Multivalent Platform for Spatial Control of Arterial Venous Differentiation of Pluripotent Stem Cells

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Statement of Purpose: Vascular endothelial cells differentiated from pluripotent stem cells have potential in a variety of therapeutic areas such as tissue engineered vascular grafts and re-vascularization of ischemic tissues. However, there remain limitations in the control of stem cell differentiation into the desired functional phenotypes with current methods. During vascular development, it has been demonstrated that the early arterial-venous cell fate is genetically programmed, with the expression of transmembrane proteins ephrinB2 and ephB4 as the first distinction, prior to hemodynamic cues [1]. Little is known on whether the appearance of ephrinB2 and ephB4 at the early stage of differentiation plays a role in determine the subsequent arterial venous cell fate decision. Here, we hypothesize that arterial venous differentiation is influenced, not only from the Notch activation pathway, but also from the bidirectional signaling of these transmembrane ligand-receptor proteins. Based on my previous work, ephrinB2/ephB4 signaling has been shown to influence arterial venous differentiation in mouse embryonic stem cells (mESCs) (Figure 1A). However, ephrins are known to require receptor clustering to increase potency. Therefore to increase multivalent interactions, we propose to create hyaluronic acid (HA) chains conjugated with ephrinB2 peptides for photo-patterning onto polyethylene glycol (PEG) hydrogels for site specific arterial venous endothelial stem cell differentiation.

Methods: We are investigating both the extracellular domain (ECD) and the binding loop peptide, TNYL-RAW [2], as potential ephrinB2 signaling motifs. Both were designed with an additional cysteine at the N-terminus for later conjugation to HA. EphrinB2 ECD was expressed, amplified in an *E. coli* bacteria culture and purified with IMAC chromatography. Protein purity and molecular weight was confirmed by SDS-page. Bioactivity of the recombinant ephrinB2 ECD was validated through ephB4 binding assay. Both TNYL-RAWC and ECD constructs were fluorescently tagged using NHS chemistry for future quantification and next bioconjugated to 3,3´-N-[ε-Maleimidocaproic acid] hydrazide, trifluoroacetic acid salt (EMCH) activated HA-methacrylate through thiol-maleimide chemistry for 24hours at 4°C [3]. Following product purification, the final molar ratios were calculated by fluorescence readings compared to a standard curve. HA conjugates were tested for photo-patterning capability on PEG hydrogel using UV masking technique (Figure 1B), and demonstrated stability for over 2 weeks. A preliminary study was conducted using the soluble HA conjugates treatment on mESCs to evaluate the multivalent potency of the construct for vascular differentiation of stem cells.

Results: We successfully created multivalent ephrinB2 HA conjugates by controlling initial HA: ephrinB2 molar ratios. Although reaction efficiencies were low, we still were able to generate a range of multivalent conjugates (HA: ephrinB2 ratio, from 1:1 to 1:14) for soluble testing (Figure 1C). Based on our previous studies, it was expected that ephrinB2 signaling would result in an increase in mESC vascular Flk1+ differentiation. This data confirms that 1:8 multivalent TNYL-RAWC conjugate leads to an increase in RNA expression of Flk1 compared to that of a 1:3 conjugate or the untreated control group (Figure 1D).

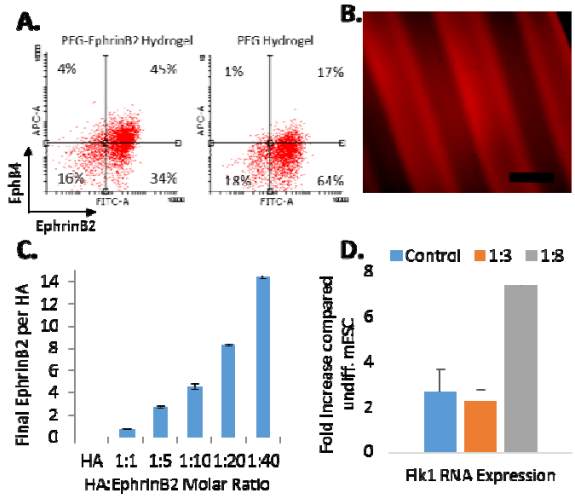

Figure 1. A) Ephrinb2 immobilized on PEG hydrogel increases ephb4 expression of Flk1+ population compared to blank PEG hydrogel control. **B)** TNYL-RAWC HA conjugate patterned onto a PEG hydrogel surface using a UV mask, scale bar 500µm. **C)** Generated library of ephrinB2 peptide HA conjugates from 1:1 to 1:14 final ratios **D)** Fold increase of vascular marker Flk1 RNA expression for 1:8 TNYL-RAWC conjugate treatment group compared to 1:3 and untreated control.

Conclusions: Preliminary data illustrates the potential of creating multivalent expressing HA chains with ephrinB2 peptide to influence stem cell fate. We can control the amount and spacing of ephrinB2 displayed on HA. Furthermore, we can then pattern HA chains onto a PEG hydrogel to guide arterial venous differentiation in a spatially specific manner. Ongoing studies are continuing to optimize the design of multivalent HA polymers. We are exploring the arterial venous differentiation effects of ephrinB2 ECD conjugates compared to that of the binding loop peptide conjugates using both flow cytometry and RT-PCR quantifications. Ultimately, stem cells differentiation on patterned PEG hydrogels will provide a mechanism for directing site specific stem cell differentiation with the goal of creating spatially defined arterial and venous endothelial cell territories.

References: [1] Wang H. Cell 1998; 93:741-753. [2] Chrencik JE. Biol. Chem. 2006;281: 28185-28192. [3] Conway A. Nat. Nanotech. 2013;8: 831-840.