Polymer 54 (2013) 2115–2122

Contents lists available at SciVerse ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Facile preparation of photodegradable hydrogels by photopolymerization

Chang Seok Ki, Han Shih, Chien-Chi Lin*

Department of Biomedical Engineering, Purdue School of Engineering and Technology, Indiana University-Purdue University at Indianapolis, Indianapolis, IN 46202, USA

ARTICLE INFO

Article history:
Received 29 November 2012
Received in revised form
31 January 2013
Accepted 11 February 2013
Available online 18 February 2013

Keywords:
Photopolymerization
Photodegradable hydrogels
Click reaction

ABSTRACT

Photodegradable hydrogels have emerged as a powerful material platform for studying and directing cell behaviors, as well as for delivering drugs. The premise of this technique is to use a cytocompatible light source to cleave linkers within a hydrogel, thus causing reduction of matrix stiffness or liberation of matrix-tethered biomolecules in a spatial-temporally controlled manner. The most commonly used photodegradable units are molecules containing nitrobenzyl moieties that absorb light in the ultraviolet (UV) to lower visible wavelengths (~280–450 nm). Because photodegradable linkers and hydrogels reported in the literature thus far are all sensitive to UV light, highly efficient UV-mediated photopolymerizations are less likely to be used as the method to prepare these hydrogels. As a result, currently available photodegradable hydrogels are formed by redox-mediated radical polymerizations, emulsion polymerizations, Michael-type addition reactions, or orthogonal click chemistries. Here, we report the first photodegradable poly(ethylene glycol)-based hydrogel system prepared by step-growth photopolymerization. The model photolabile peptide cross-linkers, synthesized by conventional solid phase peptide synthesis, contained terminal cysteines for step-growth thiol-ene photo-click reactions and a UV-sensitive 2-nitrophenylalanine residue in the peptide backbone for photo-cleavage. Photolysis of this peptide was achieved through adjusting UV light exposure time and intensity. Photopolymerization of photodegradable hydrogels containing photolabile peptide cross-linkers was made possible via a highly efficient visible light-mediated thiol-ene photo-click reaction using a non-cleavage type photoinitiator eosin-Y. Rapid gelation was confirmed by *in situ* photo-rheometry. Flood UV irradiation at controlled wavelength and intensity was used to demonstrate the photodegradability of these photopolymerized hydrogels.

1. Introduction

Hydrogels containing photodegradable linkers have great potential in controlled release and tissue engineering applications [1]. For example, drugs or cells immobilized or entrapped within a photodegradable hydrogel can be released 'on-demand' via light-mediated cleavage of photolabile linkers [2–5]. Furthermore, the cleavage of photolabile cross-links leads to the reduction of hydrogel cross-linking density and stiffness [1,6,7]. This approach has been used to study cell fate processes, such as migration of fibroblast [1], de-activation of valvular interstitial cells (VICs) [7,8], and differentiation of human mesenchymal stem cells (hMSCs) [1]. Most of the photolabile units contain an *ortho*-nitrobenzyl (*o*-NB) moiety, which is highly susceptible to ultraviolet (UV) light exposure. The absorbance spectra of macromers containing *o*-NB groups usually peak at around 280–400 nm and tail off in the lower visible light range (~430 nm) [2,5]. When exposing to lights at selective wavelengths, *o*-NB groups are effectively photo-lysed, resulting in the liberation of conjugated molecules or reduction in gel cross-linking density.

The exploration of photodegradable hydrogels thus far has focused on synthesizing photolabile groups with different light sensitivities or on controlling cellular response via photolysis-induced material property changes. Current methods to fabricate photodegradable hydrogels are restricted to non-photo-mediated reactions, such as Michael-type conjugations, redox reactions, emulsion polymerization, thermal polymerizations, or orthogonal 'click' chemistries [1,9–16]. While these gelation schemes preserve the molecular integrity of photodegradable moieties, some limitations exist. For example, redox radical polymerizations compatible with photolabile units are commonly initiated by cytotoxic components such as ammonium persulfate (APS) and tetramethylethylenediamine (TEMED) [9,12,17]. Although gels prepared from Michael-type addition reactions contain no cytotoxic components, this reaction scheme suffers from low functional group conversion and long gelation time due to significant intramolecular reactions [18–20]. Alternatively, researchers have begun to use cytocompatible orthogonal 'click' chemistries, such as the copper-

* Corresponding author.
E-mail address: lincc@iupui.edu (C.-C. Lin).

0032-3861/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.polymer.2013.02.018

free strain-promoted azide-alkyne cycloaddition reaction, to synthesize photo-tunable hydrogels [13,21–23]. However, none of the above cross-linking methods permit spatial-temporal controls during gelation because the polymerization starts as soon as the macromers are mixed together.

Photopolymerization has always been one of the most popular methods to fabricate hydrogels due to rapid and highly tunable gelation kinetics. This attractive gelation method, however, is unfortunately excluded from the polymerization of photodegradable hydrogels. We hypothesized that photodegradable hydrogels can be synthesized by photopolymerization if an appropriate and efficient photopolymerization system is available. Our laboratory has recently reported the synthesis of step-growth hydrogels by visible light-mediated thiol-ene photo-click reactions [24]. A halogen cold light lamp was used as a cytocompatible visible light source while eosin-Y (EY) was used as the sole initiator to initiate thiol-ene photo-click gelation using macromer poly(ethylene glycol)-tetra-norbornene (PEG4NB) and cross-linker dithiothreitol (DTT). This new step-growth hydrogel system not only preserves all favorable features offered by the rapid and tunable photochemistry, but also excludes the use of potentially cytotoxic components (e.g., triethanolamine) that are indispensable in the conventional visible light-mediated chain-growth photopolymerizations. We reasoned that this new photopolymerization scheme (Scheme 1A) could be used to synthesize photodegradable hydrogels since the absorbance of eosin-Y peaks at around 516 nm, a wavelength far away from the absorbance of all reported photolabile *o*-NB groups [1–3,5].

In this contribution, we report a proof-of-principle photopolymerization system using orthogonal wavelengths that are compatible for forming photodegradable hydrogels. A simple yet highly efficient visible light-mediated thiol-ene photo-click reaction was utilized to fabricate step-growth photodegradable hydrogels cross-linked by PEG4NB and a photolabile peptide containing a commercially available photolabile amino acid, L-2-nitrophenylalanine (NPA) (Scheme 1B). The model peptide Cys–Gly–NPA–Gly–Cys (CGOGC, O: NPA residue. Cysteines were added for thiol-ene photo-crosslinking) was synthesized by standard solid phase peptide synthesis (SPPS) while the formation of hydrogels was accomplished within minutes using a halogen cold light lamp that emits bright visible light. The photodegradation of these photopolymerized hydrogels was achieved by flood UV light irradiation.

2. Experimental section

2.1. Materials

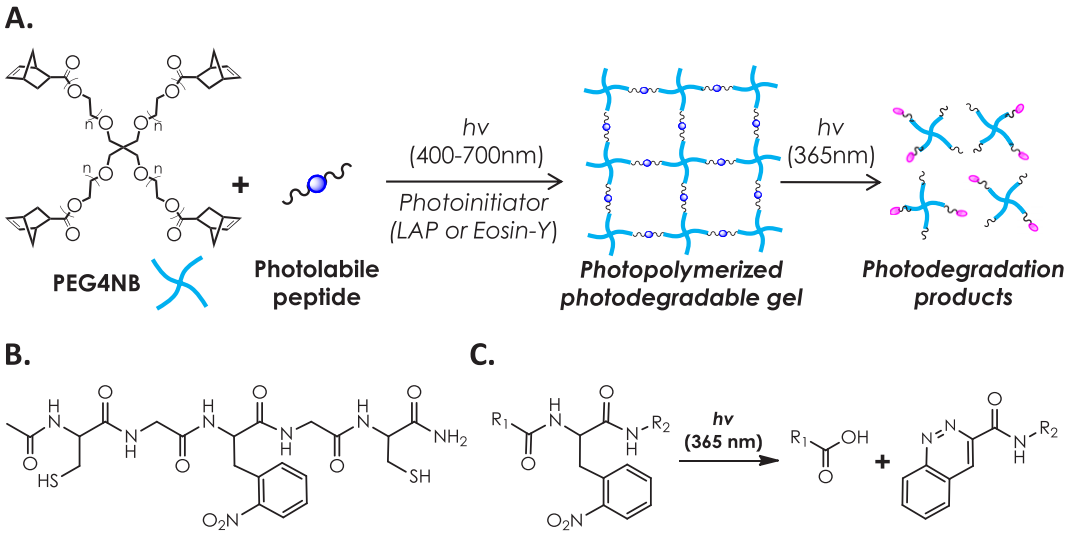
4-arm PEG (20 kDa) was purchased from JenKem Technology USA. Fmoc-Rink Amide MBHA resin, Fmoc-Cys(Trt)-OH, Fmoc-Gly-OH, Fmoc-Tyr(tBu)-OH, and reagents for peptide synthesis were acquired from Chempep, Inc. Fmoc-L-2-nitrophenylalanine-OH (Fmoc-2-NPA) was obtained from PepTech Inc. HPLC grade acetonitrile and water were obtained from Fisher Scientific and VWR International, respectively. Eosin-Y disodium salt was acquired from Fisher Scientific. All other chemicals and reagents were obtained from Sigma–Aldrich unless otherwise noted.

2.2. Macromer and peptide synthesis

PEG-tetra-norbornene (PEG4NB) and photoinitiator lithium arylphosphinate (LAP) were synthesized as described previously [25,26] and characterized by ^{1}H NMR (85–95%). Photolabile peptides (NH_2-CGOGC-NH_2 and Ac-CGOGC-NH_2) and non-photolabile peptides (NH_2-CGGGC-NH_2 and NH_2-CGGYC-NH_2) were synthesized using Fmoc-Rink-Amide HBMA resin in a microwave-assisted solid peptide synthesizer (CEM Discover SPS) following standard HOBT/HBTU coupling chemistry. Deprotection of Fmoc groups and coupling of Fmoc-amino acids (with the exception of Fmoc-Cys(Trt)-OH) were performed at 50 W, 75 °C for 3 and 5 min, respectively. Coupling of Fmoc-Cys(Trt)-OH was performed at 50 W, 50 °C for 10 min to reduce racemization. A portion of CGOGC was acetylated by reacting peptide N-terminus with acetic anhydride (0.5 M acetic anhydride and 0.125 M diisopropylethylamine in dimethylformamide) before cleavage using a cleavage cocktail (95% trifluoroacetic acid, 2.5% dH_2O, 2.5% triisopropylsilane, and 5 wt/vol % phenol). Crude peptides were purified by reverse phase HPLC (RP-HPLC, Perkin Elmer Flexar system) to at least 95% purity. The purified peptides were characterized by mass spectrometry (Agilent Technology).

2.3. Characterization of photodegradable peptides

UV/Vis absorbance spectra of the photoinitiators (eosin-Y (EY) and lithium arylphosphinate (LAP)) and photodegradable peptides (NH_2-CGOGC-NH_2 and Ac-CGOGC-NH_2) were measured using a



Scheme 1. (A) Schematic of photo-crosslinking and photodegradation of step-growth thiol-ene hydrogels. (B) Chemical structure of model photodegradable peptide Ac-CGOGC-NH_2. (C) Schematic of UV-mediated peptide photolysis.