Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Surface-Modified Protein Nanospheres as Potential Antiviral Agents

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Materials and Methods: All chemicals and reagents were of analytical grade. Bovine serum albumin, sodium 2-mercaptoethane sulfonate (MES) and *n*-dodecane were obtained from Sigma-Aldrich and used without further purification.

Synthesis of BSA microspheres (BSA-MS) and MES conjugated BSA particles (MES-CP): For preparation of BSA-MS, an aqueous solution of BSA (30 ml, 5%) was over-layered with Canola oil or *n*-dodecane (20 ml). The tip of the ultrasonic horn (Ti horn from Sonics and Materials VCX 600, 20 kHz, 600 W at 40 % efficiency) was then positioned at the interface between the aqueous and organic phases and the solution was sonochemically irradiated for 3 min in ice. After sonication, the formed emulsion was kept at 4ºC for 24 h, resulting in phase separation. The particles were segregated as a middle layer and were collected and dialyzed in 12.5 kDa dialysis membrane against deionized water for 48 h with intermittent water changes. To prepare MES-CP, different amounts of MES (up to 2×10^4 MES:BSA mole excess) were dissolved directly into the BSA solution (30 ml, 5%) at pH 8 and sonicated as above. The mixture was then kept at 4ºC overnight and dialyzed. Controls including aqueous solution of either BSA or MES, aqueous mixture of BSA and MES (all without oil) and aqueous MES overlayered with oil or *n*-dodecane were sonicated similarly.

Particle Characterization. The shape and morphology of the particles were characterized by optical-fluorescence microscopy (Apo-Tome AxioImager.z1 microscope, Zeiss, Germany), scanning electron microscopy (SEM, FEI Quanta™ 200 FEG, Hillsboro, Oregon) and confocal microscopy (Leica-SPE microscope, Mannheim, Germany). For the SEM analyses a sample (10 µl) of the particles was spotted onto a glass wafer, followed by drying and gold sputtering. The samples were then analyzed by SEM. The size and the size distribution of the particles were determined by Malvern zeta sizer nano series (Nano ZS, Malvern).

Cell culture: Vero African green monkey kidney epithelial cells were cultured in minimum essential medium (MEM)-Eagle (Biological Industries, Israel) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Biological Industries), L-glutamine (Biological Industries), and penicillin-streptomycin-amphotericin (PSA) (Biological Industries). Cells were maintained at 37ºC under 5% CO_2.

Cytotoxicity: Vero cells (2.5×10^4 per well) were grown in 96-well plates with 5% FCS in MEM. BSA-MS and MES-CP (333, 166, 83, 41.5 and 20.75 µl mL^{-1}) were then added to each well and final volume was adjusted to 150 µl. Cell survival was then determined by XTT (Biological Industries) following 24, 48 and 72 h post-particle additions. Controls consisting of untreated cells and pure medium were included. The absorbance of each well was then measured at 405 nm (680 nm background) using the TECAN Spectrafluor Plus plate reader.

Antiviral assay: Vero cells (**(**7×10^4 per well) were grown in a 24-well plate. Confluent monolayers of cells were inoculated with 250 µL of HSV-1 McIntyre strain suspensions (350 plaque forming units (pfu)/well) in the presence of different concentrations of BSA-MS (100 µl mL^{-1}) or MES-CP (100 µl mL^{-1}) at 37ºC. Controls consisting of soluble BSA, sonicated BSA, sonicated MES and sonicated MES in oil mixture were included in the assay. After 2 h, cells were overlaid with 500 µL of MEM-Eagle's medium supplemented with 2% heat-inactivated FCS and 0.1% human γ-globulin containing the particles or controls concentration to maintain the constant concentration. After 2 days of incubation, the cell monolayers were stained with Giemsa stain, and the plaques were counted.

Antiviral efficiency of BSA-MS and MES-CP: Flow cytometry (Galios, Beckman Coulter) was used to determine the concentrations of the particles (number of particles.mL^{-1}) and the final concentrations were adjusted accordingly. Antiviral activity was measured and compared for the two particles at different concentrations (100, 20, 10, 1 and 0.1 µl mL^{-1}).

Cell-to-cell spread inhibition assay: Cells were infected with 1.5×10^6 PFU mL^{-1} and incubated for 2 h at 37°C. The cell cultures were then washed and overlaid with fresh media (250 µl) containing 0.1% of human γ-globulin and 2 % FCS. After 1, 6 and 24 h, the medium was replaced with MES-CP solution at 100 µl mL^{-1} with 2% FCS and 0.1% human γ-globulin. Plaque sizes were measured using AxioVision software release 4.7.2, and averaged for 100 plaques in each set 48 h after infection. For statistical interpretation of the data, ANOVA analysis with Bonferroni multiple comparison analysis was carried out using SPSS version 15.

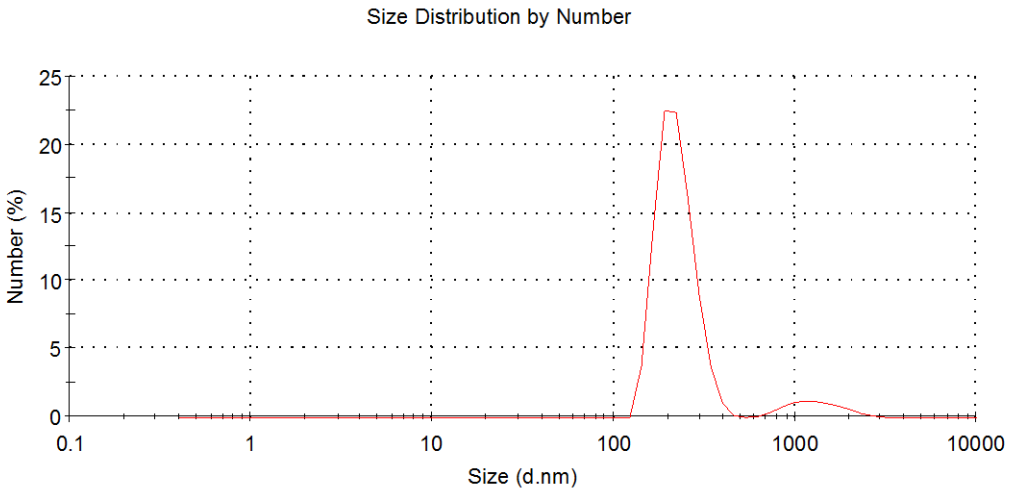

Fig. S1 Physical properties of the MES conjugated particles. Size distribution analysis of the particles according to their number as recorded by DLS.

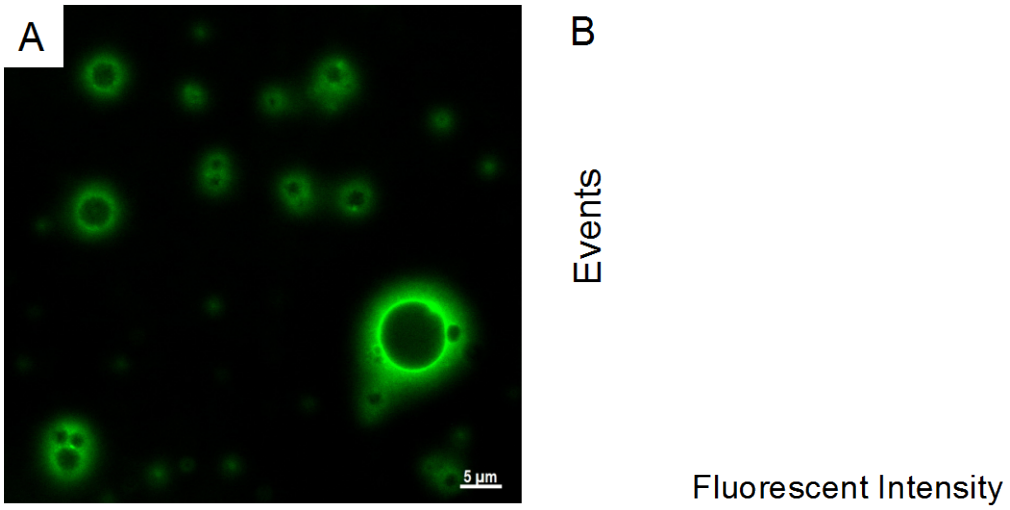

Fig. S2. Chemical reaction of fluorescein isothiocyanate (FITC) with the MES-CP suggests that the shells of the particles are built from BSA molecules. MES-CP (0.4 mL) were reacted in Tris buffer (100 mM, pH 8.5) with excess of fluorescein isothiocyanate (20 µL, 5 mM; FITC) for 1 h at room temperature. The particles were then washed extensively by centrifugation and then analyzed either by a) fluorescent microscope or by b) flow cytometry. Black line represents the fluorescent intensity parent MES-CP, while the gray line shows that of MES-CP following their reaction with FITC.