Acta Biomaterialia xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Photoinitiator-free synthesis of endothelial cell-adhesive and enzymatically degradable hydrogels

Derek R. Jones [a], Roger E. Marchant [a], Horst von Recum [a], Anirban Sen Gupta [a], Kandice Kottke-Marchant [a,b,*]

[a] *Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106, USA*
[b] *Robert J. Tomsich Pathology and Laboratory Medicine Institute, Cleveland Clinic, Cleveland, OH 44195, USA*

A R T I C L E I N F O

Article history:
Received 1 July 2014
Received in revised form 7 October 2014
Accepted 5 November 2014
Available online xxxx

Keywords:
Extracellular matrix
Cell adhesive
Enzyme degradable
Michael addition
Photoinitiator free

A B S T R A C T

We report on a photoinitiator-free synthetic method of incorporating bioactivity into poly(ethylene glycol) (PEG) hydrogels in order to control physical properties, enzymatic biodegradability and cell-specific adhesiveness of the polymer network, while eliminating the need for UV-mediated photopolymerization. To accomplish this, hydrogel networks were polymerized using Michael addition with four-arm PEG acrylate (10 kDa), using a collagenase-sensitive peptide (CSP) as a crosslinker, and introducing an endothelial cell-adhesive peptide either terminally (RGD) or attached to the crosslinking peptide sequence (CSP-RGD). The efficiency of the Michael addition reactions were determined by nuclear magnetic resonance and Ellman's assay. Successful decoupling of cell adhesivity and physical properties was demonstrated by quantifying and comparing the swelling ratios and Young's moduli of various hydrogel formulations. Degradation profiles were established by incubating functionalized hydrogels in collagenase solutions (0.0–1.0 μg ml^{-1}), demonstrating that functionalized hydrogels degraded at a rate dependent upon collagenase concentration. Moreover, it was shown that the degradation rate was independent of CSP-RGD concentration. Cell attachment and proliferation on functionalized hydrogels were compared for various RGD concentrations, providing evidence that cell attachment and proliferation were directly related to relative amounts of the CSP-RGD combination peptide. An increase in cell viability was achieved using Michael addition techniques when compared to UV polymerization, and was assessed by a LIVE/DEAD fluorescence assay. This photoinitiator-free method shows promise in creating hydrogel-based tissue engineering scaffolds allow for decoupled cell adhesivity and physical properties and that render greater cell viability.

© 2014 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

The extracellular matrix (ECM) provides mechanical support and biochemical cues to ECM-adherent cells through a complex network composed of macromolecular proteins and polysaccharides [1–4]. Cell membrane-anchored integrins bind adhesive sequences present on proteins within the ECM microenvironment [2–5]. Anchorage-dependent cells lose viability quickly when they are unable bind to the supporting matrix [6]. Additionally, integrin binding stimulates signaling processes responsible for the production of interstitial collagenase, which aids in the degradation and remodeling of the microenvironment [1–4]. This remodeling is an important biological process needed for tissue regeneration, wound healing and morphogenesis [2,3]. Thus, the ECM serves as a mechanical scaffold for cells as well as a bioactive and dynamic environment that mediates cellular function [1–4]. Tissue engineering strategies have sought to mimic many of the supportive and biochemical properties of the ECM to guide the regeneration of specific tissues after loss of function induced by trauma or chronic disease [1,7,8].

The field of tissue engineering combines techniques from both engineering and life sciences to create artificial constructs to guide tissue repair and regeneration [3,9]. Hydrogels provide a suitable platform for tissue-engineered constructs due to their highly swollen three-dimensional environment similar to soft tissues, and a highly porous structure that permits transport of nutrients and cellular waste through the biomatrix [3,4,7,9–11]. Biologically derived hydrogels have been developed from collagen and fibrin, which possess intrinsic biologically active components that mediate cell

* Corresponding author at: Robert J. Tomsich Pathology and Laboratory Medicine Institute, Cleveland Clinic, Cleveland, OH 44195, USA. Tel.: +1 216 444 2484; fax: +1 216 445 9535.

E-mail address: marchak@ccf.org (K. Kottke-Marchant).

http://dx.doi.org/10.1016/j.actbio.2014.11.012
1742-7061/© 2014 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

behavior [1,3,4,12–14]. However, biologically derived hydrogels possess obvious drawbacks, including high batch-to-batch variability, the possibility of disease transmission, as well as non-selectivity [1,3,4,7,15]. To address these shortcomings, synthetic scaffold materials, such as poly(ethylene glycol) (PEG) hydrogels, have been employed for a wide range of applications including bone, cartilage and blood vessel regeneration [1,3,9,10,16,17]. PEG hydrogels have tunable mechanical properties that permit facile manipulation of the scaffold architecture and functionalized end groups [3,7]. PEG hydrogels, however, are bioinert, resist protein adsorption and do not support cell adhesion [3,4,9,11,18,19]. In order to capitalize on the strengths of both biologically derived cell-interactive cues and tunability of synthetic hydrogels, extensive research has been performed in the conjugation of short bioactive peptides to synthetic materials to provide highly customizable bioactive hydrogels [3,4,8,9,14,18,19]. This approach provides the distinct advantage of quantitative control of cell–scaffold interactions while avoiding confounding results from ECM-bound growth factors (commonly associated with biologically derived hydrogels), and resisting non-specific adsorption of exogenous proteins that can facilitate non-specific cell attachment to the material surface [3,15]. Non-specific protein interactions with the biomaterial surface can have adverse effects such as inflammatory cell binding and foreign body responses [20,21]. Copolymerizing synthetic bioactive peptide sequences derived from type I collagen such as GPQGIAGQ or the GRGDSP sequence from fibronectin have been utilized to promote enzyme-mediated degradation and cell adhesion to the hydrogel matrix, respectively [3,4,7,14]. The peptide sequence derived from type I collagen has been shown to degrade in the presence of collagenase [3]. Combining these properties in a synthetic material allows for the potential to design a wide range of temporary tissue-engineered constructs that are degraded by collagenase from endogenous cells and gradually replaced with the body's own tissue over time.

Cell-adhesive peptides linked to a PEG-diacrylate (PEGDA) network via PEG-monoacrylates (PEGMA) have been shown to be inefficient, and result in the formation of non-network terminal ends within the hydrogel matrix [3,18]. The presence of these terminal ends results in a decrease in crosslink density, an inconsistency of the physical properties of the hydrogel network, and complicates comparisons between hydrogel formulations containing different RGD concentrations [14]. Moreover, polymerization techniques, such as UV photopolymerization can adversely impact encapsulated cell viability due to prolonged exposure to cytotoxic free radicals or unreacted small molecules, as well as the photoinitiator compounds [10,11,22,23]. To avoid these shortcomings, we investigated the copolymerization of a thiolated collagenase-sensitive peptide CGPQG↓IAGQC (CSP) (↓ indicating the cleavage site), a thiolated collagenase-sensitive and cell-adhesive bifunctional peptide GPQG↓IAGQCGRGDSP (CSP-RGD), and four-arm PEG-acrylate using a mechanism known as Michael addition (Fig. 1). The incorporation of cysteine in these sequences simultaneously served to introduce the enzymatically degradable sequence into the backbone of the otherwise bioinert PEG hydrogel, and provides a means of crosslinking the network. The location of the middle cysteine in the bifunctional sequence allows for the cell-adhesive RGD peptide to remain available for cell attachment. Under slightly basic conditions (pH 8.0), the nucleophilic thiols on the peptides react with the unsaturated acrylate groups on the four-arm PEG to form bioactive hydrogels with easily tunable parameters (cell adhesivity and enzymatic biodegradability), physical properties independent of cell-adhesive peptide concentration, and without UV-mediated photopolymerization. Hydrogels formed by Michael-type addition reactions are subject to a higher level of control since termination and radical transfer events are less relevant and there is no new polymeric species formed [8]. Moreover, hydrogels utilizing this technique can be formed under physiological conditions in direct contact with tissues, cells and biological molecules, and thus have utility as scaffolds for tissue engineering. These added benefits allow for the overall goal of designing a non-UV polymerized material that can incorporate both cell-adhesive and biodegradable functionality independent of the physical properties. Such a material has the potential of being incorporated into a wide range of tissue-engineered constructs.

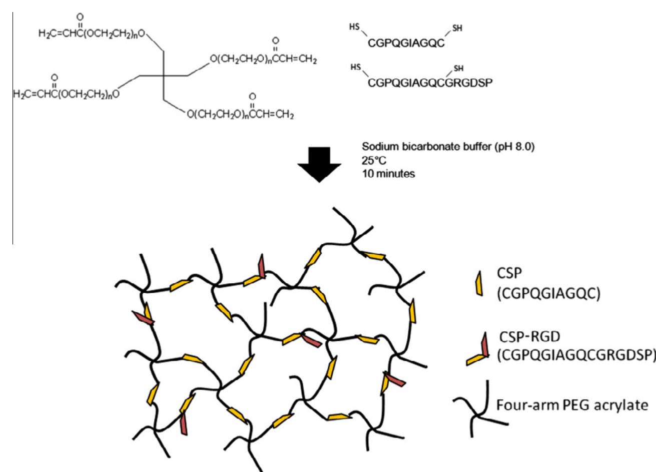

Fig. 1. Bioactive four-arm PEG hydrogels containing CSP and CSP-RGD allows for simultaneous cell attachment and enzymatic degradation. Combining four-arm PEG acrylate with cysteine containing peptides at pH 8.0 allows for stoichiometric equivalence of the acrylate and thiol functional groups, and the incorporation of the collagenase-sensitive peptide and the RGD peptide in the backbone of the hydrogel network.

2. Materials and methods

2.1. Materials

All reagents were obtained from Sigma-Aldrich (St Louis, MO) and used as received unless otherwise stated.

2.2. Instrumentation

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was performed on a Bruker Autoflex III (Billerica, MA) equipped with a standard linear detector and a gridless reflection detector. Samples were dissolved in 1:1 (v/v) ethanol and water and mixed with the matrix solution of 2,5-dihydroxybenzoic acid before deposition on the stainless steel sample plate. Mass spectra were acquired from 500 laser shots. Parallel plate dynamic mechanical analysis was performed using a Perkin Elmer DMA7e (Waltham, MA). Phase-contrast photomicrographs of cells were obtained on an inverted phase-contrast microscope (Nikon Diaphot 200, Melville, NY) with a charge-coupled display (CCD) camera using the Metamorph software package (version 6.2r6, Sunnyvale, CA).

2.3. Peptide synthesis and characterization

Thiol containing CSP and CSP-RGD were synthesized in a 1.0 mmol scale with Knorr resin with a loading of 0.89 mmol g^{-1} and 9-Fluoromethoxycarbonyl (Fmoc)-protected amino acids (Advanced Chem Tech, Louisville, KY) to produce an amide C-terminus on a solid-phase peptide synthesizer (Applied Biosystems, Model 433A, Foster City, CA) using standard Fmoc chemistry. Peptides were cleaved from the resins and deprotected using

Please cite this article in press as: Jones DR et al. Photoinitiator-free synthesis of endothelial cell-adhesive and enzymatically degradable hydrogels. Acta Biomater (2014), http://dx.doi.org/10.1016/j.actbio.2014.11.012

Download English Version:

https://daneshyari.com/en/article/6483702

Download Persian Version:

https://daneshyari.com/article/6483702

Daneshyari.com