Targeting and differentiating endothelial progenitor cells via surface engineering

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Statement of Purpose: Approximately 500,000 coronary artery bypass (CAB) graft procedures are performed annually in the United States. To overcome these challenges, we propose to investigate whether a synthetic vascular graft biomaterial coating can be used for promoting homing, attachment and differentiation of circulating EPCs. The vascular graft is based on a modified ePTFE graft using a coating of the copolymer, poly(1,8-octanediol-co-citrate) (POC) to allow for electrostatic binding of stromal cell derived factor-1α (SDF-1). SDF-1 is a potent chemotactic factor for EPCs *[1, 2]*. We aim to modify ePTFE with POC and SDF-1 such that it has the potential to recruit and differentiate circulating EPCs *in vivo* through surface engineering and immobilization of SDF-1.

Methods: The synthesis and characterization of the biomaterial, POC, has been previously studied *[3]*. SDF-1 was immobilized to POC for two hours at room temperature with or without human fibronectin (FN) followed by multiple rinses in PBS. Immobilized SDF-1 was quantified by ELISA. Early EPCs were isolated as described previously *[4]*. Briefly, whole blood is drawn from healthy human donors and peripheral blood mononuclear cells (PBMNCs) isolated by centrifugation using histopaque density gradient. PBMNCs are seeded at 5×10^5 cells/cm^2 in 6 well plates coated with fibronectin (5μg/cm^2) in endothelial culture media supplemented with 20% FBS. Non-adherent cells were removed at day 4 by complete media change and cells were used for experimentation between days 7-10. For platelet attachment and activation assays, platelet rich plasma was obtained from whole blood as described previously *[5]*.

Results: The SDF-1 immobilization efficiency to POC was relatively high (greater than 80%) with a surface density of approximately 50 ng/cm^2 (data not shown). The SDF-1 receptor, CXCR4, showed a high level of expression on isolated EPCs (Figure 1 A and B). For all POC surfaces, there was no significant difference in platelet attachment ($p > 0.05$). The concentration of soluble P-selectin, an indicator of activated platelets was also quantified by ELISA and showed no significant change for the modified POC surfaces. Preliminary static adhesion experiments also evaluated the ability for immobilized SDF-1 to promote EPC attachment (Figure 1D). EPCs blocked with CXCR4 blocking antibody had significantly reduced adhesion compared with cells incubated with isotype control antibody. POC surfaces with immobilized SDF-1 or co-immobilized with fibronectin demonstrated CXCR4 receptor mediated cell attachment.

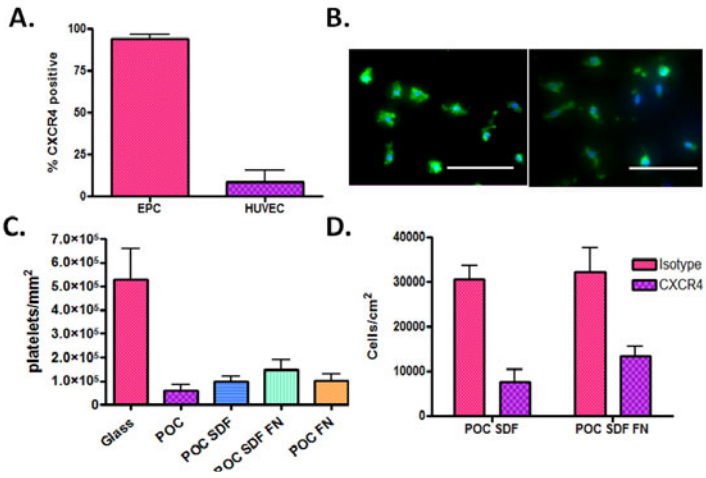

Figure 1. A) CXCR4 receptor flow cytometry analysis for EPCs (average of 3 donors) and HUVECs, B) EPC immunofluorescence characterization for CXCR4 receptor (left panel) and VE-Cadherin (right panel) (nuclei: blue, positive stain: green, scale bar: 50 microns) C) Platelet attachment D) EPC attachment to POC SDF and POC SDF FN surfaces blocked with CXCR4 function blocking antibody or isotype control. (FN = Fibronectin)

Conclusions: We have demonstrated a simple and effective way to significantly alter cell/biomaterial interactions of POC, by immobilization of SDF-1 to improve EPC attachment. Platelet attachment and activation on POC was not significantly altered by the protein modification procedures, which is important for vascular graft applications. The POC SDF material was also able to promote EPC attachment in a CXCR4 receptor / SDF-1 specific manner as demonstrated by antibody binding blocking experiments. Future experiment will focus on EPC attachment under fluid shear stress *in vitro* and an *in vivo* porcine bypass graft animal model using POC coated ePTFE vascular grafts.

References:

[1]K. Stellos, H. Langer, K. Daub, T. Schoenberger, A. Gauss, T. Geisler, B. Bigalke, I. Mueller, M. Schumm, I. Schaefer, P. Seizer, B. F. Kraemer, D. Siegel-Axel, A. E. May, S. Lindemann, M. Gawaz, Circulation 2008, 117, 206.
[2]R. Sadir, F. Baleux, A. Grosdidier, A. Imberty, H. Lortat-Jacob, J Biol Chem 2001, 276, 8288.
[3]J. Yang, A. R. Webb, S. J. Pickerill, G. Hageman, G. A. Ameer, Biomaterials 2006, 27, 1889.
[4]K. K. Hirschi, D. A. Ingram, M. C. Yoder, Arterioscler Thromb Vasc Biol 2008, 28, 1584.
[5]D. Motlagh, J. Allen, R. Hoshi, J. Yang, K. Lui, G. Ameer, J Biomed Mater Res A 2007, 82, 907.