Specificity of Adsorption in a Prototype Whole Blood Affinity Therapy Device for Removal of *Staphylococcus aureus*

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Introduction: In order to invade the human body many disease-causing bacteria and viruses bind to specific regions on the surface of cells in blood vessels and major organs, notably to heparan sulfate (HS) glycosaminoglycans[1]. We have developed and scaled up synthesis of an adsorption media with a biomimetic surface that captures pathogens[4], toxins and pro-inflammatory cytokines[2] within a disposable cartridge inserted into a dialysis-like extracorporeal circuit. Using covalently-bonded heparin, an analog of HS, together with optional supplemental ligands we mimic receptor sites on human cell membranes while presenting a blood-contacting surface known to be highly antithrombogenic. Heparin is bonded to non-porous UHMW polyethylene microspheres by a modified covalent end-point attachment process that maximizes surface concentration of (ATIII) binding sites. The single-use device consists of a transparent housing with tens of square meters of active surface area, a low pressure drop, and free blood volume of 100 mL. One important clinical indication is as a supplement to anti-infective drugs to prevent sepsis[3], e.g., in the treatment of potentially fatal *Staphylococcus aureus* bacteremia in dialysis patients[4]. Here we report the specificity of *in vitro* bacterial binding to the 'heparin-only' media.

Methods: Methicillin-resistant *Staphylococcus aureus* (MRSA) strain USA300 (ST8:5) was cultured on Brain Heart Infusion (BHI) agar to obtain an isolated colony which was grown overnight in BHI broth to give a stationary phase culture. Exponential phase MRSA was obtained by inoculating fresh BHI broth with a dilution of the culture. Optical density confirmed exponential growth at 2 hours (OD_{600} = 1). Colony forming units (CFU/mL) were determined by standard dilution plating assays. Exponential phase cultures were centrifuged, washed and re-suspended in PBS to a density that results in ~100% mice mortality. One-gram samples of heparinized media in miniature adsorption columns were equilibrated with twice their free volume of PBS, followed by 2 mL of Fetal Bovine Serum (FBS) and a PBS rinse. Two-mL aliquots of MRSA USA300 suspension were then passed through each column under gravity. Third-pass samples were dilution plated for bacterial counts. The *Pseudomonas aeruginosa* procedure was identical but Luria Broth replaced BHI media.

Results and Discussion: As shown in Fig. 1, MRSA mean concentration was reduced 67% after three passes through the heparin-only media. (MRSA removal from culture is consistent with earlier results with whole human blood which averaged 71% reduction with a single pass[4].) In comparison, reduction of *P. aeruginosa.* was only 16% under the same conditions. When the therapy is administered throughout a typical dialysis session the patient's entire circulating blood volume will be subjected to >10 passes through the apheresis cartridge (Fig. 2.), possibly reducing MRSA concentrations to undetectable levels. When the source of the bacteria is known and eliminated, the use of our whole blood affinity therapy, optionally combined with antibiotics, may accelerate the clearance of blood-borne pathogens and toxins to a level that can be handled by the patient's immune system. It is expected that reducing the pathogen load *and* shortening the duration of bacteremia will reduce the risk of metastatic complications. When the pathogen and/or the site of the infection is *unknown*, safe, broad-spectrum affinity therapy may delay the progression from bacteremia to septic shock and multi-organ failure, allowing more time for diagnosis and treatment.

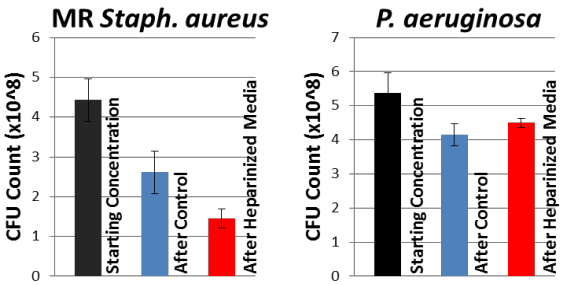

Figure 1. *In vitro* binding to 'heparin-only' adsorption media. Left: USA 300 Methicillin-resistant *Staphylococcus aureus* Right: *P. aeruginosa*

Figure 2. Seraph™ Microbind™ Affinity Blood Filter (ExThera Medical, Berkeley, CA) in series with a dialyzer during a large-animal safety study.

Conclusions: Bacterial removal by the anti-thrombogenic heparin media was much greater for MRSA, which binds to soluble heparin, relative to *P. aeruginosa* which does not. The (thrombogenic) control media also bound some MRSA non-specifically. A very long list of pathogens and toxins have been reported to bind to soluble heparin and heparan sulfate[1]. This indicates a.) the breadth of adsorbates that may be removed from whole blood by 'immobilized heparin affinity therapy' and b.) its potential utility as a clinical treatment for bacteremia, particularly when caused by drug-resistant organisms, or when no alternative treatments are available.

References: 1. Bartlett, A.H., Park, P.W. in Glycans in Diseases and Therapeutics, Berlin: Springer-Verlag, 2011, p.31 2. Axelsson, J., et al. ASAIO 56 (1): 48, 2010. 3. LaRosa, S.P. and Opal, S.M., Curr Infect Dis Rep 14 (5)474 (2012). 4. Mattsby-Baltzer, I., et al, J.Microbiol. Biotechnol. (2011), 21(6), 659-664