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Covalently-crosslinked mucin biopolymer hydrogels for sustained drug delivery

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ABSTRACT

The sustained delivery of both hydrophobic and hydrophilic drugs from hydrogels has remained a challenge requiring the design and scalable production of complex multifunctional synthetic polymers. Here, we demonstrate that mucin glycoproteins, the gel-forming constituents of native mucus, are suitable for assembly into robust hydrogels capable of facilitating the sustained release of hydrophobic and hydrophilic drugs. Covalently-crosslinked mucin hydrogels were generated via exposure of methacrylated mucin to ultraviolet light in the presence of a free radical photoinitiator. The hydrogels exhibited an elastic modulus similar to that of soft mammalian tissue and were sensitive to proteolytic degradation by pronase. Paclitaxel, a hydrophobic anti-cancer drug, and polymyxin B, a positively-charged hydrophilic antibacterial drug, were retained in the hydrogels and released linearly with time over seven days. After four weeks of drug release, the hydrogels continued to release sufficient amounts of active paclitaxel to reduce HeLa cell viability and sufficient amounts of active polymyxin B to prevent bacterial proliferation. Along with previously-established anti-inflammatory, anti-viral, and hydrocarbon-solubilizing properties of mucin, the results of this study establish mucin as a readily-available, chemically-versatile, naturally-biocompatible alternative to complex multifunctional synthetic polymers as building blocks in the design of biomaterials for sustained drug delivery.

1. Introduction

Modern drug development relies inextricably on the concomitant development of appropriate drug delivery systems. The cost, safety, and bioavailability of drugs are greatly improved when the therapeutic molecules are delivered with precise control over delivery rates and localization. Hydrogels, hydrophilic polymer networks capable of absorbing thousands of times their mass in water, have shown significant promise as vehicles for controlled drug delivery [1,2]. Drug molecules are loaded into the porous hydrogel matrix, allowing for controlled release of the drug from the hydrogel over time with limited immunogenicity. The aqueous nature of hydrogels, however, presents a challenge because water-soluble molecules may simply diffuse out of the delivery material unhindered. Loading hydrophobic drugs into hydrogels presents another difficulty because of the incompatibility between the hydrophilic hydrogel polymer network and hydrophobic drug molecules. Insoluble in the aqueous hydrogel matrix, hydrophobic drugs tend to precipitate or simply release from the hydrogel in a rapid burst [1]. Though burst release patterns are useful for certain treatments, they are most often detrimental to the pharmacological and economical performance of the drug delivery system [3].

Chemical engineers have thus far addressed problems of drug delivery control in hydrogel-based drug delivery systems by designing nanodomain-structured materials, such as block copolymers, that bind to drug molecules through electrostatic [4,5] and covalent bonds [6,7] as well as hydrophobic interactions [8,9], delaying diffusion of the drug out of the material. More complex assemblies combining electrostatic and hydrophobic interactions have been developed to co-deliver hydrophilic and hydrophobic drugs [10–13] to thereby trigger drug synergy or suppress drug resistance. For example, co-delivery of paclitaxel with an interleukin-12-encoded plasmid by nanoparticles suppresses cancer growth more effectively than the paclitaxel or the plasmid alone [13]. However, scaling up the production of such complex macromolecules is difficult and costly. Furthermore, the polymerization techniques used to create synthetic multifunctional hydrogel polymers frequently require cytotoxic catalysts, such as copper, that limit the hydrogels' biocompatibility and potential to reach the

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market [14]. In this work, we bypass the difficulty and expense of synthetic polymer engineering by exploiting a readily-available gel-forming biopolymer that naturally contains sufficiently various chemical moieties to bind diverse classes of molecules.

Mucin, a high-molecular mass glycoprotein, is the primary structural component of the mucus covering all wet epithelia, including within the nose, mouth, lungs, gastrointestinal tract, and female genital tract. Millions of years of evolution have endowed mucin the ability to bind a wide variety of molecules to protect organisms against viruses [15,16], bacteria [17,18], and small particles [19]. Mucin molecules are indeed equipped with an optimized and diverse chemistry: their protein backbone, rich in thiol groups, contains both hydrophobic and charged domains. Additionally, oligosaccharides attached to the mucin protein backbone provide intramolecular and intermolecular hydrogen bonding capabilities, high hydration and hydrophilicity, and carboxyl and sulfate groups that confer a net negative charge to mucin at neutral pH [20]. As a result of its unique chemistry, mucus constitutes a major biophysical barrier to the oral delivery of most drugs [21], binding and retaining the intended therapeutic molecules in its matrix [22]. Though the mucosal barrier presents a vexing problem for oral drug delivery, an opportunity emerges whereby mucin–drug interactions could be exploited to retain and release multiple types of drugs over long periods of time using mucin-based biomaterials.

To most effectively harness the properties of mucin to bind and release drugs, the mucin must first be assembled into a material that is easily handled and that leaves its biophysical properties intact. Mucin-based biomaterials in the form of thin films have been assembled layer-by-layer, complexing mucin with lysozymes [23], chitosan [24,25], or lectins [26,27]. Microparticles composed of mucin–alginate [28] or mucin–gelatin [29] complexes also have been created. However, the ability of solely mucin-based biomaterials to retain and release small drug molecules has not previously been investigated, nor has a robust macroscopic covalently-crosslinked mucin hydrogel previously been developed. In this work, we assemble methacrylated mucin into covalently-crosslinked hydrogels to study the drug binding and release capabilities of the mucin molecule. We characterize mucin hydrogels rheologically and investigate the loading and release from mucin hydrogels of polymyxin B, a positively-charged hydrophilic antibacterial drug, and paclitaxel, a hydrophobic anti-cancer drug. We demonstrate the sustained release from mucin hydrogels of both hydrophilic and hydrophobic model drugs, the absence of toxicity of mucin hydrogels on bacterial and mammalian cells, and the capacity of mucin hydrogels to load and retain sufficient quantities of active drug to maintain an antibacterial and anti-cancer effect over several weeks.

2. Materials and methods

2.1. Materials

Bovine submaxillary mucin (BSM, Sigma–Aldrich, Lot SLBC2523V) was dissolved at 10 mg/mL in water, filtered through a 0.45 μm filter (Bottle-Top Vacuum Filter, Corning) to remove insoluble aggregates, then further purified by dialysis (100 kDa cutoff, Spectrum Labs) against ultrapure water for 4 days to remove protein contaminants. BSM methacrylation was conducted with methacrylic anhydride (MA, Sigma–Aldrich) used as described below. Hydrogel formation was induced by free radical photoinitiator 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959, Sigma–Aldrich). Model drugs selected were paclitaxel (Invitrogen) and polymyxin B (Invitrogen) and their fluorescently-labeled versions, paclitaxel Oregon Green 488 conjugate (Invitrogen) and polymyxin B BODIPY FL conjugate (Invitrogen). Paclitaxel stock solution was prepared in dimethyl sulfoxide (DMSO) at 5 mg/mL, and polymyxin B stock solution was prepared in water at 5 mg/mL. Fluorescein isothiocyanate-labeled dextran of various molecular masses (5, 20, 40, 250, 2000 kDa) was obtained from Sigma–Aldrich. Polyglutamic acid (PGA, 25 kDa) was obtained from Sigma–Aldrich and fluorescently labeled by activating the carboxyl groups with *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (64.5 mM, Sigma–Aldrich) and *N*-hydroxysulfosuccinimide (4.6 mM, Sigma–Aldrich) for 30 min at room temperature, removing the reaction byproducts by centrifugation filtering (10 kDa cutoff, Spectrum Labs), and reacting with 5(6)-aminofluorescein (Sigma–Aldrich) in dimethylformamide solvent. After 1 h at room temperature, excess 5(6)-aminofluorescein was removed by repeated centrifugation filtering. For degradation experiments, α-chymotrypsin from bovine pancreas (Sigma–Aldrich) and protease from *Streptomyces griseus* (Sigma–Aldrich) were used.

2.2. BSM-MA synthesis

BSM was dissolved at 10 mg/mL in ultrapure water. The solution was adjusted to pH 8 using sodium hydroxide (NaOH) and put on ice. Once cooled, a solution of MA was added to the solution at an MA to BSM mass ratio of 0.832%. The solution was gently stirred for 6 h while maintaining a pH of approximately 8 using a 5 M NaOH solution, then placed overnight at 4 °C with gentle stirring. The solution was then centrifuged to remove excess MA and mucin precipitates and dialyzed for 2 days with 2 changes of water per day. The resulting methacrylated bovine submaxillary mucin (BSM-MA) was lyophilized and stored at −20 °C until use.

2.3. BSM-MA hydrogel formation

BSM-MA hydrogels were formed by dissolving BSM-MA at a concentration of 40 mg/mL in phosphate buffered saline (PBS) for 1 h at 4 °C. Then, Irgacure 2959 photoinitiator dissolved in ethanol at a concentration of 100 mg/mL was added to the solution at a final concentration of 0.5 mg/mL, and the solution was immediately mixed by vortex before being placed in a mold. Molds used were wells of a 96-well plate or custom-made molds composed of 5 mm thick polydimethylsiloxane (PDMS) punctured with 8 mm diameter holes. The solution was then exposed to 365 nm wavelength ultraviolet light with exposure power of ~10 mW/cm^2 for 10 min to allow formation of the BSM-MA hydrogel. After formation, the hydrogels were unmolded and placed in fresh PBS solution.

2.4. Scanning electron microscopy (SEM)

BSM-MA hydrogel slabs, 8 mm in diameter, were dehydrated in baths of increasing ethanol concentration (50, 70, 80, 90, 95, 99, 100% ethanol, 10 min each) followed by baths of hexamethyldisilazane (2 baths of 10 min each). The resulting dehydrated hydrogels were further air-dried overnight, cut open, mounted on a carbon tape, sputter coated with a 10 nm gold layer, and observed with a JOEL 6010LA scanning electron microscope.

2.5. BSM-MA hydrogel degradation

BSM-MA hydrogels (200 μL) were formed in 2 mL tubes, washed 3 times with 1 mL PBS, then immersed in 400 μL of either PBS (supplemented with 25 mM Ca^{2+}), α-chymotrypsin (400 μg/mL, 16 units/mL) dissolved in PBS with 25 mM Ca^{2+}, or pronase (400 μg/mL, 1.4 units/mL) dissolved in PBS with 25 mM Ca^{2+}. The

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