Electronic Supplementary Material (ESI) for Nanoscale.

SUPPLEMENTARY INFORMATION

Amine-modified Hyaluronic Acid-functionalized Porous Silicon Nanoparticles for Breast Cancer Targeting

Patrick V. Almeida[a], Mohammad-Ali Shahbazi[a], Ermei Mäkilä[a,b], Martti Kaasalainen[b], Jarno Salonen[b], Jouni Hirvonen[a], Hélder A. Santos[a]*

[a] Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, FI-00014 University of Helsinki, Finland

[b] Laboratory of Industrial Physics, Department of Physics and Astronomy, FI-20014 University of Turku, Finland

***Address correspondence:**

helder.santos@helsinki.fi

Tel.: +358 2941 59661

Fax.: +358 2941 59138

Materials and reagents

Sodium salt of hyaluronic acid (HA), anhydrous dimethyl sulfoxide (DMSO), N-hydroxysuccinimide (NHS), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC, ≥97.0%); diisopropylamine (DIPA), trifluoroacetic acid (TFA, ≥99.0%), and ethylenediaminetretraacetic acid (EDTA) were purchased from Sigma-Aldrich (Finland). The monoprotected diamine NH_2-PEG_2-NHBoc was obtained from Iris Biotech (Marktredwitz, Germany). Dulbecco's phosphate buffer saline (10× PBS) and Hank's balanced salt solution (10× HBSS) were purchased from Gibco® (Life Technologies, USA). Dulbecco's modified eagle's medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, fetal bovine serum (FBS), trypsin (2.5%), non-essential amino acids (NEAA, 100×), L-glutamine (100×), and penicillin-streptomycin (100×) were purchased from HyClone (USA). CellTiter-Glo® Luminescent Cell Viability assay kit was obtained from Promega (Madison, USA). BD Horizon™ PE-CF594 mouse anti-human CD44 antibody was acquired from BD Biosciences (USA).

Cell lines and culturing conditions

MCF-7 and MDA-MB-231 breast cancer cell lines were cultured in Dulbecco's modified eagle medium (DMEM) and Roswell Park Memorial Institute (RPMI) 1640 medium, respectively, both supplemented with 10% (v/v) fetal serum bovine (FBS), 1% non-essential amino acids (NEAA), 1% L-glutamine, and 1% streptomycin-penicillin (100 IU/mL). The cell lines were obtained from the American Type Culture Collection and incubated in 75 cm^2 culture flasks (Corning Inc. Life Sciences, USA) at 37 ºC, at an atmosphere of 95% humidity and 5% CO_2 (BB 16 gas incubator, Heraeus Instruments GmbH, Germany). For subculturing, the cells were harvested using 0.25% trypsin-PBS-EDTA solution. MCF-7 and MDA-MB-

231 with passage numbers 8–25 and 5–30, respectively, were used for the *in vitro* experiments.

Flow cytometric analysis of CD44 expression

The expression of CD44 receptor in MCF-7 and MDA-MB-231 breast cancer cell lines was evaluated by flow cytometry. The cells were suspended in 1× PBS (pH 7.4), at a concentration of 1.5×10^7 cells/mL, and 50 µL of the cell suspensions were incubated with 50 µL of PE-CF594 anti-human CD44 antibody stock solutions, with concentrations of 5.0, 10.0, and 20.0 µg/mL, for 45 min at 4 ºC in the dark. Thereafter, the samples were washed three times with 1× PBS (pH 7.4) buffer, after centrifugation at 1000 rpm for 5 min, in order to remove the unbound portion of anti-human CD44 antibody, and subsequently re-suspended in 800 µL of 1× PBS (pH 7.4) buffer. Prior to the measurements, the samples were filtered using a 70 µm nylon cell strainer (BD Biosciences, USA) and collected into test tubes. The analyses were performed using an LSR II flow cytometer (BD Biosciences, USA), with the laser excitation wavelength of 488 nm. The fluorescence was detected with a 610/620 nm detector filter and at least 10000 events per sample were recorded using FACSDiva software. The data were analyzed and plotted using Flowjo 7.6 software (Tree Star, Ashland, OR, USA).

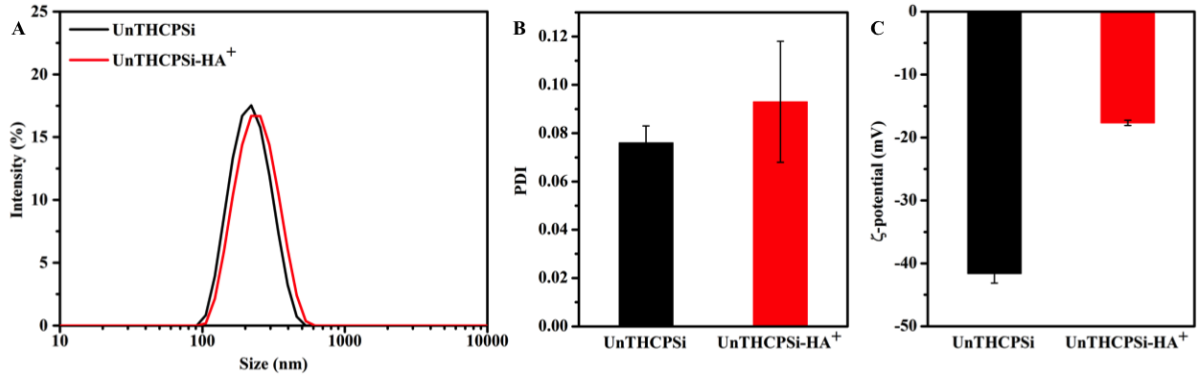

Figure S1. Physicochemical characterization of the particle size distribution **(A)**, PdI **(B)**, and ζ-potential **(C)** of the UnTHCPSi and UnTHCPSi–HA^+ nanoparticles, respectively. Bars represent the mean ± s.d. of at least three independent measurements.

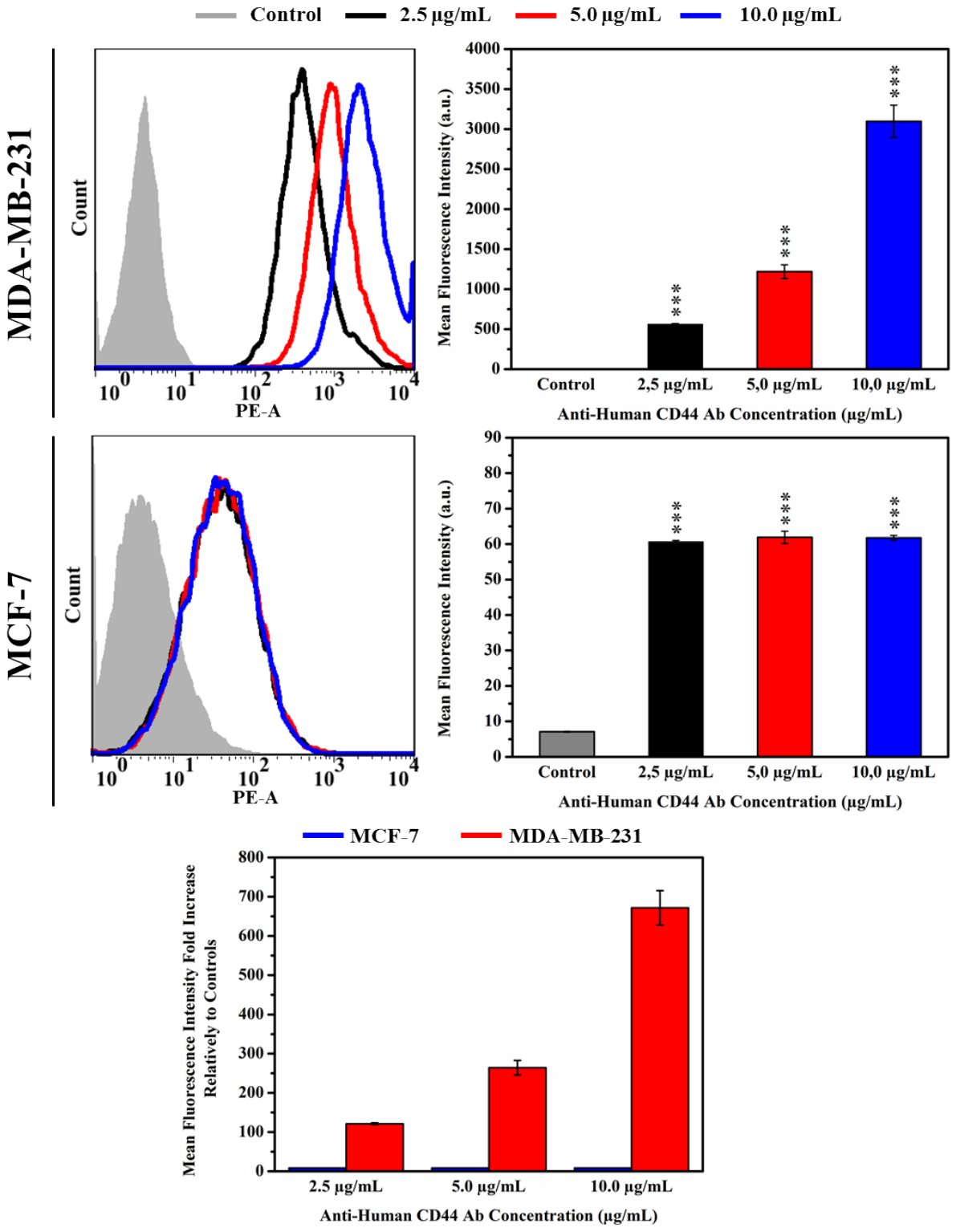

Figure S2. Flow cytometric analysis of CD44 expression in MDA-MB-231 and MCF-7 breast cancer cells. The cells were incubated with PE-CF594 anti-human CD44 antibody at the concentrations of 2.5, 5.0 and 10.0 µg/mL for 45 min at 4 ºC. The flow cytometry histograms of the unstained (control) and stained cells were obtained and the correspondent

mean fluorescence intensity was quantified, and subsequently compared to evaluate the expression of CD44 receptor in both cell lines. Error bars represent mean ± s.d. ($n \geq 3$). Statistical analysis was performed by means of Student's *t*-test with the level of significance set at probabilities of $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.