Oxygen Modulates Endothelial Tubule Formation Kinetics in Bio-functionalized Poly (ethylene glycol) Hydrogels

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Statement of Purpose: During development, vasculogenesis and angiogenesis occur in a low oxygen microenvironment ranging from 1-10% [1]. This low oxygen microenviroment induces stabilization of hypoxia-inducible factors (HIF-1 and HIF-2) and induces the expression of vascular endothelial growth factor (VEGF) [2,3], which stimulates endothelial cell migration and proliferation and initiates angiogenesis. However, standard in vitro culture conditions use a physiologically irrelevant oxygen level of 21%. Therefore, the purpose of this study is to examine the differences in tubule formation kinetics of vascular cells cultured in bio-functionalized poly (ethylene glycol) (PEG) hydrogels at 10% O_2 and 21% O_2.

Methods: Bio-functionalized PEG pre-polymers were synthesized through the following methods. Matrix metalloproteinase sensitive peptide sequence GGPQGIWGQGK (PQ) and cell adhesive ligand Arg-Gly-Asp-Ser (RGDS, American Peptide) were incorporated into PEG to allow for degradation and cell adhesion, respectively. PQ peptide sequences were generated by standard solid phase FMOC chemistry using an Apex396 peptide synthesizer (Aapptec). A diacrylate ABA block co-polymer of the PQ sequence was synthesized by reacting heterobifunctional acrylate-PEG-succinimidyl valerate (Laysan, Bio, acrylate-PEG-SVA, 3.4kDa, 1:2.2, peptide:PEG molar ratio) in anhydrous dimethyl sulfoxide. RGDS was conjugated to acrylate-PEG-SVA (1:1.1, PEG:RGDS molar ratio) under the same conditions. Polymer conjugation was verified via gel permeation chromatography with UV-vis and evaporative light scattering detectors (Polymer Laboratories). Human umbilical vein endothelial cells (HUVECs) and human pericytes (HPs) were encapsulated into bio-functionalized PEG hydrogels composed of 10% (w/v) PEG-PQ-PEG and 3.5mM PEG-RGDS. Cells (60E6/ml, 4:1, HUVECs:HPs) were first suspended in a pre-polymer solution of PEG-PQ-PEG, PEG-RGDS, 3.5 ul/ml N-vinyl pyrrolidone, 1 mM eosin Y, 1.5% triethanolamine in 10 mM HBS. This cell and pre-polymer solution was placed in a mold and exposed to white light for 35 sec to form a 300 um thick cylindrical cell-laden hydrogel. Cell-laden hydrogels were then cultured at 10% or 21% O_2. Samples were fixed at various time points, immunostained and imaged viaconfocal microscopy. Images were analyzed using the Rapid Analysis of Vessel Elements[4] program (determined vessel fraction area, vessel length density and fractal dimension). VEGF and HIF-1A gene expression was analyzed using qRT-PCR. cDNA from cell laden hydrogels for qRT-PCR analysis was obtained by first digesting the hydrogels using proteinase K (15 mg/ml). Following digestion, mRNA was extracted and used to synthesize cDNA.

Results: Following 12 hr of culture under 10% O_2 vascular cells formed more tubules than when exposed to 21% O_2 (A,B,C). Additionally, these vessels were significantly longer and more tortuous. At 24 hr differences in vessel length density between vascular cells exposed to 21% O_2 and 10% O_2 were observed (D,E,F). HUVECs and HPs exposed to 10% O_2 for 12 hours expressed higher levels of VEGF compared to HUVECs and HPs exposed to 21% O_2(G). After 24 hours HIF-1A gene expression was significantly higher in tubules formed under 10% O_2 compared to 21% O_2(H).

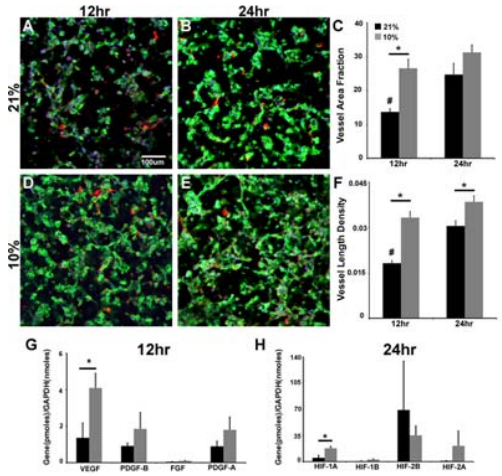

Figure 1. HUVECs(green) and HPs(red) encapsulated in bio-functionalized PEG hydrogels cultured at 10% and 21% O_2 (A, B, D, E). Vessel parameters of tubules formed under 10% and 21% O_2(mean±std err) (C, F). HUVECs and HPs co-cultured angiogenic factors(G) and HIFs gene expression(H) after 12 hr and 24 hr, cultured at 10% and 21% O_2, respectively.(*=p<0.05, #=p<0.05 compared to all groups, ANOVA, Tukey HSD)

Conclusions: Vascular cells exposed to short durations of 10% oxygen form longer and more tortuous tubules than when exposed to 21% O_2. Differences in tubule formation kinetics observed in 10% O_2 may be mediated by increased expression of angiogenic factor VEGF and transcription factor HIF-1A. Exposing vascular cells to different O_2 levels can potentially be used as a method to modulate the rate of tubule formation and properties. Additionally, these studies can be further used as a screening method to assess how tubule formation/density will change once these vascularized hydrogels are implanted into sites of injury and ischemia where oxygen levels are lower than 21% O_2.

References:

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