A Nanofibrous Bioactive Vascular Graft for Small Vessel Reconstruction

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Background

There is currently no small diameter graft (< 5mm internal diameter or ID) accepted for vascular reconstruction for patients afflicted with peripheral arterial disease (PAD), which is estimated to affect some 8-10 million people in the United States alone. An "off-the-shelf" small vessel prosthesis which emulates some of the natural biologic processes of normal arterial walls would greatly expand the surgical options in treating PAD. The goal of this study is to develop *in vitro* and *in vivo* a novel nanofibrous bioactive small-diameter (4mm ID) prosthetic vascular graft.

Methods

Electrospinning Methodology: Polyester chips along with a potent anticoagulant (recombinant hirudin or rHir) and growth-promoting agent (vascular endothelial growth factor or VEGF) were dissolved in an organic solvent prior to electrospinning. Nanofibrous polyester tubular constructs (4mm ID) were electrospun using our proprietary electrospinning technology (BioSpun-VG; n=24 grafts). Nanofibrous polyester grafts electrospun without bioactive agents and served as controls. All grafts were sterilized via ethylene oxide (EtO).

Physical Characterization: Non-drug loaded and BioSpun-VG grafts were then evaluated for tensile strength and suture retention using a Q-Test apparatus (n = 4 segments/test group). Control and BioSpun-VG grafts were also examined for water permeation.

Antithrombin Activity: Control and BioSpun-VG grafts (0.5cm X 0.5cm segments) were examined for surface antithrombin activity using a chromogenic assay for thrombin (S-2238). Thrombin concentrations of 2, 5 and 10NIHU were evaluated (n = 6 segments/test group). The assay was started by the addition of 1ml of 100μM S-2238 with the change in absorbance/minute monitored.

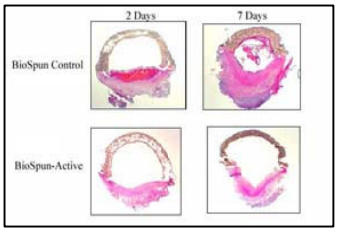
Endothelial Cell Proliferation: EtO-sterilized control and BioSpun-VG grafts were cut into 16mm diameter circular segments (n = 7 segments/test group). Custom-designed seeding chambers housing the respective materials were placed in a 24-well culture plate. Human coronary artery endothelial cells (HCAECs, passage = 6) were grown in T75cm^2 flasks, trypsinized and counted. HCAECs (20,000 cells) were then seeded into each seeding device. After 3 days, materials with cells were rinsed with HBSS, fixed with 100% methanol and cells stained with Hoechst. Photographs of the complete surface of each material (n=4/material) were taken using an Olympus microscope and analyzed in a blinded fashion via Velocity software to determine cell number on each material.

Implantation of BioSpun-VG Grafts: Through a midline incision, the carotid arteries of adult canines (n = 18 animals) were exposed for a length of approximately 10cm. An arteriotomy was performed and an end-to-side anastomosis between the side of the artery and end of a 4mm ID BioSpun-VG graft (6-7cm in length) was performed. A similar anastomosis was carried out on the distal aspect of the carotid artery. On the contralateral carotid artery, a control 4mm PET graft (no sealant) or non-drug loaded electrospun graft was implanted. Implantation periods of 2, 7, 14, 30 and 60 days were assessed.

Results/Discussion

There was no significant change in tensile strength (2.61 ± 0.63 versus 2.64 ± 0.47 kgf/mm^2) or suture retention (1.02 ± 0.14 versus 0.96 ± 0.11 kgf/mm^2) between the non-drug loaded and BioSpun-VG grafts. EtO-sterilization did not adversely affect overall physical properties. Water permeation through BioSpun-VG graft (70 ± 23 ml/min/cm^2) was comparable to the control.

BioSpun-VG grafts had significant antithrombin activity at all thrombin concentrations examined as compared to controls. BioSpun-VG grafts also had a 6.4 fold greater HCAECs within the graft material as compared to non-drug loaded materials.



BioSpun-VG grafts showed no thrombus deposition at the 2 and 7 day implantation periods and were patent (**Figure**). In contrast, the non-drug loaded control grafts showed significant thrombus deposition at 48 hours. All BioSpun-VG grafts were patent at 14 days, whereas 30 and 60 day patency rates were 50% and 0%, respectively, with hyperplasia (and not thrombus formation) being the main contributor of graft failure. Interestingly, BioSpun-VG grafts showed cellular healing which occurred throughout the mid-portion of these grafts with extensive capillary formation throughout the graft wall, a finding that has never been observed in an artificial blood vessel.

Conclusions

A novel nanofibrous bioactive vascular graft, BioSpun-VG, was synthesized and characterized both *in vitro* and *in vivo*. Future studies will focus incorporating an anti-proliferative agent into this platform technology in order to control all aspects of healing, thereby resulting in long-term graft patency.

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