Synergistic effect of dynamic flow and mesenchymal stem cell coculture on endothelial progenitor cell angiogenesis

Bao-Ngoc B. Nguyen, B.S.[1], John P. Fisher, Ph.D.[1]
[1]Fischell Department of Bioengineering, University of Maryland

Statement of Purpose: Tissue engineering efforts to grow tissue has been hindered by inadequate vasculature throughout large constructs. Dynamic *in vitro* culture environments, such as the tubular perfusion system (TPS), are bringing tissue engineering closer to feasibility by providing sufficient nutrient transport as well as shear stress, an important stimulus for human mesenchymal stem cells (hMSCs) osteogenesis[1] and human endothelial progenitor cell (hEPCs) angiogenesis[2]. Osteogenesis and angiogenesis are inherently related, necessitating the combined culture of endothelial cells and differentiating hMSCs. So far, cocultured monolayers and 3D structures have shown promising preliminary results of synergistic vascularization and differentiation in static conditions. However, to better mimic *in vivo* environments, the cells can be seeded onto three-dimensional scaffolds and dynamically cultured in the TPS bioreactor, creating a prevascularized tissue-engineered bone construct for *in vivo* applications. The angiogenic interaction between these two cell populations is studied via vascular endothelial growth factor (VEGF), which is endogenously expressed by both cells, but acts as positive feedback molecule[3]; expression is increased during angiogenesis in hEPCs, which in turn increases expression in hMSCs. This project investigated the effect of culturing ratios of hMSCs to EPCs in dynamic culturing conditions to enhance angiogenesis.

Methods: hMSCs and hEPCs were cocultured in different ratios to determine optimized angiogenic and osteogenic effects. hMSCs were encapsulated in 2% alginate beads using $CaCl_2$, while hEPCs were encapsulated in Type I collagen disks. At ratios of 3:1, 1:1, 1:0, or 0:1of hMSCs to hEPCs encapsulated scaffolds were dynamically cultured in the TPS bioreactor using 50:50 osteogenic hMSC to growth hEPC media. **Figure 1** shows a schematic of the 1:1 culture chamber setup. On days 1, 7, and 14, alginate and collagen scaffolds were collected and separately analyzed for gene expression and protein production via qRT-PCR and histology staining, respectively. For hMSCs, mRNA expression of vascular endothelial growth factor (VEGF) and alkaline phosphatase (ALP) was analyzed, while mRNA expression of VEGF and CD31 was studied for hEPCs. In both cases, fold change expression was normalized to Day 1and statistical analysis was made using $p<0.05$.

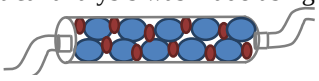

Figure 1: Schematic of 1:1 (hMSC:hEPC) coculture setup. Blue circles are alginate beads containing hSMCs. Red ovals represent collagen disks encapsulating hEPCs.

Results: hMSCs saw an upregulation of VEGF mRNA expression in all culturing ratios, with significant increase seen in the 1:1 ratios by day 14 (**Figure 2**). When cultured by themselves, hMSCs in the 1:0 ratio group showed steady increase in VEGF mRNA expression, however the 1:1 group had significantly greater expression on day 14.

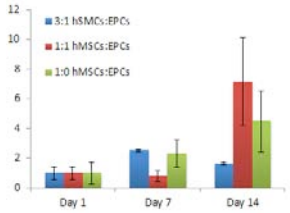

Figure 2. mRNA expression of VEGF in hMSCs over 14 days of coculture with hEPCs. Significant expression of VEGF is seen from 1:1 ratio hMSCs compared to other coculture ratios. The * represents statistical significance within the timpeoint groups.

Immunohistochemistry staining of VEGF (brown) on hEPCs sections (**Figure 3)** showed the most increased expression of VEGF over 14 days in the 1:1 coculture with hSMCs compared to other ratios. In addition, the staining indicated clustering of hEPCs on the periphery of the collagen scaffolds. The clustered cells (blue) also expressed less VEGF staining compared to unclustered cells at the center of the scaffold. Such clusters were less numerous in the hEPC-only culture and smaller in size.

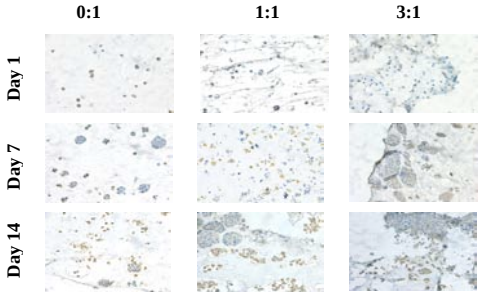

Figure 3. VEGF immunostaining of hEPCs in collagen scaffolds after 14 days of coculture with different ratios of hMSCs in the TPS bioreactor. Blue indicates hematoxylin staining of the cells, brown indicates VEGF staining. All images taken at 40x.

Conclusions: These results show the coculture ratio of 1:1 of hMSCs to hEPCs results in the highest mRNA expression of VEGF in hMSCs as well as a corresponding protein expression in hEPCs. The expression of VEGF may play an important role as a positive feedback mechanism to enhance hMSC differentiation and hEPC's angiogenesis. Therefore, a 1:1 ratio in conjunction with the dynamic flow environment may lead to overall increased angiogenic development for bone tissue engineering applications.

References: [1] AB Yeatts, *et al.* Tissue Engr 2011 Mar; 17(3):337. **[2]** EJ Lee, *et al.* Tissue Engr 2010 Oct; 16(5):1181.**[3]** BM Beckermann, *et al.* Br J Cancer 2008 Aug; 99(4).

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