Electronic Supplementary Information (ESI)

CD44-specific Supramolecular Hydrogel for Fluorescence Molecular Imaging of Stem-like Gastric Cancer Cells

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Experimental details

Materials: Branched polyethyleneimine (25,000 Da) and Cy5.5 mono N-hydroxysuccinimide (NHS) ester were purchased from Sigma-Aldrich and Amersham, respectively. Hyaluronic acid (1,000,000 Da) was obtained from the Yuhan Pharmaceutical Corporation, Seoul, Korea. Phosphate buffered saline (PBS; 10 mM, pH 7.4) and RPMI-1640 medium were purchased from Gibco. All chemicals and reagents were of analytical grade.

Preparation of the NIR-sensitive supramolecular hydrogel: To prepare the NIR-sensitive supramolecular hydrogel (Cy5.5-PEI/HA polyplex), the optimal PEI/HA molar ratios were evaluated. The PEI to HA molar ratios evaluated were 25, 12.5, 6.25, 3.125, and 1.5625. Induction of fluorescence in the PEI/HA polyplex was achieved by conjugating Cy5.5 mono NHS ester (8.3 nmol) with PEI (1.6 nmol) in PBS (pH 7.4, 10mM), followed by subsequent mixing with HA (0.3 nmol). Formation of the Cy5.5-PEI/HA polyplex was verified with gel electrophoresis (50mV, 30 min) and atomic force microscopy (Multimode Ⅴ, Veeco). Cy5.5-PEI was used as a control (Cy5.5 8.3 nmol, PEI 1.6 nmol) for gel electrophoresis. Colloidal size and zeta-potential of the Cy5.5-PEI/HA polyplex was analyzed using dynamic light scattering (ELS-Z, OTSUKA electronics). Dispersity of the Cy5.5-PEI/HA polyplex in a biological medium was confirmed using dark-field microscopy (BX51, Olympus). A schematic illustration describing the overall preparation process is depicted in Fig. 1.

*Cytotoxicity and in vitro binding affinity of NIRSH:*Six gastric cancer cell lines (MKN-1, MKN-45, MKN-28, MKN-74, AGS, and NCI-N87) were obtained from the American Type Culture Collection. These lines were cultured in medium containing 10% fetal bovine serum and 1% Antibiotic-Antimycotic at 37°C, humidified at 5% CO2 atmosphere. We evaluated

CD44 expression levels in gastric cancer cell lines (MKN-45 and MKN-28) using fluorescence-activated cell sorting (FACSCalibur, Beckman Dickson). A CD44-antibody conjugated with fluorescent isocyanate (cell signaling technology, CD44 mouse mAb) was used to label cells. The cytotoxicities induced by PEI, HA and NIRSH in the two gastric cancer cell lines (MKN-45 or MKN-28; 1×10^4 cells/well in a 96-well plate) was obtained with an MTT assay kit (Roche, Cell proliferation Kit 1, 670 nm of absorbance), respectively. The targeting efficiency of NIRSH in CD44-expressed gastric cancer cells (MKN-45 and MKN-28; 5×10^5 cells/well in a 4-well plate) was evaluated by fixing the cells with 4% paraformaldehyde in PBS for 15 minutes. The cells were treated with NIRSH (with 3.8 μM of Cy5.5) for 4 hours, and rinsed with PBS, 3 times to eliminate unbound NIRSH. The nuclei were stained with Hoechst33342 (H-3570, Molecular Probes). Fluorescent images of the gastric cancer cell lines treated with NIRSH were obtained by confocal microscopy (LSM700, Carl Zeiss).

In vivo NIR imaging: To establish the mouse xenograft model for gastric cancer, MKN-45 cells (1×10^7 cells) were implanted separately into the proximal thigh (heterotopic) and exteriorized stomach (orthotopic) of two male mice (6-week-old BALB/c-nude mice). The targeting efficiency of NIRSH to CD44 molecules was investigated by injecting NIRSH (with 7.8 mM of Cy5.5) intravenously into the tail veins of the mice (30 days after implantation in the heterotopic model and 10 days after implantation in the orthotopic model). NIR images of the xenograft mouse model treated with NIRSH were obtained with an Optical imager (eXplore Optix MX, GE Healthcare). The tumor volume in the heterotopic xenograft model was calculated using the equation: $(4/3)\times\pi\times((\text{minor axis})/2)^2\times((\text{major axis})/2)$ mm^3. All animal experiments were conducted with the approval of the Association for Assessment and Accreditation of Laboratory Animal Care International.

Statistical analyses: Statistical evaluation of data was performed with an analysis of variance test (ANOVA) and Student's t-test. A p-value of less than 0.05 was considered statistically significant.

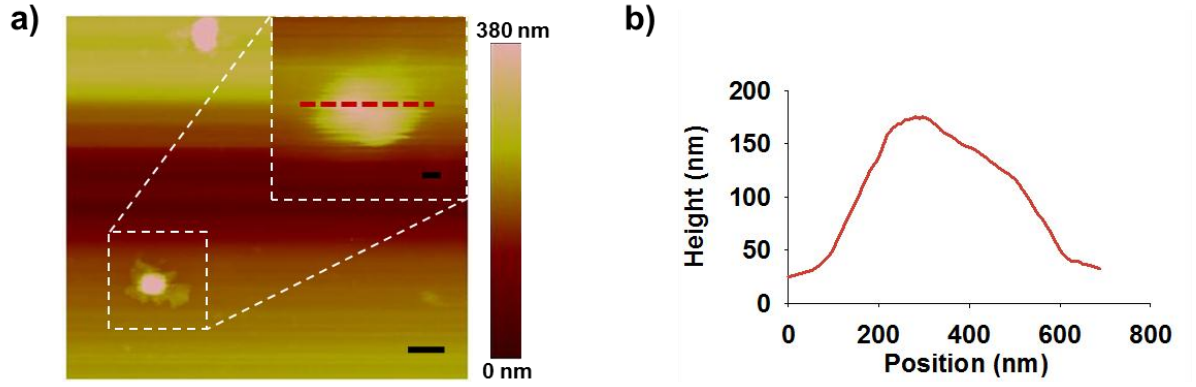

Figure S1. a) AFM image of NIRSHs. b) The height profile graph at the position of the red-dashed line of a).

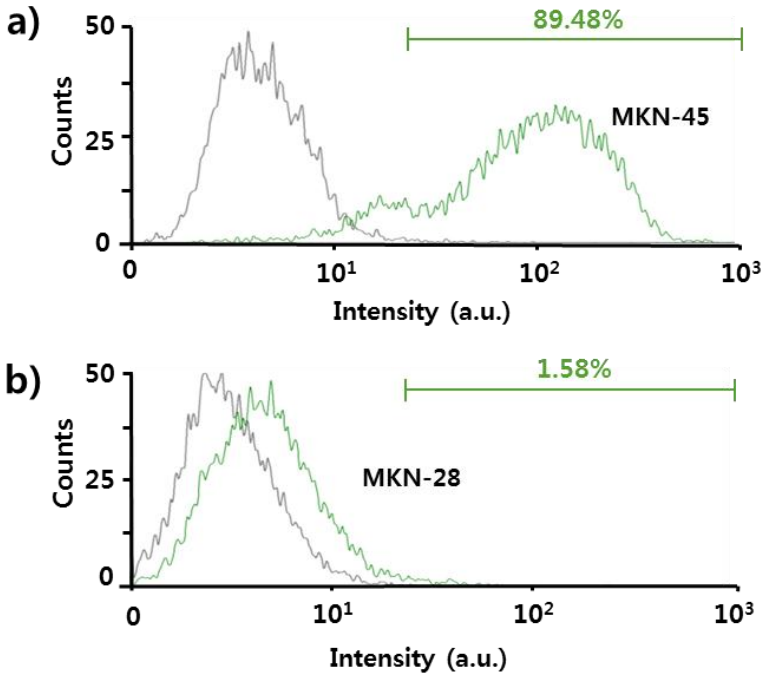

Figure S2. FACS analysis for a) MKN-45, b) MKN-28 by using CD44-FITC antibody.

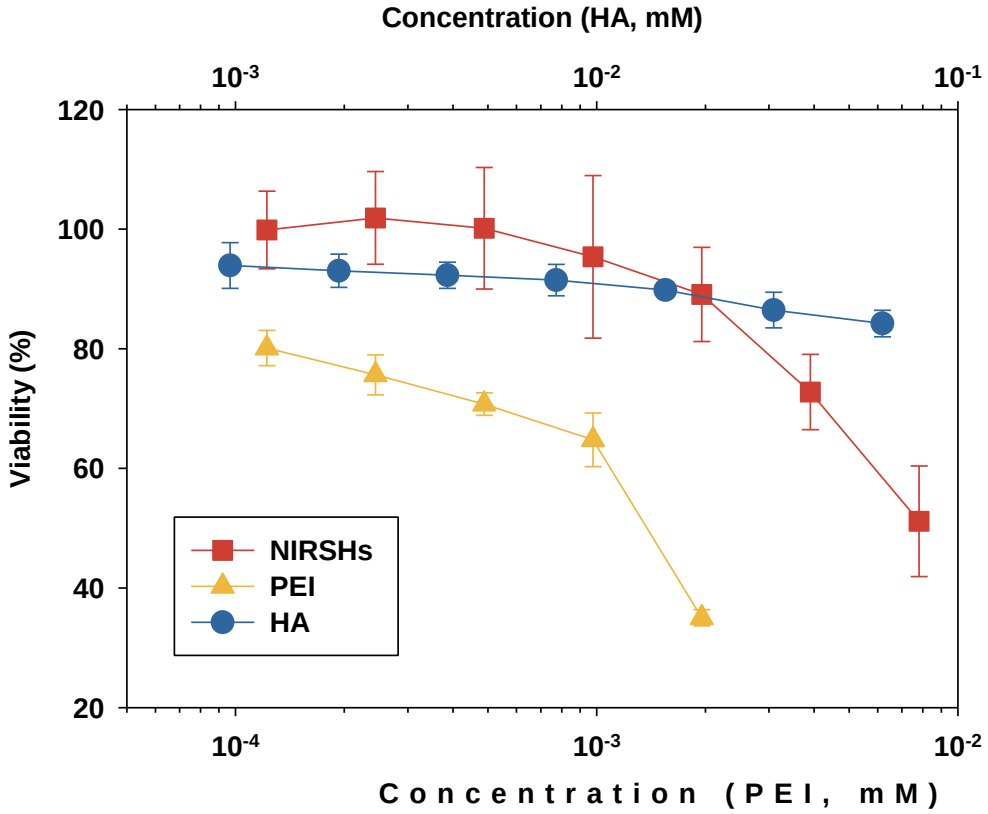

Figure S3. Cell viability of CD44-overexpressed gastric cancer cell line (MKN-45). Cell viability was observed according to HA concentrations (upper tick label) for HA, NIRSHs and PEI concentrations (lower tick label) for PEI, NIRSH.

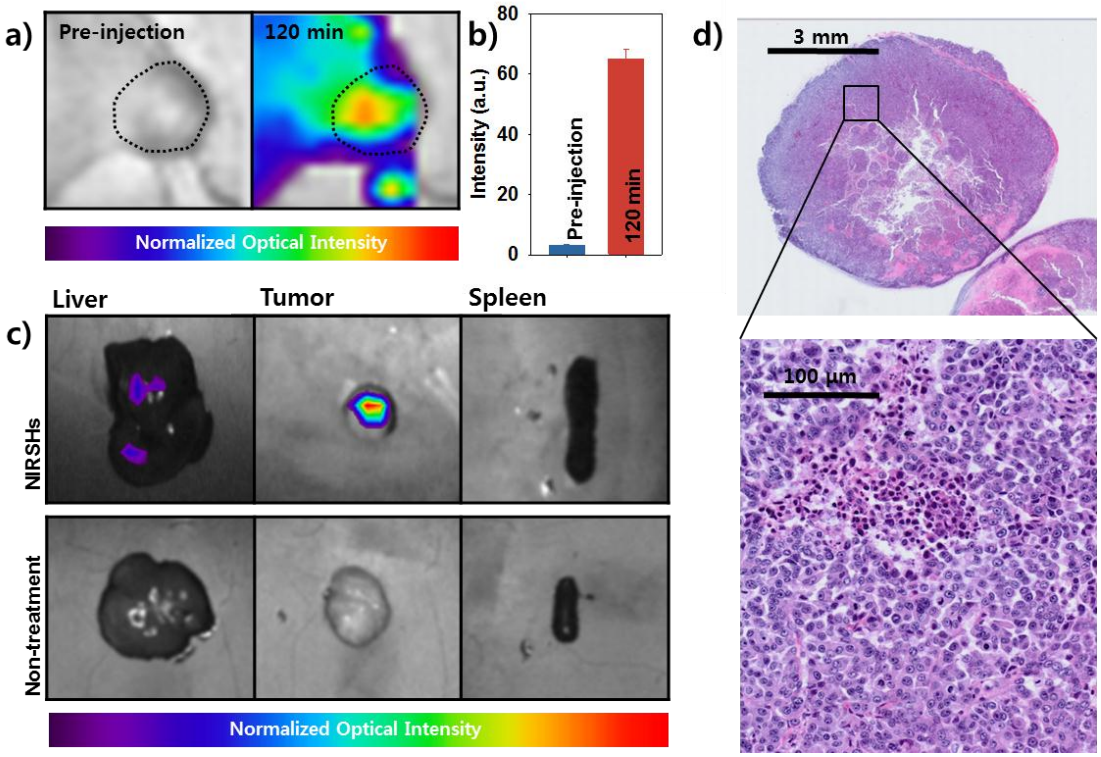

Figure S4. a) *in vivo* fluorescence images of CD44-overexpressed tumor-bearing xenograft heterotopic mouse model after the intravenous injection of NIRSHs into the tail vein and b) the fluorescence intensity graph. c) *ex vivo* fluorescence images of exercised liver, tumor and spleen of NIRSHs-treated and non-treated mouse model. d) H&E staining of stomach.

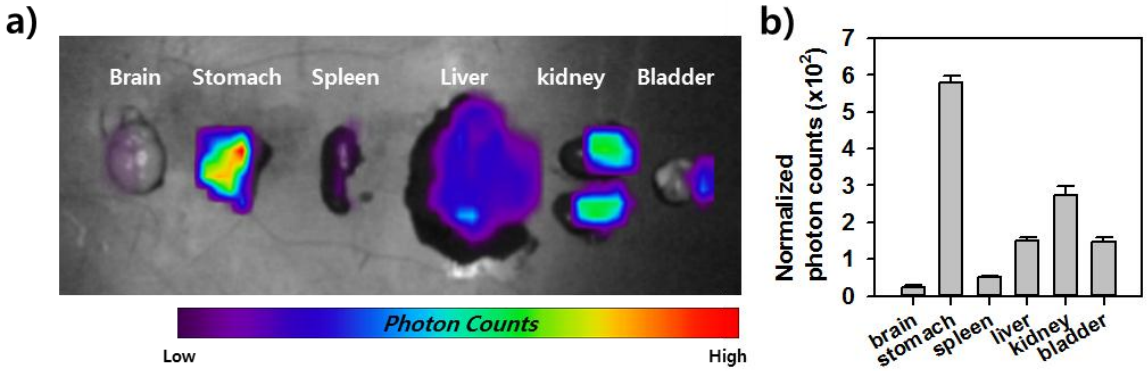

Figure S5. a) *Ex vivo* NIR fluorescence images b) normalized photon counts of extracted organs (brain, stomach, spleen, liver, kidney, and bladder) from MKN-45 tumor-bearing mouse after the intravenous injection of NIRSH.