Dual mechanism for toughening and sustained aminoglycoside elution from a polyphosphate hydrogel

Dwight D. Lane[a], Russell J. Stewart[a]

[a]Department of Bioengineering, University of Utah, 20 S. 2030 E., Salt Lake City, UT 84112, USA

Introduction: Bacteria preferentially colonize biomaterial and medical device surfaces, often leading to the formation of an exopolysaccharide matrix or biofilm, capable of increasing resistivity to antibiotics by up to 1000 fold.[1] As a result, infections in chronic wounds or on medical device surfaces may require hospital readmission, long courses of high-dose antibiotics, revision surgeries, culminating in patient discomfort, increased morbidity rates, and elevated health care costs.[2] Hydrogels have been explored as potential drug carriers with good biocompatibility. A major drawback has been the weak, brittle nature of hydrogels. Here we report on a polyphosphate hydrogel with a dual mechanism for both toughening and prolonging aminoglycoside drug release.

Materials and Methods: Polyphosphate hydrogels were formed by free radical polymerization of: methacrylated poly(phosphoethyl-methacrylate-co-acrylic acid) polymer (6.5 wt %), acrylamide and bis-acrylamide (1 wt %) with APS and TEMED as initiators. Hydrogels were then loaded with either 50 mM Ca^{2+}, 5 mM tobramycin or a combination of both. All loading solutions contained 150 mM NaCl and were pH adjusted to 7.7. Volume changes during loading were measured by photography and image J software. Hydrogels were immersed in 130 mM NaCl, plus 1.5 mM Mg^{2+} and 2.5 mM Ca^{2+} at pH 7.3, the solution was changed daily and tobramycin content measured colorimetrically to determine the release behavior. The initial modulus was measured using an Instron 3342 material test system and derived from the linear slope from the first 10% of compressive strain, errors are +/- 1 S.D., n=3. *P. aeruginosa* ATCC 27853 biofilms were grown and CFU quantified using Clinical Laboratory and Standards Institute (CLSI) standard protocols. Biofilms were exposed to tobramycin loaded hydrogels in a biomimetic flow cell with flow rate of 5 mL/h and CFU's counted after 24 and 72 h.

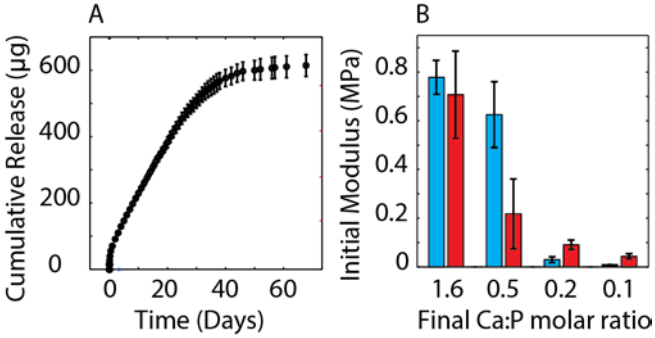

Figure 1: **A) Cumulative release of tobramycin from polyphosphate hydrogels. ~600 µg total elution with a daily release above the measured *P.Aeruginosa* minimum bactericidal concentration for greater than 50 days. B) Initial modulus of polyphosphate hydrogels with Ca^{2+} (blue) and after tobramycin loading (red). Groupings represent varying amounts of initial loaded Ca^{2+} and the axis shows the final ratios of calcium to phosphate following tobramycin loading. Error bars are +/- 1 S.D., n=3.**

Results and Discussion: Both divalent metal cations and positively charged drugs will electrostatically crosslink phosphates in the hydrogel structure.[3] Hydrogels shrank by ~50% after the addition of Ca^{2+} or tobramycin antibiotic, resulting from dense metal-phosphate or drug-phosphate crosslink formation.[3] Ca^{2+} has a toughening effect resulting in a compressive modulus of 0.8 +/-0.07 MPa, Fig. 1B. This is similar to cartilage 0.7 MPa and meniscus 0.2 MPa.[4,5] In contrast tobramycin loading has a limited toughening effect, 0.04 +/- 0.01 MPa compressive modulus, but results in prolonged release of tobramycin above the measured MBC for *P.Aeruginosa* (2 µg/ml) for greater than 50 days, Fig 1A. The prolonged release has been shown effective at total eradication of *P.Aeruginosa* biofilms (9 $\log_{10}$ reduction) in a biomimetic flow cell. Preliminary studies combining both Ca^{2+} crosslinks and tobramycin crosslinks in a single hydrogel have resulted in a hydrogel that is both tough, compressive modulus of 0.2 +/- 0.01 MPa, and maintains the prolonged release profile for tobramycin.

Conclusion: Polyphosphate hydrogels with the dual toughening and drug releasing mechanism could be capable of a structural role in arthroplasty procedures or as a protective bandage in wound care or surgery and still sustain local release of antibiotics.

References: [1] P.S. Stewart, Lancet 358 (2001) 135-138. [2] M.L. Schweizer, JAMA Surg. 149 (2014) 575-581.
[3] D.D. Lane, Soft Matter 11 (2015) 6981-6990. [4] J.S. Jurvelin, J. Biomech. 30 (1992) 235–241. [5] K.A. Sweigart, Proc. Inst. Mech. Eng. Part H J. Eng. Med. 219 (2005) 53–62.