PEO-Tethered Peptides for Capture of Circulating Bacteria and Endotoxin in Sepsis

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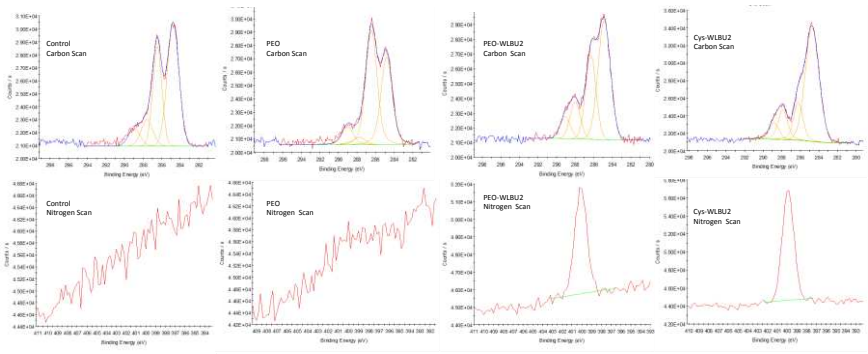
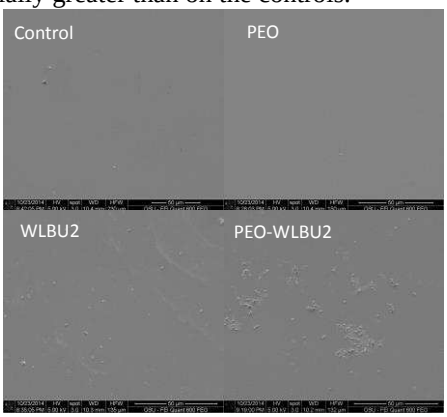
Statement of Purpose: Severe sepsis is a blood infection that affects over 750,000 people each year in the US alone, killing 28-50% (more than prostate cancer, breast cancer and AIDS combined). Symptoms result from a highly dysregulated immune response, which, if untreated, can lead to multiple organ failure and death. Currently, treatment uses wide-spectrum antibiotics, but this is hindered by the rise of antibiotic-resistant 'superbugs'. One potential novel treatment is a high-throughput microfluidic hemoperfusion device, which specifically removes circulating bacteria and cell wall fragments ("endotoxin") from blood. Microfluidics offer enhanced mass transfer and control of particle trajectory, as well as very high surface-to-volume ratios. A microfluidic device with a biocompatible and bioactive surface coating could selectively bind circulating bacteria and endotoxins from blood, enabling rapid, safe treatment of bacterial sepsis. WLBU2 is an α-helical, cationic amphiphilic peptide (CAP) with 13 positively-charged arginine and 11 hydrophobic tryptophan/valine residues oriented on opposite faces of the helix. WLBU2 has high anti-microbial against a variety of pathogens, and integrates into bacterial cell membranes (Deslouches, et al. J. Antimicrob. Chemother. 2007; 60: 669-672). WLBU2 retains its helical structure when bound to a surface, and immobilized WLBU2 binds bacteria and endotoxin. Biocompatible, non-fouling surfaces can be made by covalently tethering a dense brush of polyethylene oxide (PEO) polymer chains at the surface. Longer PEO tethers terminated with WLBU2 should enable increased mobility and solvent accessibility to tethered WLBU2, allowing it to bind bacterial cells/endotoxin, without compromising the biocompatibility of the coated surface.

Methods: Self-assembled monolayers (SAMs) were produced on gold-coated SiO_2 wafers by exposure to thiolated poly-ethylene oxide carrying a terminal carboxylic acid group (HS-PEO_{5000}-COOH). The terminal acids were activated with EDC/NHS, and WLBU2 tethered at its N-terminal amine. Poly-L-arginine and poly-L-glutamic acid served as controls for charge effects, while tethered glycine was used as a "null" peptide. Cys-WLBU2 served as a tether-free control. The surface chemistry was verified using X-ray Photoelectron Spectroscopy (XPS) and Atomic Force Microscopy (AFM). The SAMs were then challenged for 4 hours with Pseudomonas aeruginosa (strain PA14) in phosphate-buffered saline (PBS) at 2×10^5 CFU/ml. The samples were then washed with PBS and water, fixed with glutaraldehyde, and dehydrated by immersion in a series of solutions ranging from 0% to 100% ethanol. Scanning Electron Microscopy (SEM) was used to demonstrate capture of bacteria at the coated surfaces.

Results: The appearance of N_{1s} and characteristic C-N bonding peaks at 400 eV and 286 eV on the high-resolution XPS C_{1s} spectra is consistent with peptide immobilization at the surface. C_{1s} peaks associated with ether (C-O-C) bonds confirm the presence of PEO tethers. Conjugation of WLBU2 or control peptides to the terminal -COOH groups of the PEO caused an increase in nitrogen peaks, consistent with tethering of the peptides.



AFM images (not shown) demonstrated the uniform coverage of gold surfaces with PEO and peptides, which resulted in increased RMS roughness vs. controls. Greater adherence of P. aeruginosa cells was evident in SEM images of surfaces coated with PEO-tethered WLBU2 than for WLBU2 directly bound to the surface. Bacterial adhesion on the WLBU2-coated surfaces was also substantially greater than on the controls.



Conclusions: WLBU2 can be tethered on model surfaces by standard bioconjugation chemistry. Tethered WLBU2 more effectively binds P. aeruginosa than surface-bound WLBU2, presumably due to its enhanced mobility. Cells adsorbed to a much lesser extent on control wafers. Future work will focus on optimization of the coating to enable high loading of tethered bioactive molecules, without compromising surface biocompatibility. We are also developing a novel surface coating platform, using self-assembly and immobilization of PEO-based surfactants. This method shows promise in providing biocompatibility and biological function to a variety of polymers used in medical devices, without requiring expensive and toxic crosslinking reagents.