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Synthesis of photoresponsive hybrid alginate hydrogel with photo-controlled release behavior

Chien-Ying Chiang[a], Chih-Chien Chu[a,b,*]

[a] School of Medical Applied Chemistry, Chung Shan Medical University, Taichung 40201, Taiwan
[b] Department of Medical Education, Chung Shan Medical University Hospital, Taichung 40201, Taiwan

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ABSTRACT

A photoresponsive hybrid alginate hydrogel was successfully prepared by Ca^{2+}-mediated crosslinking reaction with a mixture of β-cyclodextrin-grafted alginate (β-CD-Alg) and diazobenzene-modified poly(ethylene glycol) (Az_2-PEG). The water-soluble Az_2-PEG exhibits efficient *trans*-to-*cis* isomerization of the terminal azobenzene moieties under UV-light irradiation and readily switched back to the initial *trans* state under visible light. Because of low affinity between β-CD and *cis*-Az, the host–guest inclusion complex formed by β-CD and *trans*-Az gradually dissociates under UV-light exposure. Accordingly, the bulk gel exhibits substantial photo-induced transformation in gel morphology by the appearance of significant comb-like cavities. This photosensitive behavior accompanied by the structural degradation enables the rapid release of entrapped dye molecules under UV light stimulus. Moreover, an incident light with higher power and mild acidic environment are capable of accelerating the photo-triggered release, thus allowing the potential applications toward acute wound healing.

1. Introduction

Alginate, which is commonly isolated from brown algae, is an anionic linear polysaccharide composed of two saccharides: epimeric β-D-mannuronate (M) and α-L-guluronate (G). The M and G monomers are covalently bonded through 1,4-glycosidic linkages and arranged into either homopolymeric blocks (MM and GG) or alternating blocks (MGMG) along the polymeric backbone (Martins, Sarmento, Souto, & Ferreira, 2007; Gattás-Asfura & Stabler, 2009; García-González, Alnaief, & Smirnova, 2011; Gong et al., 2011; Goh, Heng, & Chan, 2012). According to the "egg-box" model, two facing GG blocks can be coordinated with divalent ca^{2+} ions, resulting in interchain crosslinking and hydrogel formation (Sikorski, Mo, Skjåk-Bræk, & Stokke, 2007; Coleman et al., 2011; Narayanan, Melman, Letourneau, Mendelson, & Melman, 2012; Cui et al., 2013).

Alginate hydrogels have high water content, elasticity, and the ability to maintain a physiologically moist microenvironment in the wound bed; therefore, they are widely applied in tissue engineering (Patterson, Martino, & Hubbell, 2010; Sun & Tan, 2013; Bozza et al., 2014). Moreover, alginate wound dressings can accommodate drugs and gradually release the drugs during the process of gel swelling to prevent wound infection (August, Kong, & Mooney, 2006; Bencherif et al., 2012; Pereira et al., 2013). However, alginates especially rich in GG blocks can incorporate more ionic interactions between chains and usually form a gel with high mechanical integrity. Therefore, during the controlled release of drugs, a bulk alginate hydrogel is less responsive to external stimuli such as temperature, pH level, and mechanical force. Recently, Han et al. (2012) presented a pH-sensitive shape-memory alginate hydrogel prepared by crosslinking a β-cyclodextrin (β-CD)-modified alginate and a diethylenetriamine-modified alginate. Ariga and co-workers reported a controlled release system containing a β-CD-crosslinked alginate gel triggered by a mechanical stimulus (Izawa et al., 2013). The release of drugs from this gel system was enhanced through mild mechanical compression because of a change in the host–guest inclusion ability of CD moieties for accommodating drug molecules.

Semiinterpenetrating networks (semi-IPNs) that are composed of one crosslinked polymer system in which free polymer chains are dissolved are capable of modulating the bulk properties of gel networks (Matricardi, Pontoriero, Coviello, Casadei, & Alhaique, 2008; Pescosolido et al., 2011). In semi-IPN systems, both crosslinked and free polymers synergistically contribute to the physicochemical properties of hybrid gels. Based on this concept, we developed a photoresponsive hybrid alginate semi-IPN that contains crosslinked β-CD-grafted alginate (β-CD-Alg) and

* Corresponding author at: School of Medical Applied Chemistry, Chung Shan Medical University, No. 110, Sec. 1, Jianguo N. Rd., Taichung 40201, Taiwan.
Tel.: +886 4 2324 8189; fax: +886 4 2324 8189.
E-mail addresses: jrchu@csmu.edu.tw, jrchu3933@gmail.com (C.-C. Chu).

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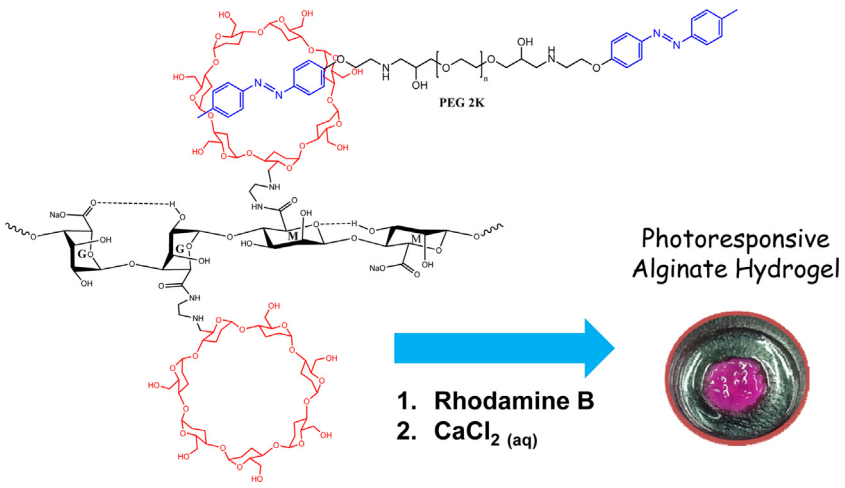

Scheme 1. Preparation protocol of a photoresponsive hybrid alginate hydrogel that contains crosslinked β-CD-grafted alginate (β-CD-Alg) and interpenetrating diazobenzene-terminated poly(ethylene glycol) (Az_2-PEG). Red-colored rhodamine B (RhB) is the mimic of entrapping drug molecules.

interpenetrating diazobenzene-terminated poly(ethylene glycol) (Az_2-PEG), as shown in Scheme 1. Because of the size and shape of the CD cavity, *trans*-Az and β-CD can form a favorable inclusion complex through host–guest affinity, whereas *cis*-Az is excluded from the complexation (Yuen & Tam, 2010; Tan et al., 2012). Therefore, the hybrid gel network features Ca^{2+} ions as cross-linkers as well as numerous junction points composed of β-CD and *trans*-Az inclusion complexes. Moreover, UV light irradiation induces efficient *trans*-to-*cis* isomerization (Peng, Tomatsu, & Kros, 2010; Tamesue, Takashima, Yamaguchi, Shinkai, & Harada, 2010; Meng et al., 2011). Accordingly, the hybrid alginate hydrogel is sensitive to the UV light used to facilitate *trans*-to-*cis* photoisomerization, which results in the dissociation of the inclusion complex and partial gel degradation. Thus, a light trigger can accelerate the release rate of small molecules entrapped within the gel. In addition to causing spontaneous drug release during gel swelling, this strategy entails using a bulk alginate hydrogel as a photocontrollable release system.

2. Experimental

2.1. General methods

All reactions were carried out under a nitrogen atmosphere. All solvents were dried following standard procedures. Sodium alginate (M_w = 1.2–1.4 × 10^5 Da) and *poly(ethylene glycol) diglycidyl ether* (M_n = 2 × 10^3 Da) were purchased from Sigma-Aldrich, and other chemical reagents were obtained as *high-purity reagent-grade* from commercial suppliers and used without further purification. Flash column chromatography was performed on spherical silica gel with 75-200 um particle dimensions. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Varian Mercury Plus 400 MHz spectrometer *at room temperature*. Spectral processing (Fourier transform, peak assignment and integration) was performed using MestReNova 6.2.1 software. *Trans*/*cis* photoisomerization for the azobenzene-containing polymers dissolved in organic solvents was carried out under the exposure of light-emitting diodes (LEDs) at 365 and 470 nm and an output power of 10 W. Ultraviolet–visible (UV–vis) absorption spectra were performed on a Thermo Genesys 10S UV–vis spectrometer equipped with a thermostatic cuvette holder. Field emission scanning electron microscopy (FE-SEM) was performed on a Jeol JSM-6700F instrument equipped with a cold-cathode field emission gun. The UV–vis measurement was carried out under a constant temperature. The relative viscosity (η_r) measurement was performed on an Ostwald–Fenske viscometer using distilled water as a standard.

The hydrogel samples containing rhodamine B (RhB) with a strong fluorescence at λ_{max} = 580 nm were irradiated with 365 nm LED, and the reflective emission from the samples were collected and induced by a fiber bundle into a CCD imaging spectrometer (USB-4000, Ocean Optics) for the spectra recording. To carry out *trans*-to-*cis* photoisomerization, the samples were also excited by 365 nm LED for a specific time interval and in situ analyzed by the same experimental apparatus (see Fig. S1 in Supporting information).

2.2. Materials synthesis and characterization

2.2.1. Synthesis of (E)-4-(p-tolyldiazenyl)phenol (**1**)

An aqueous solution of $NaNO_2$ (1.91 g, 27.7 mmol) was slowly added into a solution of p-toluidine (1.52 g, 14.2 mmol) in 30 mL of 3 M HCl, and then the mixture was stirred under 0 °C for 30 min, followed by adding an aqueous buffer solution containing phenol (1.71 g, 18.2 mmol), NaOH (0.73 g, 18.2 mmol), and Na_2CO_3 (1.93 g, 18.2 mmol). After stirred at 0 °C for 30 min, the mixing solution was extracted by ethyl acetate for 3 times. The combined organic phase was dried over anhydrous magnesium sulfate, and rotary evaporation to dryness afforded the crude product. Further purification was performed on flash column chromatography (SiO_2, ethyl acetate/hexane = 2:8, R_f = 0.4) to yield the final product **1** as orange solid (2.41 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.84 (d, J = 8.8 Hz, 2*H*), 7.78 (d, *J* = 8.3 Hz, 2*H*), 7.29 (d, *J* = 8.3 Hz, 2*H*), 6.91 (d, *J* = 8.8 Hz, 2*H*), 5.74 (bs, 1*H*), 2.42 (s, 3*H*).

2.2.2. Synthesis of (E)-1-(4-(2-bromoethoxy)phenyl)-2-(p-tolyl)diazene (**2**)

To a anhydrous THF solution of **1** (1.5 g, 7.1 mmol), K_2CO_3 (6.8 g, 49 mmol), and 18-crown-6 (20 g, 75 mmol), 1,2-dibromoethane (27 g, 0.14 mol) was added dropwisely over 30 min under N_2 atmosphere. The mixture was stirred at 45 °C for overnight and then extracted by ethyl acetate for 3 times. The combined organic phase was dried over anhydrous magnesium sulfate, and rotary evaporation to dryness afforded the crude product. Further purification was performed on flash column chromatography (SiO_2, ethyl acetate/hexane = 2:8, R_f = 0.6) to yield the final product **2** as orange solid (1.9 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.90 (d, *J* = 9.1 Hz, 2*H*), 7.79 (d, *J* = 8.3 Hz, 2*H*), 7.30 (d, *J* = 8.3 Hz, 2*H*), 7.01 (d, *J* = 9.1 Hz, 2*H*), 4.36 (t, *J* = 6.3 Hz, 2*H*), 3.67 (t, *J* = 6.3 Hz, 2*H*), 2.43 (s, 3*H*).