Self-Assembly of Hyaluronate–Cucurbituril Conjugate for Controlled Drug Delivery Applications

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Statement of Purpose: Cucurbit[6]uril (CB[6]) having six glycoluril units forms stable host-guest complexes with polyamines such as spermine (SPM) and spermidine (SPMD) with a extremely high binding affinity ($K > 10^7$ M^{-1}) in aqueous solution [1]. Hyaluronate (HA) is a natural polysaccharide and has been used for various medical applications [2,3]. In this work, we synthesized HA-CB[6] conjugate for controlled drug delivery applications. The novel HA-CB[6] conjugate could be successfully exploited for the preparation of the second generation magic bullets with various functional moieties by host-guest chemistry in a non-covalent manner.

Methods:

Synthesis & characterization of HA-CB[6] conjugate: Thiolated HA (HA-SH) was conjugated with $(allyloxy)_{12}$-CB[6] by photoreaction in DMSO. The successful synthesis of HA-CB[6] conjugate and its self-assembly with SPM were confirmed by ^{1}H NMR analysis.

Preparation of self-assembled magic bullet: HA-CB[6] conjugate solution was mixed with SPMD-FITC to form self-assembling complex. The remaining free SPMD-FITC was removed by dialysis. B16F1 cell line, which has over expressed HA receptor, was treated with FITC-SPMD and FITC-SPMD/HA-CB complex in the presence and absence of free HA. The cellular uptake was analyzed by confocal microscopy.

Preparation of injectable HA hydrogels: Each 3 wt% solution of HA-CB[6] and hexamethylenediamine grafted HA (HA-HMDA) was prepared and applied as the precursor solutions for the preparation of *in situ* forming hydrogels by self-assembly in nude mice.

Results: We confirmed the successful synthesis of HA-CB[6] conjugate and its self-assembly with spermine derivatives by ^{1}H NMR analysis (Figure 1). The insertion of hydrocarbon molecules to hydrophobic cavity of CB[6] resulted in a chemical shift of ^{1}H NMR peak of the hydrocarbon to the down field due to the electrical environment change. A novel self-assembling second generation magic bullet could be prepared by simple mixing of HA-CB[6] with various spermine derivatives. As a proof of concept, we carried out cellular uptake tests of HA-CB[6]/SPMD-FITC complex by HA receptor mediated endocytosis to B16F1 cells with over-expressed HA receptors (Figure 2). According to the confocal microscopic analysis, HA-CB[6]/SPMD-FITC complexes appeared to be well internalized to the cells, whereas SPMD-FITC remained outside of the cells. Furthermore, co-incubation tests with free HA confirmed the cellular uptake of HA-CB[6]/SPMD-FITC by HA receptor mediated endocytosis. The HA-CB[6] conjugate could be exploited to make the second generation magic bullets with various moieties including SPMD-FITC, SPMD-chemical drug, SPMD-biopharmaceuticals, and SPMD-aptamer as a targeting ligand. On the other hand, HA-CB[6] and HA-HMDA conjugate solutions were used for the preparation of injectable HA hydrogels by self-assembly. The *in situ* formation of HA hydrogels under the skin of nude mice was observed within 6 minutes after subcutaneous injection of HA-CB[6] and HA-HMDA conjugate precursor solutions.

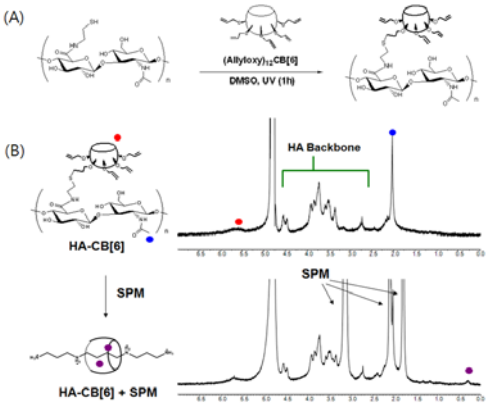

Figure 1. (A) Schematic representation for the synthesis of HA-CB[6] conjugate. (B) ^{1}H NMR analyses of HA-CB[6] conjugate and its complex with spermine.

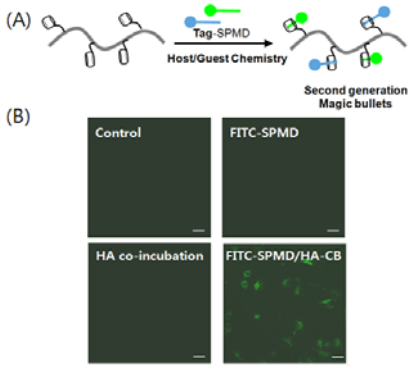

Figure 2. (A) Schematic representation of self-assembled magic bullet. (B) Confocal microscopic analysis of self-assembled magic bullet.

Conclusions: HA-CB[6] conjugates were successfully synthesized, characterized, and applied for the proof of concept of the second generation magic bullets with various functional moieties by self-assembly. In addition, HA-CB[6] and HA-HMDA conjugate solutions could be used for the preparation of injectable hydrogels, which would be used as a drug depot system and a scaffold for tissue engineering applications.

References:

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