anastomotic sites<sup>2</sup>, as a reason of hemodynamic flow perturbations, and thrombosis is a common problem. An urgent need thus exists for patent, compatible tissue engineered small-diameter (2-6 mm) vascular grafts, to which endothelial cells (EC) can adhere, form an anti-thrombogenic luminal surface, exhibit vasoactive properties and improve patency. In this study, novel nonwoven PET fiber structures for vascular scaffolds obtained from the melt blowing process<sup>3</sup> are studied. Their nonwoven structure will allow for mechanical compliance adjustment through fiber diameter and layout as well as porosity control, in contrast to woven Dacron structures. EC and smooth muscle cells (SMC) were seeded in the structures and their growth and attachment observed.

**Materials and Methods:** Nonwoven structures were obtained from melt blowing as reported elsewhere<sup>3</sup> and used as disks. These disks were pre-wetted with cell media or with 0.5% gelatin and seeded ( $10^6$  cells) with human brain EC (HBEC) and aortic SMC (AoSMC). Cells were allowed to grow for 6 days at 37°C in humidified atmosphere. They were then washed twice with warm HBSS, incubated with 10 µg/ml CFDA (fluorescent probes) at 37°C for 45 min and washed again. Fluorescence of cells grown on the structures and on the bottom of the well (after removing the disks) was measured using a cytofluorimeter reader. EC and AoSMC proliferation rates were determined from fluorescence measurements. Mechanical testing of structures was performed to assess compliance, strength (as an indicator of burst pressure) and strain at break. Ribbons of structures (10 mm wide with effective length of 50 mm) were tested in uniaxial tension at a strain rate of 0.2 min<sup>-1</sup>. Results are compared to properties of commercial vascular grafts (Hemashield<sup>TM</sup>, Boston Scientific) obtained in similar conditions.

**Results and Discussion:** SEM of the nonwoven fibrous structure, well below the 20 µm range, is shown in Fig. 1. The fluorescence measured from HBEC and AoSMC proliferation for various periods of time are reported in Fig. 2. The fibrous structure tested allowed attachment and growth of types of cells. While HBEC surprisingly grew with similar efficacy on uncoated and

gelatin-coated structures, AoSMC growth tended to be even significantly higher (20-50%) for the non-coated fibrous structure as compared to the gelatin-coated structure. HBEC tended also to follow fiber alignment.

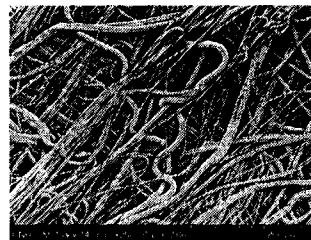


Fig. 1 Planar image of nonwoven PET fiber structure.

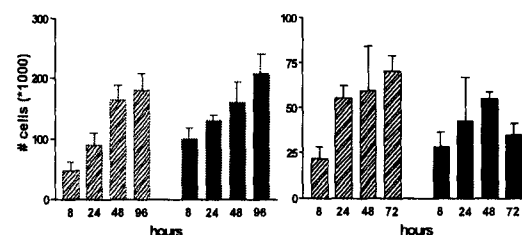


Fig. 2 Proliferation rates of HBEC (left) and AoSMC (right) grown on either uncoated (dashed) or gelatin-coated (full) structure. Bars represent mean of cells  $\pm$  std. dev. of 2 experiments performed in triplicates.

The compliance, strength and strain at break are shown in Table 1. Results are compared with thoracic aorta properties (pig)<sup>4</sup>, corrected for width and length. Results indicate that compliance of the nonwoven structure is within the same order of magnitude as the aorta compliance; while commercial grafts compliance is one order of magnitude lower. The load and strain at break values show a similar trend of too high properties for the commercial graft.

Table 1. Mechanical properties (8 tests, std dev 5%)

| Material                 | Compliance<br>$\times 10^{-3}$ (m/N) | Load at<br>break (N) | Strain at break<br>(mm/mm) |
|--------------------------|--------------------------------------|----------------------|----------------------------|
| Nonwoven                 | 1.1                                  | 8.6                  | 38                         |
| Commercial               | 0.11                                 | 240                  | 117                        |
| Aorta (pig) <sup>4</sup> | 3.5-5.0                              | 2.0-5.0              | $\approx 70$               |

**Conclusions:** Nonwoven PET fibrous structures with tightly controlled porosity and fiber diameter present promising characteristics for vascular graft fabrication such as good EC/SMC attachment and proliferation following fiber alignment, high compliance and deformation range similar to pig aorta values, contrasting with low compliance commercial grafts.

**References:** 1. Heart and Stroke Foundation of Canada Annual Report, 2006. 2. Ghosh J et al. J Vasc Surg 43, 142, 2006. 3. Ajji A, Bureau MN, Moreno M, Robitaille L., Non-Woven Mat and Method of Producing Patent pending. 4. Delgadillo J, Delorme S, Thibault F, DiRaddo R, Hatzikiriakos SG, submitted.