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Preparation and fracture process of high strength hyaluronic acid hydrogels cross-linked by ethylene glycol diglycidyl ether

Burak Tavsanli, Oguz Okay *

Istanbul Technical University, Department of Chemistry, 34469 Istanbul, Turkey

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ABSTRACT

We present a synthetic strategy to produce high-strength hydrogels based on hyaluronic acid, a unique biomacromolecule with distinctive biological functions. The hydrogels were prepared using a two-step procedure. In the first step, hyaluronic acid (HA) was chemically cross-linked in aqueous solutions using ethylene glycol diglycidyl ether (EGDE) under various experimental conditions. EGDE-cross-linked HA hydrogels containing 97–99% water were fragile, and ruptured when compressed to 25–51% strain under 0.02–0.15 MPa stresses. By applying the double-network approach in the second step, we were able to generate high strength HA/poly(*N*,*N*-dimethylacrylamide) double-network hydrogels containing 84–94% water. Tuning the ratio of the network components could result in hydrogels exhibiting a compressive modulus of 0.9 MPa that sustain 19.4 MPa compressive stresses, which are much larger than those reported before for the hydrogels derived from the methacrylated HA macromers. Thus, the hydrogels presented here are promising materials to make use the characteristics of HA in stress-bearing biomedical applications. Cyclic mechanical tests show irreversible stress-strain curves with a large hysteresis indicating that elastically effective cross-links of HA first-network are irreversibly destroyed under load by dissipating energy. This internal fracture of HA network together with the high mass ratio of the second to the first-network components are responsible for the extraordinary mechanical properties of the hydrogels.

1. Introduction

Hyaluronic acid (HA) is a natural anionic polyelectrolyte consisting of disaccharide repeat units of β-1,4-D-glucuronic acid - β-1,3-*N*-acetyl-D-glucosamine [1]. HA is the main component of extracellular matrix, and it plays an important role in wound-healing processes [2]. Because of the distinctive biological functions and lubricating properties, HA is an effective biomaterial for soft tissue regeneration [3–6]. However, rapid in vivo degradation and poor biomechanical performance of pure native HA limit its clinical applications. To generate a less degradable HA with improved mechanical properties, HA was chemically cross-linked to form HA hydrogels [7,8]. The functional groups in HA as potential cross-link points are the one carboxyl group and four hydroxyl groups on its repeat unit, which can be cross-linked via ester and ether linkages, respectively (Scheme 1).

Cross-linking of HA has been reported using several cross-linkers [9–15], including divinyl sulfone (DVS), glutaraldehyde (GTA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), ethylene glycol diglycidyl ether (EGDE), butanediol diglycidyl ether (BDDE) and poly (ethylene glycol) diglycidyl ether (PEGDE). Although the cross-linking of HA reduces its degradation rate and solubility in aqueous media, the resulting hydrogels exhibit poor mechanical properties to be used in stress-bearing applications. The lack of mechanical strength in HA hydrogels is mainly due to the absence of a viscoelastic dissipation in the chemically cross-linked HA network leading to the fracture of the hydrogels under low stresses [16,17]. Recently, high-toughness HA hydrogels with a macroporous structure have been prepared using cryogelation of aqueous HA solutions at subzero temperatures in the presence of EGDE cross-linker [18,19]. Due to the cryoconcentration of the reactants in the unfrozen micro-domains of the cryogelation system [20], HA cryogels are very tough and can completely be squeezed without any crack propagation. However, the toughness of HA cryogels is due to water flowing out of their macropores under stress and, they are very soft materials exhibiting an elastic modulus of below 2 kPa [18].

An alternative two-step approach to produce HA hydrogels is the chemical modification of HA prior to the cross-linking reactions to incorporate additional functional groups. The widely used strategy is to introduce photopolymerizable methacrylate groups on HA molecules using glycidyl methacrylate, which are subsequently photopolymerized to form HA-based hydrogels [21–23]. Another strategy is the synthesis of thiol-functionalized HA which spontaneously forms intermolecular disulfide bonds upon exposure to air [24,25]. To increase the rate of cross-linking, Michael-type addition reaction was also employed by

* Corresponding author.
E-mail address: okayo@itu.edu.tr (O. Okay).

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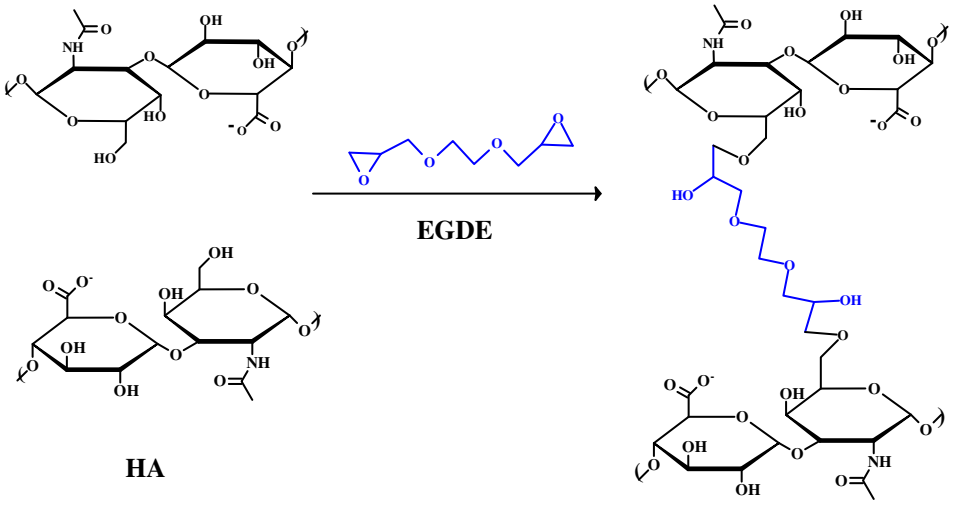

Scheme 1. Cross-linking of hyaluronic acid (HA) using ethylene glycol diglycidyl ether (EGDE).

the addition of poly(ethylene glycol) diacrylate cross-linker [24]. However, the hydrogels derived from the chemically modified HA also exhibit poor mechanical properties [26,27]. To achieve a high mechanical strength, they can be reinforced with other macromolecules [28–30], or energy dissipation mechanisms can be created to slow crack propagation within the gel network [31,32]. By using the double-network (DN) concept [33–37], Weng et al. prepared mechanically strong HA hydrogels consisting of a highly cross-linked methacrylated HA (GMHA) first-network and a lightly cross-linked ductile poly(*N*,*N*-dimethylacrylamide) (PDMA) second-network [31]. DN hydrogels exhibit a compressive modulus of 0.5 MPa and a fracture stress of 5.2 MPa. A similar procedure was recently applied by Tavsanli et al. for the synthesis of triple-network hydrogels [32]. The hydrogels consisting of GMHA/PDMA/PDMA interconnected interpenetrated network components exhibit compressive fracture stresses up to 20 MPa.

However, the chemical modification procedures of HA summarized above to prepare DN hydrogels involve macromonomer synthesis and purification steps with many chemicals including dihydrazides, trimethylamine and phase transfer catalysts. Alternatively, a simple way to produce DN hydrogels is to use native HA to create the first-network hydrogels instead of GMHA. Moreover, because GMHA is more hydrophobic than HA due to its methacrylate groups, one may expect that the single-network HA hydrogels will exhibit a higher degree of swelling in aqueous solutions as compared to GMHA hydrogels. This means that a larger amount of the second ductile PDMA network component could be introduced in HA hydrogels during double networking leading to better mechanical properties [33]. This is the main motivation of this study.

Here, we use pure native HA for the preparation of mechanically strong double-network HA hydrogels. To our knowledge, such hydrogels have not been reported before. HA hydrogels were prepared in aqueous HA solutions in the presence of EGDE cross-linker under various experimental conditions. EGDE contains epoxide groups on both ends and, has been widely used for cross-linking of biopolymers carrying hydroxyl, amino, and sulfhydryl groups [38–40]. Since the hydroxyl groups on HA molecules react with the EGDE [18,19], intermolecular cross-links form during gelation, resulting in the formation of a three-dimensional HA network (Scheme 1). We monitored the cross-linking reactions of HA with EGDE cross-linker in alkaline solutions by rheometry using oscillatory deformation tests. HA hydrogels formed were subjected to rheological and mechanical measurements to evaluate their viscoelastic and mechanical properties as a function of the cross-linker content. By applying DN approach, we were able to generate HA/PDMA hydrogels containing 84–94% water. The hydrogels sustain 0.8–19.4 MPa compressive stresses and exhibit compressive moduli up to 0.9 MPa. These values are much larger than those reported before for DN hydrogels derived from GMHA macromers [31,32]. Thus, HA hydrogels described here are promising materials for stress-bearing biomedical applications. As will be discussed below, high mechanical strength of the present hydrogels is due to the internal fracture of HA network by dissipating energy as well as due to the larger swelling capacity of the first-network hydrogels based on HA as compared to GMHA, increasing the ratio of the second-to-first network components in DN hydrogels.

2. Materials and methods

2.1. Materials

Hyaluronic acid sodium salt (HA) from Streptococcus equi was obtained from Sigma-Aldrich (impurities: ≤1 protein), and its viscosity averaged molecular weight is 1.2×10^6 g·mol^{-1} [18]. Ethylene glycol diglycidyl ether (EGDE, Polysciences, Inc., total chlorine content: 0.6%), NaOH (Merck, ≥99%), *N*,*N*-dimethylacrylamide (DMA, Sigma-Aldrich, 99%, contains 500 ppm monomethyl ether hydroquinone as inhibitor), 2-oxoglutaric acid (Fluka, ≥99%), and N,N -methylene(bis)acrylamide (BAAm, Merck, ≥99%), were used as received.

2.2. Hydrogel preparation

HA solutions were prepared by dissolving HA in 1 w/v% NaOH solution by gently stirring at 25 °C for 1 h. To ensure a complete dissolution, HA solutions were kept at 4 °C for 24 h. After adding EGDE cross-linker, the solution was stirred for 15 min before being transferred into plastic syringes (inner diameter = 6 mm, length = 70 mm) to conduct the cross-linking reactions. The reactions were carried out both at 25 °C and 50 °C for 4 days and 4 h, respectively. DN hydrogels were synthesized by immersing HA hydrogels in as-prepared states in aqueous solutions containing the monomer DMA (30 w/v%), the second-network cross-linker BAAm (0.05 and 0.10 mol% of DMA), and the photo-initiator 2-oxoglutaric acid (0.1 mol% of DMA). The volume of the aqueous solution was much larger than the gel volume (around 120 mL solution per gram of hydrogel sample), so that the concentration of the solution was practically unchanged. After the swelling equilibrium was established within 4 days, the solution containing the hydrogel was transferred into syringes of 10 mL in volume and the polymerization was carried out 25 °C under UV light at 365 nm for 24 h.