Direct Co-Culture of Endothelial Progenitor Cells with Human Mesenchymal Stem Cells on Nanopatterned Surfaces

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Statement of Purpose: Traditional treatment of Coronary Artery Disease (CAD) involves an autograft of the internal mammary artery or saphenous vein, requiring multiple surgeries and posing an additional risk for the patient. Synthetic vascular grafts are a viable alternative, although grafts less than 5 mm in diameter have been shown to exhibit increased thrombogenicity and late intimal hyperplasia. Tissue engineered blood vessels (TEBVs) may provide a more biocompatible alternative to synthetic small-diameter vascular grafts by culturing a patient's own cell supply in vitro and seeding them onto synthetic or tissue-engineered scaffolds. The development of a TEBV comprised entirely of autologous cells would be ideal for reduction of immune response against synthetic materials. Ultimately, a TEBV should match the mechanical and antithrombogenic properties of a native vessel.

Human bone marrow-derived mesenchymal stem cells (hMSCs) are pluripotent and express potential to be used as a source of smooth muscle cells (SMCs) to recreate a vascular wall due to their ability to differentiate into multiple cell lineages through environmental cues[1]. Furthermore, hMSCs have been shown to elicit minimal immunological response, providing potential for use in allogeneic transplants[2]. Seeding endothelial progenitor cells inside the lumen of a TEBV comprised of hMSCs as depicted in Figure 1 should reduce thrombosis. Direct co-cultures of hMSCs with endothelial cells (ECs) have shown increased SMC differentiation in hMSCs, indicated by the expression of SMC markers such as smooth muscle α-actin, smooth muscle 22α, and smoothelin[3]. Finally, nanopatterned substrates have been shown to increase the organization of collagen on the surface of hMSCs[4], which is desirable for enhancing the mechanical strength of the blood vessel. This study compares the effects of direct co-culture of hMSCs with EPCs on nanopatterned and smooth poly(dimethylsiloxane) (PDMS) substrates. Co-culturing hMSCs with EPCs on nanopatterned substrates should increase differentiation of the hMSCs to the SMC phenotype, which would in turn provide greater mechanical strength to the TEBV.

Figure 1: EPCs Seeded Inside TEBV Comprised of Aligned hMSCs.

Methods: Nanopatterned PDMS substrates will be made using a previously studied nanopattern generated with electron beam lithography and replicated on PDMS with soft lithography. The patterned PDMS surfaces will have gratings measuring 250 nm in depth, 350 nm in width, and 700 nm in pitch[4]. Smooth PDMS substrates will be used as controls. PDMS surfaces will be coated with bovine collagen I. Stained hMSCs will be seeded onto the surface of the PDMS substrates at a low cell density and allowed to become confluent on the surfaces of the PDMS, after which EPCs will be seeded onto the surface of the hMSCs at a confluent density. Immunofluorescent staining and Western blotting will be used to evaluate cell alignment and the expression and morphology of ECM proteins fibronectin, elastin, and collagen I. The differentiation of the hMSCs into the SMC phenotype will be evaluated using Western blotting and real time RT-PCR to determine the expression of SMC markers calponin and smooth muscle α-actin.

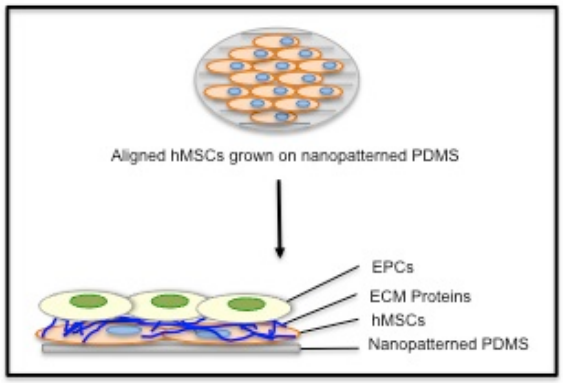

Figure 2. Seeding of EPCs on Aligned hMSCs Grown on Nanopatterned PDMS

Results: Previous studies have shown that the growth of hMSCs on a nanopatterned substrate increases cell alignment and greater organization of ECM proteins fibronectin and collagen I[4]. Co-culture of hMSCs with EPCs should exhibit greater production of ECM proteins collagen and elastin. Nanopatterned substrates should exhibit greater organization of the collagen, elastin, and fibronectin than smooth PDMS substrates. Previous direct co-culture studies of hMSCs with ECs showed increased expression of the SMC phenotype[3]. Co-culture of hMSCs with EPCs should cause increased expression of calponin and smooth muscle α-actin.

Conclusions: Co-culturing hMSCs with EPCs should increase differentiation of the hMSCs to the SMC phenotype. The use of nanopatterned substrates should increase the organization and production of the ECM proteins. Increasing the amount of ECM proteins produced in a TEBV created from aligned hMSCs should promote mechanical properties similar to those of a native vessel.

References: 1. Jiang Y. Nature 2002;418:41-49. 2. Ren G. Cell Stem Cell 2008;2:141-150. 3. Lozito T.P. J Cell Biochem. 2009;107:714-722 4. Zhao F. Mol Ther. 2010;18:1010-1018.