Surface Modification by Antibiotic-Loaded Microgels Inhibits Bacterial Colonization

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Introduction: Multiple strategies to mitigate biomaterials-associate infection have been developed. Many involve continuous coatings that elute antibiotics or antibiotic grafting to a surface. We have been exploring surface modifications using microgels (1,2). Discontinuous coatings of anionic microgels can be deposited on positively charged substrates by one-step non-line-of-sight electrostatic self assembly (Fig. 1 A-B). When the average inter-gel spacing is ~1 μm, these coatings can significantly reduce bacterial adhesion (1,3) while maintaining desirable cell (e.g. osteoblast) interactions (1,4). The microgels can, however, be further loaded with small cationic molecules by a second self-assembly step (Fig. 1 B-C). We have previously shown that a nanofiber tissue-scaffold can be modified with PEG-AA co-polymer microgels, which are subsequently loaded with an antimicrobial peptide (L5), to inhibit colonization by staphylococci throughout the scaffold, including its interior, while preserving conditions for osteoblast adhesion and spreading at the scaffold surface (5). Here we expand this approach to include microgels made of PEG-PAA [poly(ethylene glycol) – poly(acrylic acid)] semi IPN structures with post-deposition loading by an FDA-approved cationic antibiotic (amikacin).

Methods: Semi-IPN PEG/PAA microgels were synthesized via inverse emulsion polymerization using PEG diacrylate (Mw = 575 Da), PAA (Mw = 450 kDa), and photo-initiator dissolved in water. Using Span 80 surfactant, this precursor solution was dispersed by sonication in cyclohexane and exposed to UV for 15 min. Microgels were harvested by centrifuging/resuspending 3 times each in ethanol and water, dispersed in 10 mM phosphate buffer, and electrostatically deposited onto poly (L-lysine) primed Si substrates. After deposition, amikacin was loaded by immersing the microgel-modified substrates in an amikacin solution. A short-term anti-bacterial assay (2 h) was performed using *S. aureus* cultures (1×10^6 CFU/mL) followed by live/dead staining.

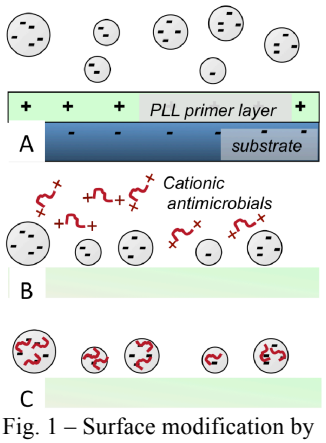

Fig. 1 – Surface modification by anionic microgels with post-deposition antimicrobial loading.

Results: Figure 2 shows an SEM image of PEG-PAA microgels (dry) after deposition on PLL-primed Si. The average inter-gel spacing can be controlled by the deposition time and the microgel concentration in the parent suspension (3). Loading of amikacin into the deposited microgels was confirmed by FITC labeling of the amikacin (Fig. 2 bottom inset). Identical microgel-modified substrates exposed to amikacin-free buffer show no FITC staining (Fig. 2 top inset). The effect of microgel modification on the short-term colonization by *S. aureus* is illustrated by Fig. 3. The amikacin-free microgel-modified surface has ~22x more live bacteria than the amikacin-loaded modified surface. There is an undetectable amount of dead bacteria on the control surface. In contrast, amikacin loading leads to significant bacterial killing despite the short culture time.

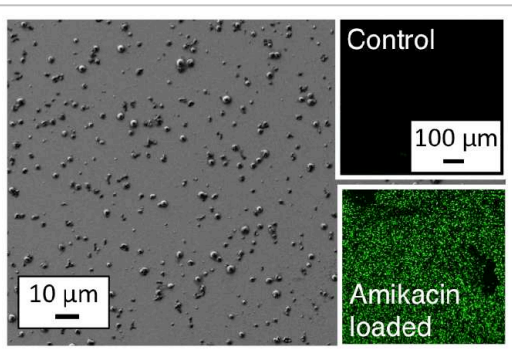

Fig. 2 – PEG-PAA semi-IPN microgels electrostatically deposited on Si. Insets: FITC staining confirms loading when the microgel-modified Si is exposed to an amikacin solution.

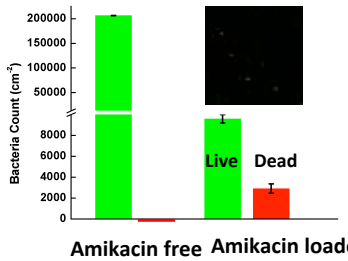

Fig. 3 – Live/dead staining shows that *S. aureus* aggressively colonizes amikacin free surfaces whereas amikacin-loaded surfaces inhibit adhesion and promote active killing.

Conclusions: The hierarchical modification of a synthetic surface by anionic microgels followed by antibiotic loading is an effective and straightforward means with which to enhance the resistance of biomedical device surfaces to bacterial colonization and subset biomaterials-associated infection.

References:

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