Heparin-decorated, Hyaluronic Acid-based Hydrogel Particles for the Controlled Release of Bone Morphogenetic Protein 2

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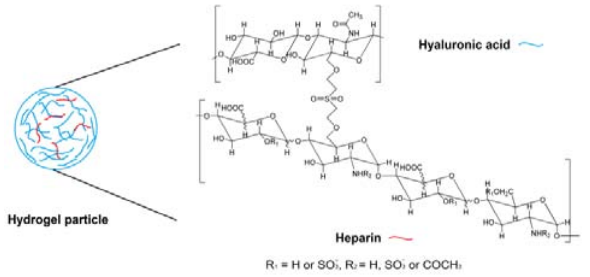
Statement of Purpose

Our group has developed hyaluronic acid (HA)-based hydrogel particles (HGPs) with large surface area, defined pore size and enhanced stability. These particles that are very promising as growth factor release depots for tissue regeneration [1,2]. To further enhance the biological activities of HA HGPs, heparin (HP) was covalently integrated into HA HGPs during the particle synthesis (Scheme 1). We demonstrate that, compared to HA HGPs, the HA-HP composite hydrogel particles exhibit an enhanced binding capacity for bone morphogenetic protein 2 (BMP-2) and improved control over the BMP-2 release. The chondrogenic efficacy of BMP-2-loaded HA/HP HGP system was evaluated based on high density micromass culture of multipotential stem cells (MSCs).



Scheme.1 Schematic illustration of heparin-incorporated hyaluronic acid-based hydrogel particles.

Materials and Methods

Hyaluronic acid (HA, sodium salt) was a gift from Genzyme Corporation (Cambridge, MA, USA). All chemicals were purchased from Aldrich (Milwaukee, WI). BMP-2 and BMP-2 Quantikine ELISA kit were obtained from R&D System (Minneapolis, MN).

Synthesis of Heparin Incorporated Hyaluronic Acid based Particles. Four different particle formulations were prepared based on previously reported inverse emulsion methods [1] and the product were designated as HA-HPx, with x being the amount of HP in feed (micrograms of HP per milligram of HGPs, x=0, 1, 10, 100).

Toluidine Blue Assay for Heparin Quantification. The assay was performed based on previous procedure [2]. The amount of heparin incorporated in HGPs was derived from a series of standards containing 5-80 μg heparin.

Morphological Characterization. Scanning electron microscope (SEM) was used to obtain the morphological feature of the hydrogel particles.

Pore Size Analysis. Pore size analysis was performed by solute exclusion method using Fluorescein isothiocyanate labeled dextran (FITC-DX) as the molecular probe [2]. The retention of FITC-DX by HGPs was used to reflect the average mesh size of HGPs.

Quantification of BMP-2 Release. BMP-2 release was analyzed according to a reported procedure [2].

In vitro Chondrogenesis Evaluation based on High Density Micromass Culture. Micromass culture of C3H10T1/2 multipotential stem cells was performed in the presence of BMP-2-loaded HGPs. The chondrogenesis differentiation was assessed by histochemical analysis and quantitative polymerase chain reaction (qPCR).

Results

Particle Synthesis and Characterization. HA-based, HP-decorated hydrogel particles were successfully synthesized *via* an inverse emulsion polymerization technique. Toluidine blue assay revealed a linear relationship between the amount of HP added during the synthesis and that actually incorporated in the final product. The actual HP content in HA-HP1 HGPs was estimated to be 0.5μg/mg. SEM imaging (data not shown) revealed that the particles were spherical in shape with a bimodal particle size distribution (around 1μm and 100nm). Solute exclusion experiment showed that the average pore size of HGPs is around 22-28 nm.

Quantification of BMP-2 Release. A close to zero release kinetics of BMP-2 was obtained when the heparin content was optimized to 0.5 μg/mg (HA-HP1 HGPs). In contrast, burst release profile was detected when no heparin was incorporated (HA HGPs) and hindered release kinetics was found in cases with high HP content (HA-HP100 and HA-HP10 HGPs).

In vitro Chondrogenesis. Compared to cells treated with of BMP-2-loaded HA HGPs and BMP-2 carriers alone, MSCs cultured in the presence of BMP-2 loaded HA-HP1 HGPs exhibited the most robust chondrogenic differentiation, as evidenced by the strongest Alcian blue staining and the highest chondrogenic marker mRNA expression.

Conclusions

Hyaluronic acid-based hydrogel particles containing covalently immobilized heparin were successfully synthesized *via* inverse emulsion polymerization. The controlled release of BMP-2 combined with the inductive role of HA in chondrogenesis make the current system very promising for growth factor therapy. *In vivo* assessment of the hybrid BMP-2 delivery system is currently underway.

References

[1] Jha A.K., et.al. Macromolecules. 2009: 42:537-546.
[2] Jha A.K., et.al. Biomaterials. 2009: 30: 6964-6975.