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Stretchable, anti-bacterial hydrogel activated by large mechanical deformation

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ABSTRACT

Hydrogels have been used extensively to deliver functional molecular cargos in response to external mechanical force. However, the intrinsic brittleness of gels restricts the applicable range of strain to 0.1, thus limiting the range of molecular release rate that may be controlled. Also, uncontrollable molecular diffusion, which is especially prominent in small molecules, reduces the role of mechanical stimulus on the release rate. As such, we hypothesized that these challenges would be resolved by combining cyclodextrin, which may form guest-host complexes with small molecular cargos, with a stretchable hydrogel system. We examined this hypothesis by synthesizing cyclodextrin acrylate and incorporating it into a polyacrylamide gel that can be stretched by 100% of its original length. In the absence of external stretching, hydrogels containing cyclodextrin acrylate with a degree of acryloyl group substitution (DS_A) of 2.3 presented a lower molecular release rate than hydrogels without cyclodextrin acrylate. More interestingly, the polyacrylamide-cyclodextrin hydrogel system displayed an increased molecular release rate corresponding to the degree of stretching, particularly in the gels containing cyclodextrin acrylate with a DS_A of 2.3. As such, this stretchable gel loaded with quinine was used to inhibit the growth of *E. coli* in lysogeny broth only when the gel was stretched. We believe the results of this study would be valuable for improving the quality of controlled molecular delivery and subsequent efficacy of molecular cargos.

1. Introduction

In living organisms, mechanical forces play crucial roles in the transportation of bioactive molecules (e.g., growth factors, cytokines, and hormones) responsible for development, homeostastis, and regeneration [1–4]. In particular, compressive and tensile stress exerted during daily activities and exercise promote the pulsatile release and transportation of bioactive molecules which are sequestered within the extracellular matrix (ECM) [5,6]. For example, static pressures ranging from 80 to 140 mmHg induce apoptosis in human mesangial cells via increased connective tissue growth factor expression [7]. Transport of ribosomes towards focal adhesions in cells can be induced by the application of mechanical stress on the order of tens of dynes per cm^2 [8]. Through the process of mechanical stimulation, molecules can regulate phenotypic activities of biological cells within the same or distant tissue. Inspired by this biotransport phenomena, efforts have emerged to assemble materials that release molecular cargos of interests, such as pharmaceuticals, nutrients, anti-bacterial agents, food supplements, and pesticides, in response to external forces [9–12].

Within this class of materials, hydrogels formed from the cross-linking or self-assembly between hydrophilic molecules have garnered much attention because of the ability to tune the chemical and mechanical properties [13,14]. These features make the material useful for interfacing with biology and other soft matter. Mechanical forces can deform the cross-linked structure of these gels and subsequently prompt molecular release. In hydrogels, it is possible to control the molecular release rate in response to a stress by adjusting parameters such as the elastic modulus of the gel, molecular structure of the cross-links, and the applied force [15–17]. However, many hydrogel systems are fragile under the external forces necessary to induce a substantial change in the molecular release rate. As such, many of hydrogels can only withstand a limited degree of strain [18]. Additionally, most small molecular cargos loaded in the gel passively diffuse out rather than being controlled by external forces, unless there are molecular cues that can

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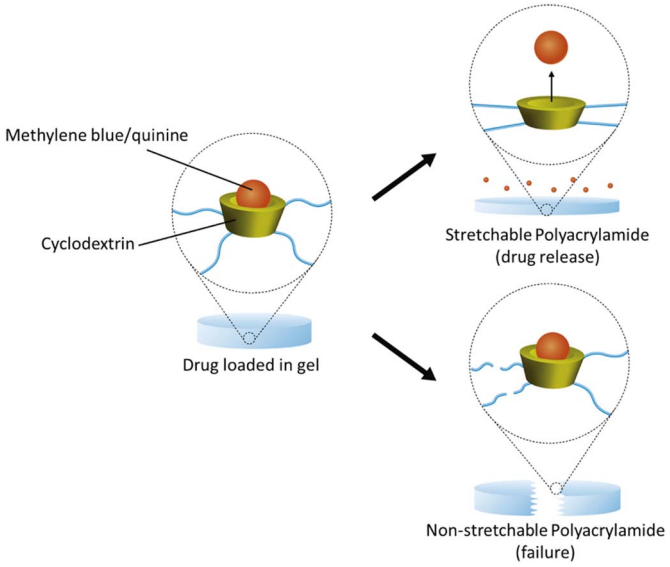

Scheme 1. Schematic depicting the controlled release of molecular cargos from polyacrylamide-cyclodextrin hydrogel by mechanical stretching. The molecules (i.e., methylene blue and quinine) are sequestered by cyclodextrin units in the gel, forming guest-host complexes. The tough polyacrylamide-cyclodextrin gel can be utilized to release the molecular cargos upon stretching, while the non-stretchable polyacrylamide gel fractures when stretched.

sequester and retain them [16,19].

To this end, other attempts have been made to harness mechanical forces to stimulate controlled molecular release profiles. One example is a bi-layered material formed by placing a buckled polystyrene layer on poly(dimethylsiloxane) (PDMS) with molecules entrapped in the resulting microcapsules. The bi-layered material showed an increased molecular release when the material was stretched [20]. Efforts were also made to address this challenge by assembling a silicone rubber-based elastomer loaded with drug-releasing microgel depots [21]. A major limitation in each of these strategies is that drug molecules loaded in the microcapsules or microgel particles are still released by diffusion even without external stretching.

To resolve these challenges, we hypothesized that by combining a soft and stretchable hydrogel system with molecular sequestration depots, we could regulate molecular release rate over a broad range by utilizing varying magnitudes of external force (Scheme 1). To examine this hypothesis, we used a polyacrylamide gel formulated with the ability to be stretched more than ten times its original length as a model stress-responsive hydrogel. β-cyclodextrin was used as a model depot in which molecular cargos are hydrophobically entrapped [22–24]. These cyclodextrins were modified with a controlled number of acryloyl groups in order to be linked to the gel during the polymerization reaction. By doing so, external forces would result in a conformational change in cyclodextrin, thus prompting the molecular release. In the past, there have been studies to examine stress-dependent molecular release from a gel chemically coupled with cyclodextrin. However, due to the limited deformability of the gel, only a small range of compressive strain (i.e., < 0.5) was applied to deform the rigid cyclodextrin in a gel [25]. Other studies used cyclodextrin to limit drug release from a viscous poly(ethylene oxide)–poly[(r)-3-hydroxybutyrate]–poly(ethylene oxide) gel without the influence of strains [26].

We first examined the extent that the cyclodextrin with controlled degree of acryloyl substitution modulates stiffness, ultimate strength, and ultimate strain of the stretchable polyacrylamide gel. Next, the gel's capability to release molecular cargos in response to varying strains up to 1.0 was examined by loading methylene blue and quinine as model drug molecules that form guest-host interactions with cyclodextrin [27,28]. For all these experiments, we utilized a polyacrylamide gel free of cyclodextrin as a control since the loaded molecules would diffuse out, regardless of the external force. Finally, the large strain-induced efficacy of the molecular releasing system was evaluated by loading the anti-bacterial molecule, quinine, into the stretchable polyacrylamide-cyclodextrin gel and by examining bacterial growth in solution with the gel while varying the degree of stretching. Overall, the results of this study will serve to greatly improve the quality of molecular delivery for a wide array of applications related to health science and the environment.

2. Materials/methods

2.1. Synthesis of β-cyclodextran acrylates

Cyclodextrin was modified with various amounts of acryloyl groups following a simple, previously reported procedure [29]. Briefly, a 0.43 M solution of KOH (1.51 g) in deionized water (62.5 mL) was prepared. β-cyclodextrin (5 g, Sigma-Aldrich) was added to the solution at 0.08 g per mL. Once dissolved, the solution was brought to 0 °C by placing it in an ice bath. Then, acryloyl chloride was added to the solution dropwise, slowly, and with mixing in a molar excess to cyclodextrin at 1.5×, 3×, and 5× depending on the desired degree of substitution of acryloyl groups on cyclodextrin (DS_A). After the addition, the solution was stirred at 40 °C for 6 h. The white precipitate that formed during this process was filtered off and disposed. The remaining filtrate was added to a large amount of acetone, which yielded another white precipitate. This precipitate was dried and retained until usage.

2.2. Synthesis of the stretchable polyacrylamide-cyclodextrin gel

β-cyclodextrin acrylate was conjugated to the polyacrylamide gel via radical polymerization by cross-linking the molecule along with acrylamide monomers to form a gel. The desired amount of β-cyclodextrin acrylate (from 0 to 50 mg) was dissolved in 1 mL of 40% w/v aqueous solution of acrylamide. Next, 4 μL of 2% w/v methylene bis-acrylamide and 10 μL of 10% w/v ammonium persulfate were added into the solution of acrylamide and β-cyclodextrin acryate followed by addition of 2 μL of tetramethylene diamine to make the stretchable gel. The molar ratio of methylene bis-acrylamide to acrylamide was kept constant at either 1:63 for the preparation of the non-stretchable gel or 1:10800 for the stretchable gel. After mixing for 10 s, the solution was quickly poured and sandwiched between two glass plates separated by glass slides used as spacers with separation of 1.5 mm. The resulting gel was removed and incubated in water overnight.

2.3. Analysis of physical properties of the polyacrylamide-cyclodextrin gel

Polyacrylamide-cyclodextrin gels were cut into strips of 1 cm in width and placed on a mechanical tensile tester (MTS Insight 1 kN, MTS Systems Corporation) to characterize the mechanical properties of the gel. The clamps were positioned 1 cm apart and the gel was stretched at a rate of 1 cm/min until it failed. The recorded tensile stress vs. strain data obtained from the measurement was used to quantify the elastic modulus, the ultimate strength and strain, and the fracture energy. Briefly, the tensile elastic modulus was measured from the slope of the stress vs. strain curve for the first 10% of strain (i.e., elastic region). The ultimate strength and strain were simply recorded as the final, measured stress and strain before a tear or fracture of the gel. The fracture energy was evaluated as the total area under the stress vs. strain curve until the point of fracture. The degradation of the hydrogels was evaluated by incubating gels with and without cyclodextrin acrylate in PBS with shaking over 2 weeks and measuring the swelling ratio of the gels at various time points.