Antimicrobial Smart Materials: From Responsive Hydrogels to Polymer-Drug Conjugates
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Statement of Purpose: The Centers for Disease Control and Prevention reports that at least 2 million cases of infection by drug resistant bacteria occur annually in the United States alone, leading to a minimum of 23,000 deaths. The World Health Organization recently warned of an impending “post antibiotic era” in which common infections are lethal due to rising antimicrobial resistance. Microbes inherently evolve resistance mechanisms over time, the rates of which can be exacerbated by frequent use of broad-spectrum antimicrobials and prolonged exposure. This issue is also concerning for fungi, such as Candida albicans, which is the most common cause of fungal infections (U.S. CDC, 2013). Compounding this rise in resistance is a lack of discovery and approval of new antimicrobial compounds (Taubes G. Science. 2008;321:356-361.). There is a need for smart, triggered drug delivery systems that limit exposure to antimicrobials, helping prevent resistance. To address these issues, we have developed several smart materials for effectively treating local and systemic infections before further resistance can develop, including microbe responsive hydrogels and novel polymer-drug conjugates.

Methods: Antimicrobial hydrogels: Gellan (1 to 4 wt %) hydrogels were formulated using calcium chloride (1 to 7 mM) in ultrapure water via ionotropic gelation. Solutions of gellan and salt were heated above approximately 120 °C and cooled, while mixing in up to 0.06 % w/v vancomycin hydrochloride. Enzyme-responsive hydrogels were developed using photo-polymerization of acrylated poly(ethylene glycol) (PEG) modified with a fungi responsive peptide (LRF(p-NO2))FLAPK (LFFK)) or gellan conjugated to CENTA™ while incorporating vancomycin or anidulafungin. Rheological properties of these hydrogels were characterized. Release studies: Hydrogel samples were released in physiologic and simulated infection conditions (37 °C, 1× phosphate buffered saline (PBS), HEPES buffered saline (HBS), pepsin, or penicillinase solutions). Drug release was monitored using a BioTek Cytation 3 UV-visible spectrophotometer. Polymer-antibiotic conjugate synthesis: Colloidal carbodiimide chemistry was used to conjugate several β-lactam antibiotics (oxacillin, nafcillin, carbencillin, penicillin G, and ampicillin) to hyaluronic acid (at three average molecular weights: 39, 150, and 1650 kDa) via an ester bond. Products were characterized using size exclusion chromatography and Fourier transform-infrared spectroscopy. In vitro and in vivo efficacy: Modified Kirby-Bauer assays and microdilution assays were used as previously reported (Shukla A. Small. 2010; 6:2392-404) to examine hydrogel activity against S. aureus 25923 (ATCC) and C. albicans 28366 (ATCC). Polymer-antibiotic conjugate activity was also tested using microdilution and Galleria mellonella assays against various methicillin resistant S. aureus (MRSA) strains.

Results: We have formulated highly tunable gellan- and PEG-based hydrogels for the encapsulation and release of antibiotics (e.g. vancomycin) and antifungals (e.g. anidulafungin) in response to virulence and resistance-conferring enzymes (e.g. beta-lactamases and secreted aspartic proteases) and other non-specific stimuli. Gellan hydrogels, were formulated to release drug over 6 to 9 days, with greater swelling of more tightly cross-linked gels leading to more rapid drug release. Gellan modified with a cephalosporin, CENTA™, was rendered β-lactamase responsive, as shown in Figure 1A.

Figure 1. Antimicrobial hydrogel response. A) β-lactamase responsive gellan-CENTA hydrogel (15 mm X 15 mm X 5 mm) in buffer versus penicillinase. B) Secreted aspartic protease responsive PEG-LFFK hydrogel drug release in pepsin versus buffer.

The LFFK peptide is cleaved by secreted aspartic proteases, produced by virulent Candida. PEG-LFFK hydrogels containing anidulafungin remained stable in HBS, while being rapidly degraded in the presence of pepsin, a model aspartic protease. These gels were stable for up to 4 weeks under acidic conditions (pH 2) with approximately 0.4% of the total loaded anidulafungin being released. In the presence of pepsin, the hydrogels released 2.1±1.3% of the total loaded drug within one hour, 64.9±10% after two hours, and 88.4±0.02% after twenty-four hours. Non-peptide containing hydrogels (i.e. pure PEG hydrogels) remained intact in pepsin for at least 24 hours, releasing just 0.05±0.003% of the total loaded drug.

We also developed versatile hyaluronic acid-β-lactam conjugates, which respond to pH changes and hyaluronidases, secreted by virulent bacteria, by releasing functional antibiotic. The conjugates were able to increase survival of Galleria mellonella injected with MRSA, compared to non-conjugated therapeutics for 5 days over 1 day. The conjugates can be injected, ingested, or used topically, while the hydrogels can fit any wound configuration.

Conclusions: We have developed multiple responsive hydrogels and polymer-drug conjugate systems that have the potential to control the release of antimicrobial agents and thus, lower the susceptibility of resistance. The smart antimicrobial materials we have developed could greatly improve infection treatment options. These materials can detect and treat infections locally, potentially eradicating infections more effectively and promoting rapid wound healing compared to current options.