Recombinant Elastin Analogues as Cell-Adhesive Matrices

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Statement of Purpose: Integration of biologic and structural functions of the extracellular matrix provides an important design strategy for generating a durable vascular substitute that stimulates arterial wall regeneration. We have previously reported the synthesis of a new class of recombinant elastin-mimetic triblock copolymers that mimic the elastomeric properties of native elastin, a dominant structural protein in the arterial wall.[1] However, the ability of this material to direct cellular responses is limited. Therefore, the long-term goal encompassing this work is to introduce cell adhesive peptide motifs within the elastomeric domains of the recombinant protein polymer in order to promote integrin-mediated responses to the scaffold, such as cell adhesion, proliferation, spreading, and migration. To this end, the $\alpha_v\beta_3$ integrin-binding peptide sequence V2[2], isolated from the cysteine rich connective tissue growth factor CCN1, was genetically engineered into the triblock copolymer **LysB10**.

Methods: Assembly of the bioactive protein polymer triblock was performed via genetic engineering methods, with protein purification enabled by a polyhistidine tag. SDS-PAGE and amino acid composition analysis revealed protein purity. Differential scanning microcalorimetry was used to assess the inverse temperature transition of the elastin-like biopolymer. Biological characterization of the modified construct included 2 hour HUVEC adhesion studies in low serum conditions, comparing **V2** and **LysB10** proteins adsorbed at 37ºC onto non-tissue culture treated plates (n=4). A Cyquant cell proliferation assay kit was utilized to quantify adhesion. Integrin specificity was determined with the use of blocking antibody LM609, as well as EDTA. Furthermore, a haptotactic migration assay was performed with modified Boyden chambers. The lower surfaces of filter membranes were coated with appropriate concentrations of the proteins and cells were allowed to migrate across inserts for 5 hours. HUVECs were also immunostained for the cytoskeletal components vinculin and actin in order to demonstrate their well-spread morphology on modified **V2** surfaces.

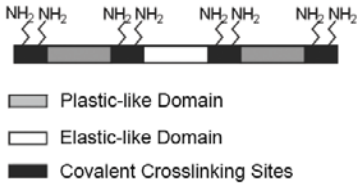

Figure 1. Design of **V2** triblock. The cell-binding sequence V2 was placed in the center of the elastic-like domain.

Results: SDS-PAGE analysis yielded one protein band at about 250 kDa (theoretical molecular weight of **V2** is 233.1 kDa). Furthermore, DSC confirmed the presence of a single endothermic transition peak at 21ºC, which is comparable to the coacervation temperature of **LysB10**.

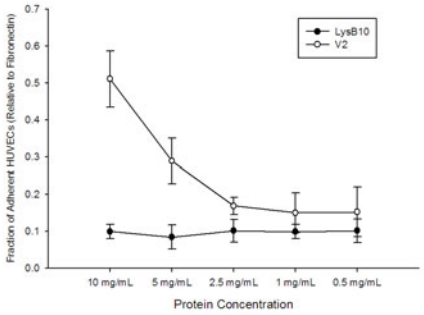

Figure 2. HUVEC adhesion to protein polymers that were adsorbed from solutions ranging from 10 mg/mL to 0.5 mg/mL.

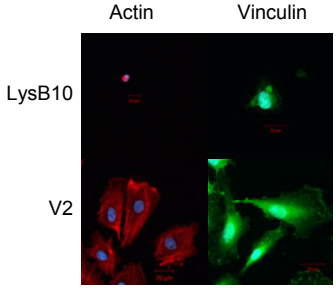

Figure 3. Cytoskeletal components of HUVECs on protein coatings.

As seen in Figure 2, HUVEC adhesion to **V2** increased in a dose-dependent manner. However, this response was muted when cells were incubated with the $\alpha_v\beta_3$ integrin-blocking antibody LM609, as well as the integrin-stripping EDTA solution. Furthermore, the haptotaxis assay demonstrated that HUVECs readily migrated toward immobilized **V2**. This migratory response was approximately four times that of **LysB10**. In addition, confocal microscopy images of fluorescently labeled actin and vinculin indicated well-spread HUVECs on **V2** as compared to **LysB10** (Figure 3).

Conclusions: Cell adhesion, migration, and morphology were evaluated as markers of surface functionality on recombinant protein polymers incorporating various bioactive domains. The findings above indicate the potential of these biopolymers in promoting endothelialization of a surface. This study also lays the groundwork to synthesize triblock proteins with other ECM peptide sequences in order to direct cell fate. Future studies will focus on the *in vivo* response to the recombinant elastin-mimetic protein polymers.

References:

(1) Sallach RE. Biomater. 2008. 30(3):409-422. (2) Chen N. JBC. 2004. 279(42): 44166-44176.