In Vivo Real-Time Bioimaging of Hyaluronic Acid Derivatives Using Quantum Dots

[1,2,*] Sei Kwang Hahn, [1] Ji Seok Kim, [2] Hyungu Kang

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

Statement of Purpose: 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 dot (QDot). QDot is advantageous for long time imaging since they do not photo-bleach and can be easily conjugated with biomolecules [2]. This novel HA-QDot conjugate was characterized and applied for long-term real-time monitoring of HA derivatives in the body without sequential sacrificing animals.

Methods: 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.* [3]. HA-ADH was dissolved in phosphate buffered saline (PBS, pH=7.4, 10 mM) and then mixed with QDot containing carboxyl terminal ligands which were activated with HOBt and EDC. When the concentration of HA-ADH solution was increased to higher than 4 wt%, HA-ADH hydrogels incorporating QDot were synthesized in 30 minutes. The emission of HA-QDot conjugate and HA hydrogel incorporating QDot were observed with a digital camera after excitation with UV-lamp (365nm). HA-QDot conjugate and the precursor solution of HA hydrogels incorporating QDot were injected to the subcutaneous of nude mouse and long term real-time monitored to elucidate the behavior of HA derivatives in the body.

Results/Discussion: HA-Quantum dot conjugates were successfully synthesized for long term real-time imaging of HA derivatives in the body. Figure 1 shows the schematic representation of the formation of HA-QDot conjugates. HA-ADH with ADH content of *ca.* 70 mol% was synthesized and conjugated with QDots containing carboxyl terminal ligands which were activated by the addition of HOBt and EDC. When the concentration of HA-ADH solution was higher than 4 wt%, HA-ADH hydrogels incorporating quantum dots were synthesized in 30 minutes. The HA-QDot conjugate and the precursor solution of HA hydrogels incorporating QDot were injected to the subcutaneous of nude mouse for real-time monitoring of HA derivatives in the body. Figure 2-A shows the formation of HA-ADH hydrogels crosslinked with QDot and Figure 2-B shows *in vivo* real time imaging of HA-QDot hydrogels by the injection of their precursor solution. Depending on the size of quantum dots, the emitting color was different, for example, red, yellow, blue, and so on. Recently, HA has been reported to be degraded at the HA receptor of LYVE-1 on lymphatic endothelial cells [4]. Tumor growth and lymphangiogenesis might be real-time imaged using HA-QDot conjugates which would selectively bind to LYVE-1. The overall *in vivo* test results will be presented and discussed in detail.

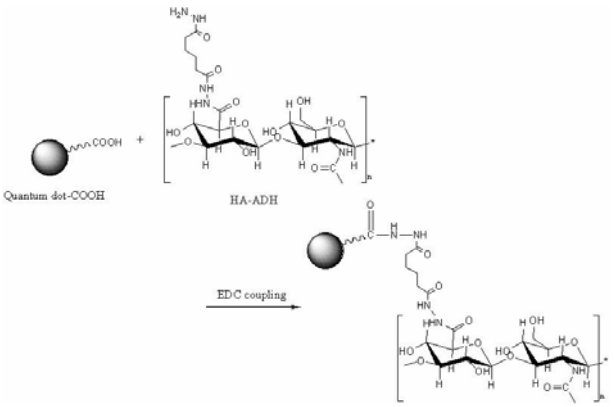

Figure 1. Schematic representation of the formation of HA-Quantum dot conjugates.

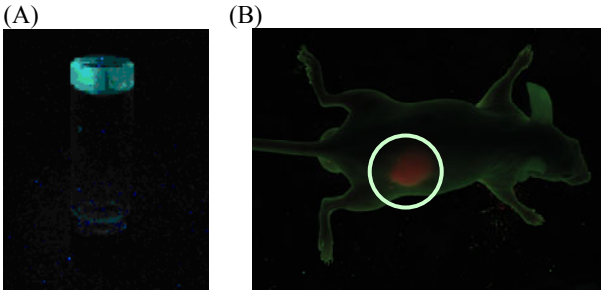

Figure 2. (A) HA-ADH hydrogels crosslinked with quantum dot in a capsized glass vial. (B) *In vivo* real time imaging of HA-QDot hydrogels by the injection of their precursor solution in a nude mouse.

Conclusions: A real time bio-imaging of HA derivatives using QDot was successfully carried out in a nude mouse. The novel HA-QDot conjugate will be used to investigate the biological roles of HA in the body for various future tissue engineering applications.

References:

[1] Laurent TC. The chemistry, biology and medical applications of hyaluronan and its derivatives; Portland press: London and Miami, 1998; Chapter 3.
[2] Medintz IL. Nat Mater. 2005;6:435-446.
[3] Luo Y. J Control Rel. 2000;69:169-184.
[4] Prevo R. J Biol Chem. 2001;276:19420-19430.