Electronic Supplementary Material (ESI) for ChemComm.

Rock the nucleus: significantly enhanced nuclear membrane permeability and gene transfection by plasmonic nanobubble induced nanomechanical transduction

Xiuying Li [1], Peiyuan Kang [1], Zhuo Chen[2], Sneha Lal[3], Li Zhang [3], Jeremiah J. Gassensmith[2,4], and Zhenpeng Qin [1,4,5]*

[1] Department of Mechanical Engineering, [2] Department of Chemistry and Biochemistry, [3] Department of Biological Sciences, [4] Department of Bioengineering, University of Texas at Dallas, Richardson, Texas 75080, United States; [5] Department of Surgery, The University of Texas Southwestern Medical Center, 5323 Harry Lines Blvd, Dallas, Texas 75390

Table of Contents

Materials and Methods

1.1 Materials

Dipalmitoylphosphatidylcholine (DPPC) and cholesterol were purchased from Avanti Polar Lipids, Inc. DMEM medium, trypsin, Dulbecco's phosphate buffered saline, live/dead cell imaging kit (R37601), and Hoechst 33342, were purchased from Thermo Fisher Scientific. Calcein sodium salt was purchased from Alfa Aesar. Gold chloride was purchased from Sigma-Aldrich. All other chemicals were analytical grade. pEGFP-VSVG was a gift from Jennifer Lippincott-Schwartz (Addgene plasmid # 11912),

1.2 Preparation and characterization of plasmonic liposomes

Liposomes were prepared by thin-film hydration followed by extrusion. Briefly, A lipid mixture of dipalmitoylphosphatidylcholine (DPPC) and cholesterol (mol/mol 8/2) was dissolved in chloroform and subsequently the solvent was removed under nitrogen, followed by evaporation under vacuum overnight. The resulting lipid film was hydrated with phosphate buffered saline (PBS) under 50 °C for 1 h. The resulting multilamellar vesicles were then extruded 21 times through 200 and 100 nm pore sized polycarbonate membrane using an Avanti Mini Extruder. Gold nanoparticles were decorated onto the liposome surface following a previously reported method with minor modification [1]. Aqueous solutions of gold chloride (10 mM) and ascorbic acid (40 mM) were prepared. Gold chloride solution was added and gently mixed with liposome suspension (1.5 mM lipid concentration) in a 1:4 molar ratio until uniformly distributed, followed by addition of the same volume of ascorbic acid solution. Following reduction, plasmonic liposomes samples were dialyzed against PBS for 2 h under room temperature to remove unreacted gold chloride and ascorbic acid. Plasmonic liposomes and uncoated liposomes were stored at 4°C.

The sizes of plasmonic liposomes and uncoated liposomes were determined by dynamic light scattering measurement (Malvern ZetaSizer Nano ZS). Liposomes were diluted with PBS to 150 μM before measurement. Extinction spectrum of plasmonic liposomes and uncoated liposomes in PBS were obtained with a spectrophotometer (DU800, Beckman Coulter). The morphology of the plasmonic liposomes was observed by a transmission electron microscope (TEM, JEM-2100F, 200 keV). Briefly, a droplet of plasmonic liposomes at a lipid concentration of 100 μM was placed on a carbon support film, and the excess liquid was evaporated at room temperature for 1 h before imaging.

1.3 Plasmonic nanobubble detection

We used an optical pump-probe technique to measure plasmonic nanobubbles, which are transient vapor bubbles generated from plasmonic liposomes when activated by an ultrafast picosecond laser pulse. Plasmonic liposome solution was placed between two glass slides and irradiated with laser pulses at fluence values (30, 60, 150 mJ/cm^2). Plasmonic liposomes absorb the near-infrared laser pulse (*i.e.*, pump, 750 nm) and create nanobubbles. Nanobubbles strongly scatter a continuous laser beam (*i.e.*, probe, 633 nm) and lead to a decrease in the transmitted laser intensity. The probe beam laser intensity was recorded with a fast photodetector (FPD510-FV, Thorlabs) and an oscilloscope (LeCroy WaveRunner204Xi-A).

1.4 Nuclear delivery of 40 kDa FITC-dextran (FD-40)

Raw 264.7 cells were released from cell culture flask using a cell scraper and centrifuged at 150 g for 2 min. Cells were then re-suspended in serum-free DMEM medium at a concentration of 5×10^6 cells/mL. Cell suspensions (380 μL) were transferred into electroporation cuvettes and kept on ice. FD-40 in serum-free DMEM medium (20 μL, 2 mg/mL) was added to cell suspension before electroporation. The electroporation was carried out using the same conditions as gene transfection experiments [2], using a voltage of 260 V and an infinite internal resistance value. Cell suspensions were discharged for 30 s. The electroporated cells were diluted with DMEM/FBS, transferred to 25-mm glass bottom dishes, and then kept in a humidified incubator at 37°C and 5% CO_2. After 3 h, Raw 264.7 cells were incubated with serum-free DMEM medium containing 150 μM empty plasmonic liposomes or non-coated liposomes for 3 h, and then treated with near-infrared

pulsed laser (60 mJ/cm^2). Cells with or without laser activation were stained with Hoechst 33342 and observed by a confocal microscope using 100 × oil objective.

1.5 In vitro gene transfection

Raw 264.7 cells were harvested when the cells reached approximately 80% confluence, followed by resuspending in serum-free DMEM medium at a 5×10^6 cells/mL. Cell suspensions were mixed with pEGFP-VSVG (5 ug/mL) and transferred into electroporation cuvettes, which were then kept on ice. The electroporation was immediately carried out following a previously reported protocol[2], using a voltage of 260 V and an infinite internal resistance value. Cell suspensions were discharged for 30 s. The electroporated cells were diluted with DMEM/FBS. For CLSM imaging, cells were transferred to 25-mm glass bottom dishes. For spectrofluorimetry analysis, cells were transferred to black 96 well plates. Cells were kept in a humidified incubator at 37°C under 5% CO_2. After 3 h, Raw 264.7 cells were incubated with serum-free DMEM medium containing 150 μM empty plasmonic liposomes or non-coated liposomes for 3 h, and then activated with the near-infrared pulsed laser (60 mJ/cm^2). After 48 h, cells with or without laser activation were stained with Hoechst 33342 and observed by a confocal microscope using 100 × oil objective. For spectrofluorimetry analysis [3], transfected cells were washed three times with PBS and lysed in radioimmunoprecipitation assay buffer (RIPA) at 4°C. The lysate was then used to measure the EGFP-VSVG expression by fluorescence plate reader (λ_{ex} = 485 nm, λ_{em} = 528 nm). Background and autofluorescence were determined using cells treated under the same condition but without pEGFP-VSVG. The total protein content in cell lysate from each well was determined by Bradford Protein Assay Kit. The EGFP-VSVG expression efficiency was calculated by subtracting background values and normalized against protein concentration after cell lysis.

1.6 Cell viability

To understand the effect of laser irradiation on the cell, cell viability was assessed by a live/dead staining kit, which consists of calcein-AM and a cell impermeable red dye (R37601, Thermo Fisher Scientific). Calcein-AM is a cell membrane-permeable fluorescence dye, which is hydrolyzed to cell membrane-impermeable, green-fluorescent calcein by cellular esterase in living cells. The membrane-impermeable dye can pass through disrupted cell membranes emitting red fluorescence, thus labeling dead cells. Raw 264.7 cells with endocytosed plasmonic liposomes were treated under the same condition with the nuclear delivery studies. Immediately after laser exposure, live/dead assay working solution was added to each dish and incubated for 20 mins, followed by confocal imaging.

Supplementary Figures

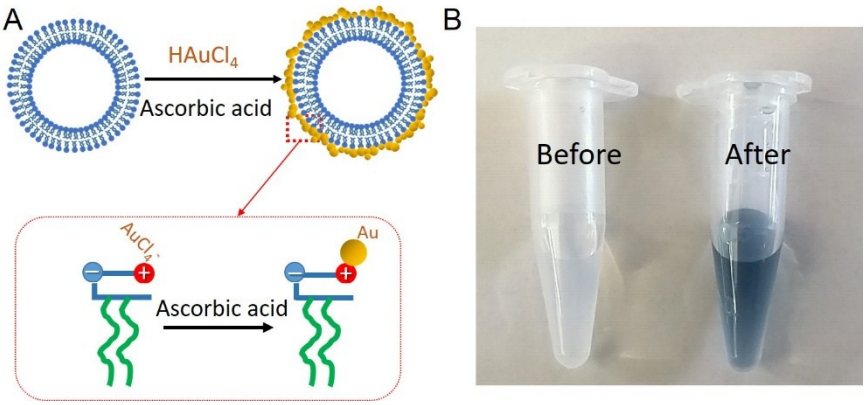

Figure S1 (A) Schematics of plasmonic liposome formation and (B) photograph of liposomes before and after gold decoration.

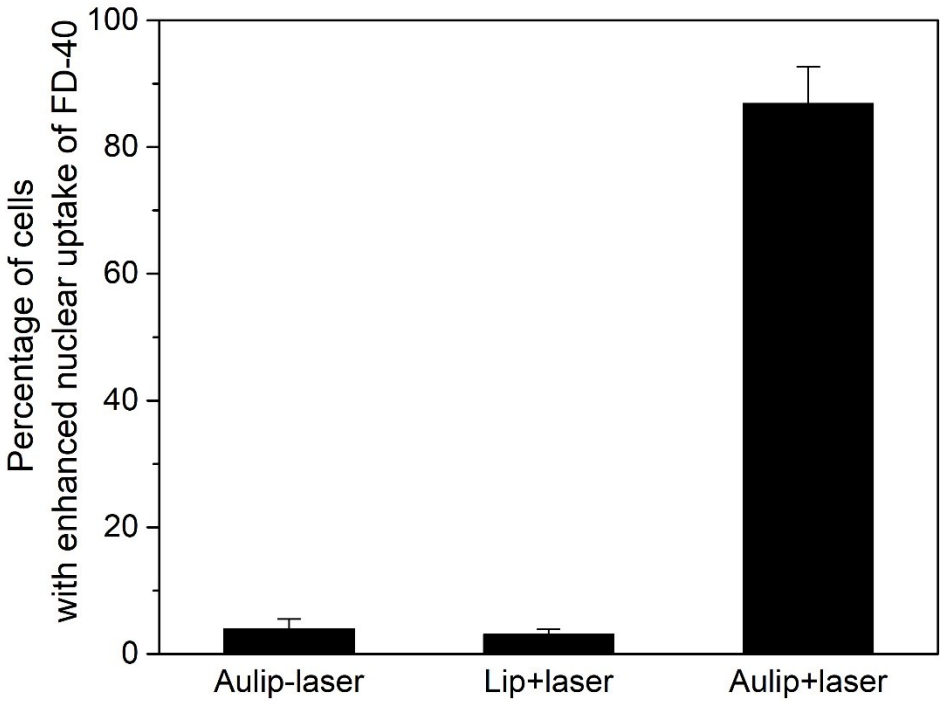

Figure S2 Percentage of cells with enhanced nuclear uptake of FD-40 calculated from around 80 cells for each group. Data are represented as the mean ± SD of three independent experiments. Under current experimental conditions, 87% Raw 264.7 cells showed enhanced nuclear uptake of FD-40 after nanomechanical transduction. In contrast, when using standard liposomes without gold coating or plasmonic liposomes without laser activation, no mechanical transduction occurred and less than 4% cells exhibited enhanced nuclear uptake of FD-40.

Supplementary Tables

Table S1 Particle size, and polydispersity index (PDI) of different liposomes

Samples	Particle size	PDI
Empty liposomes	118.9±7.1	0.058±0.008
Empty plasmonic liposomes	135.2±4.6	0.131±0.017

Table S2 Influence of nanomechanical transduction on different cell components

Cell components	Affected by nanomechanical transduction or not	Notes
Cell membrane	No	Contains the dye in the cytosol
Nucleus	Yes	Finding of the current study
Endo/lysosomes	Yes	From others and our previously reported work [4, 5]
Endoplasmic Reticulum	Unknown	Spreads its cisternae and tubules across the entire cytoplasm
Mitochondria	Unknown	Size is smaller than optical microscope resolution [6]
Golgi	Unknown	Same as above

References

(1) Leung, S. J.; Kachur, X. M.; Bobnick, M. C.; Romanowski, M. Wavelength-Selective Light-Induced Release from Plasmon Resonant Liposomes. *Adv Funct Mater* **2011,** *21* (6), 1113-1121.

(2) Transfection of mammalian cells by electroporation. *Nat Methods* **2006,** *3* (1), 67-68.

(3) Jiang, M.; Gan, L.; Zhu, C.; Dong, Y.; Liu, J.; Gan, Y. Cationic core-shell liponanoparticles for ocular gene delivery. *Biomaterials* **2012,** *33* (30), 7621-30.

(4) Li XY, Che ZF, Mazhar K, Price TJ, Qin ZP. Ultrafast Near-Infrared Light-Triggered Intracellular Uncaging to Probe Cell Signaling. *Adv Funct Mater* **2017**, 27, 11, 1605778.

(5) Lukianova-Hleb, E. Y.; Belyanin, A.; Kashinath, S.; Wu, X.; Lapotko, D. O. Plasmonic nanobubble-enhanced endosomal escape processes for selective and guided intracellular delivery of chemotherapy to drug-resistant cancer cells. *Biomaterials* **2012**, 33 (6), 1821-6.

(6) Xu H, Su W, Cai M, Jiang J, Zeng X, Wang H. The asymmetrical structure of Golgi apparatus membranes revealed by in situ atomic force microscope. *PloS One* **2013**;8, e61596.