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Supplemental Data

Inorganic Mercury Detection

and Controlled Release of Chelating Agents

from Ion-Responsive PEG-Liposomes

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Materials

The lipids 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-n-[methoxy(polyethyleneglycol)-2000] (ammonium salt) (PEG-PE) were purchased from Avanti Polar Lipids. Stock solutions of lipids in chloroform at 30 mg/ml were freshly prepared. Column buffer was prepared by addition of 24 ml of 500 mM HEPES buffer at pH 7.4 and 15 ml of 2 M NaCl into 160 ml of distilled water. 1 mM, 100 µM and 10 µM stock solutions of $Hg(ClO_4)_2$ were prepared. 200 mM and 1 mM stock solution of divalent metal ions were prepared for further use.

Liposome preparation

The PEG-PE and DOPE were mixed at the 20% w/w ratio. The solution was dried under N_2 and desiccated under vacuum overnight. The dried lipids are rehydrated with 1 ml of 50 mM of fluorescein in 100 mM of NaCl and 50 mM of HEPES buffer at pH 7.4. The hydrated mixture is incubated at 37 °C for one day. The resultant solution is extruded 11 times in a mini-extruder through 1 µm polycarbonate membrane. The resulting vesicles are purified by PD-10 desalting column against column buffer. 1300 µl of liposome solution is collected from PD-10 column. The liposome solution is diluted to 4500 µl in column buffer.

Detection of mercury by fluorescence

2 µl of stock solution of 1, 2.5, 5, 10 or 100 µM of $Hg(ClO_4)_2$ was added into 200 µl of fluorescein encapsulated liposomes for detection of final concentration of 10, 25, 50, 100 or 1000 nM Hg^{2+}. The fluorescence change with the addition of inorganic mercury is monitored by fluorimeter (λ_{ex} = 495 nm and λ_{em} = 520 nm) over 15 minutes using disposable glass cuvettes. In order to check the effect of other divalent metal ions, 2 µl of stock solution of various concentrations of metal salts was added into 200 µl of fluorescein encapsulated liposomes. The change in fluorescence is monitored using the same parameters in monitoring the effect of Hg^{2+}. The fluorescent measurements are performed in triplicate.

Encapsulation of *meso*-DMSA and fluorescein in liposomes

The DOPE and PEG-PE were mixed at the four to one ratio, dried under N_2 and desiccated under vacuum overnight. The dried lipids are rehydrated with 1 ml of 50 mM fluorescein with 0, 100, 1000 or 2000 µM *meso*-DMSA in 100 mM of NaCl and 50 mM of HEPES buffer at pH 7.4 for in vitro experiments. The dried lipids are rehydrated with 1 ml of 2 µM *meso*-DMSA in 100 mM of NaCl and 50 mM of HEPES buffer at pH 7.4 for HeLa cell experiments. The hydrated mixture is incubated at 37 °C for one day. The resultant solution is extruded 11 times in a mini-extruder through 1 µm polycarbonate membrane. The resulting vesicles are purified by PD-10 desalting column against column buffer. 1300 µl of liposome solution is collected from PD-10 column. The liposome solution is diluted to 4500 µl in column buffer.

HeLa cell experiments

HeLa cells are cultured following ATCC's recommendation, in Eagle's Menimum Essential Medium, supplemented with 100 units/mL aqueous penicillin G, 100 µg/mL streptomycin, and 10% fetal bovine serum.The HeLa cells were grown in 96-well plates with medium at concentrations to allow 70% confluence in 24 h. At the experiment day, cells were washed with PBS buffer and incubated with prewarmed Opti-MEM medium (phenol red reduced) for 30 minutes at 37 ^{o}C. After the addition of different formulations of liposomes (with or without *meso*-DMSA (2µM, 80µL)), cells was incubated for 8 hours and then washed with 100 µL/well PBS twice. Cells were further treated with 0, 0.2, 0.4, 0.6, 0.8 or 1.0 µM final concentrations of Hg using stock solution of 20, 40, 60, 80 or 100 µM of $Hg(ClO_4)_2$ in prewarmed fresh growth medium for 48 h. Cell viability was assessed colorimetrically with the MTT reagent (Sigma-Aldrich) following the standard protocol provided by the manufacturer. The absorbance was read with a microplate reader at 570 nm.