Removal of Drug-Resistant Bacteria and Ebola Virus from Whole Blood with a Sorbent Hemoperfusion Device: Carbapenum-Resistant Enterobacteriaceae

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Introduction: Many pathogenic bacteria and viruses invade the body by binding to specific regions on the surface of vascular endothelial cells. Heparan sulfate (HS) glycosaminoglycan[1] is a common attachment site**.** We have developed a biomimetic adsorption media that captures many pathogens[2], toxins and pro-inflammatory cytokines[3] within a disposable cartridge, during a dialysis-like extracorporeal therapy. Using covalently-bonded heparin, an analog of HS, together with supplemental adsorbents, we mimic receptor sites on human cell membranes while presenting a highly anti-thrombogenic blood-contacting surface. The single-use device (Fig. 1) has tens of square meters of active surface area and low priming volume. One potential clinical indication is as a supplement or alternative to anti-infective drugs in the prevention of sepsis[4], e.g., by *early* treatment of often fatal bloodstream infections caused by viruses and drug-resistant bacteria[2]**.** Here we report the removal of carbapenum-resistant Enterobacteriaceae ('CRE Superbugs'), and a rationale for removing Ebola virus, both of which cause bloodstream infections with mortality rates that can exceed 50%.

Methods: Heparinized UHMWPE beads were produced as described by Larm[6]. The beads were rendered hydrophilic prior to surface coupling. Reactive amino functions were introduced, followed by a modified end-point attachment to the aminated surface of heparin by reductive amination, utilizing the aldehyde function in the reducing terminal residue of partially (nitrous acid) degraded native heparin. The resulting PE-beads, with covalently end-point attached heparin, were sterilized with ethylenoxide.

Results and Discussion: We have previously reported methicillin-resistant *Staph. aureus* (MRSA) removal from whole human blood averaging 71% reduction with a single pass[2,5]**.** One option is to administer hemoperfusion therapy in a dialysis session (Fig. 1) during which the patient's entire circulating blood volume is typically subjected to 10-15 passes through the extracorporeal circuit, likely reducing bloodstream pathogen concentrations to undetectable levels. As shown in Table 1., both the efficiency of removal *and* the binding capacity of the media are very high. Therefore the use of this whole blood affinity therapy, optionally combined with anti-infective drugs (when available), should 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 CRE bacteremia and Ebola viremia will significantly improve outcomes[8]. When the pathogen and/or the site of the infection are *unknown*, a safe, broad-spectrum affinity therapy should delay the progression from bacteremia to septic shock and multi-organ failure, allowing more time for diagnosis and treatment.

	Test Medium	Starting Concentration (CFU/ml)	% Removed	Capacity (CFU/g media)
E. coli ATCC 8739	2 mL Defibrinated Blood	6.15E+05	99.75	2.04E+06
K. pneumoniae ATCC 13883		4.02E+05	36.43	4.88E+05
E. coli ATCC BAA-2469 (CRE)		2.57E+05	99.93	8.56E+05
K. pneumoniae ATCC BAA-2146 (CRE)		1.40E+05	99.94	4.66E+05

Table 1. *In vitro* binding to heparin-functional media showing high efficiency of removal *and* high binding capacity per gram of adsorption media: typical bacteria concentration during bacteremia is ca. 10^2 to 10^3 CFU/mL (in 5 liters of blood.)

Figure 1. Seraph® Microbind® Affinity Blood Filter, (ExThera Medical Corp., Berkeley, CA) in series with a dialyzer.

Conclusions: Many pathogens and toxins have been reported to bind to heparin and heparan sulfate[1], including Ebola and other filoviruses that cause hemorrhagic fever[7]. This and our binding study results indicate 1.) the breadth of adsorbates that may be removed from whole blood by 'immobilized-heparin affinity therapy' and 2.) its potential utility as a treatment for bacteremia, and viremia when efficacious anti-infective drugs are not available.

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