Mechanical Improvement of Hyaluronic Acid (HA) Hydrogels and Incorporation of Polyethylene Glycol (PEG)

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Statement of Purpose: Hyaluronic acid (HA) is a naturally derived polymer as a major component of the extracellular matrix, mediating various cellular activities, and has been widely used as tissue engineering scaffolds [1, 2]. Having many advantages, however, rapid chemical and mechanical degradation of HA by hyaluronidase in the physiological condition has been regarded as a major challenge to be resolved. A range of HA modifications have been proposed for its long-term stability, where cross-linking HA chains via a cross-linker has been commonly used [3]. In this study, we have introduced polyethylene diglycidyl ether (PEGDE) to HA as a cross-linker in order to enhance mechanical and chemical stabilities of HA hydrogels as compared to one of commonly used cross-linkers, 1,4-butanediol diglycidyl ether (BDDE). Moreover, the material properties of HA-PEG hydrogels have been explored in terms of PEG contents in the composite hydrogel.

Methods: HA hydrogels were cross-linked using either polyethylene glycol diglycidyl ether (PEGDE) (M.W. = 500) or 1,4-butanediol diglycidyl ether (BDDE) by varying the concentrations of each cross-link agent. 10 wt% HA solution was prepared by dissolving 1 g HA (M.W. = 1500~2200 kDa in 10 mL 0.25 N NaOH aqueous solution. 200, 300, and 400 μL BDDE were added to the HA solution, respectively, so called as 1x, 1.5x and 2x BDDE. Likewise, the molar equivalent amount of PEGDE to BDDE for three different concentrations was added to the HA solution, resulting in 1x, 1.5x and 2x PEGDE. All reactions were performed in oven at 50 ºC for 2 h. Fourier-transformed Infrared (FT-IR) measurement was carried out with cross-linked HA hydrogels as compared to pure HA. Swelling test was performed with fully swollen samples in PBS at 37 ºC. Rheology measurement was carried out using parallel plate rheometer.

Results: PEG-cross-linked HA hydrogels were fabricated at different PEGDE concentrations as shown in **Fig. 1a**. The FT-IR spectra of PEG-cross-linked HA indicate that intensity of peaks around 2875, 1108 cm^{-1} are significantly increased as the degree of cross-linking increases along with increased PEGDE concentrations (**Fig. 1b**). Swelling behavior of PEG-cross-linked HA hydrogels were investigated as compared to cross-linked HA by BDDE (**Fig. 2**). At the same molar concentration, PEG-cross-linked HA was less swollen than BDDE-cross-linked HA. In the meantime, as the concentration of each cross-linker increases, the degree of swelling is degreases. Rheological behavior of cross-linked HA clearly exhibits that PEG-cross-linked HA hydrogels has improved elasticity as compared to BDDE-cross-linked HA at the same molar concentrations (**Fig. 3a**). At 1 Hz, shear moduli of all cross-linked HA were compared. With PEGDE, the shear modulus of the cross-linked HA was found to increase up to ~2700 Pa.

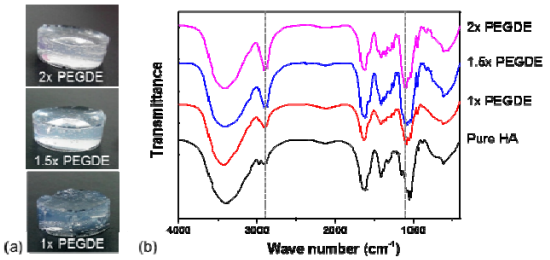

Figure 1. (a) Optical images and (b) FT-IR spectra of PEG-cross-linked HA hydrogels at different PEGDE concentration.

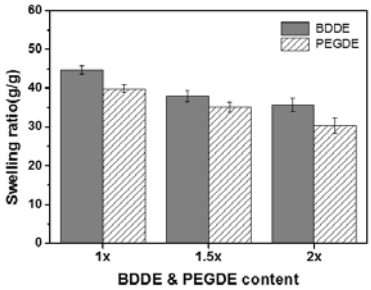

Figure 2. Swelling ratios of cross-linked HA hydrogels by two cross-linking agents at different concentrations.

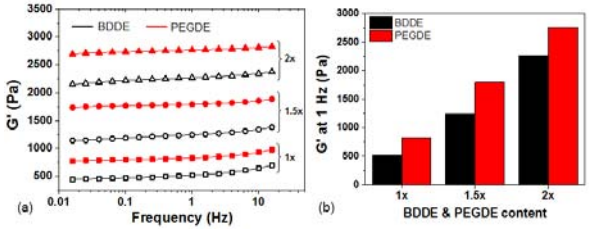

Figure 3. (a) Rheological behavior and (b) shear modulus (at the frequency of 1 Hz) of cross-linked HA hydrogels by two cross-linking agents (BDDE and PEGDE) at different concentrations

Conclusions: PEG-cross-linked HA hydrogels have been successfully fabricated using PEGDE without additional modification. When compared to cross-linked HA by BDDE, PEG-cross-linked HA exhibited decreased swelling ratios and increased elasticity, indicating improved mechanical performance. Moreover, the amount of PEGDE controls swelling behavior and elasticity of the cross-linked hydrogels by means of degree of cross-linking, which can optimize HA hydrogels to meet the needs of various tissue engineering applications.

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