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Branched polyrotaxane hydrogels consisting of alpha-cyclodextrin and low-molecular-weight four-arm polyethylene glycol and the utility of their thixotropic property for controlled drug release

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ABSTRACT

In this work, we developed a new class of branched polyrotaxane hydrogel made of 4-arm polyethylene glycol (4-PEG) and α-cyclodextrin (α-CD) using supramolecular host-guest interactions as a cross-linking strategy. Because of the dynamic nature of the non-covalent host-guest cross-linking, the resulting supramolecular α-CD/4-PEG hydrogels show thixotropic behavior and undergo a reversible gel-sol transition in response to shear stress change. We loaded the antiglaucoma drug brimonidine into the α-CD/4-PEG gel and found the drug release kinetics was controlled by shear stress. This thixotropic shear thinning property makes the supramolecular hydrogels highly attractive in drug delivery applications and suitable for preparation of injectable drug formulations.

1. Introduction

Supramolecular hydrogels based on host-guest complexation interactions have been recognized as a fascinating class of soft materials with many potential applications in drug delivery and tissue engineering [1–5]. Unlike conventional polymer hydrogels typically cross-linked with covalent bonds, supramolecular hydrogels display a wide range of stimuli-responsive behaviors, mechanical properties, and even self-healing capacities in that the host-guest complexation is dynamic and reversible [6–11]. Cyclodextrin (CD) is a series of cyclic oligomers with 6–12 D-glucopyranose units. Of most common CDs are α-CD, β-CD and γ-CD, which contain 6, 7, and 8 glucopyranose units, respectively [12–15]. The first reported host-guest complexation utilizing α-CD is an inclusion complex formation between α-CD and polyethylene glycol (PEG) [16]. PEG chains penetrate into the cavities of α-CDs to form pseudopolyrotaxanes, which are further strengthened via hydrogen bonds between neighboring α-CDs on the same PEG chain [17–19].

PEGylated nanoparticles, PEO-grafted polymers, and micelles made of PEG-based amphiphilic copolymers have been successfully utilized as multi-valent building blocks to form supramolecular hydrogels with α-CD [20–28]. These supramolecular hydrogels are polyrotaxane complexes formed through the interaction of α-CD and PEG chains [29]. The sliding of polyrotaxane rings makes polyrotaxane gels have interesting mechanical properties [30]. Takeoka group reported a series of extremely stretchable and tough thermosensitive hydrogels by introducing polyrotaxane cross-linkers into the polymer network [31,32]. In these systems, labor-intensive synthesis and purification are often needed as a variety of chemicals such as catalysts, initiators as well as solvents are used. Nonetheless, low-molecular-weight linear PEGs (M_n < 2 kDa) hardly form hydrogel with α-CD because the inclusion between α-CD and PEG has to engage both ends of PEG chains, requiring non-complexed PEG chain portions to be long enough to form a network [23]. While high-molecular-weight linear PEGs can more effectively interact with α-CD to form pseudopolyrotaxanes and then gels, their inefficient systemic clearance due to large hydrodynamic radius make themselves less pharmacokinetically appealing [33]. Therefore, a facile green method to prepare α-CD/PEG supramolecular hydrogels using low-molecular-weight PEG derivatives would be most desirable.

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Table 1
α-CD and 4-PEG mixed at varied host/guest (H/G) molar ratios.

	H/G molar ratio	α-CD (mg/mL)	4-PEG (mg/mL)
TH^{50}	50/1	100	21
TH^{20}	20/1	100	52
TH^{10}	10/1	100	103
TH^{4}	4/1	100	257

In this work, we studied whether we could employ the inclusion complexation mechanism to form a supramolecular hydrogel using α-CD as a host molecule and low-molecular-weight 4-arm polyethylene glycol (4-PEG) as a guest molecule to form branched polyrotaxanes. 4-PEG contains four short-chained PEGs, each of which has a molecular weight of 2500 Da. The ratio of host molecule to guest molecule was varied, and its effect on rheological properties and solidification time was studied. Supramolecular hydrogels formed on the basis of host-guest complexations may show thixotropic shear-thinning properties in that inclusion complexation is driven by dynamic and reversible noncovalent interactions [25,34,35]. Such a property enables pre-formed supramolecular hydrogels with injectability, an attractive feature in gene/drug delivery applications [36–42]. To this end, we studied the thixotropic behavior of the resulting supramolecular hydrogels. The antiglaucoma drug brimonidine was loaded into α-CD/4-PEG hydrogel, and the drug release kinetics was investigated by subjecting the drug formulation to shear force change.

2. Experimental section

2.1. Materials

α-CD (≥98%), brimonidine (free base) and tartrate solutions were purchased from Sigma-Aldrich. Four-arm polyethylene glycol (4-PEG, M_n = 10,000 g/mol) was purchased from Xiamen SINOPEG Biotech (Fujian, China).

2.2. Preparation of supramolecular hydrogels

α-CD (34 mg) was dissolved in 340 μL of PBS, and the solution was shaken for hours for complete dissolution. 4-PEG was then added to the solution. The host/guest (H/G) molar ratio of α-CD to 4-PEG varied from 50/1, 20/1, 10/1, to 4/1 (Table 1). The mixture was vortexed for 30 s and then allowed to stand still for solidification.

2.3. Microscopy

The morphology of the supramolecular hydrogels (H/G 50/1, H/G 20/1, and H/G 10/1) was investigated with scanning electron microscopy (SEM, JEOL LV-5610) or field emission SEM microscopy (Hitachi FE-SEM Su-70). Lyophilized supramolecular hydrogel samples were coated with platinum for 90 s using an ion sputter.

2.4. Rheology

Rheological measurements at 25 °C were carried out on Discovery Hybrid Rheometer HR-3 (TA Instruments) and a 20 mm parallel plate geometry was used. An amplitude sweep was first performed at a constant angular frequency of 1 rad/s in the strain range of 0.1%–10%. Within the linear viscoelastic region (LVR), oscillatory frequency sweeps were then carried out under a constant strain of 0.5% in the frequency range of 0.1–100 rad/s. To investigate the sol-gel transition of the supramolecular hydrogel, continuous step strain measurements at alternate strain 0.5% (within LVR) and 20% (outside LVR) at a frequency of 1 Hz and 150 s time intervals were performed. A flow sweep cycle (viscosity *vs.* shear rate) was carried out from 0.01 s^{-1} to 1000 s^{-1}.

2.5. Cytotoxicity

The cytocompatibility of the supramolecular hydrogel was studied. NIH3T3 fibroblasts cells were seeded in a 96-well plate at a density of 1×10^4 cells/well. After 24 h of cell attachment, the culture medium was replaced with 200 μL of fresh medium containing the gel at different concentrations. Cell viability after 48 h-incubation was determined by using WST-1 assay.

2.6. In vitro drug release kinetics

The saturated solubility of brimonidine in PBS and 10 wt% α-CD PBS solution was first determined. Briefly, 2 mg brimonidine was added to 1 mL PBS or 1 mL PBS solution containing10 wt% α-CD. The mixtures were then sonicated for 1 h and allowed to stand overnight. The supernatants were diluted 25-fold with PBS, and drug concentrations in the dilutions were analyzed with HPLC. Brimonidine was in-situ loaded to TH^{50} at its saturated concentration. Specifically, 34 mg α-CD, 7 mg 4-PEG, and 238 μg brimonidine were mixed in 340 μL PBS in a 20-mL centrifuge tube and thoroughly vortexed. After standing for 5 min, a solid brimonidine-loaded gel formed at the bottom of the tube. To the tube was added 10 mL of PBS. The tube was positioned on a MaxQ2000 orbital at a speed of 0, 100, or 300 rpm to generate orbital shear stress at various levels on a relative scale [43]. Drug release kinetics in response to shear stress variation was then investigated. At each pre-determined time point, 1 mL of supernatant was taken out, and drug concentration in the supernatant was quantified with a Waters reverse phase HPLC system. An equal volume of fresh PBS (1 mL) was added to the tube to maintain a constant volume of release medium. The experiment was repeated three times.

3. Results and discussion

3.1. Preparation and characterization of the supramolecular hydrogel

In general, when α-CD and linear PEG are mixed, α-CD molecules are threaded by a single PEG chain, resulting in the formation of a polyrotaxane. However, using branched PEGs to form branched polyrotaxanes with α-CD has not been tested before. In this work, we used this supramolecular interaction as a cross-linking strategy to form branched polyrotaxane hydrogel on the basis of α-CD and 4-PEG (Fig. 1**a**). A solid hydrogel formed within 2 min after mixing α-CD and 4-PEG when the host/guest molar ratio was 50/1 (Fig. 1**b**). The SEM images illustrate an interconnected porous 3D network structure (pore size: 2 ~ 5 μm) of TH^{50} (Fig. 1**c**). TH^{20} (Fig. 1**d**) and TH^{10} (Fig. 1**e**) also show a macroporous morphology; however, the pore size increased to tens of micrometers, indicating cross-linking density decrease. Keeping the concentration of α-CD constant at 100 mg/mL, we tested three other host/guest molar ratios as shown in Table 1 and tested the oscillatory frequency sweep of the mixture formulations. Table 1 also shows that the solid content varied with the host/guest ratios: 121 mg/mL gel for TH^{50}, 152 mg/mL gel for TH^{20}, 203 mg/mL gel for TH^{10}. TH^{50}, TH^{20} and TH^{10} not only possess storage modulus (G') higher than their loss modulus (G"), their G' are also frequency-independent (Fig. 2). Such rheological behaviors are typical of a hydrogel. However, TH^{4} exhibited a rheological behavior of solution as its G' was lower than its G" (Fig. 2). This result suggested that the host/guest ratio of 4:1 is not high enough to form an effective cross-linked network. Although 20/1 host/guest ratio can form a hydrogel network, the modulus is still lower than TH^{50}.