Development of Tissue Engineered Small Diameter Vascular Grafts Employing Autologous Progenitor Cells

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Statement of Purpose: Existing endothelialization strategies designed to improve blood compatibility of small diameter vascular grafts (SDVG) often face obstacles like dearth of suitable graft materials, lack of reliable cell sources, prolonged and tedious cell culture procedure. To address this, a novel strategy was developed to generate in vivo endothelialized SDVG by triggering localized homing of endothelial progenitor cells (EPC) into inner lumen of SDVG without complex *in vitro* technique.

Methods: Crosslinked urethane doped polyester (CUPE) was used to fabricate SDVGs as in our earlier publications.[1,2] The physical and mechanical characterization of the grafts was done as earlier. The inner lumen of such grafts were coated with various cytokines like erythropoietin (Epo), stromal derived factor-1α (SDF-1α) and their combinations based on our pilot studies that have found these cytokines to be potent recruiters of various progenitor cells, including endothelial progenitor cells (EPCs). Such cytokine-loaded SDVGs were implanted in the peritoneal space for various time points (1 and 2 weeks). At the end of each time point, these grafts were histologically analyzed for the presence of EPCs (CD34+CD133+), endothelial cells (CD31+) and inflammatory cells (CD11b+CD45+).

Results: Biphasic CUPE SDVG had a porous outer and non-porous inner phase (Fig 1A). These grafts have excellent physical and mechanical characteristics (tunable burst pressure 1500-2602 mm Hg; suture retention values - 2.45±0.23 N). The inner lumen of SDVG was loaded with various cytokines like Epo and Epo+SDF-1α that were released for up to 2 weeks. By implanting these in mice peritonea, we found that within 1 week, CD34+ CD133+ EPCs were found in greater numbers in the luminal space of SDF-1alpha+Epo coated grafts followed by Epo coated grafts, with negligible number of cells in the control (Fig 1B). This was almost 2X the number of cells in the Epo group and more than 5X that in control. In fact at the end of 2 weeks, we saw a large number of CD31+ endothelial cells in both the cytokine loaded groups (Fig 1C). Interestingly, the combination of SDF-1α + Epo led to almost 3X more endothelial cells in the lumen as compared to Epo loaded grafts and more than 9X as compared to untreated controls. The presence of almost 4X more E-cadherin, an endothelial cell adhesion molecule, in SDF-1 α +Epo group as compared to control indicates the suitability of this time point and the combination of chemokines for *in situ* endothelialization (Fig 1D).

Conclusions: Our studies have led to the development of a graft material that has excellent physical and mechanical properties that closely resemble the natural vasculature.

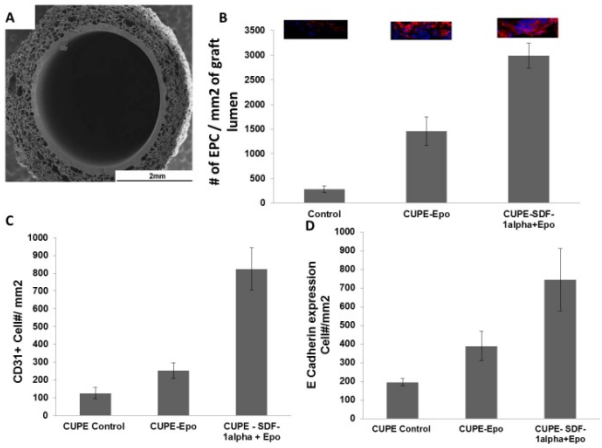

Fig 1. Cross-sectional view of CUPE SDVG (A). CD34+CD133+ endothelial progenitor cells at 1 week (B). CUPE vascular grafts coated with Epo or SDF-1alpha+Epo were implanted in the peritonea. CD31+ endothelial cells (C) and E-Cadherin expression (D) was assessed and quantified at the end of 2 weeks.

By incorporating EPC recruiting and differentiating cytokines, we have shown that the lumen of the SDVGs can be endothelialized by selective recruitment of EPCs and their differentiation into endothelial cells. In future, specific cytokines capable of recruiting EPCs as well as facilitating smooth muscle cell proliferation can be incorporated in the SDVGs to develop a fully functional, transplantable SDVG that is developed using the patient's own cells.

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References:

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