# Enhanced Material Thromboresistance with Endothelial Progenitor Cells Overexpressing Thrombomodulin

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**Statement of Purpose:** Thrombus and platelet deposition is a significant problem for synthetic vascular materials, particularly small diameter (<6mm) synthetic vascular grafts arising from a thrombogenic graft surface. Endothelial cell (EC) sodding of the luminal surface of synthetic vascular grafts is known 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 and anti-inflammatory properties. Endothelial progenitor cells (EPCs) were isolated from patients with coronary artery disease. Experimental groups included native EPCs, EPCs transfected via an adenoviral vector containing TM (AdTM), and EPCs transfected via a control adenoviral vector containing β-galactosidase (AdCV). Transfected cells were assessed for their functional ability to produce activated protein C (APC) and inhibit neutrophil adhesion.

**Methods:** EPC Isolation and transfection: Late outgrowth EPCs from CAD patients were isolated and grown as previously described [2]. Replication deficient adenoviral TM was gift from Dawn Bowles; adenoviral β-galactosidase was purchased from Eton Biosciences. Cells were transfected for 4 hr at a multiplicity of infection of 100 and all experiments were performed the following day.

APC production: 24-well plates were seeded with EPCs and transfected. 5nM thrombin (Haematologic Technologies Inc., HTI) was added to a confluent monolayer of EC for 5 minutes. 400nM human protein C (HTI) was added and duplicate 50μL supernatant aliquots were removed at 1, 2, 5, 10, 15, 20, 25, 30, and 40 minutes. Free thrombin was inhibited with equal volumes of TM stop solution [5U/mL hirudin (Calbiochem), 1μM human antithrombin III (HTI), and 5U/mL porcine heparin (Sigma) in HBSS]. The APC formed was quantified with the addition of 100mL/well of 400mM Spectrozyme PCa (American Diagnostica) in a microtiter plate reader (BioTek Instruments) by the change in absorbance with time at 405nm.

Neutrophil adhesion: HL-60 cells were stained with Cell Tracker Orange (Invitrogen). Slideflasks (Nunc) were seeded with transfected EPCs and incubated for 4 hr with EPC media or EPC media with IL-1β (10 μg/mL). Slides were placed in a parallel plate flow chamber and HL-60 cells ($2x10^6$ cells/mL) were infused over the EPCs for 5 minutes at a shear rate of $55s^{-1}$ and shear stress of 0.5 dynes/$cm^2$ using a syringe pump (Harvard Apparatus). The slide was then washed by infusing DPBS for 5 minutes at a shear rate of 110 $s^{-1}$ and the slide was fixed with 3.7% paraformaldehyde. A series of 12 images per slide were captured on a fluorescent microscope (Nikon, TE2000U) and the number of adherent HL-60 cells were quantified.

**Results:** The APC assay measures the rate protein C is cleaved to form APC when thrombomodulin complexes with thrombin. Native EPCs expressed lower levels of TM than a control population of HAECs (Figure 1). Transfection of EPCs with AdTM increased the rate of APC formation over 3-fold ($p<0.05$). EPCs with AdCV similar levels of APC production to native cells.

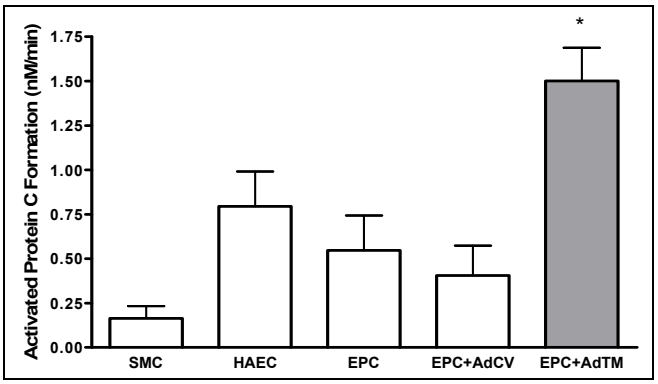


**Figure 1.** APC production for smooth muscle cells (SMCs), human aortic ECs (HAECs), native EPCs, EPCs transfected with AdCV, and EPCs transfeced with AdTM.

While TM is best known for its role in the anticoagulant pathway, TM also has anti-inflammatory properties. When cells were pre-stimulated with IL-1β, EPCs transfected with TM had 35% fewer neutrophils per $cm^2$ ($p<0.05$) (Figure 2). Without IL-1β stimulation, few HL-60 cells were able to adhere to EPCs with AdTM or EPCs with AdCV.

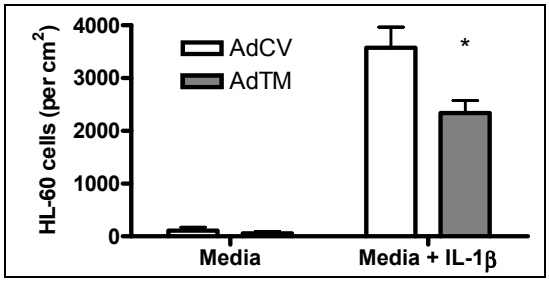


**Figure 2.** Neutrophil adhesion to unstimulated and IL-1β stimulated EPCs.

**Conclusions:** Here we show that EPCs are capable of overexpressing TM leading to increased production of APC and reduced neutrophil adhesion to stimulated EPCs. Synthetic vascular grafts and other endothelialized materials with augmented TM expression may have enhanced anti-coagulant and anti-inflammatory properties while avoiding the risk of systemic administration of anti-coagulant or anti-inflammatory molecules.

**References:**

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2. Broxmeyer HE. Methods in Enzymol. 2006;419:439-73.