Hyaluronic Acid – Calcium Phosphate Nano Composite via *in-situ* Precipitation

Seol-Ha Jeong[a], Tae-Sik Jang[a], Hyun-Do Jung[a], Min-Ho Kang[a], Hyoun-Ee Kim[a],

[a] Department of Materials Science and Engineering, Seoul National University, Seoul, Korea

Statement of Purpose:

Hyaluronic acid (HAc) exhibits excellent biocompatibility and hydrophilicity, whereas it has limitations on biomedical applications due to poor biomechanical properties as well as fast in vivo degradation through enzymatic reaction [1]. In this study, we have introduced in-situ precipitation method for fabrication of HAc-calcium phosphate (CaP) nanocomposite hydrogel in order to improve mechanical and biological behaviors of HAc under physiological conditions by adding calcium phosphate nanoparticles. In particular, in-situ precipitation enables homogeneous and fast incorporation of nanoparticles into a polymer matrix. The nanocomposite hydrogels with various CaP contents were evaluated to define optimal conditions of the hydrogels.

Methods:

Glycidyl-methacrylate-HAc conjugates were crosslinked under UV light for 15 min [2]. The fabricated HAc hydrogels were immersed in the mixed solution with calcium chloride and phosphoric acid for 1day and then were dipped in ammonium hydroxide solution for 3hours to precipitate calcium phosphate within the hydrogels. Morphology of the composite hydrogels and size analysis of nanoparticles in the hydrogels were evaluated by SEM and TEM. Both the swelling and *in vitro* degradation tests of the hydrogels were performed in PBS at 37℃. Mechanical behavior of hydrogels was assessed using a controlled strain rheometer where. Elastic moduli (G') were measured in the range of frequency, 0.1 to 100 rad/s. Cell morphology was observed by CLSM and cell proliferation was examined by MTS assay 10days after seeding fibroblast cell (L929) on the hydrogels.

Results:

The CaP nanoparticles were distributed homogeneously within HAc matrix, increasing surface roughness of the hydrogels, and appeared uniform with the size of ~200 nm (Fig. 1). The degradation of the composite hydrogels were significantly retarded as compared to that of pure HAc hydrogels in hyaluronidase solution, showing improved chemical stability of the composite(Fig. 2A). Moreover, composite hydrogels exhibit improvement on rheological behaviors, indicating the shear moduli of composite hydrogels are 2.5-4 times as great as pure HAc hydrogel depending on the CaP contents (Fig. 2B). Fig. 3(A) represents that fibroblast cells on the nano composite hydrogels show more advanced progress of cell adhesion as compared to HAc hydrogel. The level of cell proliferation behaviors on nanocomposite hydrogels after 5days was around 8 times higher than that on HAc hydrogel, implying the enhanced biocompatibility of composite hydrogels. . Cells were also found to penetrate up to ~ 60μm depth from the surface of the composite hydrogels, whereas only 10 μm from the surface of HAc hydrogel.

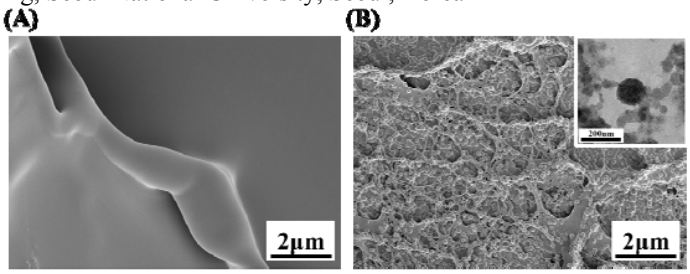

Fig 1. Surface morphology of (A) HAc hydrogel and (B)HAc-CaP nano composite hydrogel

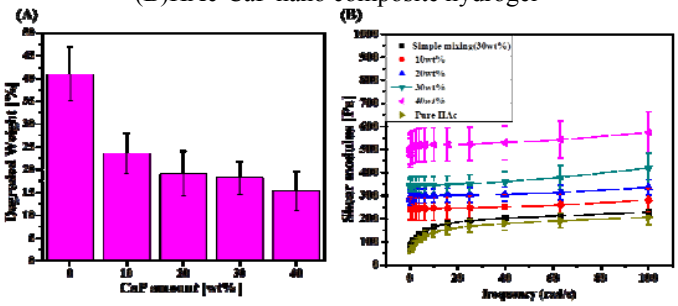

Fig 2. (A) Degraded weight of various hydrogels (B) Rheological behaviors of various hydrogels

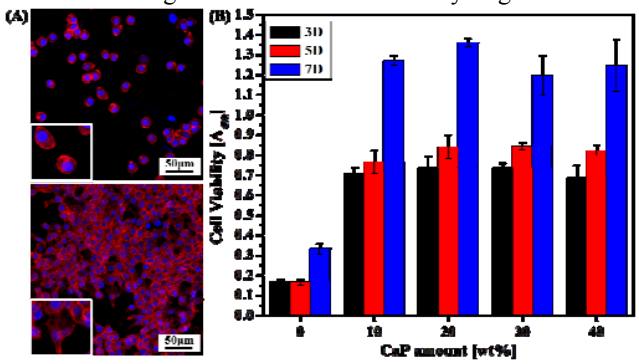

Fig 3. (A) Cell morphology of HAc hydrogel (Top) and 30 wt% CaP composite hydrogel (Bottom) (B) Cell proliferation after 3, 5 and 7 days of various hydrogels

Conclusions:

From the in-situ precipitation, CaP-HAc nanocomposite hydrogels were successfully fabricated, where uniformly precipitated CaP nanoparticles were strongly entangled with HAc polymer chain. The composite hydrogels exhibit significantly reduced degradation rates and dramatic improvement of shear modulus. The *in vitro* cell test results also demonstrated significantly enhanced biocompatibility of the composite hydrogels. By controlling the CaP amounts, mechanical and biological behaviors of the nanocomposite hydrogel can be optimized, exhibiting great potential for various biomedical applications.

References:

1. Li,Z.Y., J. Mater. Chem. B, 2013; 1(12); 1755-1764.
2. Schramn, C., IOVS, 2012;53(2); 613-621.