A High Throughput Assay for Optimization of Endothelialization of Mechanical Heart Valve Prostheses

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Statement of Purpose:

Heart valve disease is a serious and increasing clinical problem. Approximately 100,000 heart valve replacements are performed each year in the US, Mechanical prosthetic valves (MPV) are very durable substitutes, but necessitate lifelong anticoagulation therapy because of the risk of thromboembolism formation on the pyrolytic carbon surfaces (Hammermeister et al, 2000). Our objective is to functionalize the surface of the mechanical valve to enable endothelial specific adhesion, which may reduce the blood thinner burden for these patients (Fig. 1).

This study investigates the selective adhesivity and shear resistance of adhesion peptides to better anchor endothelial cells and more closely match their 3D biological environment surrounded by extracellular matrix proteins.

Methods:

The biological matrix proteins Collagen I, Fibronectin, and Laminin in addition to the adhesion ligands FN7-10 (recombinant fragment of FN) and GFOGER were tested. A high throughput bioreactor was developed to test the shear resistances. The device consists of a top plate with flow inlet holes, a spacer to maintain the proper channel height for fluid dynamics, a poly-dimethyl siloxane (PDMS) gel to hold the valves in position during the tests, and a bottom shell to hold the gel and enclose the device (Fig. 2).

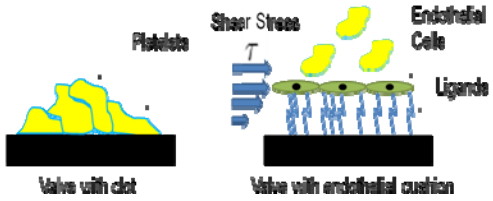

Figure 1. Proposal schematic

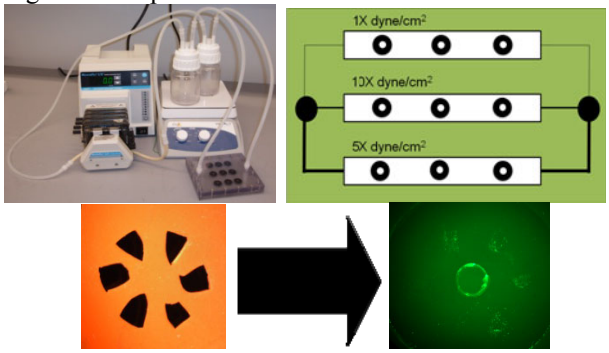

Figure 2. Assembled device, spacer schematic, and well isolator

The ligands were seeded onto the tilting disc valve surfaces (Donated by Medtronic, Inc.) in 7µm droplets at 10µg/µL and incubated for 2.5 hrs. The surfaces were then blocked with 5% powdered milk in PBS for 1 hr. Porcine aortic valve endothelial cells (PAVECs) tagged with Calcein-AM were then added to the blocked surface and incubated for 2.5 hrs. The reactor was then assembled and was perfused at progressively increasing rates for 5 minute intervals while imaging. ImageJ was used to quantify the number of cells present.

Results:

The time-dependence studies show that FN7-10 binds cells faster than the other ligands tested (Fig. 3). The shear experiment data (Fig. 4) shows that Collagen I is about two times as shear resistant as other ligands tested at shear rates up to 60 dynes/cm^2. The control group has the least shear resistance and has few or no cells bound after a shear rate of 30 dynes/cm^2.

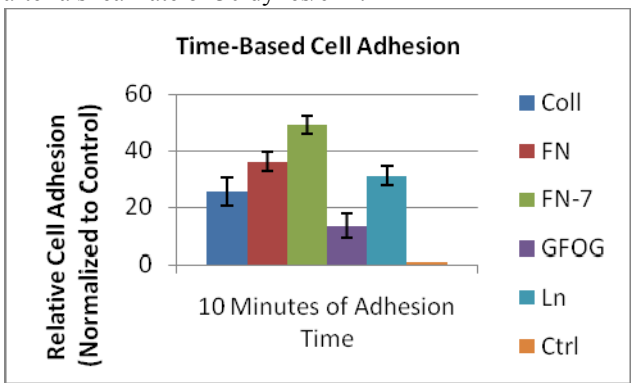

Figure 3. Time-based adhesion results

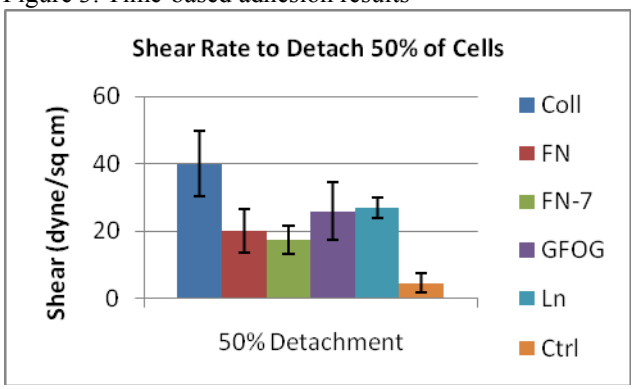

Figure 4. Data from shear experiment

Conclusions:

The shear resistance of an endothelial cell layer seeded on top of a valve can be enhanced by functionalizing the surface with extra-cellular matrix ligands. Shear-induced detachment could take place at the surface-ligand interface or at the cell-ligand interface. Ongoing studies are evaluating the efficacy of covalently tethering adhesion peptides to the valve surface.

References:

1. Weston, MW. Annals of Biom Eng. 1999;27:572-579.
2. Gulbins, H. Thorac Cardiov Surg. 2005; 53: 96–102.
3. Hammermeister, K. J Am Coll Cardiol. 2000; 36: 1152-1158.