Collagen Biomatrices for Enhanced Endothelial Progenitor Cell Differentiation, Proliferation and Survival

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Statement of Purpose: Biomaterials that have the ability to augment angiogenesis are highly sought-after for applications in regenerative medicine, particularly for revascularization of ischemic tissue. Collagen type I has been implicated in supporting angiogenesis, and collagen type IV, which is part of the basement membrane surrounding vasculature, has been suggested to augment angiogenesis. Angiogenesis can be enhanced and initiated from circulating progenitor cells, termed Endothelial Progenitor Cells (EPCs). EPC use in regenerative medicine is limited by the poor availability of sufficient cell numbers *ex vivo*, and the rapid death of cells post-transplantation. We developed and tested injectable thermoresponsive collagen I-based matrices, with and without the addition of collagen type IV, as candidate biomaterials for future use in revascularization techniques and regenerative medicine as EPC microenvironments.

Methods: EPCs were isolated from healthy human donor blood and cultured for 4 days on fibronectin-coated plates. Matrices comprised mainly of collagen type I in a low or high concentration were created, with and without the addition of collagen type IV. Chondroitin sulfate-C was added, and all polymers were cross-linked with glutaraldehyde. Excess cross-linker was quenched with glycine, then matrices were thermogelled at 37˚C. Collagen IV's presence was confirmed using immuno-histochemistry. Four matrices and fibronectin controls were used in all assays, and flow cytometry was employed to correlate cell surface marker expression with functional observations. Antibodies to progenitor markers (CD34, CD133) and endothelial markers (CD31, CD144) were used. Proliferation was monitored by the incorporation of EdU into cells plated on matrices for 24 hours. EdU is taken up, similarly to BrdU, and incorporated into newly synthesized DNA. Apoptosis was induced using serum starvation, and cells were harvested after 48 hours. 7-AAD incorporation was used to determine dead and/or dying cells. After 4 days on matrices, cell populations were examined for EPC and mature endothelial cell (EC) markers.

Results: Differences were not observed between collagen matrices. The addition of type IV collagen did not modify the ability of EPCs to proliferate, respond to pro-apoptotic signals, or induce significantly different differentiation responses. However, when compared to fibronectin, 1.64-fold more cells were proliferating on the matrices (P=0.0001). Specifically, $CD133^+$ and $CD34^+CD133^+$ cells had increased proliferation 2.42- and 1.27-fold (P=0.002 and P=0.02, respectively). $CD34^+CD133^-$ cells had a reduced proliferation on the matrices, by 24% (P=0.01). During serum starvation, there was a 21% reduction in viable cells cultured on matrices, as compared to fibronectin (P=0.002). However, viability was enhanced 3.91-fold for progenitor cells positive for CD34 (P=0.00005) and 10.41-fold for CD133 (P=0.0001). After 4 days in culture, two populations of endothelial lineage emerged: 4.0-fold more $CD31^+CD144^+$ cells (P=4x10^{-7}) and 3.8-fold more $CD34^+CD133^+$ cells (P=5x10^{-5}).

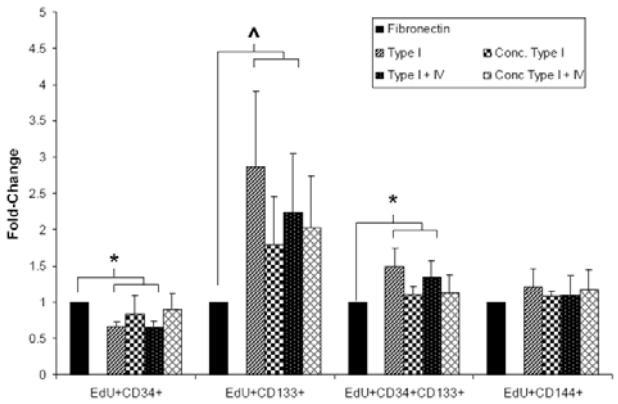

Figure 1 – Fold-changes in the number of proliferating cells, indicated by EdU incorporation, that were $CD133^+$, $CD34^+$, $CD34^+CD133^+$, and $CD144^+$ after 24 hours of culture on different substrates; *P<0.05 and ^P<0.005.

Conclusions: Although we were not able to show effects of collagen IV on EPCs *in vitro*, it has been suggested that collagen IV requires "activation" by matrix metalloproteinases *in vivo* in order for it to be pro-angiogenic (1). Regardless, our developed matrices have been shown to support the survival of both EPC and EC populations *in vitro*. In a pro-apoptotic environment, more cells died on our matrices; however, our matrices conferred pro-survival signals to $CD133^+$ and $CD34^+$ cells. Most notably was the observation that the matrices supported $CD133^+$ EPC proliferation, which has not yet been reported in literature to the best of our knowledge. Our matrices provide a novel microenvironment for EPC viability, differentiation, and proliferation. In the context of an ischemic treatment, our injectable biomaterials may have the potential for supporting revascularization by providing an ideal niche for EPCs, and overcoming two of the main impediments in cell therapy: rapid cell death and insufficient progenitor cell numbers.

References:

(1) Xu J. J Cell Biol. 2001;154:1069-79.