Surface Modification of a Poly(Glycolic-co-Lactic Acid) - Poly(DL-Lactide-co-Caprolactone) Small-Diameter Vascular Graft via CD34 Antibody Immobilization to Enhance Cell Attachment

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Statement of Purpose: Endothelialization is crucial to small-diameter vascular graft success, as the development of a healthy endothelium regulates hemostasis, decreasing the risk of thrombogenicity and intimal hyperplasia.[1] While endothelial cells (ECs) are natural targets for tissue engineered vascular grafts (TEVGs), endothelial progenitor cells (EPCs) have also been targeted for their endothelial differentiation, regenerative, and repair capabilities.[2] CD34 is expressed on the surface of circulating EPCs, making it a viable target for antibody capture onto a vascular graft to promote endothelialization.[3] Thus, such an antibody was covalently linked to a biodegradable TEVG in a new, modified graft system to enhance EPC attachment.

Methods: Graft fabrication consisted of rolling 5x7 mm woven poly(glycolic-co-lactic acid) felt (PGLA) (Biomedical Structures, Warwick, RI), followed by saturation with a poly(DL-lactide-co-caprolactone) (50:50 ratio) (PLACL) solution. Grafts were frozen and freeze-dried. Graft dimensions were found via scanning electron microscopy. Antibody immobilization followed a procedure similar to a previous method of RGD immobilization.[4] Grafts were aminolyzed by immersion in a 1,6-hexanediamine solution. A quantitiative ninhydrin assay was performed to determine available NH_2 groups. Grafts were then immersed in a sulfosuccinimidyl-4(N-maleimidomethyl)cylcohexane-1-carboxylate (sulfo-SMCC) solution to provide a linking arm for the antibody, followed by incubation in a solution of primary goat polyclonal IgG CD34 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). For cell studies, human umbilical vein endothelial cells (HUVECs) and endothelial progenitor cells (EPCS) were cultured. Anti-goat IgG antibodies with FITC were used to confirm CD34 antibody immobilization via fluorescence microscopy and fluorescence intensities obtained by spectroscopy. Grafts were cut to fit a 96-well tissue culture plate and sterilized. Both antibody-coated and uncoated control grafts were used in separate HUVEC and EPC cultures. Cells were seeded at a concentration of 5×10^3 cells/well. After 4 hrs, 1 day, 3 days, and 7 days, a Live/Dead assay was performed to assess cell attachment and proliferation via microscopy.

Results: Grafts of length 7.0 mm, wall thickness 0.3 mm and inner lumen diameter 1.0 mm were fabricated. Quantitative ninhydrin assay results yielded an available NH_2 concentration at 5.85×10^{-6} moles/mm^3 of approximated graft volume. Fluorescent intensity of control and antibody-coated grafts was measured before and after incubation with anti-goat IgG FITC. While there was a -0.01 ± 0.18 fold change for control grafts, antibody-coated grafts showed a fluorescent intensity change of 1.99 ± 0.36 fold change after incubation with the anti-goat IgG FITC, demonstrating CD34 antibody immobilization. Cell culture studies demonstrated initial EC attachment rates of 5.77 ± 0.39 % for uncoated grafts and 13.59 ± 3.23 % for antibody-coated grafts and EPC attachment rates of 3.72 ± 1.86 % and 13.19 ± 4.21 %, respectively. Cell proliferation is demonstrated in Figure 1 for HUVECs and Figure 2 for EPCs.

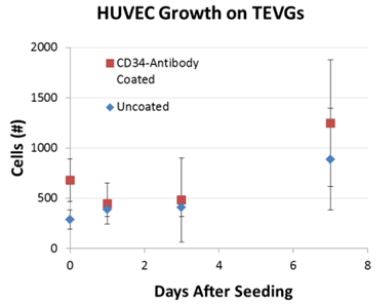

Figure 1: HUVECs adhered to CD34 antibody coated and uncoated grafts.

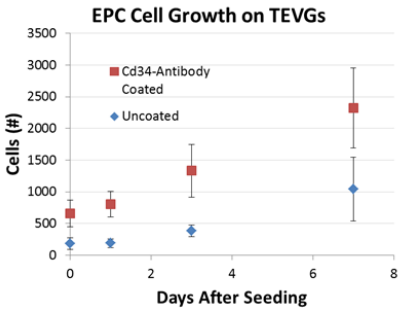

Figure 1: EPCs adhered to CD34 antibody coated and uncoated grafts.

Conclusions: A successful technique to covalently immobilize CD34 antibodies and enhance cell attachment to a biodegradable PGLA-PCLLA graft has been demonstrated. Qualitative visualization of NH_2 groups on the graft indicated widespread aminolysis, crucial for subsequent antibody immobilization via the sulfo-SMCC linker arm. The sulf-SMCC linker is thought to interact with the disulfide bonds of the CD34 antibody, maximizing availability of antigen-binding sites. While the coated grafts appeared to experience increased initial HUVEC and EPC attachment, more modest differences in EPC proliferation rates between uncoated and coated grafts were observed. Thus, immobilized CD34 antibodies appear to enhance initial cell attachment to the TEVG and exert only modest influence over attached cell proliferation. Ongoing work includes implantation in a mouse model to determine significant *in vivo* effects on endothelialization of the graft.

References: 1) Rubanyi GM. *Cardio Pharma.* 1993:22:S1-S14. **2)** Rafii, S, Lyden D. *Nature Med.* 2003:9:6:702-712. **3)** Avci-Adali M. *Biotec.* 2010: 28:119-129. **4)** Zhang H. *Biomat.* 2009:30:4063-40069.