Towards a Bioselective Surface for Treatment of Sepsis in a Hemoperfusion Device
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Introduction: Sepsis is a life-threatening dysregulated immune response to circulating bacteria and bacterial cell wall fragments (endotoxin). Sepsis (aka “blood poisoning”) afflicts more than 750,000 people in the U.S. alone each year, with many more cases worldwide and a mortality rate between 28-50%. One promising option is extracorporeal hemoperfusion (“blood filtration”) in an adsorbent device, to eliminate pathogens and endotoxin from the blood. However, state-of-the-art hemoperfusion devices have not been widely adopted, mainly due to a lack of convincing evidence of their efficacy. The Japanese Toraymyxin hemoperfusion columns are based on a packed bed of immobilized polymyxin B (PMB), a cationic amphiphilic peptide widely used in antibiotic ointments (e.g. Neosporin). Clinical results with Toraymyxin have been inconclusive, with reports of damage to blood cells and protein loss due to the unprotected hydrophobic surface, and the known membrane-disrupting activity of PMB which may further fragment captured endotoxin vesicles. We address these fundamental shortcomings by a biocompatible, highly-branched hydrophilic surface coating with engineered endotoxin-binding proteins based on bacteriophage binding proteins. Bacteriophage are innocuous to mammalian cells, so we expect this approach to reduce host cell damage and immune response while increasing endotoxin capture.

Materials and Methods: Immobilized hydrogel nanofilms are grown in situ by surface-initiated atom-transfer radical polymerization (SI-ATRP) of highly-branched hydrophilic polyhydroxyethylmethacrylate (pHEMA) brushes. Surfactant-based initiators can be immobilized on polymers used in medical devices, tubing, etc. We are using Genetic Code Expansion (GCE) to express “click-ready” phage proteins with non-canonical amino acids (ncAA’s) at various sites, which react with exquisite specificity at reactive groups engineered at the periphery of the protein-repellent branched surface brush layer. This technique also allows for controlled orientation of binding proteins, and (importantly) requires no prior purification because of the highly-specific bioorthogonal immobilization. Polymer brushes are characterized by 1H-NMR, XPS, AFM, etc. Protein and endotoxin binding are analyzed by QCM-D, ELISA, etc.

Results/Discussion: We have demonstrated expression of bacteriophage proteins with click-ready groups at various sites, as well as production of click-reactive branched hydrophilic polymers. Preliminary experiments indicate that brush-tethered phage proteins do indeed repel fibrinogen (a model blood protein) while specifically capturing endotoxin.

Conclusions: The combination of GCE and SI-ATRP to produce click-ready proteins and branched polymer brushes on a variety of biomedical polymers enables production of simultaneously biocompatible and bioactive surface coatings for myriad applications. We will present up-to-date results for the model application of treatment of bacterial sepsis, although numerous other applications are readily apparent.

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