Biomaterials 101 (2016) 217–228

Contents lists available at ScienceDirect

Biomaterials

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

Mechanically resilient, injectable, and bioadhesive supramolecular gelatin hydrogels crosslinked by weak host-guest interactions assist cell infiltration and in situ tissue regeneration

Qian Feng [a, b, 1], Kongchang Wei [a, b, c, 1], Sien Lin [a, g], Zhen Xu [h], Yuxin Sun [g], Peng Shi [h], Gang Li [g], Liming Bian [a, c, d, e, f, *]

[a] Division of Biomedical Engineering, The Chinese University of Hong Kong, Shatin, New Territories 999077, Hong Kong
[b] Department of Mechanical and Automation Engineering, The Chinese University of Hong Kong, Shatin, New Territories 999077, Hong Kong
[c] Shun Hing Institute of Advanced Engineering, The Chinese University of Hong Kong, Shatin, New Territories 999077, Hong Kong
[d] Shenzhen Research Institue, The Chinese University of Hong Kong, Hong Kong
[e] China Orthopedic Regenerative Medicine Group (CORMed), Hangzhou, China
[f] Centre of Novel Biomaterials, The Chinese University of Hong Kong, Hong Kong
[g] Department of Orthopaedic and Traumatology, The Chinese University of Hong Kong, Prince of Wales Hospital, New Territories 999077, Hong Kong
[h] Department of Mechanical and Biomedical Engineering, City University of Hong Kong, Tat Chee Avenue, Kowloon 999077, Hong Kong

ARTICLE INFO

Article history:
Received 8 March 2016
Received in revised form
9 May 2016
Accepted 24 May 2016
Available online 2 June 2016

Keywords:
Supramolecular hydrogel
Host-guest complexation
Biomaterial carrier
Drug delivery
Tissue regeneration

ABSTRACT

Although considered promising materials for assisting organ regeneration, few hydrogels meet the stringent requirements of clinical translation on the preparation, application, mechanical property, bioadhesion, and biocompatibility of the hydrogels. Herein, we describe a facile supramolecular approach for preparing gelatin hydrogels with a wide array of desirable properties. Briefly, we first prepare a supramolecular gelatin macromer via the efficient host-guest complexation between the aromatic residues of gelatin and free diffusing photo-crosslinkable acrylated β-cyclodextrin (β-CD) monomers. The subsequent crosslinking of the macromers produces highly resilient supramolecular gelatin hydrogels that are solely crosslinked by the weak host-guest interactions between the gelatinous aromatic residues and β-cyclodextrin (β-CD). The obtained hydrogels are capable of sustaining excessive compressive and tensile strain, and they are capable of quick self healing after mechanical disruption. These hydrogels can be injected in the gelation state through surgical needles and re-molded to the targeted geometries while protecting the encapsulated cells. Moreover, the weak host-guest crosslinking likely facilitate the infiltration and migration of cells into the hydrogels. The excess β-CDs in the hydrogels enable the hydrogel-tissue adhesion and enhance the loading and sustained delivery of hydrophobic drugs. The cell and animal studies show that such hydrogels support cell recruitment, differentiation, and bone regeneration, making them promising carrier biomaterials of therapeutic cells and drugs via minimally invasive procedures.

1. Introduction

Among many biomaterials developed for applications in regenerative medicine, hydrogels have received increasing attention due to their similarity to biological tissues in terms of their high water content, tunable physical and biological properties [1–9]. Up to date, great success has been achieved in using hydrogels to emulate stem cell microenvironments for controlling stem cell differentiation and tissue regeneration [10–13]. A number of hydrogels for both *in vitro* and *in vivo* studies have been developed in order to reveal fundamental understanding of cell-material interactions and their roles in directing tissue regeneration [14–16]. However, it is still challenging to prepare hydrogels that can simultaneously fulfill the stringent requirements for real applications in tissue engineering. Such requirement usually include

* Corresponding author. Division of Biomedical Engineering, The Chinese University of Hong Kong, Shatin, New Territories 999077, Hong Kong.
E-mail address: lbian@mae.cuhk.edu.hk (L. Bian).
[1] Theses authors contributed equally.

http://dx.doi.org/10.1016/j.biomaterials.2016.05.043
0142-9612/

facile preparation, easy surgical application, robust mechanical properties, tissue adhesion, excellent biocompatibility and bioactivity, etc [17–22]. Herein, we describe the facile preparation of supramolecularly engineered gelatin hydrogels that can meet such challenging requirements in real applications.

Gelatin has been extensively used to fabricate hydrogels for tissue engineering, due to its good biocompatibility, intrinsic bioactivity and abundancy [23–26]. However, chemical crosslinking is often necessary to stabilize gelatin hydrogels, because the native gelatin hydrogels spontaneously formed at low temperatures (<30 °C) are not stable under the physiological conditions [27]. The physical crosslinking of gelatin triple helix will be disrupted at temperatures above 30 °C [28]. Therefore, to prepare stable hydrogels for biomedical applications, gelatin is usually chemically functionalized by polymerizable groups, yielding the crosslinkable gelatin macromers such as the methacrylated gelatin (MeGel) [29–31]. Subsequent crosslinking of such macromers produces stable gelatin hydrogels that have been used in many cell studies [32]. However, most of these chemically crosslinked gelatin hydrogels are brittle and hard for post-gelation processing, thus precluding it from widespread surgical applications [33]. For example, the MeGel hydrogels fails at a low compressive or tensile strain and are not capable of self-healing, and this makes the MeGel hydrogels not suitable for application in load bearing sites such as joint cartilage. The inability to inject the MeGel hydrogels in the gelation state makes the use of the MeGel hydrogels in minimal invasive procedures difficult.

To address these challenges, we propose a novel "Host-Guest Macromer" approach to prepare mechanically robust gelatin hydrogels with desirable supramolecular properties and biological functions. Instead of the chemically functionalized gelatin macromer, the host-guest supramolecular macromer (HGM) is used as the hydrogel precursor. Free from chemical modifications of the biopolymers, the HGM is formed via the efficient host-guest complexation between aromatic residues of gelatin (e.g., phenylalanine, tyrosine, and tryptophan) and the free diffusing photocrosslinkable acrylated β-cyclodextrins (Ac-β-CDs). Subsequent UV-initiated polymerization of the Ac-β-CDs produces the mechanically robust hydrogels with gelatin polymers physically crosslinked by host-guest interactions (termed HGM hydrogel). Such supramolecularly engineered gelatin HGM hydrogels can form highly deformable and resilient 3D constructs under the physiological condition. They can be injected through 18G needles without compromising the viability of the encapsulated human mesenchymal stem cells (hMSCs) due to the reversible nature of the host-guest interactions. In addition, many hydrophobic small molecules such as dexamethasone and kartogenin are effective inducting agents of stem cell differentiation, which is important to the regeneration of the injured tissues [34,35]. However, these hydrophobic small molecules are difficult to be effectively loaded in the conventional hydrophic hydrogel network with a sustained long term release [36,37]. The excess β-CDs not only engender the adhesion of the hydrogels to biological tissues by the coupling to the aromatic groups of the native proteins and but also allow the delivery of hydrophobic drugs via the hydrophobic cavity. Furthermore, *in vitro* and *in vivo* studies show that such hydrogels support cell infiltration, differentiation and *in situ* bone regeneration, making them promising carriers of therapeutic cells and drugs via minimally invasive procedures. This simple but effective strategy opens up a new route to develop biopolymer-based supramolecular hydrogels with enhanced physical and biological functionalities as drug and/or cell carriers for regenerative medicine.

2. Materials and methods

2.1. Materials

β-cyclodextrin (β-CD), acrylate chloride and hydrogen peroxide (H_2O_2) were bought form Aladdin. Gelatin (type A, from porcine skin, isoelectric point: 7–9, Cat. No.: G1890-500G, Sigma), methacrylic anhydride, dimethylmalenic acid (DMMA), deuterium oxide (D_2O), dimethylsulfoxide-d6 (DMSO-d_6), dexamethasone (Dex), 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (I2959), 4′, 6-diamidino-2-phenylindole (DAPI), 3-(Trimethoxysily) propyl methacrylate, sliver nitrate, paraformaldehyde, Triton X-100, sodium thiosulfate, Triethyl amine (TEA), ethidium bromide, hyaluronidase were purchased from Sigma. Dimethyl Formamide (DMF), dimethylsulfoxide, acetone, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from Fisher Scientific. Poly (ethylene glycol) diacrylate (PEGDA) were purchased from Jenkem. Phosphate buffered saline (DPBS), α-minimum essential medium (DMEM), penicillin, streptomycin, L-glutamine, calcein AM, fetal bovine serum (FBS), and Trizol were obtained from Gibco. BCA protein assay kit, calcium colorimetric assay kit, and revertAid First strand cDNA synthesis kit were obtained from Thermo. Peroxidase substrate kit DAB and vectastain ABC kit were purchased from Vector Lab. Human mesenchymal stem cells (hMSCs) from Lonza.

2.2. Synthesis of Acrylate β-cyclodextrin (Ac-β-CD)

10 g β-CD was added into 150 mL DMF with 7 mL TEA added into the solution. The mixture was stirred and cooled down to 0 °C before 5 ml acrylic acid was added into the solution. After stirring for 12 h, the mixture was filtrated to remove trimethylamine hydrochloride and the obtained clear solution was concentrated to about 20 ml by vacuum rotary evaporation. Then the solution was dripped into 600 ml acetone to precipitate the modified cyclodextrin. The precipitate was washed several times with acetone and vacuum dried for 3 days. The substitution degree (DS) of CD as 1 is confirmed by ^{1}H NMR (Bruker Advance 400 MHz spectrometer). It was recorded with DMSO-d_6 as the internal reference at 37 °C. In order to confirm the complexation between the Ac-β-CD and the benzene ring of gelatin, two dimensional nuclear overhauser effect spectroscopy (2D NOESY) was performed in D_2O with a Bruker Advance 400 MHz spectrometer at 37 °C.

2.3. Synthesis of methacrylated gelatin (MeGel)

10 g gelatin (type A) was dissolved in 100 mL PBS at 50 °C. A total of 12 mL methacrylic anhydride was then added to the 10% gelatin solution and stirred for 4 h at 50. The resulting mixture was dialyzed against DI water for one week at 45 °C to remove unreacted reagent (6 KDa cut-off dialysis membrane). Then, the liquid was lyophilized for 4 days at −104 °C. The degree of methacrylation as 3.17×10^{-4} mol per gram was determined by ^{1}H NMR (Bruker Advance 400 MHz spectrometer). It was recorded in D_2O with DMMA as the internal reference at 37 °C.

2.4. Preparation of hydrogels

2.4.1. HGM supramolecular gelatin hydrogel

Gelatin and Ac-β-CD was dissolved in PBS at 37 °C to produce mixture solutions with fixed concentration of gelatin (8% (w/v)) and varying amount of Ac-β-CD (0% (w/v), 4% (w/v) and 10% (w/v)). Then initiator I2959 was added at 0.05% (w/v). The mixture was pipetted into PVC molds at 37 °C, cooled down to 25 °C, and then exposed to 365 nm ultraviolet (UV) light (10 mW/cm^2, 10 min) at