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Gamma ray-induced synthesis of hyaluronic acid/chondroitin sulfate-based hydrogels for biomedical applications

Linlin Zhao [a], Hui-Jeong Gwon [b], Youn-Mook Lim [b], Young-Chang Nho [b], So Yeon Kim [a,c,*]

[a] Graduate School of Energy Science and Technology, Chungnam National University, Daejeon 305-764, South Korea
[b] Radiation Research Division for Industry & Environment, Korea Atomic Energy Research Institute, Jeongeup-si, Jeollabuk-do, 580-185, Republic of Korea
[c] Department of Chemical Engineering Education, College of Education, Chungnam National University, Daejeon 305-764, South Korea

HIGHLIGHTS

- HA/CS/PAAc hydrogels were synthesized by gamma-ray irradiation.
- HA/CS/PAAc hydrogels exhibited 91–93% gel fractions under 15 kGy radiation.
- All of the HA/CS/PAAc hydrogels exhibited high water contents of over 90%.
- The hydrogel samples showed relatively high cell viabilities of more than 82%.

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ABSTRACT

Hyaluronic acid (HA)/chondroitin sulfate (CS)/poly(acrylic acid) (PAAc) hydrogel systems were synthesized by gamma-ray irradiation without the use of additional initiators or crosslinking agents to achieve a biocompatible hydrogel system for skin tissue engineering. HA and CS derivatives with polymerizable residues were synthesized. Then, the hydrogels composed of glycosaminoglycans, HA, CS, and a synthetic ionic polymer, PAAc, were prepared using gamma-ray irradiation through simultaneous free radical copolymerization and crosslinking. The physicochemical properties of the HA/CS/PAAc hydrogels having various compositions were investigated to evaluate their feasibility as artificial skin substitutes. The gel fractions of the HA/CS/PAAc hydrogels increased in absorbed doses up to 15 kGy, and they exhibited 91–93% gel fractions under 15 kGy radiation. All of the HA/CS/PAAc hydrogels exhibited relatively high water contents of over 90% and reached an equilibrium swelling state within 24 h. The enzymatic degradation kinetics of the HA/CS/PAAc hydrogels depended on both the concentration of the hyaluronidase solution and the ratio of HA/CS/PAAc. The *in vitro* drug release profiles of the HA/CS/PAAc hydrogels were significantly influenced by the interaction between the ionic groups in the hydrogels and the ionic drug molecules as well as the swelling of the hydrogels. From the cytotoxicity results of human keratinocyte (HaCaT) cells cultured with extracts of the HA/CS/PAAc hydrogels, all of the HA/CS/PAAc hydrogel samples tested showed relatively high cell viabilities of more than 82%, and did not induce any significant adverse effects on cell viability.

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1. Introduction

Due to their unique biocompatibility, flexible methods of synthesis, range of constituents, desirable physical characteristics, and capability to respond to their environment, hydrogels have been extensively used in a variety of biomedical applications (Deligkaris et al., 2010; Hoare and Kohane, 2008). Over the past three decades, chemically and physically diverse hydrogels have become standard materials for drug delivery, contact lenses, corneal implants, tissue scaffolds, encapsulation of cells, and wound dressings (Hoffman 2002; Kraehenbuehl et al., 2009; Hamidi et al., 2008).

The fabrication of hydrogels can be approached using several methods, including irradiation, thermal annealing, freezing–thawing, and the use of chemical crosslinking agents to generate the physically or chemically crosslinked hydrophilic polymer networks (Deligkaris et al., 2010; Hoare and Kohane, 2008; Hoffman 2002; Kraehenbuehl et al., 2009; Hamidi et al., 2008).

Specifically, in recent years, there has been a growing interest in hydrogel systems prepared by the gamma-irradiation technique. The irradiation of an aqueous polymer solution results in the formation of radicals on polymer chains. Also, the radiolysis of

* Corresponding author at: Department of Chemical Engineering Education, College of Education, Chungnam National University, Daejeon 305-764, South Korea. Tel.: +82 42 821 5892; fax: +82 42 821 8864.
E-mail address: kimsy@cnu.ac.kr (S.Y. Kim).

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water molecules results in the formation of hydroxyl radicals, which also attack the polymer chains, resulting in the formation of macro-radicals. The recombination of the macro-radicals on different chains results in the formation of covalent bonds, so finally, a cross-linked structure is formed (Abd El-Rehim et al., 2013; Abd Alla. et al., 2012; Magda et al., 2014). Living free radical polymerization via gamma-ray irradiation is a promising process for practical applications because of its many advantages, such as simple process control, being environmentally friendly, the possibility of achieving hydrogel formation and sterilization in one technological step, ability to run at room temperature, and high penetrating ability (Gottlieb et al., 2005; Jha et al., 2010). In addition, the radiation technique is clean, because it does not require additional chemical initiators and cross-linkers, which could be harmful and difficult to remove from the polymer networks, and does not need any further purification (Jha et al., 2010; Rosiak et al., 1995). For these reasons, the gamma-ray irradiation method is very useful for preparing hydrogels for biomedical applications, for which even a small degree of contamination is undesirable, and is often used to sterilize biomedical devices for medical and veterinary applications (Gottlieb et al., 2005; Jha et al., 2010; Rosiak et al., 1995; Gad 2008; Juby et al., 2012).

Glycosaminoglycans (GAGs), a large family of heterogeneous polysaccharides, are linear sulfate-substituted polymers (with the exception of hyaluronic acid (HA)) composed of alternating hexuronic acid and hexosamine units that play important roles in all living organisms (Hu et al., 2011). They represent the main source of the high water binding capacity of cartilage. The swelling is based on the binding of water to the polar groups of GAG (carboxylate and sulfate), on the electrostatic repulsion between the GAG molecules, and on the entropic contributions resulting from the mixing of water and counterions (Hu et al., 2011; Lam et al., 2014; Kim et al., 2011).

HA and chondroitin sulfate (CS) belong to the most important GAGs found in extracellular tissues in many parts of the body. HA is a material of increasing importance to biomaterials science and is found in applications in diverse areas ranging from tissue culture scaffolds to cosmetic materials (Lam et al., 2014; Kim et al., 2011; Leach and Schmidt, 2005). HA plays a prominent role in lubrication, cellular processes, and wound healing, and it is naturally angiogenic when enzymatically degraded to small fragments (Leach and Schmidt, 2005; Oudshoorn et al., 2007; Toh et al., 2012). CS can bind with core protein to produce the highly absorbent aggregan, which is a major structure inside cartilage and acts as a shock absorber, or it can produce sydecan, which is a cell receptor that can interact with adhesion proteins, cells and the extracellular matrix (Hu et al., 2011; Strehin et al., 2010; Wang et al., 2003; Piai et al., 2009). It was also reported that inflammatory reactions at injury sites were reduced by CS due to the accelerated metabolism of cells and the ability to sustain a normal microenvironment for cell growth (Yan et al., 2013).

There have been developed various natural polymer hydrogel system which prepared using radical initiators and cross-linkers (Hoare and Kohane, 2008; Hoffman 2002; Hu et al., 2011; Lam et al., 2014). Recently, it has been shown that irradiation of highly concentrated solutions (paste-like state) of carboxymethyl cellulose or carboxymethyl starch can lead to cross-linking without the need for any additives (Yoshii et al., 2003; Nagasawa et al., 2004; Kume et al., 2002). However, natural polymer hydrogel system crosslinked by gamma-ray irradiation methods has been rarely reported because the treatment of polysaccharides and other natural polymers with ionizing radiation either in the solid state or in aqueous solution leads to degradation (Al-Assaf et al., 2007).

The main purpose of this study was to develop biocompatible hydrogel systems prepared by gamma-ray irradiation of aqueous polymer solution without any radical initiators or crosslinkers to serve as scaffold materials with appropriate water-binding capacity, degradation rate and drug release properties for skin tissue engineering applications. In this study, we designed hydrogels composed of biocompatible natural polymers, HA and CS, and the synthetic polymer, poly(acrylic acid) (PAAc), including their carboxylic acid side groups. These groups are readily ionizable and sensitive to the effects of the pH and ionic strength of the surrounding medium. The HA/CS/PAAc hydrogels were synthesized by gamma-ray irradiation, and their physicochemical properties were characterized to evaluate their feasibility as artificial skin substitutes. The degree of gelation, swelling properties, enzymatic degradation kinetics, and *in vitro* drug release behaviors of the HA/CS/PAAc hydrogels were determined. In addition, the *in vitro* cytotoxicity of the HA/CS/PAAc hydrogels was investigated.

2. Materials and methods

2.1. Materials

HA (Mw 1.6×10^6 Da), CS (chondroitin sulfate A sodium salt from bovine trachea), PAAc (Mw 89,000–98,000), methacrylic anhydride (MAA), glycidyl methacrylate, and triethylamine were purchased from Sigma (St. Louis, MO, USA). Hyaluronidase, cefazolin, theophylline, and sodium hydroxide were obtained from Aldrich (Milwaukee, WI, USA). Dulbecco's Modified Eagle Medium (DMEM; high glucose, with L-glutamine and pyridoxine hydrochloride without sodium pyruvate), Dulbecco's phosphate-buffered saline (PBS), and heat-inactivated fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). Distilled and deionized water was prepared using a Milli-Q Plus System (Millipore, Bedford, MA, USA). All other chemicals used were reagent grade and were used as purchased without further purification.

2.2. Methods

2.2.1. Synthesis of HA and CS derivatives

HA and CS derivatives with polymerizable residues were synthesized according to a previously reported method (Oudshoorn et al., 2007). Briefly, 1 g HA was dissolved in 100 ml distilled water. Next, 2.2 ml triethylamine and 2.2 ml glycidyl methacrylate were added separately and thoroughly mixed for 1 h at 60 °C, and were then stirred overnight at room temperature. Then, the reaction solution was precipitated in a 20-fold volumetric excess of acetone and dissolved twice in distilled water to remove excess reactants. The methacrylated hyaluronic acid (MA-HA) solution was lyophilized and stored desiccated at 4 °C.

After 0.5 g CS was dissolved completely in 25 ml distilled water, 8 ml MAA was added dropwise into the CS solution over 30 min. Then, the 5 N NaOH solution was carefully added to control the reaction mixture to ~pH 8.0 (mol ratio of MAA/NaOH was 1/1.12). The reaction solution was stirred at room temperature for 2 h, then at 4 °C for 8 h, and was finally moved to a refrigerator for another 14 h. After the reaction, the reaction mixture was precipitated in cold methanol and the precipitate was centrifuged and washed several times with a large amount of cold methanol until no MAA residue was detected by nuclear magnetic resonance (NMR) measurement. The resulting methacrylated chondroitin sulfate (MA-CS) was dried in a freeze-dryer. The procedures for the synthesis of MA-HA and MA-CS are illustrated in Fig. 1. The chemical structures of the HA and CS derivatives were characterized by 400 MHz ^{1}H NMR measurement (JNM-AL400, JEOL, Tokyo, Japan).

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