Controlled release of novel anti-biofilm agents from a poly (2-hydroxyethyl methacrylate) Scaffold for the treatment of medical device associated bacterial biofilm infections

Hongyan Ma, Lei zhang, Min zhang, James D. Bryers*
Department of Bioengineering, University of Washington, Seattle, WA 98195-1720

Statement of Purpose: It is estimated that over 5 million artificial or prosthetic devices are implanted per annum in the U.S. alone. However, 70% of hospital-acquired infections are associated with implants or indwelling medical devices causing >$4.5 billion medical costs annually. Systemic antibiotic therapy to control medical device-associated infections typically fails to clear biofilm, promotes antibiotic resistance, and inevitably requires removal of devices. The goal of this proposed research is to develop a new non-antibiotic based concept in biomaterials design where the biomaterial promotes healing while preventing biofilm colonization and subsequent infection. In this study, we developed a model porous "template" constructs (PCTs) of poly(2-hydroxyethylmethacrylate) (pHEMA) hydrogels encapsulated with two complementary therapies: (a) an EPS polysaccharide dispersant and (b) a Ga-siderophore based antibacterial agent to enhance drug transport and uptake for the treatment of biofilm infection diseases. Enzyme-based therapies in combination with antimicrobial Ga-complexes produced synergic effects of reducing biofilm formation.

Methods: Two Gallium (Ga) complexes drugs were synthesized using a chelation reaction. HEMA monomer was mixed with tetraethylene glycol dimethacrylate (TEGDMA), ethylene glycol and UV photo initiator. Each drug was dissoved in diH_2O and then added to above monomer mixture. Un-crosslinked poly(methyl methacrylate) (PMMA) microspheres of a desired diameter are ultrasonically packed into a mold. The mold is gently heated, which leads to sintering (fusion) of the spheres at their contact points. Next, the above mixed pHEMA monomer is vacuum-drawn in liquid form into the mold, surrounding the sintered beads. Monomer is UV polymerized in-place into a solidified crosslinked network. Finally, the PMMA microspheres are solubilized from within the crosslinked network, leaving a porous, interconnected structure. The solidified pHEMA sca were punched into one-centimeter disks, and vacuum dried. The absorbances of the drug release from samples were measured by UV spectrophotometer at 576 nm wavelength periodically for 3 months. The effectiveness of the various pHEMA biomaterials in reducing bacterial colonization of *S.aureus* and *P. aeruginosa* were quantified *in vitro* and *in vivo*.

Results: We have successfully formulated complexes of Ga. Unlike free gallium, they show significant bacterial killing effects on both gram-positive (*Staphylococcus epidermidis, SE*) and gram negative (*Pseudomonas aeruginosa, PA*) bacteria at a concentration as low as 2.5 µg/mL. We also found using Ga complexes to work synergistically in combination with enzyme-based dispersant therapies *in vitro*, such as DNase or Dispersin^{TM} (**Fig. 1**).

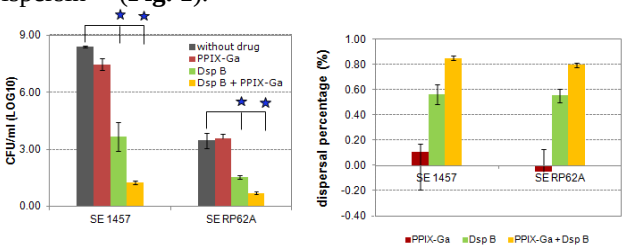

Fig 1 Enzyme-based therapies in combination with antimicrobial PPIX-Ga: Synergic effects of reducing biofilm formation.

Here, we developed pHEMA scaffolds to delivery combination drugs functional to kill bacteria and devastate biofilm matrix (**Fig. 2**). PHEMA scaffolds encapsulated with these novel drugs produced the constant and effective drug release kinetics over 3 months. All drug loaded pHEMA scaffolds showed significant reduction of adherent *SA* and *PA* cells versus control scaffolds in vitro. More importantly, our preliminary data indicated pHEMA scaffolds releasing Ga-complex drugs show great promise as a novel non-antibiotic drug delivery system to prevent medical devices associated biofilm infections in vivo.

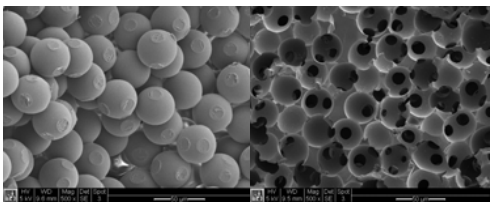

Fig 2 SEM images of PMMA templates and pHEMA scaffolds

Conclusions: Incorporation of novel drugs into pHEMA polymers achieved constant drug release rates throughout an extended period of time up to 3 months. Total amounts of drugs released from pHEMA scaffolds in a controlled-sustained manner are sufficient to kill bacteria growth in the liquid phase, as well as to reduce bacteria adhesion on surfaces. Enzyme-based therapies in combination with antimicrobial Ga complexes produced Synergic effects of reducing biofilm formation. Gallium complexes and Dispersin^{TM} were promising non-anticbiotic therapeutic drugs both *in vitro* and *in vivo* that could be released from the pHEMA scaffolds, thus enhancing the treatment effects to remove bacterial biofilm infections associated on medical devices.

Acknowledgements:
This work was supported by NIH/NIBIB 1R01EB007575-01.