Engineering the Vascular Niche:
Development of a Basement Membrane Model for Studying Megakaryopoiesis In Vitro

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Introduction
Thrombocytopenia occurs when a patient suffers from an abnormally low platelet count in the peripheral blood; usually a result of disease, trauma, or cancer treatment. To treat these patients, it is estimated that two million platelet transfusions are performed in the U.S. each year. This high demand for platelets has created a clinical demand for studying the causes of thrombocytopenia and alternative routes for treatment. Platelets are anuclear cells that are released into the bloodstream in the bone marrow by megakaryocytes (MKs) via the extension of long filaments called proplatelets. It is hypothesized that the vascular niche in the bone marrow, namely the basement membrane and vascular endothelium, plays a pivotal role in directing megakaryopoiesis. The goal of this project was to create a bone marrow basement membrane model that mimics the vascular niche for functional in vitro platelet production. We hypothesized that a silk film porous membrane with tunable material properties that resembles the physiologic basement membrane would support physiologic megakaryopoiesis.

Materials and Methods
Porous silk films (SFs) were prepared as previously reported [1]. Human MKs and endothelial cells (ECs) were isolated from newborn cord blood. MK attachment and proplatelet formation (PPF) were counted with fluorescent microscopy, staining for α-tubulin and CD61. ECs were stained with CD144. Platelet count was determined using flow cytometry and TruCount™ beads (BD Biosciences). Platelets were activated with 3 U/mL thrombin and analyzed for PAC1 binding. Results were statistically analyzed using ANOVA. Statistical significance was reported if p<0.05.

Results
Porous SFs supported 75% MK attachment and 50% PPF compared to glass coverslip controls. MK attachment and PPF on SFs was improved by altering the SF properties. SFs functionalized by entrapping proteins (e.g. fibrinogen, collagen IV, fibronectin, and laminin) within the film resulted in >100% MK attachment and PPF compared to unfunctionalized SF controls. SF surface roughness improved MK attachment compared to the control but did not affect PPF. Decreasing the SF stiffness improved PPF but did not significantly affect MK attachment. Co-culture with endothelial cells improved MK attachment while maintaining a high level of PPF. Additionally, MK and EC co-culture on the SF basement membrane model resulted in an approximate 4-fold increase in platelet production compared to MKs cultured alone (Fig. 1).

Discussion and Conclusions
The goal of this project was to develop an in vitro model of megakaryopoiesis using a tissue engineering approach. We report the development of a basement membrane model using a porous SF. Using human megakaryocytes and endothelial cells, we demonstrate the following advanced features of the silk-based basement membrane model: (1) immobilization of extracellular matrix components within the membrane, (2) tunable surface topography, (3) tunable mechanical properties, (4) physiologically relevant thickness for appropriate MK proplatelet extension, and (5) controlled localization of a vascular endothelium. Future work will test this basement membrane model with perfusion culture. The broader impact of this work offers a versatile new tool for studying MK development and platelet production in vitro and testing drug efficacy for treating thrombocytopenia related diseases using patient-derived cells.

References

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Disclosure
The authors declare that there are no conflicts of interest.