Enhanced Vascular Graft Thromboresistance with Endothelial Progenitor Cells Overexpressing Thrombomodulin

John D Stroncek, Jeffrey H. Lawson, George A. Truskey, and William M. Reichert (reichert@duke.edu)
Duke University, Durham, NC, United States

Introduction
Endothelial cell (EC) sodding of the luminal surface of small diameter synthetic vascular grafts is acknowledged to improve graft patency. However, ECs are known to down regulate their expression of key anti-thrombotic and anti-inflammatory molecules immediately after implantation [1]. This study investigated the potential of augmenting EC expression of thrombomodulin (TM), a glycoprotein expressed on the surface of endothelial cells (ECs) that has potent anti-coagulant properties. Endothelial progenitor cells (EPCs) were isolated from patients with coronary artery disease (CAD). Transfected EPCs were sodded onto grafts and assessed for their functional ability to produce activated protein C (APC).

Materials and Methods

EPC Isolation and transfection: Late outgrowth EPCs from patients with CAD were isolated and characterized as previously described [2]. Replication deficient adenoviral human TM (AdTM) was gift from Dawn Bowles; adenoviral β-galactosidase (AdCV) was purchased (Eton Biosciences). Cells were transfected for 4 hr at a multiplicity of infection of 100. Graft sodding: 1mm ID ePTFE grafts (Atrium Medical) were coated with fibronectin and seeded with EPCs from CAD patients (2x10⁶ CAD EPCs per cm²). The following day, cells were transfected with AdCV, stained with X-gal, and imaged. Activated Protein C (APC): 24-well plates were seeded with EPCs and transfected. 10 ng/mL IL-1β or TNF-α was added to media 4 hr prior to APC measurement. Ability to generate APC was measured as previously described [3]. Statistical analysis was carried out using ANOVA; P values <0.05 were considered to indicate a statistically significant difference.

Results
EPCs isolated from patients with CAD were seeded onto the inner lumen of an ePTFE graft (Fig.1A). EPCs successfully transfected with AdCV stained blue (Fig.1B). Transfection of EPCs with AdTM increased the rate of APC formation 4.75-fold over native cells (p<0.05). Ad-CV EPCs pre-stimulated with IL-1β or TNF-α had approximately 30% lower TM expression EPCs versus unsimulated cells (p<0.05).

Discussion and Conclusions
Here we show EPCs are capable TM over-expression leading to increased functional production of APC. Endothelialized vascular grafts with augmented TM expression may have enhanced anti-coagulant properties while avoiding the risks associated with systemic administration of anti-coagulants.

References

Acknowledgments
This work was supported by NIH R01HL-44972.

Disclosures
No competing financial interests exist.