Colloids and Surfaces B: Biointerfaces 170 (2018) 64–75

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Characterizations of hyaluronate-based terpolymeric hydrogel synthesized via free radical polymerization mechanism for biomedical applications

Dipankar Das[a,b], Thi Thu Hien Pham[a,b], Insup Noh[a,b,*]

[a] Department of Chemical and Biomolecular Engineering, Seoul National University of Science and Technology, Seoul 01811, Republic of Korea
[b] Convergence Institute of Biomedical Engineering and Biomaterials, Seoul National University of Science and Technology, Seoul 01811, Republic of Korea

ARTICLE INFO

Keywords:
Hyaluronate
Hydrogel
Dimethyloxalylglycine
Drug delivery
Tetracycline

ABSTRACT

In the present study, a novel terpolymeric hydrogel was developed using sodium hyaluronate (HA), 2-hydroxyethyl acrylate (2-HEA), and poly(ethylene glycol) diacrylate (PEGDA) via free radical polymerization for biomedical applications. To achieve elasticity, swelling ability, porous architecture and sufficient gel strength, hyaluronate was chemically modified by grafting and crosslinking methods using 2-HEA and PEGDA, respectively. The structure and compositions of the fabricated terpolymer (HA-g-p(2-HEA)-x-PEGDA) were verified by FTIR, ^{1}H HR-MAS-NMR, and TGA analyses. The surface morphology and cross-section of the hydrogel was detected by SEM analysis. The gel nature of terpolymer in aqueous medium at 37 °C was confirmed from swelling study, and rheological experiment. Non-cytotoxicity and biocompatibility of the HA-g-p(2-HEA)-x-PEGDA hydrogel were ascertained by *in vitro* mouse osteoblastic cells (MC3T3) proliferation, and viability studies. Hematoxylin and eosin Y, and Masson's trichrome stainings were performed to show tissue regeneration ability on the prepared hydrogel. *In vitro* release results of proangiogenic drug-dimethyloxalylglycine (DMOG), and antibiotics-tetracycline (TCN) showed sustained release behaviour from the prepared hydrogel under different pHs at 37 °C. The mathematical models fitted data imply that both DMOG and TCN release follow first order kinetics, while, the release mechanism is primarily controlled by diffusion as well as erosion process. Finally, the novel biocompatible HA-g-p(2-HEA)-x-PEGDA gel, which showed sustained drugs release, and regeneration ability of extracellular matrix and collagen, could be employed in biomedical applications, especially, for the delivery of DMOG/TCN, and in tissue engineering.

1. Introduction

Hyaluronic acid is a biopolymer composed by repeating units of D-glucoronic acid and N-acetyl-D-glucosamine [1–3]. It is the major components of the skin [4] and also found in extracellular, pericellular, intracellular tissues of the body [1,3,5] and even in nuclear localization [1]. Hyaluronic acid participates in numerous biological activities, such as cell growth, migration and diferentiation [6]. It is hydrophilic, biocompatible, non-immunogenic and degraded by hyaluronidases enzymes [7,8]. Moreover, the presence of hydroxyl and carboxylic acid groups in the HA moiety creates it an excellent biomaterial for chemical modifications [3,8]. These characteristics boosted the use of HA in biomaterials science ranging from tissue engineering [5,6,9–13], cosmetics [5] and drug delivery [8,14,15]. In contrast, one of the major drawbacks of single component hyaluronate is its solubility in physiological solution, which limits it to be an ideal matrix for biomedical applications such as tissue engineering and drug delivery. Hence, it is essential to modify hyaluronate by other chemical reagents to get better efficiency for biomedical applications.

In past few years, hyaluronic acid and/or hyaluronate (HA) have been functionalized with natural polymers, synthetic polymers or nanoparticles in terms of composite materials, hydrogels or hydrogel-nanocomposites to modulate its biological and mechanical properties for better efficiency towards numerous biomedical applications [1,3,16–24]. However, taking into consideration of the biocompatibility and mechanical properties, synthetic polymers have also been widely explored in biomedical applications including tissue engineering and drug delivery [25–28]. For bone tissue engineering, the mechanical properties of bone such as elastic modulus, compressive, and tensile strength are vital [29]. These properties are highly dependent on the position of the bone and the condition of the individual. According to structure, bone tissue is classified into two types i.e. cancellous bone (trabecular) and cortical bone (lamellar) [29]. The tensile strength, compressive strength, and Young's modulus of cancellous bone are in the ranges of 10–100 MPa, 2–12 MPa, and 0.02–0.05 GPa, respectively [29]. While, the tensile strength, compressive strength, and Young's

* Corresponding author. Tel.: +822-970-6603; fax: 02-977-8317.
E-mail address: insup@seoultech.ac.kr (I. Noh).

https://doi.org/10.1016/j.colsurfb.2018.05.059
Received 6 April 2018; Received in revised form 19 May 2018; Accepted 26 May 2018
Available online 31 May 2018

modulus of cortical bone are 50–150 MPa, 130–230 MPa, and 7–30 GPa, respectively [29]. Typically, HA-based hydrogels have been developed for various biomedical applications using oxidized/thiolated/acrylated/methacrylated HA, which were produced by chemical modification of −OH/−COOH group of HA using organic solvents and/or toxic chemicals [1,3,30–35]. Hydrogels are three-dimensional, hydrophilic, physically or chemically crosslinked polymer networks, having ability to absorb large amounts of water [36]. Compared to physical crosslinking, chemical crosslinking offers greater stability to HA-based hydrogels [36]. On the other hand, one of key characteristics of hydrogel is porosity through which interaction between gel, cells and the surrounding tissues occur [37–39]. Modification of the micro-architecture and the porosity of a hydrogel could always be vital concerns in biomaterial applications to deliver bioactive signals to cells growing within the developed hydrogel [37–39]. The controlled porosity endorses cellular penetration and new tissue formation within the three dimensional construction of the hydrogels [38,39]. Moreover, porosity of the hydrogel also regulates the rate swelling and drug/bioactive molecule delivery from the hydrogel [36,38,39]. Although there are several reports on hyaluronate-based hydrogel for biomedical applications [1,3,16–23]. But, keeping on mind the requisite demands of biomaterials such as sufficient strength and stability at physiological medium, higher mechanical properties, presence of interconnected pores, and excellent biocompatibility, researchers are continuously developing better and competent alternative biomaterials for biomedical applications. Our aim was synthesis of functionalized hyaluronate-based chemically crosslinked hydrogel with elastic property by avoiding the use of toxic organic solvents and reagents, which may create toxicity issue for its biological applications. For this purpose, keeping constant the structural integrity of hyaluronate, potassium persulphate was used to make hyaluronate-macroinitiator, which further initiated the polymerization of synthetic monomers followed by grafting and/or chemical crosslinking in presence of heat. 2-hydroxyethyl acrylate (2-HEA) was first grafted on the hydroxyl groups of the HA to import elasticity [40,41]. And then graft networks were crosslinked with bi-functional poly(ethylene glycol) diacrylate (PEGDA) to tune the microstructure properties like swelling, porosity and mechanical strength [42]. There are reports on methacrylated/thiolated HA and PEGDA or HEA-based hydrogels or semi-interpenetrating network for 3-D fibroblasts spreading and migration [43], to tune cell adhesion [1], and for the release of sodium benzoate and chlorpromazine [44,45]. However, the use of three components hydrogel such as HA, 2-HEA and PEGDA towards dimethyloxalylglycine (DMOG) and tetracycline (TCN) release, and osteoblast tissue regeneration has not been reported so far.

In the present study, HA, 2-HEA and PEGDA based terpolymeric hydrogel (HA-g-p(2-HEA)-x-PEGDA) has been synthesized by grafting and chemical crosslinking process through free radical polymerization using potassium persulfate as initiator. The amount of PEGDA has been varied to get better microstructure properties and interconnected porous network structure. Details *in vitro* cell proliferation and cytotoxicity studies using osteoblastic cells (MC3T3) have been performed by CCK, live/dead assay as well as MTT, neutral red and BrdU assays. The *in vitro* release studies of dimethyloxalylglycine as proangiogenic drug [46,47], and tetracycline as antibiotic [48] have been carried out at pH 7.0/7.4 and 37 °C. Histological analyses such as hematoxylin and eosin Y, and Masson's trichrome staining have been carried to investigate *in vitro* tissue regeneration ability. The experimental results showed that HA-g-p(2-HEA)-x-PEGDA hydrogel contains excellent interconnected porous architecture, showed excellent biocompatibility, controlled release property of DMOG/TCN, and *in vitro* regeneration of ECM and collagen. Hence it could be used in potential biomedical applications, specifically as controlled release matrix for DMOG/TCN delivery, and tissue engineering. To our best of knowledge, this is the first report on HA, HEA and PEGDA based terpolymeric hydrogel, which is examined as a carrier of DMOG/TCN drugs, and as a matrix for osteoblast tissue regeneration.

2. Experimental

2.1. Materials

Sodium salt of hyaluronic acid (HA, Mw = 1659731 Da, PDI = 3.974) was kindly donated by Hanmi Pharm. Co. Ltd., Korea. Dimethyloxalylglycine (DMOG, Cayman Chemical Company, USA), fetal bovine serum (FBS, Biotechnics Research, Mission Viejo, CA, USA), penicillin-streptomycin (Lonza, Seoul, Korea), cell counting kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan), live & dead viability/cytotoxicity kit for mammalian cells (Invitrogen, Carlsbad, CA, USA) and bromodeoxyuridine (BrdU, Roche, Germany) were purchased and used for experiment. All other chemicals such as potassium persulfate (KPS), 2-hydroxyethyl acrylate (2-HEA), poly(ethylene glycol) diacrylate (PEGDA, average MW ~575), tetracycline (TCN), α-MEM and all staining reagents were purchased from Sigma Aldrich (St. Luis, MO, USA, Germany and China). Distilled water (DW) was employed for all experiments.

2.2. Synthesis of terpolymeric gel

At first, 0.25 g HA (0.623×10^{-3} mol taking into consideration of molecular weight of one unit as shown in Scheme 1) was dissolved in 60 mL of DW in a 2-neck round bottom flask (RB) by stirring overnight at room temperature. After that, the solution was placed in digital glass oil bath (LK Lab Korea, Korea) and stirrer at 75 °C and with 400 rpm. After 2 h, nitrogen gas was pursed through the RB for 30 min to make the atmosphere inert. Afterwards, 5 mL aqueous solution of KPS (2.5×10^{-3} g, 0.0092×10^{-3} mol) as an initiator was added to the HA solution. After 20 min, 2 mL of 2-HEA (17.41×10^{-3} mol) as a monomer was mixed to the solution. When the solution turned into more viscous, three different volumes such as 250 µL (0.487×10^{-3} mol), 500 µL (0.973×10^{-3} mol), and 750 µL (1.460×10^{-3} mol) of PEGDA as a crosslinker were separately added for the synthesis of three grades terpolymeric crosslinked hydrogel. After addition of PEGDA, the reaction was processed for another 3 h. Lastly, the product was dialyzed in distilled water at 25 °C for 48 h. Then the purified samples were dried at lyophilizer at −56 °C for 7 days, called as dry HA-g-p(2-HEA)-x-PEGDA sample.

2.3. Characterizations

Molecular weight of the hyaluronic acid sodium salt (HA, Hanmi Pharm. Co. Ltd., Korea) was determined on a gel permeation chromatography (Model: Tosoh EcoSEC HLC-8320 GPC) equipped with 2 × Tskgel GMPW × l + Tskgel G2500PW × l and RI detector at Korea Polymer Testing and Research Institute (KOPTRI, Seoul, Korea). FTIR spectra were recorded using ATR-FTIR spectrometer (Model: Travel IR, Smiths Detection, USA). The wavelength range of spectra was 650–4000 cm^{-1}. The ^{1}H NMR spectra were executed with nuclear magnetic resonance (NMR) spectrometer (Model: DD2 700, Agilent Technologies-Korea, USA). The HR-MAS NMR spectrum (proton) of HA-*g*-p(2-HEA)-*x*-PEGDA gel was recorded in Korea Basic Science Institute (Seoul, Korea) using AVIII 700 MHz NMR spectrometer (Bruker Instruments, Inc., Germany) using D2O as solvent. The thermogravimetric analyses were performed using thermogravimetric analyser (TGA, Model: DTG-60, Shimadzu, Japan) at nitrogen atmosphere, where scan rate was 5 °C/min. The surface and cross-section morphology of samples were observed by SEM (Model: SEM, TESCAN VEGA3, Tescan Korea). The pore size was measured by ImageJ software, and porosity was determined using liquid displacement method using hexane as solvent [49]. The method of porosity determination is discussed in detail in the Supporting information.