A Novel Molecular Monitoring of Hyaluronic Acid Degradation Using Quantum Dots

Ji Seok Kim,[1] *Sei Kwang Hahn,*[*,1] *and Sung Jee Kim* [2]

[1] Department of Advanced Materials Science and Engineering,
[2] Department of Chemistry, Pohang University of Science and Technology (POSTECH), Pohang, 790-784, Korea
skhanb@postech.ac.kr

Introduction

Hyaluronic acid (HA) is a biodegradable, biocompatible, non-immunogenic, and non-inflammatory linear polysaccharide [1]. Because of the excellent physicochemical properties, HA has been widely used for arthritis treatment, ophthalmic surgery, drug delivery, and tissue engineering. A lot of strategies for chemical modification of carboxyl and hydroxyl groups of HA were developed to elongate the half-life of HA in the body. In this work, adipic acid dihydrazide grafted HA (HA-ADH) was synthesized and conjugated with quantum dots, which was additionally crosslinked to synthesize HA hydrogels incorporating quantum dots. Quantum dots are advantageous for long time imaging since they do not photo-bleach and can be easily multiplexed [2]. This novel HA-Quantum dot conjugate was characterized and applied for a real-time imaging of HA degradation in the body to understand the biological role of HA. Conventionally, the bio-imaging of temporal evolution of HA degradation can be only monitored by sequentially sacrificing many animals.

Experimental

Synthesis of HA-ADH. HA-ADH was synthesized and purified as described elsewhere [3]. The degree of substitution by ADH was determined with ^{1}H-NMR according to the analysis by Luo *et al.* [4].

Conjugation to quantum dots. HA-ADH was dissolved in phosphate buffered saline (PBS, pH=7.4, 10 mM) and then mixed with quantum dots containing carboxyl terminal ligands. The addition of HOBt and EDC in DMSO to the mixed solution resulted in successful conjugation of HA-Quantum dots. When the concentration of HA-ADH solution was higher than 4 wt%, HA-ADH hydrogels incorporating quantum dots were synthesized in 30 minutes.

Emission of the quantum dots. The emission of HA-Quantum dot conjugate and HA hydrogel incorporating quantum dot were observed with a digital camera after exposing to UV-lamp (365nm).

Bioimaging of HA-quantum dot conjugates. HA-Quantum dot conjugate and the precursor solution of HA hydrogels incorporating quantum dots were injected to the subcutaneous of nude mouse and real-time monitored to elucidate HA degradation in the body.

Results and discussion

HA-Quantum dot conjugates were successfully synthesized for real-time-imaging of HA degradation and the following biological phenomena. Figure 1 shows the schematic representation of the formation of HA-Quantum dot conjugates. HA-ADH with ADH content of ca. 70 mol% was synthesized and conjugated with quantum dots containing carboxyl terminal ligands which were activated by the addition of HOBt and EDC in dimethylsulfoxide (DMSO).

When the concentration of HA-ADH solution was higher than 4 wt%, HA-ADH hydrogels incorporating quantum dots were synthesized in 30 minutes. Figure 2-A shows the formation of HA-ADH hydrogels crosslinked with quantum dots and Figure 2-B shows the emission of quantum dots after exposing to UV-lamp (365nm). Depending on the size of quantum dots, the emitting color was different, for example, red, yellow, blue, and so on.

The HA-Quantum dot conjugate and the precursor solution of HA hydrogels incorporating quantum dots were injected to the subcutaneous of nude mouse and real-time monitored to elucidate HA degradation in the body and the following biological phenomena. Recently, HA has been reported to be degraded at the HA receptor of LYVE-1 on the lymphatic endothelial cells [5]. Tumor growth and lymphangiogenesis might be real-time imaged using HA-Quantum conjugates which would selectively bind to LYVE-1. The overall *in vivo* test results will be presented and discussed in detail.

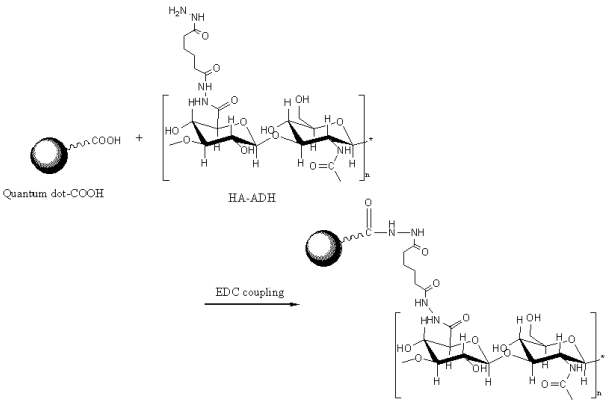

Figure 1. Schematic representation of the formation of HA–Quantum dot conjugates.

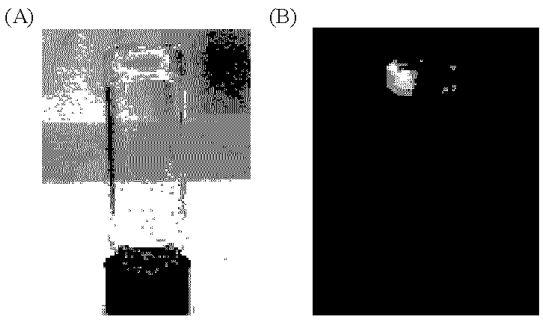

Figure 2. (A) The formation of HA-ADH hydrogels crosslinked with quantum dots. (B) The emission of quantum dots after exposing to UV (365 nm).

Conclusions

A real time bio-imaging of HA degradation was successfully carried out using HA-quantum dot conjugates. HA-ADH with ADH content of *ca.* 70 mol% was synthesized and conjugated with quantum dots containing carboxyl terminal ligands which were activated by the addition of HOBt and EDC in DMSO. When the concentration of HA-ADH solution was higher than 4 wt%, HA-ADH hydrogels incorporating quantum dots could be synthesized in 30 minutes. These novel HA-quantum dot conjugates and the precursor solution of HA hydrogels incorporating quantum dots were injected to the nude mouse and investigated to elucidate the biological roles of HA in the body for various future tissue engineering applications.

References

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