Functional Materials: Functionalization of Ultrabithorax Materials with Vascular Endothelial Growth Factor Enhances Primary Human Endothelial Cell Survival and Activation

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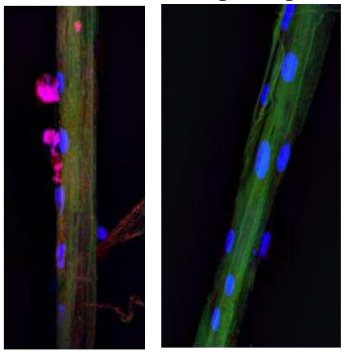
Statement of Purpose: Advances in tissue engineering will lead to new therapies that restore, maintain, or enhance function in damaged tissues. However, the most successful applications have been in thin (~2 mm) tissues, in which delivery of essential nutrients occurs by diffusion (Tian et al., 2011). Tissue engineering of more complex tissues cannot be supported by simple diffusion and will require vascular networks to support cellular function.

In this study, we have tested the ability of materials derived from the Drosophila melanogaster Hox protein Ultrabithorax (Ubx) (Greer et al., 2009) because they can be readily functionalized with full-length proteins by fusing DNA encompassing the functional protein to the ubx gene (Huang et al., 2011). Because Ubx self-assembles rapidly in mild aqueous buffers, proteins fused to Ubx are incorporated into the resulting materials in a native, fully functional material that is not toxic to human vascular cells and promotes sustained cellular interactions in culture (Patterson et al., 2014). Our initial studies have focused on whether vascular endothelial growth factor (VEGF) fused to Ubx provides a functional scaffold that can influence endothelial cell survival, activation, and sprouting.

Methods: Ubx materials were made from monomers of his-tagged Ultrabithorax (Ubx), GFP-Ubx, VEGF-Ubx, and GFP-VEGF-Ubx (Tsai et al., 2014) produced in E. coli and purified as previously described (Patterson et al., 2015). Primary human umbilical vein endothelial cells (Lonza) were cultured with Ubx fibers wrapped around inoculation loops. Immunofluorescence and western blotting were with primary antibodies raised against ERK, phosphorylated ERK, and VEGFR-2 (Cell Signaling Technology) to demonstrate VEGF-induced signaling. To determine how cells respond under starvation conditions, Ubx loops containing attached cells were starved for 8 hours in M199 without serum and then fixed using paraformaldehyde. A TUNEL assay (Abcam) was used per manufacturers' instructions and imaged using confocal microscopy on a Nikon Eclipse Ti equipped with NIS Elements AR 4.10.01 software. To examine Ubx materials ex vivo mouse aortic tissue segments were cultured with Ubx materials in a three-dimensional collagen matrix for 6 days, and the interactions between endothelial sprouts and Ubx fibers were examined.

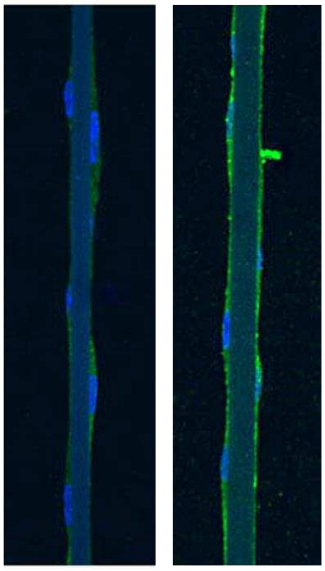


GFP-Ubx GFP-**VEGF**-Ubx

Figure 1. TUNEL assay after 8 hours of starvation showing DAPI stained viable cells on VEGF-Ubx and apoptotic cells (red) on Ubx.

Results: Ubx materials functionalized with VEGF induce endothelial cell attachment in low serum conditions (data not shown) and cells cultured on VEGF-Ubx are viably sustained after 8 hours of starvation (Figure 1).

To confirm that VEGF fused to Ubx signals through the correct pathway, we probed for changes in ERK phosphorylation (PERK) using immunofluorescence (IF) and western blotting. Figure 2 shows that IF of cells cultured on VEGF-Ubx display a much stronger PERK signal than cells on Ubx control.



Ubx **VEGF**-Ubx

Figure 2. IF of cells cultured on Ubx and stained for PERK (green)

Finally, in an ex vivo aortic ring assay, endothelial sprouts interacted with and followed VEGF-Ubx fibers, while control Ubx fibers did not elicit a change in extending endothelial sprouts (not shown).

Conclusions: Ubx is a self-assembling protein that can incorporate functional proteins such as VEGF through gene fusion. These functional materials enhanced primary human endothelial cell attachment, activation, and survival which will be conducive for instructing endothelial cells and building vascular scaffolds.

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