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Sustained tobramycin release from polyphosphate double network hydrogels

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ABSTRACT

Sustained local delivery of antibiotics from a drug reservoir to treat or prevent bacterial infections can avoid many of the drawbacks of systemic administration of antibiotics. Prolonged local release of high concentrations of antibiotics may also be more effective at treating bacteria in established biofilm populations that are resistant to systemic antibiotics. A double network hydrogel comprising an organic polyphosphate pre-polymer network polymerized within a polyacrylamide network de-swelled to about 50% of its initial volume when the polyphosphate network was crosslinked with polycationic tobramycin, an aminoglycoside antibiotic. The antibiotic-loaded hydrogels contained approximately 200 mg/ml of tobramycin. The hydrogels continuously released daily amounts of tobramycin above the *Pseudomonas aeruginosa* minimal bactericidal concentration for greater than 50 days, over the pH range 6.0–8.0, and completely eradicated established *P. aeruginosa* biofilms within 72 h in a flow cell bioreactor. The presence of physiological concentrations of Mg^{2+} and Ca^{2+} ions doubled the cumulative release over 60 days. The polyphosphate hydrogels show promise as materials for sustained localized tobramycin delivery to prevent post-operative *P. aeruginosa* infections including infections established in biofilms.

Statement of Significance

Polyphosphate hydrogels were loaded with high concentrations of tobramycin. The hydrogels provided sustained release of bactericidal concentrations of tobramycin for 50 days, and were capable of completely eradicating *P. aeruginosa* in established biofilms. The hydrogels have potential for localized prevention or treatment of *P. aeruginosa* infections.

1. Introduction

Infection prevention or eradication is a pivotal concern in management of chronic or deep wounds, at surgical sites, and in invasive medical procedures [1–4]. This is especially true at the surfaces of indwelling medical devices, such as catheters, vascular grafts, orthopedic fixtures, periodontal implants, wound dressings, and contact lenses. Bacteria preferentially adhere to and colonize biomaterial and medical device surfaces, often leading to the formation of biofilms [5]. Biofilms are communities of bacteria with exopolysaccharide matrices secreted by adhered bacterial cells that increase their antibiotic resistance by up to 1000 fold [6–8]. Some resistance may stem from frustrated penetration of antibiotic into the biofilm, as in the case of positively charged aminoglycosides that absorb to negatively charged biofilm components [9,10]. Additionally, nutrient depletion across the biofilm induces a transition to anaerobic metabolism with far less susceptibility to aminoglycosides [11]. Along with these growth state changes it is known that some subset of the population (~1%) will experience a phenotypic change resulting in increased resistance to antibiotics. It is thought that these "persister" cells reseed the colony following antibiotic regimens [12,13]. As a result, infections in chronic wounds or on medical device surfaces may require hospital readmission, long courses of high-dose antibiotics, and revision surgeries [4,14,15]. All of this culminates in patient discomfort, increased morbidity rates, and elevated health care costs [15,16]. While rates of infection are low, for example 1.2% following

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arthroplasty and 3–6% in revision surgeries [17], both the large number of procedures performed annually and an aging population drive predictions for a significant increase in procedure acquired infections [17–20]. Thus, preventing and eliminating infections remains a pressing healthcare problem.

The traditional approach to preventing and treating infections is systemic delivery of antibiotics. Adverse effects of systemic antibiotic use include organ toxicity, reduction of beneficial bacteria populations, and overgrowth of naturally resistant organisms, like Candida and *Clostridium difficile* [21]. Sustained local delivery of antibiotics from a delivery device avoids many of the pitfalls of systemic delivery [22–24]. Hydrogels have been extensively explored as vehicles for localized drug delivery due to their high water content, excellent biocompatibility, and ease of drug loading [24–26]. Passive loading and release of antibiotics from hydrogels generally does not provide for significantly prolonged release [27,28]. Numerous strategies have been devised for sustained release from hydrogels [28] that include controlling microstructure and porosity to slow diffusion [29–32], coupling release to the slow degradation of the hydrogel matrix [33], covalent conjugation to the hydrogel matrix with degradable linkers [29,33–35], environmentally triggered volume changes that modulate diffusion kinetics [31,32,36,37], and thin surface films that restrict diffusion [30,31]. Diffusion of charged molecules can also be restricted and release prolonged by electrostatic interactions with charged groups in the hydrogel matrix [38–44].

Here, we report prolonged release of tobramycin, a polycationic aminoglycoside antibiotic, from a double network polyphosphate hydrogel [45]. The hydrogel provided sustained release of tobramycin above the minimal bacteriocidal concentration (MBC) for the *P. aeruginosa* strain tested and eradicated established biofilms. The reported hydrogels show promise as materials for delivering aminoglycoside antibiotics during knee arthroplasty or revision surgery, and other indications [17,46–48].

2. Materials and methods

2.1. Materials

Phosphorus(V) oxychloride, 2-hydroxyethyl methacrylate, triethylamine, and glycidyl methacrylate were purchased from Alfa Aesar (Ward Hill, MA). Methacrylic acid, 2,2′-azobis(2-methylpropionitrile), acrylamide, *N*,*N*′-methylene-bisacrylamide, and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine were purchased from Sigma Aldrich (St Louis, MO). Ammonium persulfate, FilmTracer™ LIVE/DEAD Biofilm Viability kit, Brain heart infusion (BHI) broth and cation adjusted Mueller Hinton broth (CAMHB) were purchased from Fischer Scientific (Pittsburgh, PA). Tobramycin (97%) was acquired from Acros Organics (New Jersey, USA). *P. aeruginosa* ATCC 27853 was purchased from the American Type Culture Collection (Manassas, VA). This strain was chosen because it is well-characterized and representative of pathogenic *P. aeruginosa*. *In vitro* results with ATCC 27853 have been shown to correlate with *in vivo* pathogenicity [49–52]. Columbia blood agar plates were purchased from Hardy Diagnostics (Murray, UT).

2.2. Synthesis of MOEP monomer and polyPEMA-MA copolymers

2-(Methacryloyloxy)ethyl phosphate (MOEP) was synthesized as previously described [53]. Briefly, phosphorus oxychloride (33.9 g, 220 mmol) and hydroxylethyl-methacrylamide (HEMA) were mixed at a 0.7:1 M ratio in dry toluene (480 mL) under argon. The reaction was stirred at 4 °C while triethylamine (TEA) (77 mL) was added in three increments over 10 m. Following addition of TEA, the reaction was stirred at 22 °C for 6 h under argon, then filtered to remove precipitated salt. The reaction was cooled to 4 °C before the addition of deionized water (480 mL), then stirred under argon at 22 °C for 12 h. The reaction was extracted twice with diethyl ether (100 mL) and the organic layers were discarded. The aqueous layer was extracted using tetrahydrofuran (THF) and diethyl ether (1:2, 12 × 225 mL), then dried over anhydrous sodium sulfate before evaporating the solvent. The monomer structure was verified by ^{1}H and ^{31}P NMR (Fig. S1A and B).

Poly(phosphoethyl-methacrylate-co-acrylic acid) (pPEMA-AA) was synthesized by free radical polymerization of MOEP (85 mol %), and methacrylic acid (15 mol%) in methanol (12.5 mL mg^{-1} MOEP). MOEP and methacrylic acid were degassed by bubbling with argon for 1 h, followed by addition of azo-bis-isobutyronitrile (AIBN, 4.5 mol%) at 55 °C to initiate polymerization. The reaction was stirred for 15 h before precipitation with acetone, then dissolved in water (200 mL H_2O per 17 g pPEMA-AA). Subsequently, methacrylate groups (MA) were grafted onto the methacrylic acid sidechains with glycidyl methacrylate in 9-fold molar excess relative to the carboxylate sidechains. The methacrylated pPEMA-AA (pPEMA-MA) was adjusted to pH 7.3 with NaOH and purified by tangential flow filtration using a Millipore Pellicon 3 cassette filter with an Ultracel 10 kD membrane. The polymer product was lyophilized, and stored at −20 °C.

The molecular weight (Mw) and polydispersity index (PDI) of pPEMA65-MA23 were determined by size exclusion chromatography (SEC) on an Agilent Infinity 1260 HPLC system with a PL-Aquagel-OH Mixed-M column (8 μm, 300×7.5 mm) column. The column buffer was 0.1 M $NaNO_3$/0.01 M NaH_2PO_4, pH 8.0 with a flow rate of 1 mL m^{-1}. The average M_m and PDI, 46 kg mol^{-1} and 1.67, respectively, were estimated using sodium polymethacrylic acid standards. The structure and composition of the polymers were verified by ^{1}H and ^{31}P NMR (Fig. S1C). The phosphate prepolymer contained 65 mol% phosphate sidechains, 12 mol% HEMA, and 23 mol% MA sidechains. The copolymers are referred to as pPEMA65-MA23 to indicate the mol% phosphate and methacrylate sidechains.

2.3. Hydrogel synthesis: copolymerization of polyPEMA65-MA23 and acrylamide

Hydrogels were formed by free radical polymerization of the pPEMA65-MA23 prepolymer with acrylamide (Aam) and N,N-methylenebisacrylamide (bis-Aam), a bifunctional crosslinker, in 150 mM NaCl (Fig. 1A and B). The wt% of pPEMA65-MA23 and Aam/bis-Aam were 6.5% and 1.0%, respectively. The molar ratio of Aam to bis-Aam was 60:1. Polymerization was initiated by adding 10% ammonium persulfate (APS) and tetramethylethylenediamine (TEMED) to final concentrations of 70 μg ml^{-1} and 2.4 μl ml^{-1}, respectively. Following addition of APS and TEMED, 250 μl aliquots were placed in wells of a 48 well plate with 200 μl of 2-propanol floated on top to limit oxygen exposure. After 90 m, polymerized hydrogel disks were removed and soaked in 150 mM NaCl for 24 h to remove unreacted reagents.

2.4. Hydrogel loading and measurement of tobramycin concentrations

Uniform 250 μl hydrogel disks were immersed in 2 M NaCl and 5 mM tobramycin adjusted to pH 7.7 with HCl for 8 h. The salt concentration was then diluted to 150 mM NaCl while maintaining the 5 mM tobramycin concentration at pH 7.7 for a total of 24 h (Fig. 1C). Initial loading in 2 M NaCl prevented cracks and defects in the hydrogels caused by rapid deswelling. Time lapse videos of hydrogel deswelling during tobramycin loading were analyzed in Image J to measure hydrogel diameters. Isotropic shrinking was assumed to calculate volume changes from the diameter.