Biomaterials Science

[Supplementary Information]

Cationic amphiphilic polysaccharide nanoballs: protein stabilization and intracellular delivery by nano-encapsulation

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Scheme S1. Synthetic route of DEAE modified ESG and CHESG

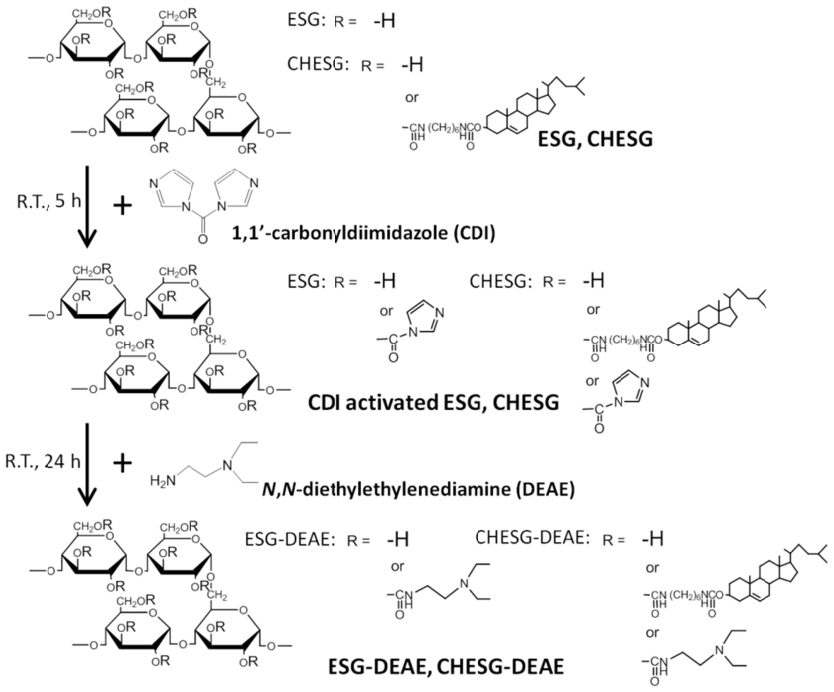

Figure S1. Elution profiles and molar-mass distribution curve of ESG derivatives in PBS (pH 7.4) determined by SEC-MALS

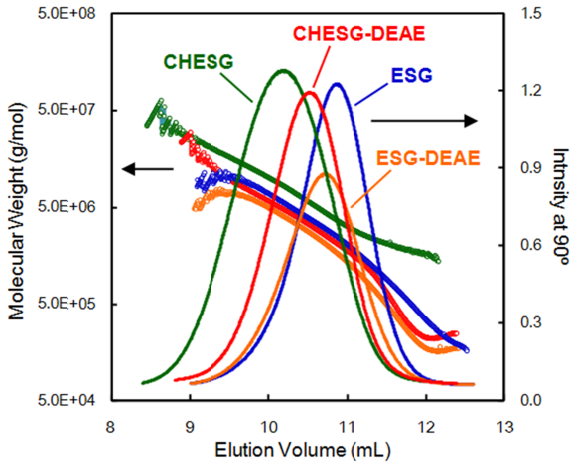

Figure S2. AFM images of (A) CHESG-DEAE and (B) CHESG-DEAE–BSA complex.

To prepare samples, FITC-BSA at a concentration of 0.132 mg mL^{-1} was dissolved in the absence or presence of CHESG-DEAE solution (6.0 mg mL^{-1} in PBS) and incubated at 25°C for 24 h. Then sample solutions were diluted 1000× by buffer without salt. A mica on a slide glass (Φ12) was freshly cleaved and the diluted sample solution (150 μL) was placed on the mica. Images were obtained by using AC-mode (fR=150.2 kHz, ampl. = 5 nm) with a cantilever USC-NM (NanoSensors) on a NanoWizard3 ultra (JPK Instruments, Berlin, Germany).

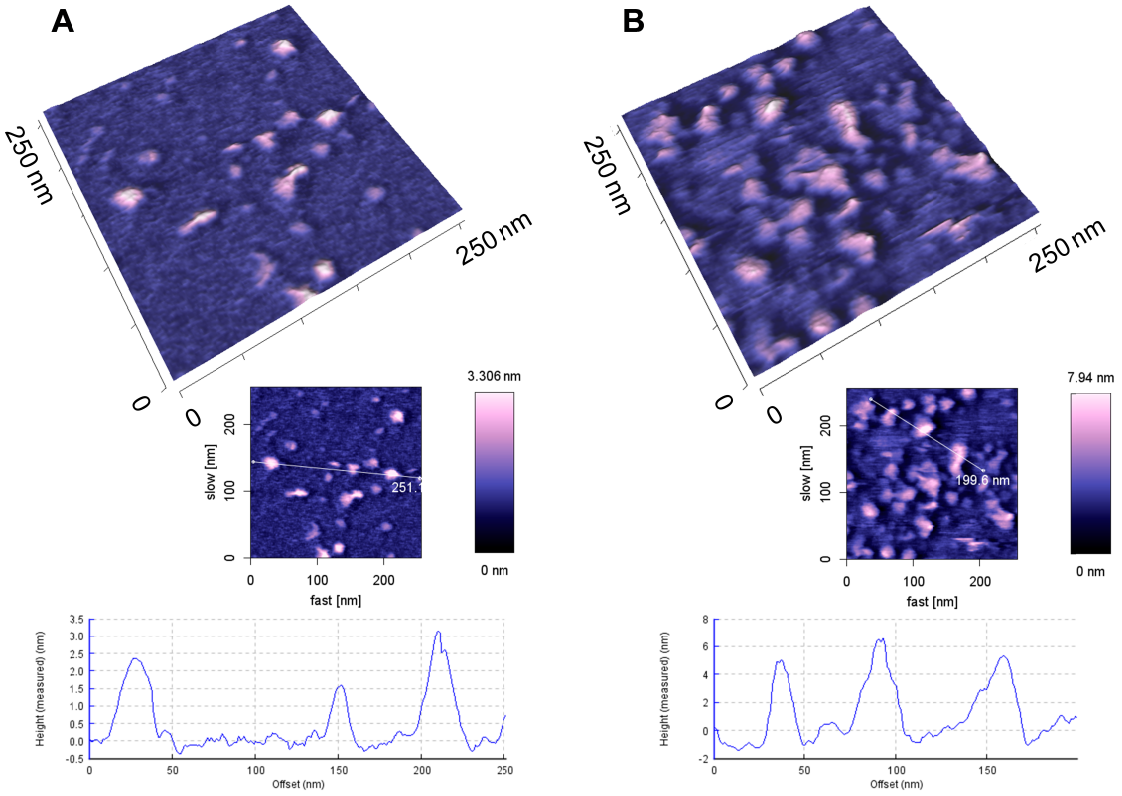

Figure S3. HeLa cell cytotoxity assay of nonionic and cationic amphiphilic ESG derivatives.

HeLa cells were plated in 96 well plates at 3.0×10^3 cells per well (100 μL) and cultured for 20 h at 37°C in 5% CO_2. Then, 11 μL of CHESG and CHESG-DEAE solutions with various concentrations (0.01–20.0 mg mL^{-1})) in PBS (pH7.4) were added to the wells (final concentration of ESG derivatives were 0.001–2.0 mg mL^{-1}) and co-incubated 48 h at 37°C in 5% CO_2. After 48 h incubation, 10 μL of the cell counting kit-8 reagent (Dojindo Laboratories, Kumamoto, Japan) was added to each wells and incubated for 40 min at 37°C in 5% CO_2. The UV-Vis absorbance at 450 nm was obtained using a microplate reader (SH-1000 Lab, Corona Electric Co., Ltd., Ibaraki, Japan). Data are mean and standard error of the means for four experiments (n=4).

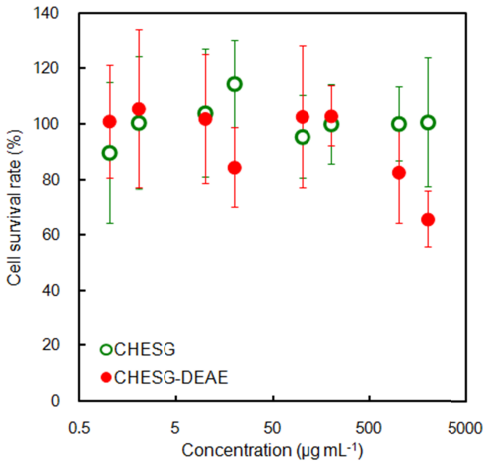

Figure S4. Histograms of the fluorescence intensity of uptake of BSA to HeLa cells by CHP-DEAE and CHESG-DEAE evaluated by flow cytometry.

HeLa cells were cultured in MEM with 10% FBS and 1% antibiotic. Cells were plated in a six-well culture dish at a density of 2.0×10^4 cells per well and cultured for 24 h at 37°C in 5% CO_2. The CHESG-DEAE or DEAE modified CHP (CHP-DEAE, 17 DEAE groups per 100 glucose units of pullulan) and Alexa Fluor 488-labeled BSA complexes were added to fresh MEM (total volume= 1.0 mL, final concentration of CHESG-DEAE and CHP-DEAE= 120 μg mL^{-1} and BSA= 2.64 μg mL^{-1}), and replaced with the pre-culture medium of cells. After co-incubation for 4 h at 37°C in 5% CO_2, the cells were washed with medium, trypsinized, and suspended in BD PharMingen™ stain buffer. The fluorescence intensity was measured by flow cytometry for evaluation of intracellular uptake of BSA into HeLa cells.