Hyaluronic Acid Silica Sol-gel Nano-hybrids by Biomimetic Approach

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Statement of Purpose: Hyaluronic acid (HA)-based hydrogels have attracted widespread interest for biomedical applications due to their excellent biocompatibility and viscoelastic properties [1]. However, HA hydrogels have poor mechanical properties that has been regarded as the main drawback to be resolved. Although extensive researches have been made to develop mechanically robust hydrogels [2], most of the strong composite systems suffer from challenges such as biodegradability and biocompatibility. Among the candidates for inorganic reinforcement of hydrogels, silica is known to be non-toxic and bioactive with high stiffness [3]. In marine organisms such as diatoms and sponges, silica is generated on the proteins by aqueous silica precursors, co-assembling to form rigid cell walls [4]. Driven by this inspiration, in this study, we have developed a biomimetic approach by using HA hydrogel itself for the formation of silica network, eventually, fabricating homogeneous and mechanically strong HA-silica organic/inorganic hybrids.

Methods: HA hydrogels crosslinked by butanediol diglycidyl ether were prepared. This HA hydrogels were swollen in ethanol/water solution then infiltrated by tetramethyl orthosilicate (TMOS) up to 10 (v/v) %. Silica network formation was followed by the sol-gel reaction through ageing process then the swelling medium was squeezed out with PBS and ethanol treatment. The densified HA-silica hybrids by the squeezing were re-swollen in PBS for evaluation. Internal structures of the HA-silica nano-hybrids were characterized by TEM and their mechanical properties were assessed using rheological measurement. Moreover, their bioactivity was evaluated using MTS cell proliferation assay.

Results and Discussion: The internal structures of the HA-silica hybrids by biomimetic sol-gel approach are shown in **Fig. 1.** About 5-10 nm sized silica nanoparticles were inter-connected to form a porous and homogeneous network structure throughout the hybrid matrix. The storage moduli (G') dramatically increased by two-order range from 1,000 to 160,000 Pa, with varying silica contents **(Fig. 2A)**. Although silica has been regarded as brittle material, the HA-silica hybrids displayed flexibility enduring high deformation by knotting **(Fig. 2B)**. *In vitro* cellular response was examined using colorimetric assay (MTS), culturing L929 for 3 days as shown in **Fig. 3**. The degrees of proliferation of HA-silica hybrids were significantly higher than those of pure HA hydrogels, which means that the HA-silica hybrids facilitate the cell growth without any sign of toxicity originating from silica.

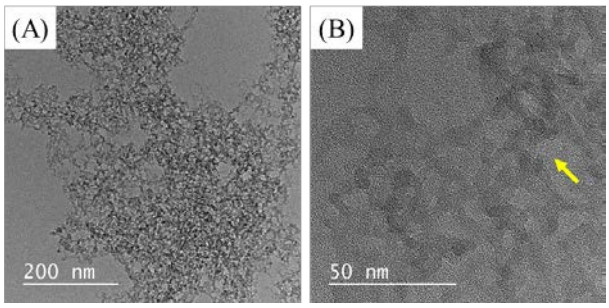

Fig. 1. TEM images of the HA-silica hybrids at different magnifications (A), (B).

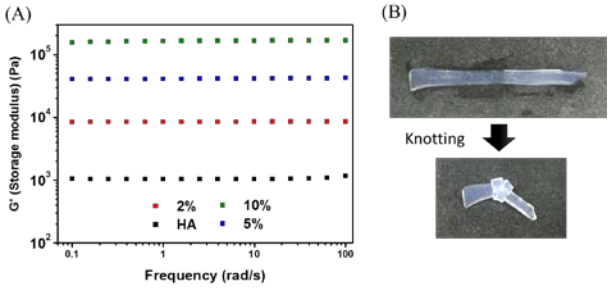

Fig. 2. Storage moduli of the HA and HA-silica hybrids with different silica contents (A), and optical images of HA-silica hybrid with knotting (B).

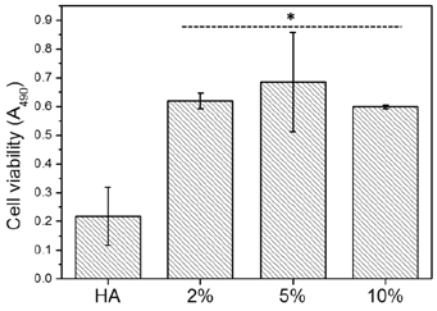

Fig. 3. Fibroblast (L929) cell viability of the HA and HA-silica hybrids with different silica contents. (*$p < 0.05$).

Conclusions: HA-silica hybrids were successfully fabricated using a biomimetic silica sol-gel process. Remarkably enhanced mechanical properties of HA-silica hybrids were achieved by an effective method that facilitates the formation of the rigid and homogeneous silica network. The mechanical strength of HA-silica hybrids can be tailored along with bioactivity by adjusting the silica contents. These findings indicate that HA-silica hybrid system would be a promising substitute for tissue engineering applications.

Reference

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