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Stevens Institute of Technology
1 Castle Point on Hudson

Hoboken, NJ 07030 -5991

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Matthew Libera
UU	UU	UU	UU		19b. TELEPHONE NUMBER 419-530-8267

RPPR Final Report
as of 03-Oct-2018

Agency Code:

Proposal Number: 58455MS **Agreement Number: W911NF-12-1-0331**
INVESTIGATOR(S):

Name: Ph.D Matthew Walter Libera
Email: matthew.libera@stevens.edu
Phone Number: 4195308267
Principal: Y

Organization: **Stevens Institute of Technology**
Address: 1 Castle Point on Hudson, Hoboken, NJ 070305991
Country: USA
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Begin Performance Period: 20-Jul-2012 **End Performance Period:** 30-Jun-2016
Report Term: 0-Other
Submitted By: Ph.D Matthew Libera Email: matthew.libera@stevens.edu
Phone: (419) 530-8267
Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: **STEM Participants:**

Major Goals: The central goal of this research project is to understand the fundamental materials science underlying the design and development of triggered drug-delivery systems that can prevent infection associated with tissue-contacting biomedical devices. This is important to the Army and other DoD agencies, because the treatment of traumatic battlefield injury is often unavoidably compromised by device-associated infection. Such complications can lead to long recovery periods, multiple surgeries, reduced limb function, amputation, or even death. Materials that can avoid device-associated infection will thus substantially enhance the recovery of injured soldiers. The strategic goal of this project is thus to develop materials using a family of multifunctional polymeric microgels with which to control the physico-chemical surface properties of tissue-contacting biomedical devices and enhance their infection resistance while preserving their ability to promote healing.

The project's specific objectives center on controlling the self-assembly and complexation phenomena associated with antimicrobial loading into, sequestration within, and triggered release from anionic microgels. We are:
- synthesizing microgels by suspension copolymerization of different acrylate-based monomers, each bringing control over microgel charge, hydrophobicity, and functionality.

- characterizing the average microgel properties using zeta potential measurements and dynamic light scattering (DLS).

- quantifying individual microgel properties using various microscopies including cryo-electron microscopy and wet-cell AFM.

- Assessing bacteria–material interactions using gram positive/negative species implicated in biomaterials-associated infections and assessing cell-material interactions using in vitro osteoblast monoculture experiments.

Important metrics of successful microgel design are their ability to: (A) electrostatically load FDA-approved antibiotics, e.g. amikacin, vancomycin, and colistin, after electrostatic deposition on a synthetic surface; (B) sequester the antibiotics for extended times (~28 days) under physiologically relevant conditions (pH 7.4, 0.15 M salt); and (C) after this time remain able to interact responsively to a bacterial challenge.

Accomplishments: Please see attached pdf.

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Training Opportunities: This project has thus far involved one graduate student - Ms. Xixi Xiao. She completed her Masters degree in Materials Science and Engineering at Stevens in May 2017. Her masters research centered on developing a polymer-based approach to replicate nano rough surfaces used to control cell-material interactions. She started her doctoral program in August 2017 working under ARO support. This project is providing key training in areas of polymer synthesis and self-assembly.

Results Dissemination:
PI Libera gave four invited presentations on infection-resisting biomaterials, a topic that includes ARO-funded research results:

10/3/17 CEMS Dept seminar, Stevens Institute of Technology

11/14/17 ASTM workshopWorkshop on Antimicrobial Combination Devices

4/9/18 Syracuse University, Dept of Chemical and Biomedical Engr

4/20/18 J&J Ethicon Somerville, NJ

In addition, a manuscript was drafted and submitted to the journal Biomaterials, the leading peer-reviewed journal in this field. The title is: "Self-Defensive Biomaterial Surfaces by Contact Transfer of Antimicrobials." We received comments and constructive criticisms from five different reviewers, and we are responding to those comments now. We anticipate submission of a revised manuscript in October 2018.

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: PD/PI
Participant: Matthew Richardson Libera
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Xixi Xiao
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
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International Travel:
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Other Collaborators:

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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Soft Matter
Publication Identifier Type: DOI Publication Identifier: 10.1039/c1sm06702h
Volume: 8 Issue: 11 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Surface-patterned microgel-tethered molecular beacons
Authors:
Keywords: microgel, PEG, electron-beam lithography, bacteria, detection, patterning
Abstract: Focused electron beams are used to pattern biotinylated PEG microgels on a solid surface. Molecular beacon DNA probes are grafted to these microgels via biotin-strepavidin binding. Each microgel binds approximately 12,000 beacons. Monte Carlo simulation of electron-polymer interactions show that the structure of each microgel is highly nonuniform and that the crosslink density approaches zero near the microgel surface. This liquid-like structure preserves the hairpin conformation of the molecular beacon probes and is responsible for their extremely good performance when exposed to complimentary targets characteristic of methicillin-sensitive and methicillan-resistant staphylococcu aureus.
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Journal: Journal of Polymer Science Part B: Polymer Physics
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Date Submitted: Date Published:
Publication Location:
Article Title: Poly(ethylene glycol) as a biointeractive electron-beam resist
Authors:
Keywords: antifouling, hydrogels, lithography, microgel, PEG, Monte Carlo Simulation
Abstract: Poly(ethylene glycol) can serve as an electron-beam resist to modulate protein adsorption and cell adhesion to surfaces. PEG preferentially crosslinks under e-beam irradiation to create microgels with controllable properties. Here, atomic-force, scanning electron, and confocal microscopies are used to study discrete microgels formed from solvent-cast PEG thin films by focused e-beams with energies between 2 and 30 keV and point doses between 10 and 1000 fC. Consistent with experimental findings, Monte Carlo simulation of electron energy deposition identifies three structures within each microgel: a highly crosslinked core near the point of electron incidence; a lightly crosslinked near corona; and a far corona at the PEG-Si interface. The nature and sizes of these three regions and, hence, the microgel-protein interactions depend on the incident electron energy and dose. The far corona creates protein-repulsive surface hundreds of nanometers or more from the microgel core. The hi
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Publication Identifier Type: DOI Publication Identifier: 10.1016/j.micron.2011.05.007
Volume: 43 Issue: 1 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Delocalized radiation damage in polymers
Authors:
Keywords: EELS, radiation damage, polymers, delocalization
Abstract: We present and discuss measurements of electron-irradiation damage in polystyrene and other polymers, based on fading of the 7-eV energy-loss peak. These measurements suggest a large increase in characteristic dose as the electron-beam diameter is reduced from 1 micron to below 1 nm. This finding is discussed in terms of secondary-electron production and delocalization of the inelastic scattering, both as it affects the volume of specimen in which the energy is deposited and the volume giving rise to the inelastic signal used to assess the damage.
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Journal: Macromolecular Symposia
Publication Identifier Type: DOI Publication Identifier: 10.1002/masy.201200106
Volume: 329 Issue: 1 First Page #: 35
Date Submitted: Date Published:
Publication Location:
Article Title: PEG-Based Microgels to Modify Biomaterials Surfaces
Authors:
Keywords: biomaterials, drug delivery, infection,microgels, poly(ethylene glycol)
Abstract: Microgels are hydrogel particles ~0.1 - 10 microns in size which have been increasingly explored for biomaterials applications. They can be designed to sequester drugs based on both hydrophobic and electrostatic interactions, and different strategies exist to trigger drug release from them. Microgels based on poly(ethylene glycol) are particularly attractive because of their intrinsic antifouling properties, the flexibility they bring to microgel design and synthesis, and the regulatory precedents set by PEG's use in a number of FDA-approved applications. This paper briefly reviews progress in the field of PEG-based microgels. We give examples illustrating their electrostatic deposition onto biomaterials surfaces and their ability to sequester antimicrobials for applications involving biomaterials-asociated infection.
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Journal: The Analyst
Publication Identifier Type: DOI Publication Identifier: 10.1039/C4AN01220H
Volume: 0 Issue: 0 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Dip-Pen Microarraying of Molecular Beacon Probes on Microgel Thin-Film Substrates
Authors:
Keywords: Molecular diagnostics, e-beam patterning, microgel, poly(ethylene glycol), DNA
Abstract: The integration of microarray-based nucleic acid detection technologies and microfluidics is attractive, because the combination of small sample volumes, relatively short diffusion distances, and solid-phase detection enhances the development of multiplexed assays with improved sensitivity and minimal sample size. However, traditional microarray spotting methods typically create probe spot sizes of ~50-100 m diameter, comparable to the dimensions of many microfluidic channels. In addition, detection of hybridization events typically requires a post-hybridization labeling step. We address both issues by exploring the use of dip-pen nanolithography (DPN) to pattern linear oligonucleoti des and self-reporting molecular beacon (MB) probes on streptavidin-functionalized poly(ethylene glycol) microgel thin-film substrates. In contrast to many systems involving DPN deposition, the fluorescence of the labeled probes enables their amount and spatial distribution to be characterized by optical m
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DISSERTATIONS:

Publication Type: Thesis or Dissertation
Institution:
Date Received: 09-Nov-2015 Completion Date:
Title: Directed Antimicrobial Loading into and Release from Gels and Microgels
Authors:
Acknowledged Federal Support:

Summary Highlights from Year 1

Figure 1 schematically illustrates key aspects of this project. Anionic microgels are synthesized and electrostatically deposited onto synthetic substrates (e.g. silicon wafers). They are then immersed in solutions containing small-molecule cationic antimicrobials, which load by complexation-driven self-assembly into the microgels. As a consequence of the loading the microgels deswell. The microgels then need to be able to sequester the loaded antimicrobials for extended periods of time. Contact with bacteria can trigger the release of the sequestered antimicrobials. This topic - bacteria-triggered contact release - is the subject of a manuscript drafted and currently under revision for Biomaterials, the leading journal in the field. This first manuscript focuses on the complexation interactions between microgels and antimicrobial peptides with relatively high amounts of electrostatic charge. The current ARO project concentrates on controlling the loading, sequestration, and release properties of FDA-approved cationic antimicrobials, all of which have less electrostatic charge than the peptides we have studied previously.

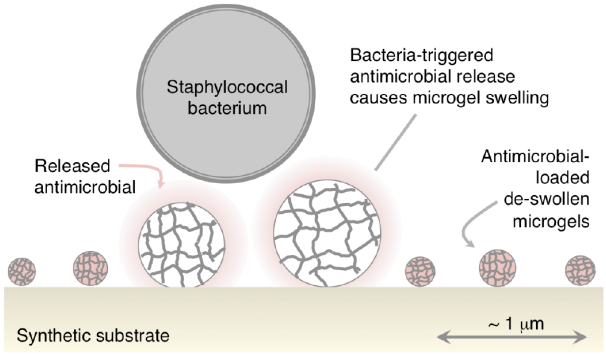

Figure 1 – Biomaterials surfaces modified by antimicrobial-loaded microgels responsively interact with bacteria to locally release antimicrobials and mechanically protect the surface by swelling. Unchallenged surface areas remain loaded and de-swelled to confront later challenges.

A central hypothesis of the project is that the complexation interaction between an antimicrobial and the microgel can be controlled both by the electrostatic charge of the microgel as well as by the extent of hydrophobic moieties it contains. Our early experiments centered on microgels consisting of poly(acrylic acid) [PAA]. We continue to use PAA microgels as a reference system against which to compare more recent microgel designs. To introduce controlled amounts of hydrophobicity, we have made microgels by copolymerizing acrylic acid [AA] and hydroxypropylmethacrylate [HPMA]. We have studied a range of synthesis conditions associated with the solvents/co-solvents and composition. Figure 2 present typical SEM images of dry

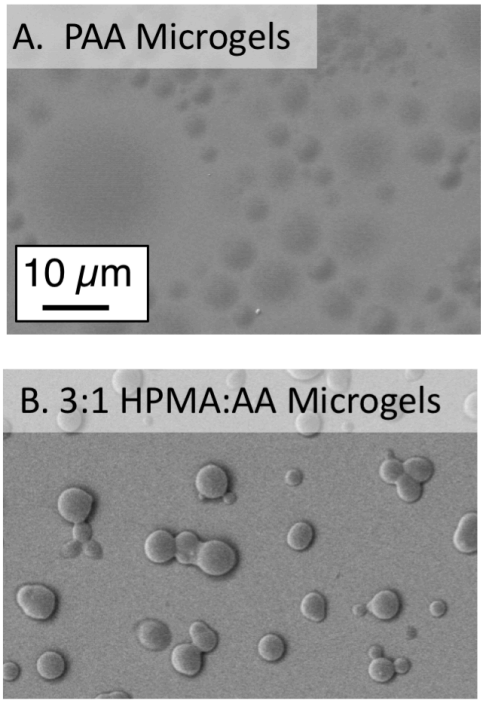

Figure 2 - SEM images of microgels electrostatically deposited onto hard substrates. Copolymerization with HPMA increases microgel modulus and strength.

microgels of: (A) PAA; and (B) 3:1 HPMA:AA. In contrast to the PAA microgels, which are relatively soft and conform to the substrate, the copolymer microgels are mechanically more robust and maintain their spherical shape, even after drying.

Most significant is that the introduction of hydrophobic moieties into the microgels affects antimicrobial sequestration. The clearest demonstration thusfar of this effect involves the FDA-approved antibiotic tobramycin. Antimicrobial release into a buffer can be followed by measuring the microgel diameter in situ as a function of time. Antimicrobial release is manifested by microgel swelling. Figure 3 shows that tobramycin-loaded PAA microgels deswell in tobramycin-free 0.01M phosphate buffer. Deswelling indicates tobramycin release. In the case of PAA microgels, much of this release occurs within a timescale of minutes. In contrast, the diameter of tobramycin-loaded PAA-HPMA microgels does not change when loaded microgels are exposed to identical tobramycin-free phosphate buffer for the duration of this 24 h experiment. This finding clearly indicates that the tobramycin-microgel complexation thermodynamics is affected by the hydrophobicity introduced by the HPMA, consistent with the underlying hypothesis of the project. Ongoing work during year 2 will elaborate further on this phenomenon, including experiments in buffer with higher ionic strength where electrostatic shielding can affect the complexation strength.

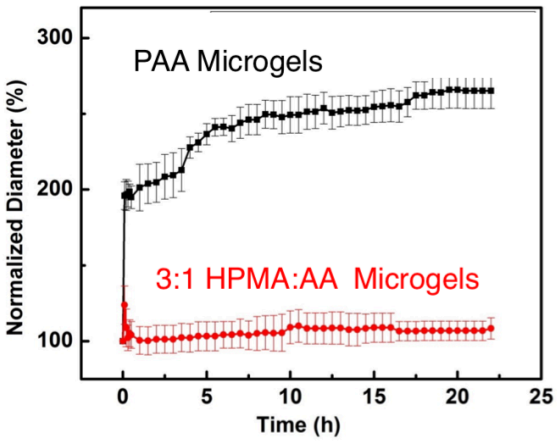

Figure 3 - Microgel deswelling followed by in-situ optical microscopy. Pure PAA microgels are unable to sequester complexed tobramycin when immersed in tobramycin-free 0.01 M phosphate buffer. Hydrophobicity introduced into the microgels by copolymerizing with HPMA increases the complexation strength so there is no tobramycin release under otherwise identical conditions.