Tunable Antibacterial Gellan Hydrogels for Burn Wounds

Shashank Shukla, Anita Shukla
School of Engineering, Center for Biomedical Engineering, Brown University

Statement of Purpose: Topical burn wound infections caused by bacterial pathogens affect approximately 100,000 people each year in the United States alone with a 50% mortality rate if left untreated (Wiersinga WJ. Virulence. 2014; 5:36-44). Current clinical treatments involve the repetitive administration of silver sulfadiazine through a variety of delivery systems daily. However, silver is highly toxic to keratinocytes and fibroblasts, key components of wound healing (Abedini F. Int. Wound. J. 2013; 10:573-8, Atiyeh BS. Burns. 2007, 33:139-148).

To address many of the complications associated with current treatments, we have developed an advanced hydrogel drug delivery system with tunable mechanical and release properties to treat burn infections. Hydrogels, with their high water content, have the potential to enhance tissue repair, by promoting cell viability and enhancing autolytic debridement. To effectively eradicate typical bacteria found in burn wounds, such as *Staphylococcus aureus (S. aureus)* and *methicillin resistant S. aureus*, we developed hydrogels containing vancomycin, a Food and Drug Administration (FDA) approved, "last resort" antibiotic. There is significant interest in development of effective local vancomycin delivery mechanisms in order to enable use of the drug while preventing resistance. To deliver vancomycin, we used gellan gum hydrogels. The structure of these hydrogels ranged from amorphous to sheet by simply altering polymer and ion concentration. To maximize drug loading and release duration, we fabricated gellan hydrogels containing vancomycin and vancomycin loaded activated carbon particles. Activated carbon is a highly porous form of carbon used often for molecular absorption. We demonstrated a longer vancomycin release from our constructs than currently available topical hydrogels such as AnaSept®, which release sodium hypochlorite for 24 hours and require daily reapplication (Anacapa Technologies, San Dimas, CA).

Methods:

Hydrogel fabrication: Vancomycin hydrochloride, Carboxen™ 1000 activated carbon, calcium chloride ($CaCl_2$) dihydrate, and Gelzan™ CM gellan gum were purchased from Sigma-Aldrich. Varying gellan and $CaCl_2$ concentrations generated amorphous and sheet hydrogels. Hydrogels were loaded with pure vancomycin and vancomycin loaded-activated carbon.

Mechanical properties: The Young's modulus of the sheet hydrogels was measured with a Bose EnduraTec ELF 3200 linear motion test instrument using 10% strain.

Release studies: Hydrogel samples along with activated carbon were loaded with 0.2 and 1 mg/mL vancomycin respectively for 3 hours and released in physiologic conditions (37°C, 1X PBS). Vancomycin was quantified with a BioTek Cytation 3 UV-visible spectrophotometer.

In vitro 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).

Results: To characterize our antibacterial hydrogel system, we examined drug release, mechanical tunability, and *in vitro* efficacy against *S. aureus*. An increase in both gellan and $CaCl_2$ concentration resulted in sheet hydrogels (Figure 1a), whereas lower concentrations resulted in an amorphous delivery system (Figure 1b). A 1:10 (w/w) vancomycin:activated carbon ratio resulted in 72.8 ± 0.1% loading with above minimum inhibitory concentration (MIC) (>1 μg/mL) release for 6 days. Drug release studies with activated carbon loaded hydrogels resulted in daily absolute release above MIC for 4 days, shown in Figure 1e. We are currently examining the effects of particle pore size and loading conditions on drug release.

Young's moduli of the sheet gellan, vancomycin loaded gellan, and vancomycin-loaded gellan with vancomycin-loaded activated carbon were as follows: 22.36 ± 13.7 kPa, 31.51 ± 17.8 kPa, and 36.18 ± 21.5 kPa, respectively. No statistical significance was observed indicating that vancomycin and activated carbon particles do not affect the structural integrity of the hydrogels.

In vitro modified Kirby-Bauer assays shown in Figure 1c-d demonstrated a clear zone of inhibition indicating that the hydrsogels (S) are effective against *S. aureus* after 18 hours, compared to a pure vancomycin control (+), and negative unloaded hydrogel control (-).

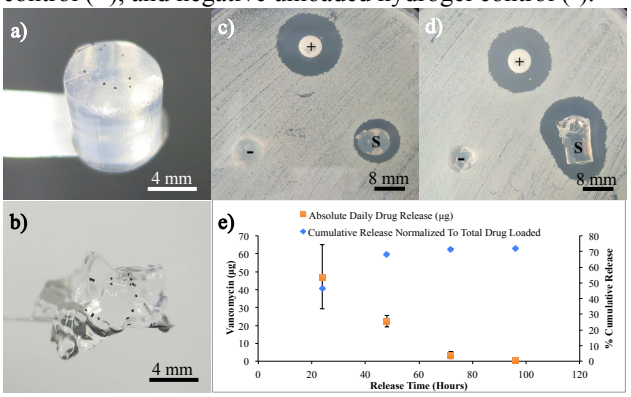

Figure 1: Sheet (a) and amorphous (b) hydrogels. Hydrogel efficacy against *S. aureus* in sheet (c) and amorphous (d) hydrogels. % Cumulative and absolute daily release of vancomycin from sheet hydrogels ($n = 3$, $p < 0.05$ using a two-tailed t-test) (e).

Conclusions: We have developed a unique antibiotic delivery system using entirely FDA approved materials and without covalent crosslinking modifications. Vancomycin is successfully released above MIC for varying durations of time as a result of the inclusion of drug-loaded activated carbon particles. Our hydrogel system eliminates the need for daily reapplication, and has the potential to improve patient compliance. We are currently testing *in vivo* efficacy of our hydrogels in a murine burn infection model.