A Nanofibrous Self-Sealing Bioactive Hemodialysis Access Graft

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Statement of Purpose

The use of currently available prosthetic grafts and arteriovenous fistulas utilized for hemodialysis access to treat End-Stage Renal Disease (ESRD) is associated with thrombosis, infection and uncontrolled cellular growth. The goal of this study is to develop a self healing nanofibrous bioactive hemodialysis access graft (BioAccess) via electrospinning technology that will provide early access to dialysis, while avoiding those complications.

Methods

Electrospinning of BioAccess Graft: Polyester and polyurethane chips were dissolved in an organic solvent together with a potent anticoagulant (recombinant hirudin or rHir), antimicrobial (moxifloxacin or Moxi) and anti-proliferative (paclitaxel or Pac) agents. Nanofibrous tubular constructs (6mm ID) were synthesized using our proprietary electrospinning technology (BioAccess; n = 10 grafts). Control grafts were spun without the drugs. All grafts were sterilized via ethylene oxide (EtO).

Material Characterization: The graft surface was analyzed by SEM. The crystallinity of the drugs within the graft and determining the removal of residual solvent were measured using Differential Scanning Calorimetry (DSC) and Thermogravimetic Analysis (TGA), respectively.

Physical Testing: Control, BioAccess and ePTFE (current standard) grafts were evaluated for tensile strength, suture retention and burst strength using a Q-Test apparatus (n = 4 segments/test group).

Permeability and Self Sealing: Control, BioAccess and ePTFE grafts were cut in circular segments (15mm diameter; n = 5 segments/test group). Segments were exposed to distilled water at 120mm Hg pressure and the mass of water (per 60s) that passed through the sample determined. The segments were then punctured using 19.5, 17 and 15 gauge needles to replicate punctures required for dialysis. After each puncture (3/segment), water passage was again determined.

Wash Studies: Control and BioAccess grafts were cut into 5mm length pieces (n = 3 segments/test group/time period) and washed in 5ml of sterile PBS at 37°C in a rugged rotator inversion mixer for 30 days, with segments and wash solutions removed at selected intervals. Unwashed segments served as the t=0 controls.

Antimicrobial Activity: Moxi release from the segments was measured using UV/VIS spectroscopy as well as by spectrofluorometry. Antimicrobial activity of the washed segments was measured using a zone of inhibition assay.

Antithrombin Activity: rHir concentration in the wash solution was measured using Lowry Protein Assay. Washed segments were examined for surface antithrombin activity using a chromogenic assay for thrombin (S-2238) and a whole blood assay.

Anti-Proliferative Activity: Human coronary smooth muscle cells (HCASMCs, passages = 5-7) were seeded into 24-well plates (10,000 cells/well) and incubated for 24 hours. HCASMCs were exposed to wash solutions (100µl) to determine changes in proliferation. Washed segments were placed into SmGM-2 medium for 24 hours, with the medium transferred each day onto the cells to determine Pac activity in the grafts. Alamar Blue (AB) assay was run to determine cell activity.

Results BioAccess grafts were electrospun in 100mm lengths with an average wall thickness of 0.3mm. The grafts had a yellow hue and fluoresce under UV light, which was a direct result of Moxi distribution throughout the fibers (**Figure 1B** left). SEM analysis revealed the fibrous morphology with fiber sizes ranging from 100nm to 5µm. It was evident from the permeability/puncture studies that the BioAccess grafts self-sealed without any visible holes (**Figure 2**). In contrast, the puncture holes were intact in the ePTFE grafts. The BioAccess grafts had comparable suture retention (0.32 ± 0.1 kgF) and burst strength (4.9 ± 1.7 kgF) when compared to ePTFE grafts (0.5 ± 0.11 and 6.2 ± 1.4 kgF, respectively; p = 0.1). Elastic strain of the BioAccess grafts was 2.12mm/mm, which is approximately 7-fold greater than that of ePTFE grafts (0.31mm/mm). BioAccess grafts have a lower elastic modulus (3.2 ± 0.9 kgF/mm^2) as compared to ePTFE (5.3 ± 1.3 kgF/mm^2). Wash studies revealed a consistent release and activity of all three active agents over the time of 30 day. Additionally, BioAccess grafts had significant bioactive agents remaining within the graft at all time periods assessed (Zone of Inhibition > 10mm and antithrombin activity > 0.80 NIHU through 30days)

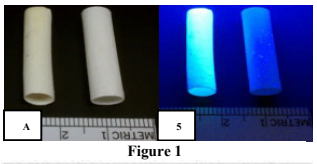

Figure 1

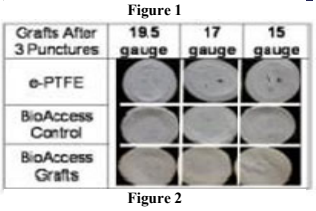

Figure 2

Conclusions

The BioAccess graft possesses excellent strength and unique self-sealing properties making it a candidate for immediate hemodialysis access. Additionally, anti-thrombin, antimicrobial and anti-proliferative properties are present after extensive washing. Future studies will evaluate this novel graft *in vivo* using a canine arteriovenous grafting model.

Acknowledgments

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