Collagen Increases Surface Tissue Factor Activity in Baboon Endothelial Progenitor Cells

Brandon D. Markway, Stephen R. Hanson, and Monica T. Hinds
Oregon Health & Science University

Statement of Purpose: Recently, there has been a growing interest in utilizing endothelial progenitor cells (EPC) as a lumenal surface on vascular devices.[1,2] Mature endothelial cells (EC) normally exist in a quiescent state *in vivo* with very little tissue factor (TF) activity on the cell surface, thus maintaining a non-thrombogenic substrate for blood.[3] However, the extracellular matrix (ECM) proteins on which cells adhere interact dynamically with the cells and have important functions in cell signaling. In this study, we isolated endothelial progenitor cells from baboon peripheral blood (BaEPC) and compared the effect of substrate on late passage BaEPC surface TF activity. We investigated the effects of Type I collagen, α-elastin, and fibronectin as substrates adsorbed at high, intermediate, and low concentrations.

Methods: Peripheral blood mononuclear cells (PBMC) were isolated from baboon blood by centrifugation over Histopaque-1077 (Sigma-Aldrich) density gradient and maintained in endothelial cell basal medium-2 (EBM-2; Cambrex Bio Science) supplemented with EGM-2 SingleQuots® (Cambrex) and 20% FBS (HyClone). All cells were grown at 37°C in a humidified incubator with 5% carbon dioxide and expanded upon endothelial progenitor cell outgrowth.

Tissue factor is the primary initiator of the extrinsic coagulation cascade through binding of activated factor VII (FVIIa) leading to activation of factor IX and factor X (FX). Thus, a measurement of the amount of activated FX (FXa) released by cells can be used to measure the activity of TF on the cell surface. FXa was measured with Spectrozyme® FXa (American Diagnostica), a chromogenic substrate cleaved by FXa generated in its presence. Type I rat tail collagen (BD Bioscience), α-elastin (isolated from porcine aortic elastin), and fibronectin (Sigma-Aldrich) were allowed to adsorb for one hour onto wells of a 96-well plate at concentrations of 50 μg/ml, 1 μg/ml, and 0.001 μg/ml. BaEPC (passage 7 to 9) were plated on each substrate at cell concentrations of 15,000 cells/well, grown for 24 hours in EGM-2 (20% FBS) and then quiesced for 24 hours in EBM-2 (0.5% FBS). To measure FXa, cells were rinsed with Hanks buffer (HBSS) prior to adding 20 nM FVIIa (Novo Nordisk) and 200 nM bovine FX (Enzyme Research Laboratories) in Hanks buffer and incubating at 37°C for 15 minutes. With mixing, samples were taken from each well and following addition of 0.667 mM Spectrozyme® FXa, kinematic absorbance of the samples was read at 405 nm for 20 minutes at 37°C on a Versamax microplate reader (Molecular Devices). Standard curves were prepared using serial dilutions of bovine FXa (American Diagnostica) in the 96-well plate, adding Spectrozyme® FXa, and reading with cell samples.

Results / Discussion: In the current study, we evaluated the effects of type I collagen, α-elastin, and fibronectin on surface TF activity of BaEPC at three adsorbed concentrations. Previous studies have shown that the amount of ECM protein that is adsorbed may play an important role in the phenotype of adhered cells. Ito and colleagues report that α-elastin inhibits EC proliferation when present in high concentrations (10 mg/mL) but increases proliferation at lower concentrations (0.1 mg/mL).[4,5] While ECs normally exist in quiescent state *in vivo*, they can be induced into a state of increased proliferation and migration during injury, accompanied by several differences in gene expression including increased TF; *in vitro* ECs are in a phenotype more similar to the wound healing state.[6] For this reason and the increasing interest in EPCs as a source of autologous cells for vascular device coating, we sought to determine how the thrombogenic potential of BaEPCs is modulated by the substrate to which they adhere. For a given coating concentration, the amount of FXa released by BaEPCs was significantly higher on collagen than on elastin and fibronectin at all concentrations (Figure 1). Additionally, adsorbing 50 μg/ml of collagen or fibronectin resulted in significantly higher FXa production compared to 0.001 μg/ml of the same protein. FXa release was not significantly greater for BaEPC on fibronectin than elastin at any concentration.

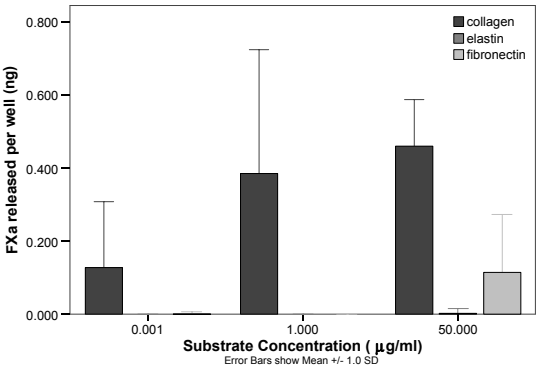

Figure 1. FXa released per well for baboon endothelial progenitor cells adhered to collagen, elastin, and fibronectin adsorbed at 0.001, 1, and 50 ug/ml.

Conclusions: In this study, we investigated the effect of substrate type and amount on surface tissue factor activity of BaEPCs isolated from peripheral blood. The results indicate that collagen and fibronectin may increase surface TF expression in BaEPCs in a concentration-dependent manner and that at equivalent ECM protein concentrations, surface activity is significantly greater for BaEPCs adhered to collagen than to elastin and fibronectin. Furthermore, BaEPCs grown on elastin (all concentrations) and fibronectin (less than 50 μg/ml) had background level expression of FXa.

References:

1. Rotmans JI. Circulation. 2005;112:12-18.
2. Kaushal S. Nature Medicine. 2001;7(9):1035-1040.
3. Grignani G. Haematologica. 2000;85:967-972.
4. Ito S. Cardiovasc Surg. 1997;5(2):176-183.
5. Ito S. Angiology. 1998;49(4):289-297.
6. Beck, Jr. LH. Differentiation. 2004;72:162-170.