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NONWOVEN PET FIBER STRUCTURES FOR VASCULAR GRAFT APPLICATIONS

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Abstract

Synthetic grafts from woven polyethylene terephthalate fibers or expanded polytetrafluoroethylene have been used for decades to replace diseased arteries. However, their thrombogenicity and low compliance (*vs.* arteries) inevitably lead to their occlusion. A need exists for new synthetic grafts with an anti-thrombogenic surface and high compliance to improve long-term patency. Grafts from nonwoven PET fiber structures obtained from melt blowing are evaluated. Contrary to commercial woven Dacron™, they allow for mechanical compliance adjustment through control of fiber diameter, porosity and graft structure configuration. Their low thrombogenicity, when properly surface-treated using polyethylene glycol functionalization, artery-matching compliance and promising burst pressure are reported.

Introduction

Vascular diseases are the leading mortality cause in our sedentary life-style societies [1]. The use of bypass grafting with autologous veins or arteries [2] is currently the standard procedure for treating patients with atherosclerosis-related vascular diseases for example, but such autologous vessels (*e.g.*, mammary artery or saphenous vein) are unfortunately not always readily available or of sufficient quality, and they only lead to mid-term patency [3]. Artificial vascular grafts are thus being developed to overcome these limitations.

Synthetic vascular grafts made from non-resorbable materials such as expanded polytetrafluoroethylene (ePTFE) porous membranes and polyethylene terephthalate (PET) woven fibers (Dacron™) are currently the gold standard references for large diameter arteries replacement (> 6 mm diameter). On the other hand, for smaller diameter vessel substitution, these grafts have not demonstrated efficacy as their thrombogenicity and dimensionally instability are well documented [4]. Another limitation of their clinical outcome originates from their considerably low mechanical compliance when compared to native arteries. This compliance mismatch has been shown to disturb the blood flow pattern at the anastomosis site (i.e., junction between the graft and the artery) that stimulates neointimal hyperplasia [5].

Bioengineered artificial vascular substitutes have been developed to reproduce the two main arterial layers to ensure their proper behavior: the *intima*, consisting of an EC (endothelial cells) monolayer that confers a thrombo-resistant surface, and the *media*, consisting of SMC (smooth muscle cells) responsible for the vascular contractibility. However, the poor bioactivity (*i.e.*, lack of beneficial cell-material interactions, leading to platelet adhesion) of most of the synthetic biomaterials and the unresolved compliance mismatch have prevented the successful development of such vascular substitutes.

In an attempt to address the compliance and thrombogenic issues, novel nonwoven structures made of PET microfibers obtained from a melt blowing process were studied as vascular substitutes. It is proposed that through precise control of the graft architecture (porosity, pore and fiber size, and configuration of fiber web stacking), the graft compliance and cellular growth can be optimized. This paper investigates the thrombogenicity of PET fiber structures functionalized with polyethylene glycol (PEG) as well as mechanical properties of our novel nonwoven structures for vascular grafts.

Materials and Method

Fabrication of PET vascular grafts

The nonwoven fibers were made of a PET grade with inherent viscosity of 1 (Dupont™). They were produced by melt blowing, which consists in extruding fibers through a 230-hole (300 µm diameter) die into a stream of hot air blown at high speed (close to the speed of sound) resulting in fiber stretching. The resulting fiber webs were then consolidated into the fibrous structures used in this study. Planar structures (2D) were used for thrombogenicity and mechanical testing, whereas tubular structures (3D) were prepared for compliance and burst pressure testing were prepared. They were fabricated by stacking individual fiber layers with alternating orientations (0°/90°) onto a plate or a 6-mm diameter mandrel. The plate was placed into an molding autoclave and the mandrel was inserted in a vacuum bag placed in an oven and under vacuum conditions, both at 100°C and 100 kPa for 20 min for consolidation.

Morphological and mechanical evaluation of grafts

A scanning electron microscope (FEG-SEM, Hitachi S-4700) was used for observation of the fibrous structures. Fiber diameter and pore size range were measured by image analysis. The overall porosity of the structures was typically 90%.

Tensile tests were performed on planar nonwoven specimens using an electromechanical microtester (Instron, model 5548) at a crosshead speed of 5 mm/min on 10 mm wide and 50 mm long specimens. The tensile strength (yield stress), stiffness and elastic modulus of the specimens were measured from the load-displacement curves obtained. The stiffness (S) and modulus (E) were calculated as follows:

$$S = \frac{\Delta F}{\Delta L}; E = \frac{\Delta F \cdot L_o}{w \cdot b \cdot \Delta L} \quad (1)$$

where $\Delta F/\Delta L$ is the slope of the elastic region of the load-displacement curves, and L_o, w and b are respectively the initial length, width and thickness of the specimen.

Compliance was measured on tubular structures on a custom-made tester consisting in a rod-mounted inflatable balloon connected to a pressure-regulated nitrogen gas line. The pressure was monitored through a pressure transducer (AP-34K, Keyence Canada) and diameter was measured using a laser scanner (LS-3100, Keyence Canada). The tubular specimens tested were 5 cm in length and 6.35 mm in diameter. They were submitted to pressure cycles between 0 and 200 mmHg, at an approximate rate of 100 mmHg per sec. Measurements of compliance, C, were made from the pressure-diameter curves according to Equation 2:

$$C = \frac{\Delta D}{D_o \cdot \Delta P} \quad (2)$$

where ΔD refers the variation of diameter when a variation of pressure ΔP is applied to a structure with an initial diameter D_o. Burst pressure measurements were performed on the tubular structures and commercial grafts by increasing the internal pressure at a rate of 100 mmHg per s. Both measurements were performed according to ISO 7198 standard for cardiovascular implants and tubular vascular prostheses.

Grafts functionalization

Reagents of 1,4-diaxone, potassium chloride (KCl), sodium hydroxide (NaOH), methanol, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), N-hydroxy succin-imide (NHS), ethanolamine 99%, hydrochloric acid (HCl), phosphate buffer (PBS) and boric acid were purchased from Sigma Aldrich. Poly(vinylamine)HCl was purchased from Poly-sciences. PEG~COOH was obtained from Rapp Polymere GmbH.

For amino-modification, PET fibers were washed in successive baths of water, methanol and water. Samples were placed in individual vials containing 1 ml of 0.1 M NaOH, 0.1 M KCl buffer, 100 µl of 750 mM poly(vinylamine)HCl solution and 400 µl of 1,4-diaxone, sonicated for 20 min and placed in a water-bath at 70°C for 24 h. Samples were rinsed with water and ethanol and dried with nitrogen gas.

For PEG functionalization, a solution of PEG~COOH in borate buffer (0.2 M, pH 8) was transferred and mixed in tubes containing EDC. This solution was then transferred and mixed in tubes containing NHS. Proportions of PEG~COOH, EDC, NHS and borate buffer are shown in Table 1, as weight to volume ratios. The amino-modified PET fibers were then placed in 295 µl of each final PEG solution After 6 h of reaction, PET fibers were washed with water. 400 µl of 1M ethanolamine solution pH 9.5 in PBS were added on the PET samples. After 30 min of reaction, samples were washed with water and ethanol and dried with nitrogen gas.

Hemocompatibility evaluation of grafts

The interactions between the blood and samples were assayed by a platelet adhesion assay (n = 6). With the approval of the Research Ethics Committee of the Montreal Heart Institute, fresh human blood containing an ACD anticoagulant was centrifuged to obtain platelet-rich plasma (PRP). This PRP was then centrifuged to obtain platelet poor plasma (PPP) and platelet clot. Isolated platelets were suspended in 5 ml buffer containing EDTA and 1 µg/ml of prostacyclin (PGI2) (Sigma, Canada).

For radio-labeling, the platelets were centrifuged, collected and re-suspended in 2 ml of EDTA-PGI2 buffer and then 300 µCi of ^{51}Cr were added in the suspension for 40 min. Radio-labeled platelets were then centrifuged, the supernatant was removed and platelets were suspended again in PPP to obtain PRP. The platelet concentration was adjusted to 250 x 10^6 platelets per ml by adding PPP in PRP. 10-mm disks of PET fiber structures and commercial grafts were place in 24-well plates and 1 ml of PRP was added on each sample. Samples were incubated in radio-labeled PRP for 1 h with gently agitation. The disk samples were washed in saline solution to remove non adherent platelets. Radioactivity was measured in a gamma counter (Minaxi 5000, Packard Instrument). Numerical data were analyzed statistically using independent Student t tests with significance at $p < 0.05$.

For microscopic observations, non-radiolabeled platelets were allowed to adhere as described in the platelet adhesion experiments. Adhered platelets were then examined microscopically. Part of the platelets were fixed with tissue fix solution for 45 min. Samples were washed, placed in mepacrine solution (10 mM) for 90 min

in obscurity and then washed again and observed with a confocal microscope (Zeiss LSM 510). The remaining platelets were fixed with 1% glutaraldehyde for 30 min at room temperature and over night at 4°C. Fixed samples were washed in PBS and dehydrated in ethanol solutions. Samples were dried and the platelets adhered to the tissue surfaces were examined by a scanning electron microscope (SEM; model S-4700, Hitachi, Canada).

Comparison with existing grafts

Two commercial vascular grafts were purchased to provide a comparison baseline for thrombogenicity, compliance and burst pressure results. The first one was made of ePTFE (IMPRA Carboflo®, 6 mm, Bard Peripheral Vascular Inc, USA) and the second one of Dacron™ (Hemashield Platinum, Woven Double Velour, 6 mm, Boston Scientific Medi-Tech, USA).

Results

Nonwoven fiber structures were obtained by stacking individual layers of fibers (webs) with alternating orientations (0°/90°) prior to their 2D or 3D consolidation. SEM images (Fig. 1) were used to determine the fiber diameter and pore size range of the nonwoven structures. Image analysis showed that the 20-layer structure had 80% of its fibers with diameters of 2-6 µm while the remaining 20% of fibers had a diameter range of 6-11 µm. Pore size measurements showed that 90% of pores had a size of 4-16 µm and remaining 10% a size of 16-30 µm. Image analysis was not performed on 10-layer structures.

Tensile testing of the 2D structures was performed for 10-layer and 20-layer structures and in two orientations to evaluate their mechanical properties, *i.e.,* in parallel and at 45° with respect to the longitudinal direction of the structure. Their elastic modulus, stiffness and strength are shown in Figure 2. As expected, this figure indicates that, for the same testing direction, the stiffness is directly related to the number of layers in the structure, and is for the 20-layer structure twice as high as for the 10-layer structure. Their elastic modulus remains unchanged, since the specimen thickness is taken into account in its calculation. The results also show that the elastic modulus is greatly affected by the testing direction, decreasing by 50% when tested at 45° instead of in parallel to the longitudinal direction. Accordingly, the tensile strength was the same for the 10-layer and 20-layer structures but was reduced by 60% when tested at 45° axis instead of in parallel to the longitudinal direction. These results indicate that the specific configuration of fiber web stacking and web orientation has an effect on the mechanical properties of the structures.

The thrombogenicity of the nonwoven fiber 2D structures was assessed by platelet adhesion count. The non-modified PET structure was compared to the PEG grafted structures and the commercial ePTFE and Dacron™ grafts. The results, shown in Figure 3, reveal that platelet adhesion on the nonwoven PET structures was significantly higher than ePTFE, but statistically similar to Dacron™. When PEG was grafted to the nonwoven structures, platelet adhesion steadily decreased with increasing PEG concentrations. For 5 and 7.5% PEG, platelet adhesion was not statistically different from that of non-modified nonwoven structures, despite the decrease noted. However, a significant decrease was noted at 7.5% PEG in comparison with Dacron™. At higher concentrations of 10% and 15% PEG, platelet adhesion was significantly reduced in comparison with Dacron™ and non-modified PET. Indeed, the low level of platelet adhesion after 10 and 15% PEG treatment was statistically similar to the level observed on the low thrombogenic ePTFE surface.

In order to confirm the adhesion results obtained with the radio-labelled platelets and visualise the morphology of the adhered platelets on the different structures, mepacrine dying was performed. As shown in Figure 4, adherent platelets were seen on the fibres of non-modified PET nonwoven structures, whereas 10%PEG grafting resulted in almost complete inhibition of platelet adhesion. The morphology of the adherent platelets was observed by SEM as shown in Figure 5. Adherent platelets were spread onto the surface of non-modified nonwoven fibers, indicating a late stage activation of platelets; whereas the adhered platelets on PEG-grafted fibers were fewer and showed far less spreading than on non-modified fibers. These findings are in agreement with the results of radio-labeled platelet adhesion and the observation made after mepacrine dying

Lastly, the compliance and burst pressure of the nonwoven 3D tubular structures were measured to confirm the main mechanical features of the novel nonwoven structure, its compliance matching characteristic and mechanical reliability. The tubular structures were subjected to 100 0-200 mmHg pressure cycles. The compliance was measured using Eq. 2 for cycle 1, 5, 10, 30, 50 and 100 in the 80-120 mmHg range. Average compliance and standard deviation are reported in Table 2 for the non-modified nonwoven structure ($[0°/90°]_{10}$) and the ePTFE and Dacron™ grafts. Commercial grafts were not tested in 2D due to their very small size.

Interestingly, the compliance values of the nonwoven structures were approximately constant for the 100 cycles imposed. Their radial deformation ranged between 8 and 15% approximately in the 0 to 200 mmHg range. The external diameter remained constant to the fourth digit upon applying a pressure of 200 mmHg for 10 min (data not shown). The compliance of the nonwoven structure was also significantly higher than the compliance of Dacron™ (8 times) and ePTFE (10^3 times)

grafts and was within reported range for human arteries. Finally, the nonwoven structure shows a lower burst pressure than ePTFE and Dacron™ grafts and human arteries. However, it remains of similar magnitude range than the burst pressure values reported for other experimental synthetic grafts [6,7].

Discussion

The ideal vascular grafts for small artery replacement should be non-thrombogenic in nature and mechanically compliant in order to prevent their occlusion and maximise their patency rate. In that goal, novel nonwoven PET fiber structures were fabricated by stacking and consolidating individual webs of aligned fibers into a $[0°/90°]_{20}$ configuration. The fibers were produced by melt blowing, a reasonably cheap, already at the pilot-plant scale and allowing a high control of the structure morphology (fiber size, pore size, porosity). Mechanical testing of the novel nonwoven 2D structures has shown that their elastic modulus and strength can be adjusted through the fiber orientation and number of layers of individual plies. Therefore, using the processes presented above, synthetic vascular grafts can be fabricated with a wide range of well controlled mechanical and morphological properties. Indeed, the nonwoven tubular structures made of PET fibers had a compliance that closely matches the compliance of human arteries and presented burst pressures that, while not as high as those of currently used vascular grafts, are still close to 10 times higher than normal systolic arterial pressure.

However, since PET is thrombogenic in nature [7] as shown by the obtained results, the surface chemistry of the novel nonwoven PET-based structures must be modified in order to present an acceptable level of thrombogenicity and patency rate. Polyethylene glycol (PEG) grafting was thus performed at the surface of the PET fibers to decrease their inherent thrombogenicity. The choice of PEG was motivated by its biocompatibility, its known hydrophilicity and related ability to prevent platelet adhesion. It was introduced on the PET fibers by amino-modification using polyvinyl amine (PVA) and EDC/NHS chemistry. Upon PEG grafting, the thrombogenic response of the nonwoven PET-based structures (radio-labelled platelet adhesion, adhesion of mepacrine stained platelets and change in the platelet morphology by SEM observation, was considerably reduced. In fact, for the nonwoven PET-15%PEG fiber structure, platelet adhesion levels were not significantly different from those of commercial ePTFE grafts, the industry's standard in terms of thrombogenicity.

Conclusions

This study demonstrates that the novel nonwoven structures made of PET fibers and fabricated from multi-layer stacking and consolidation of melt blown fiber webs present structural and morphological properties that can be controlled and tailored through control of the graft architecture to reproduce the mechanical properties of human arteries. The compliance obtained from the structures closely matches that of human arteries and burst pressures are close 10 times higher than normal systolic arterial pressure. However, burst pressure obtained should be increased to levels comparable to commercial grafts, through graft architecture optimization and consolidation conditions.

Using PEG grafting, the thrombogenicity of these nonwoven PET fiber structures can be significantly reduced to levels as low as those of commercial ePTFE grafts that are the current industry standards in terms of thrombogenicity. The effect of the structural and morphological properties of the structures on their thrombogenicity still needs to be addressed. These promising findings pave the way to further development of synthetic vascular grafts based on this novel nonwoven PET fiber structures.

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References

1. Heart Disease and Stroke Statistics-2005 Update. Dallas, Texas: American Heart Association, 2005.
2. Lysaght MJ, Reyes J. *Tissue Eng.*, **7**, 485(2001).
3. Ghosh J, Baguneid M, Khwaja N, Murphy MO, Turner N, Halka A, et al. *J. Vasc. Surg.*, 43, 142 (2006).
4. Tiwari A, Salacinski HJ, Hamilton G, Seifalian AM. Eur *J. Vasc. Endovasc. Surg.*, **21**, 193 (2001).
5. Kakisis JD, Liapis CD, Breuer C, Sumpio BE. *J. Vasc. Surg.*, **41**, 349 (2005).
6. Chen JH, Laiw RF, Jiang SF, Lee YD. *J. Biomed. Mater. Res.* **48**, 235 (1998).
7. Rubin BG, Toursarkissian B, Petrinec D, Yang LY, Eisenberg PR, Abendschein DR. *J. Vasc. Surg.*, **24**, 865 (1996).

Table 1. Respective reagents quantity used

Reagents	PEG~COOH content			
	5%	7.5 %	10 %	15 %
PEG~COOH (mg)	45	67.5	90	135
EDC (mg)	24	36	48	72
NHS (mg)	6	9	12	18
Borate buffer (µL)	900	900	900	900

Table 2. Compliance and burst pressure measured on 6-mm nonwoven structures and commercial 6-mm Dacron™ and ePTFE grafts and reported for human arteries. Number of replicates (n) is indicated.

Materials	Compliance (% $mmHg^{-1}$)	Burst pressure (mmHg)
20-layer $[0°/90°]_{10}$ Nonwoven structure (n=3)	$8.4 \pm 1.0 \times 10^{-2}$	1187 ± 137
Dacron™ (n=2)	$1.1 \pm 0.2 \times 10^{-2}$	4145 ± 207
ePTFE (n=2)	$9.2 \pm 6.0 \times 10^{-5}$	2890 ± 145
Human artery [7,7]	$\approx 8 \times 10^{-2}$	≈ 4000

Fig. 1. SEM image of nonwoven fibrous structure made of 20 layers of fibers with alternating orientations (0°/90°).

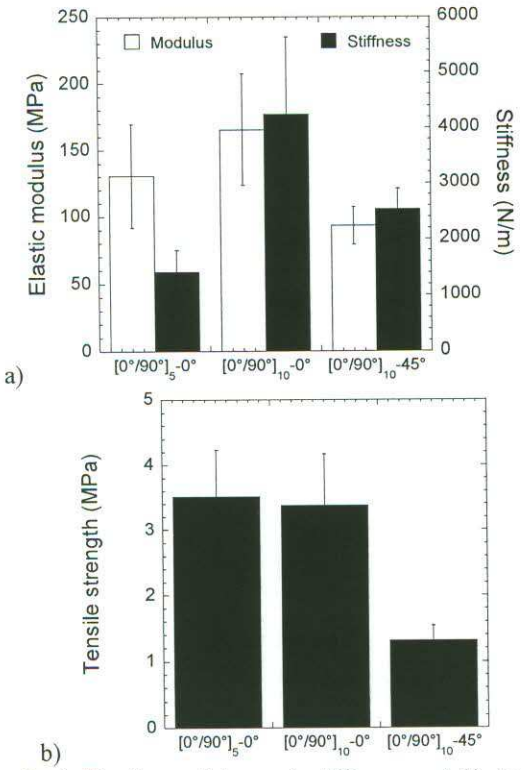

Fig. 2 a) Elastic modulus and stiffness, and b) tensile strength of the nonwoven fiber structures tested in parallel ($[0°/90°]_5$ and $[0°/90°]_{10}$) and at 45° with respect to the longitudinal direction ($[0°/90°]_{10}$ only).

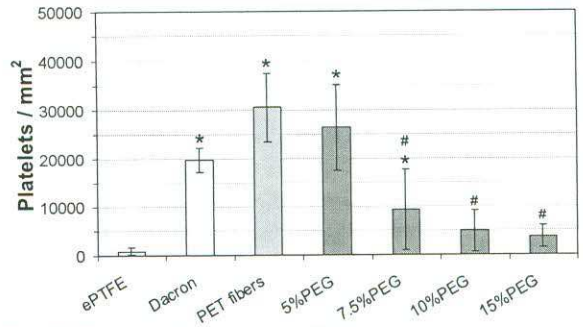

Fig. 3 Platelet adhesion on ePTFE and Dacron™ grafts, used as controls, and on non-modified nonwoven structures (designated as PET fibers) and nonwoven structures with 5%PEG, 7.5%PEG, 10%PEG and 15%PEG. Symbol * indicates significantly different from ePTFE ($p < 0.05$) and # significantly different from Dacron™ ($p < 0.05$).

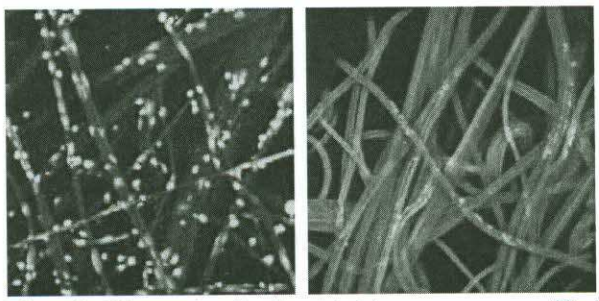

Fig. 4 Meparcrine-dyed platelets on non-modified nonwoven structures (left) and nonwoven fibers functionalized with 10% PEG (right). Fluorescence of PEG-grafted fibers is due to the presence of PEG.

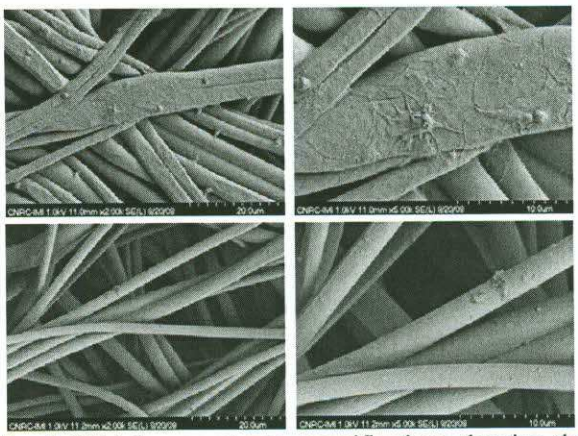

Fig. 5 SEM images at two magnifications showing the morphology of platelet adhesion on the non-modified nonwoven PET fiber structure (top), and nonwoven 15%PEG-grafted PET fiber structure (bottom).

Key words: Vascular grafts, nonwoven PET, thrombogenicity, compliance and burst pressure.