Mechanically Enhanced Hyaluronic Acid-Based Hydrogels via Dual-Crosslinking Process

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Statement of Purpose: Hyaluronic acid (HAc) have been widely used for various tissue engineering scaffolds because of excellent biocompatibility and hydrophilicity, whereas poor biomechanical properties as well as fast in vivo degradation through enzymatic require additional modifications of HAc for their clinical use [1]. In our previous study, we introduced *in situ* precipitation of calcium phosphate (CaP) to chemically cross-linked HAc [2]. Despite significant mechanical improvement of HAc-CaP nano-composite (HAc-CaP) hydrogels, further optimization is still required, in particular, when the inorganic content should be minimized for soft tissue scaffolds. Therefore, in this study, we have proposed the ionic crosslinking method using various cations in conjunction with chemical crosslinking or *in situ* CaP precipitation of HAc to enhance mechanical stability.

Methods: All HAc hydrogels were photo-crosslinked via glycidyl methacrylation synthesis. The CaP *in situ* precipitation was carried out in the crosslinked HAc hydrogel as described in our previous study [2]. The ionic crosslinking process was then introduced to both cross-linked HAc and HAc composite hydrogels by immersing them in the aqueous solutions containing multivalent cations (Ca^{2+}, Al^{3+}, Ba^{2+}, Sr^{2+} and Fe^{3+}) for 6 hrs. Mechanical behavior of hydrogels was assessed using a controlled strain rheometer in the range of frequency, 0.1 to 100 rad/s. Inorganic phases of the composite hydrogels were further evaluated by TEM and EDS after ionic crosslinking.

Results: Mechanical behavior of pure HAc and HAc/CaP nano-composite hydrogels crosslinked by both chemical and ionic crosslinking (Ca^{2+}) (called dual-CL) was found to be significantly improved as compared to the photo-crosslinked hydrogels without ionic crosslinking (called single-CL) as shown in **Fig. 1a**. In particular, **Fig. 1b** shows that ionic crosslinking increased shear modulus of both pure and composite HAc hydrogels by a factor of ~1.5 (pure HAc) and ~2.5 (HAc/CaP). To explore the efficiency of ionic crosslinking using different cations (**Fig. 2a**). Even though all six cations appear to work as effective ion crosslinkers, composite hydrogels exhibit different mechanical improvements depending on ions as opposed to pure HAc. Interestingly, Ca ions exhibits the highest mechanical improvement of HAc/CaP hydrogels, whereas Al^{3+} and Fe^{3+} were less effective because they decomposed CaP particles of the composite gel due to their acidic environments. Pre-treatment of HAc at pH 11 was also found to increase the efficiency of ionic crosslinking as compared to pH 7 (**Fig. 2b**). In the case of composite gels, *in situ* precipitation was carried out at pH 11, thus additional pH treatment was not required. After ionic crosslinking, CaP nanoparticles were observed to verify the structural or compositional changes due to additional ion incorporation (**Fig. 3**). While smaller CaP particles were additionally formed with Ca^{2+}, there was no composition change during ionic crosslinking by either Ba^{2+} or Sr^{2+}.

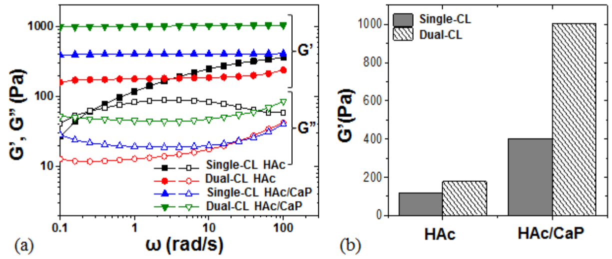

Fig.1. (a) Rheological behaviors (G', G'') and (b) G' (at 1 rad/s) of HAc and HAc/CaP hydrogels crosslinked by either single- (photo-crosslinking) or dual-crosslinking.

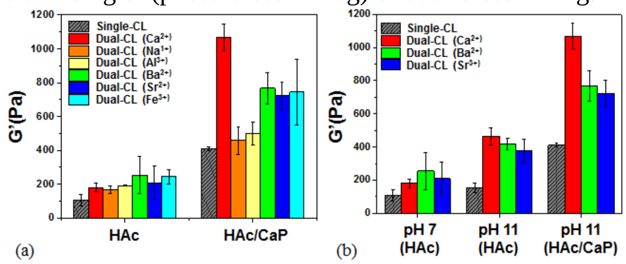

Fig.2. (a) Ionic effects and (b) pre-treatment effects at different pH of dual-CL HAc and HAc/CaP hydrogels.

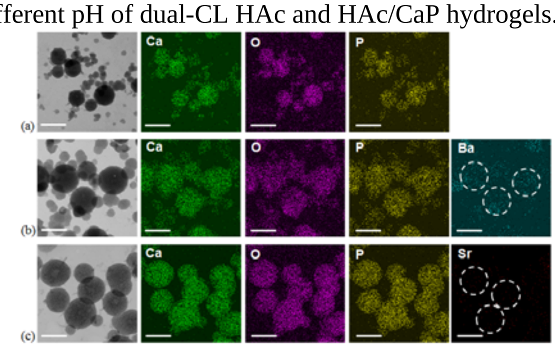

Fig.3. TEM and EDS mapping images of inorganic CaP nanoparticles in HAc/CaP hydrogels after dual-CL using different ionic solutions with (a) Ca^{2+} (b) Ba^{2+}, and (c) Sr^{2+} (Scale bar = 250 nm).

Conclusions: We have successfully crosslinked pure and nanocomposite HAc hydrogels by ionic crosslinking in addition to photo-crosslinking using various multivalent cations. All dual-crosslinked hydrogels achieved remarkable mechanical enhancement, where ionic crosslinking showed better efficiency with Ca ions and pre-treatment at pH 11. The dual crosslinking process for HAc and its composite hydrogels enables improved and reliable performance of HAc for biomedical applications.

References:

1. Li,Z.Y., J. Mater. Chem. B, 2013; 1(12); 1755-1764.
2. S.-H. Jeong et al., Society for Biomaterials, 2014, Abstract #271,