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Electronic Supplementary Information

Hyaluronate Modified Upconversion Nanoparticles for Near Infrared Light-Triggered On-Off Tattoo Systems

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EXPERIMENTAL SECTION

Materials

Upconversion nanoparticles (UCNPs) of $NaYF_4$:Yb/Er (Y: Yb: Er = 69: 28: 3) were synthesized as reported elsewhere[1]. Yttrium (III) chloride hexahydrate (YCl_3•$6H_2O$), ytterbium (III) chloride hexahydrate ($YbCl_3$•$6H_2O$), erbium chloride hexahydrate ($ErCl_3$•$6H_2O$), ammonium fluoride (NH_4F), octadecene-1, cyclohexane, poly(allylamine) (PAAm) solution (20 wt. % in H_2O) and N-hydroxysuccinimide sodium salt (NHS) were purchased from Sigma Aldrich. Sodium hydroxide (NaOH) was obtained from Samchun Pure Chemicals. Hyaluronate (HA, MW = 150 kg/mol) was obtained from Lifecore. N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) was obtained from Tokyo

Chemical Industry.

Preparation of UCNP, UCNP-PAAm and HA-UCNP

NaYF$_4$:Yb/Er nanoparticles were synthesized using the aqueous solution of LnCl$_3$ [0.78 ml of YCl$_3$•6H$_2$O (1 M), 0.2 ml of YbCl$_3$•6H$_2$O (1 M), 0.2 ml of ErCl$_3$•6H$_2$O (0.1 M)] in a three-necked 250 mL flask. The flask was kept in a spherical heating mantle and heated up at 110 ^{o}C to evaporate the water from LnCl$_3$•xH$_2$O with magnetic stirring. Then, 6 ml of oleic acid and 15 ml of 1-octadecene were added and heated up at 150 °C for 30 min to make a homogeneous solution. After cooling down to 60°C, 5 ml of methanol solution containing NH$_4$F (0.148 g) and NaOH (0.1 g) was added drop by drop. The solution was heated up at 110 ^{o}C for 20 min to evaporate the methanol and residual H$_2$O. The neck of flask was sealed with a rubber stopper and connected to the nitrogen-filled balloon with a syringe for stabilization at high pressure. Another neck of flask was connected to a dual manifold schlenk to keep the solution under vacuum for 10 min and nitrogen atmosphere by switching the stopcock. The solution was heated to 300 ^{o}C under nitrogen for 1 h at a heating rate of 10 ^{o}C/min and cooled down to room temperature. The mixture was rinsed with 40 ml acetone and transferred to a 50 ml conical tube. The solution filled tube was centrifuged at 6,654g for 10 min at 20 ^{o}C. After removal of the supernatant, the pellet was dissolved in 20 ml of cyclohexane. After centrifugation again at 1,000g and 20 ^{o}C for 5 min, the supernatant containing UCNP was collected in a glass vial.

The produced hydrophobic UCNP was surface-modified by ligand exchange using PAAm according to a previously published procedure[2]. The PAAm solution (10 μl, 20 wt% in H$_2$O) was dispersed in 4 ml ethanol by sonication for 20 min. The hydrophobic UCNP (~ 4 mg) solution in 2 ml cyclohexane was added into the PAAm solution dropwise and stirred vigorously at room temperature for 36 h. The obtained nanoparticles were re-dispersed in water after centrifugation at 10,400g and room temperature for 20 min. The covalent

conjugation between amine groups of UCNP-PAAm and carboxyl groups of HA was carried out by the EDC chemistry. One ml of aqueous solution containing 0.5 mg HA and *ca.* 0.5 mg UCNP-PAAm stirred homogenously for 30 min. Then, 20 molar excess of EDC and sulfo-NHS sodium salt to a single chain of HA were added into the mixture and stirred vigorously at room temperature for 24 h. The HA-UCNP conjugate solution was dialyzed in deionized (DI) water for 1 day to remove unreacted reagents and catalysts, and then freeze-dried for a day.

Characterization of UCNP, UCNP-PAAm and HA-UCNP

The core structure and compositions of UCNP were analyzed by transmission electron microscopy (TEM, JEM-1011, JEOL Co.) and inductively coupled plasma atomic emission spectroscopy (ICP-AES). The uniform hexagonal crystal lattice of UCNP was observed by high resolution transmission electron microscopy (HRTEM, 2200FS with Cs-corrected TEM). The size distribution and zeta potential of nanoparticles were analyzed by dynamic light scattering (DLS, Zetasizer Nano ZS90, Malvern Instruments Co.). The emission spectra were collected with a fluorolog modular spectrofluorometer (Horiba Scientific Co.) using the excitation light source of infrared diode laser at 980 nm (SDL-980-LM-2000T, Shanghai Dream Lasers Technology Co.).

HiLyte labelling of HA for the analysis of HA concentration

Five mg of HA was dissolved in 1 ml of DI water with magnetic stirring. The solution was mixed with 20 molar excess of EDC and sulfo-NHS and 3 molar excess of HiLyte^{TM} to a single chain of HA. The mixed solution stirred vigorously at room temperature for 24 h, and dialyzed in DI water and NaOH solution overnight. Then, the resulting HA-HiLyte was obtained by freeze drying. The fluorescence of HiLyte was measured with a microplate

fluorometer (Thermo Scientific Ltd, Fluoroskan Ascent^{TM}) at the excitation/emission wavelength of 584/650 nm. After that, HA-HiLyte was conjugated to UCNP-PAAm by the EDC chemistry. The fluorescence of supernatant containing unreacted HA-HiLyte was measured with the microplate fluorometer to determine the concentration of conjugated HA to UCNP-PAAm.

Biocompatibility of UCNP-PAAm and HA-UCNP conjugate

The biocompatibility of UCNP-PAAm and HA-UCNP conjugate was assessed by MTT assay for the viability of NIH3T3 cell, a mouse embryo fibroblast cell line. The cryopreserved cells were seeding in a 75T flask for a day and transferred into 96 well at 5×10^4 number. The cells were incubated with the control (medium), UCNP-PAAm and HA-UCNP for a day (n = 4). Then, each well was mixed with 50 μl of MTT solution (5 mg/ml) for 1 h and then 100 μl of dimethyl sulfoxide (Sigma aldrich). Optical density of samples was measured with a microplate spectrometer (Life Science Equipment, EMax Endpoint ELISA microplate reader) with the excitation at 540 nm.

***In vivo* transdermal delivery of UCNP-PAAm and HA-UCNP conjugate**

In vivo transdermal delivery was performed with 50 μl of control (PBS), 0.5 mg/ml UCNP-PAAm and HA-UCNP conjugate for 40 min on 1 cm × 1 cm dorsal skin of 6-week-old balb/c mice. After sacrifice, the penetration depth of samples into skin was analyzed by two-photon microscopy with 1050 nm light excitation.

Table S1. ICP-AES results of UCNP.

Compositions	Y	Yb	Er
Mass	690	360	35
Molar ratio	69	28	3

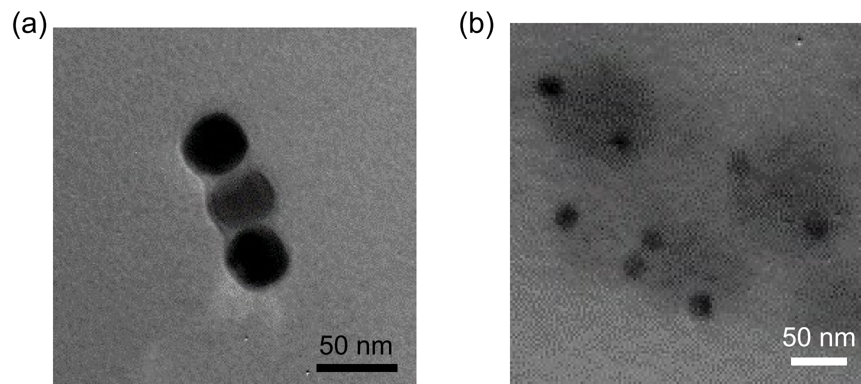

Fig. S1. TEM images of (a) UCNP-PAAM and (b) HA-UCNP conjugate.

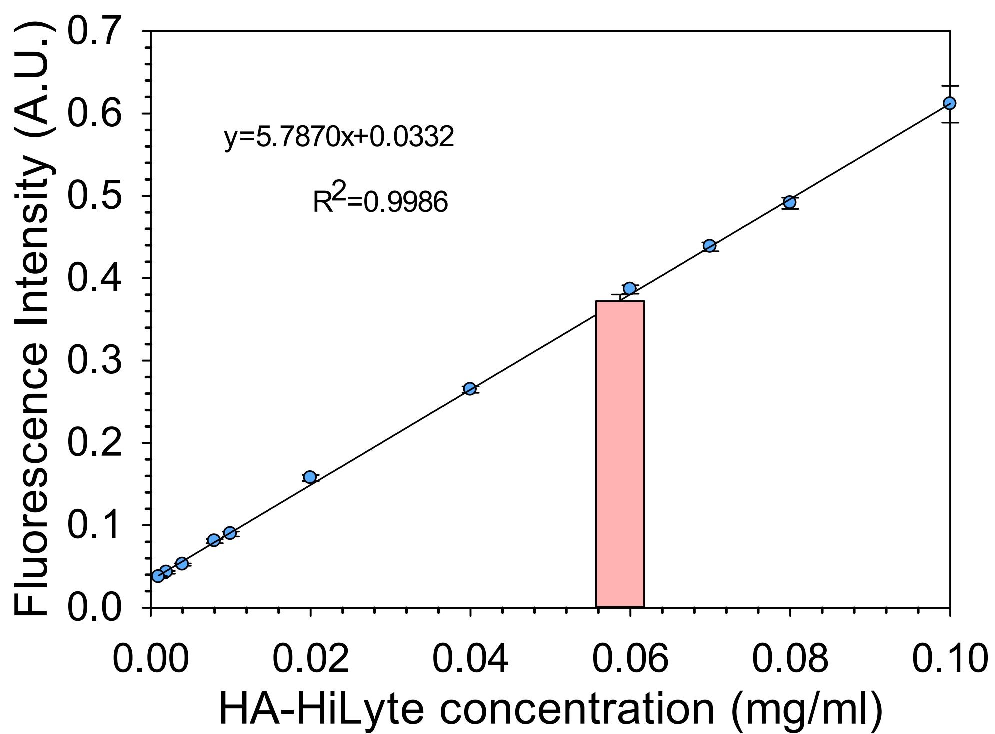

Fig. S2. The standard curve of HA-HiLyte™ to estimate the amount of conjugated HA to UCNP-PAAM.

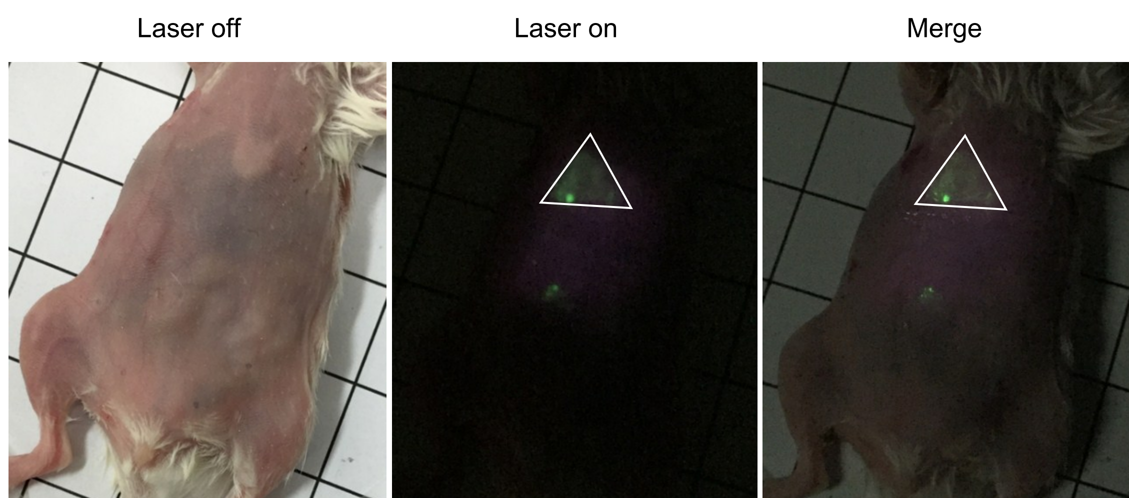

Fig. S3. *In vivo* on-off tattoo system in a triangle shape by 980 nm laser irradiation after transdermal delivery of HA-UCNP conjugate.

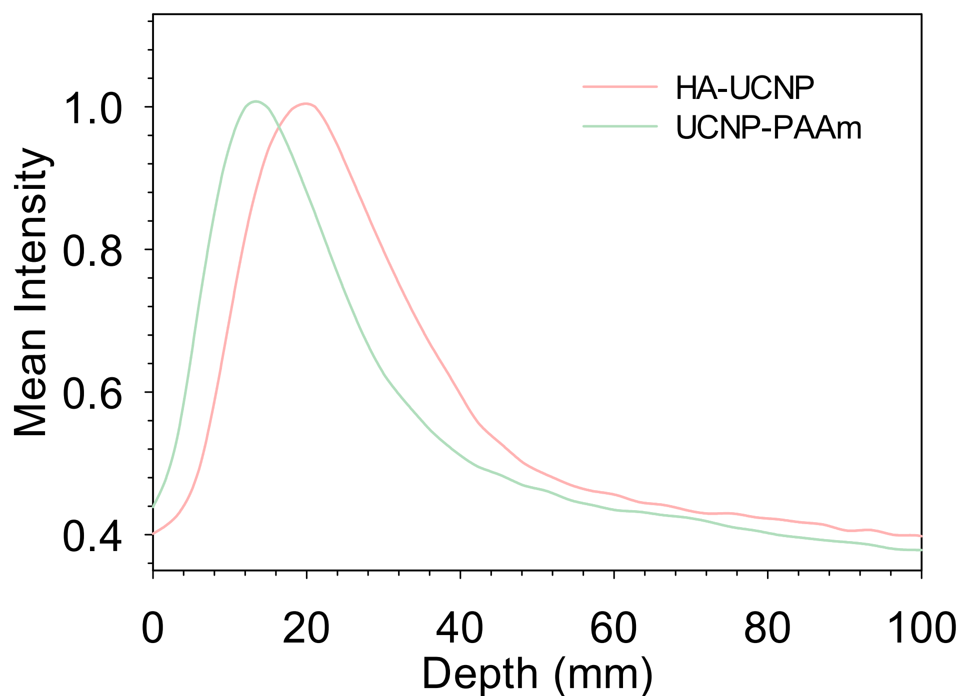

Fig. S4. The quantitative comparison for the transdermal delivery of UCNP-PAAm (green) and HA-UCNP conjugate (pink) with increasing penetration depth by two-photon microscopy.

References

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