

Using endometrial-inspired cues to drive angiogenic processes in collagen-glycosaminoglycan scaffolds

Jacquelyn Pence, Brendan Harley, Kathryn Clancy.

University of Illinois at Urbana-Champaign, Urbana, IL USA

Statement of Purpose: Poor re-vascularization remains a major bottleneck for the design of biomaterials in tissue engineering. The endometrium, the tissue lining the uterus, undergoes cyclical growth and shedding during the menstrual cycle and is tightly regulated by hormone signals. Notably, the balance of estradiol (E2) and progesterone has been implicated in crosstalk between endometrial epithelial, stromal, and endothelial cells to mediate the production of VEGF, a known pro-angiogenic factor [1]. Here we are exploring whether endometrial-inspired signals can be used to drive angiogenic processes within a 3D collagen-GAG (CG) scaffold. We hypothesized co-culturing of endometrial epithelial cells (Ishikawa clone 3-H-12) and Human Umbilical Vein Endothelial Cells (HUVECs) in the presence of E2 can support angiogenic processes within the CG scaffold. As the kinetics of these signals may be important, we also hypothesize that increasing the degree of sulfation of the GAG content of the scaffold (heparin sulfate, HP vs. chondroitin sulfate, CS) will lead to a more favorable angiogenic environment via non-covalent sequestration of pro-angiogenic molecules (*e.g.*, VEGF) within the matrix [2]. Here we examine the effects of E2 and VEGF on endothelial and endometrial epithelial cells, using metrics of cell bioactivity and markers or pro-angiogenic activity.

Methods: HUVECs were cultured to passage 4 with Media 200 supplemented with Low Serum Growth Supplement, 1% penicillin/streptomycin, and 1% L-glutamine. Ishikawas were cultured with DMEM/F-12 supplemented with 10% carbon stripped FBS, 1% penicillin/streptomycin, and 1% L-glutamine. Scaffolds were fabricated via lyophilization from a slurry of type I collagen and GAG (CG-CS, CG-HP) in acetic acid. Scaffolds were then cross-linked via EDC/NHS. 100, 000 or 250,000 cells per scaffold were subsequently seeded into scaffolds via a previously established static seeding method, and maintained in culture for up to 2 weeks. Metabolic activity was determined via Alamar Blue ($n=6/\text{time}$); proliferation was quantified ($n=6/\text{time}$) via Hoechst DNA assay. Ishikawa VEGF production was quantified via ELISA. E2 Receptor α ($\text{ER}\alpha$) phosphorylation was assessed via western blot. Statistics were performed using ANOVA and Fisher's LSD.

Results: HUVEC viability ($p<.001$) and metabolic activity ($p<.001$) was significantly increased in CG-HP scaffolds versus CG-CS scaffolds. Addition of VEGF (100 ng/mL) led to increased metabolic activity in HUVECs. Similarly, CG-HP scaffolds were found to support increased metabolic activity of Ishikawas ($p<.001$), though CG-CS scaffolds showed an increase in total cell number ($p<.001$). E2 supplementation was not found to impact HUVEC activity in CG scaffolds.

E2 supplementation was found to significantly increase both Ishikawa metabolic activity and cell count ($p<.05$) as well as an increase in $\text{ER}\alpha$ phosphorylation (Figure 1).

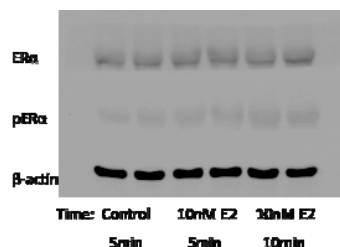


Figure 1: Early $\text{ER}\alpha$ phosphorylation in endometrial epithelial cells with E2 in CG scaffolds.

Endogenous production of VEGF by endometrial epithelial cells in CG scaffolds (Fig. 2) increased with both time in culture ($p<.001$) and 10 nM E2 supplementation ($p=.042$). No effect was found with GAG type ($p=.067$). We found an E2 dose-dependent (0, 10, 100, 1000 nM) increase in endometrial epithelial cell production of VEGF after 24 hours in culture.

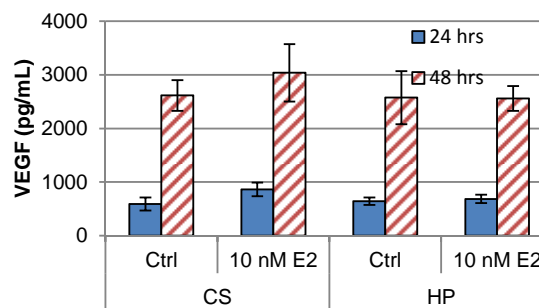


Figure 2. VEGF production by 250,000 endometrial epithelial cells in CG scaffolds (24 hrs).

Conclusions:

We are developing a biomaterial platform to explore the impact of endometrial-inspired signals on pro-angiogenic processes. Increasing the degree of GAG sulfation in the CG scaffolds led to increase in metabolic activity of both HUVECs and endometrial epithelial cells. VEGF and E2 supplementation resulted in increased metabolic activity in HUVECs and Ishikawa cells respectively. Further, E2 supplementation led to increases in VEGF production by epithelial cells. Ongoing efforts are using co-culture approaches to examine the use of template E2 and progesterone within the CG scaffold to regulate feedback between endometrial epithelial cells, HUVECs, and resultant pro-angiogenic processes.

References:

- [1] Koos RD. *Endocrinology*. 2011; 152(12): 4481-8.
- [2] d'Angelo I., et al. *Acta Biomater*. 2013; 9(7):7389-98.