One-step preparation of injectable hyaluronic acid based hydrogel at physiological condition

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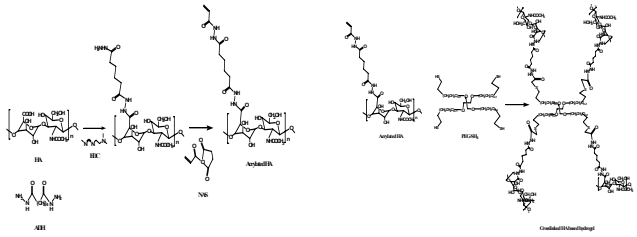
Statement of Purpose: This report presents an approach to fabricate hyaluronic acid (HA) based hydrogels to permit *in vivo* engineering of new tissues. HA based hydrogels is cross-linked network structure that is similar to that of the extracellular matrix (ECM) and allows for maintenance of tissue-like elastic properties. Especially we synthesized injectable HA based hydrogels that can be prepared at physiological condition via Michael type addition reaction. We characterized physiochemical properties of hydrogels including swelling ratio, mechanical strength, and degradation by hyaluronidase. Human mesenchymal stem cells are cultured in this HA based hydrogel and evaluated cell morphology and viability *in vitro*.

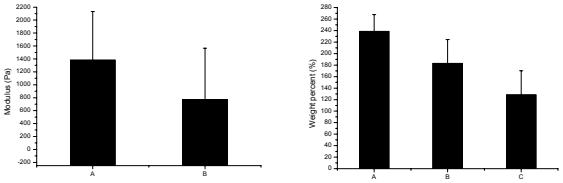
Methods: HA was derivatized by introducing acryl group using N-acryloxysuccinimide and adipic acid dihydrazide. HA derivatives and PEG-SH_4 were used for the preparation of hydrogel. Mechanical properties of hydrogels was modulated by varying concentrations of acrylated HA and PEG-SH_4. Elastic modulus(G') of the hydrogel was measured using rheometry. Swelling ratio was measured by comparing the initial gel weight and the water-swelled hydrogel weight after overnight incubation in water. Human mesenchymal stem cells (hMSCs) from human bone marrow($1X10^6$cells/construct)were put in different samples and cultured to evaluate the cellular reactions. Cell viability was analyzed by Live and Dead assay.

Results / Discussion: Using adipic acid dihydrazide and N-acryloxysuccinimide, hyaluronic acid (HA) was acrylated successfully(*Fig.1(A)*) In suitable conditions (HA: NAS: EDC is 1:12:5 and 1:12:10), HA was acrylated and identified by Fourier Transform Infrared Spectroscopy(FT-IR) and Nuclear Magnetic Resonance(NMR). The degree of acrylation of HA was about 17%. When 1%(w/v) HA-Ac solution and PEG-SH_4 was mixed together, HA based hydrogel was synthesized via Michael type reaction at 37□ without any further treatment(*Fig.1(B)*). Gelation time was analyzed by measuring elastic (G′) and viscous (G″) modulus of the HA hydrogel with different ratio of HA-Ac and PEG-SH_4(A=1:1,B= 1:2). Gelation became complete at 20min in sample A. After gelation was completed, elastic modulus was maintained from 2800 to 3100(Pa). This gelation kinetics shows that this HA based hydrogels can be injectable. As the contents of PEG-SH_4 increased, the rate of cross-linking reaction is slower and the complex modulus was smaller. (*Fig.2(A)*) and the swelling properties of hydrogel is smaller(*Fig.2(B)*). hMSCs were cultured in the gel with or without BMP-2 (Fug 3). Cellular morphology in the gel was almost similar with or without BMP-2. Cells in the BMP-2 containing hydrogel showed higher viability compared to controls. Hydrogels have been extensively used in tissue engineering and drug delivery fields. HA based hydrogel could be used for regenerating medicines as an injectable agent with growth factors or cells. Especially, hyaluronic acid is negatively charged. Positively charged growth factors can be effectively delivered without further treatment using HA based hydrogels.



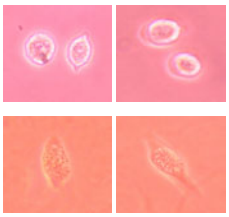
(A) (B)

Figure1. Synthetic scheme of acrylation to the HA backbone (A). Preparation of HA based hydrogel via Michael type addition reaction (B).



(A) (B)

Figure2. Mechanical properties of hydrogel (A) complex modulus, (B)swelling ratio (swelling for 2days) HA-Ac: PEG-SH(A=1:1,B= 1:2,C=1:4).



(A)

(B)

Figure3. Cellular morphology hMSCs were cultured in the HA hydrogel (A) and BMP-2 incorporated hydrogel (B) for 2 days. (x400). Cellular morphology was observed by light microscopy.

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Conclusions

1. Derivatization of Hyaluronic acid (HA) was achieved by introducing acryl group using N-acryloxysuccinimide and adipic acid dihydrazide. The degree of acrylation of HA which is measured by Nuclear Magnetic Resonance (NMR) is 17%.
2. *In situ* polymerizable hydrogels were prepared using acrylated HA as a basic monomer and thiolated poly(ethylene glycol) as a cross-linker.
3. Mechanical properties of hydrogels were modified by varying concentrations of acrylated HA and thiolated poly(ethylene glycol).
4. Human mesenchymal stem cells were cultured in this hydrogels and evaluated cell morphology and viability *in vitro*.

References:

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