Endothelial Cell Retention on Teflon-AF Using Biotin Functionalized Fibronectin

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Statement of Purpose: Essential to the production of synthetic vascular grafts (SVG) is the formation of a functional neointima prior to implantation through endothelialization. The neointima will mimic native vessel functionality and mitigate the thrombotic nature of the current materials available for SVG[1]. Current methods to achieve endothelial coverage mostly use single ligand models optimized on glass surfaces. This system uses a dual ligand method consisting of biotinylated fibronectin (bFN) and HUVECs incubated with RGD-SA (RGD-SA-HUVEC) on Teflon-AF, an amorphous copolymer of 2, 2-bistrifluoromethyl-4, 5-difluoro-1, 3-dioxole (PDD) and tetrafluoroethylene (TFE). This model couples the strength and specificity of the biotin-streptavidin interaction with the intrinsic FN-integrin relationship to enhance cell adhesion and retention on an SVG surrogate[2]. The current study aims to validate bFN as a viable protein for cell attachment, determine affinity of bound biotin for wild type streptavidin (WT-SA) and a mutant strain streptavidin (RGD-SA), and measure cell retention under flow.

Methods: Fibronectin was biotinylated with Sulfo-NHS-LC-Biotin with a spacer arm of 22 Angstroms. The biotin attaches to accessible amines on the protein. The amount of biotin molar excess was varied from 10-500. An ELISA using the antibody to the FN cell binding domain was used to determine availability of RGD cell binding site. SPR was used to measure amount and kinetics of WT-SA or RGD-SA bound to immobilized bFN. Briefly, gold cover slips with a self assembled monolayer (SAM) of 16-mercaptohexadecanoic acid were inserted into the Biacore instrument and standard EDC/NHS chemistry was used to immobilize the bFN. BIAevaluation software was used to calculate the surface density and affinity constants of WT-SA and RGD-SA flowed over the immobilized bFN. A laminar flow chamber was used to perturb cells seeded on Teflon-AF at 60dynes/cm^2 and percent cell retention was calculated using pre and post flow images in Image J.

Results/Discussion: Biotins per FN ranged from 2-21 and saturated around the 500 molar excess (Figure 1). ELISA confirmed that biotin functionalization of FN had no adverse affects on the accessibility of the RGD binding site when compared native to protein (not shown). The absorbance units for bFN by ELISA were the same as those for un-modified FN. This is promising because it means we can get the benefit of the high affinity interaction between biotin and streptavidin as well as the integrin-FN linkages with one protein. SPR surface density data showed that immobilized bFN decreased precipitously at the level of 10 biotins per FN. This is primarily due to depletion of amines necessary for EDC/NHS coupling. This decrease, however, did not negatively affect the binding of WT-SA or RGD-SA (Figure 2a). Contrary to the initial hypothesis, SPR kinetic data showed no increase in affinity due to increased biotins per FN (Figure 2b).

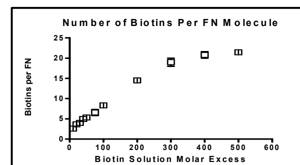

Figure 1. Biotinylation of FN

This means that though biotin density is increasing, there was no positive cooperativity from the proximal biotins; each event is an individual binding event. The data proved that the affinity of biotin for SA was not diminished by coupling to fibronectin. The consistency of the binding affinity and surface density levels between WT-SA and RGD-SA was a good sign that the high affinity binding was not adversely affected.

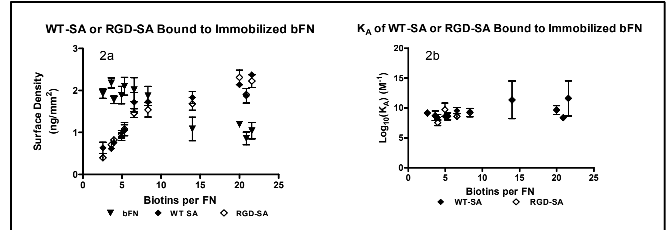

Figure 2a. Surface density of WT-SA or RGD-SA bound to immobilized bFN with different values of biotins per FN; 2b. Equilibrium association constants for binding of WT-SA or RGD-SA to immobilized bFN.

Flow experiments (Figure 3) showed high percent retention at 60 dynes/cm^2; the percent retention was 3x the value seen without protein treatment (---). However, there was no difference due to increased biotins per FN. This is consistent with the kinetic data from SPR.

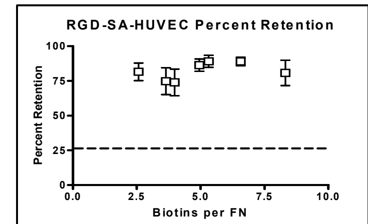

Figure 3. Percent cell retention of RGD-SA incubated HUVEC on Teflon-AF slides with bFN

Conclusions: Biotinylation of fibronectin does not mask accessibility to the cell binding domain for integrins. Kinetic analysis by SPR showed that affinity of bound biotin was the same for WT-SA as well as RGD-SA. Additionally, no enhanced kinetic effect was seen from having higher biotins per FN. This means that there was no positive cooperativity in the system. This finding was supported by the flow experiments because there was no increased adhesion strength due to higher biotin numbers. The strong binding strength means that this system may be used to enhance cell adhesion and mitigate thrombotic effects in synthetic vascular grafts.

References: 1.Zilla P. Curr. Opinions in Card. 1991. 6(6):877-86. 2. Anamelechi, CC et al. Biomaterials. 26(34):6887-96