Supplementary Information for

Magnetic Response of Mitochondria-Targeted Cancer Cell with Bacterial Magnetic Nanoparticles

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Preparation of BMPs

BMPs were obtained from *Magnetospirillum* sp. AMB-1 (ATCC® 700264) which was cultured for 5 days in shaking incubator at 27 °C and 120 rpm under anaerobic condition. A revised magnetic spirillum growth medium (MSGM) was used as growth medium.[3] Grown *Magnetospirillum* sp. AMB-1 were centrifuged for 25 min at 5000 rpm and then lysed by sonication (VCX500, Sonics&Materials, USA) for 30 min. BMPs were separated from the lysate using a neodymium-iron-boron (NdFeB) magnet and washed 5 times with 1X PBS. Collected BMPs were dispersed in 1X PBS and sterilized with autoclave (121°C, 15 min). Concentration of extracted BMPs was determined by inductive coupled plasma-atomic emission spectrometer (ICP-AES, ICPS-7500, Shimadzu, Japan).

Preparation of BMP-Cyt c apyamer complex

The material of cytochrome c aptamer is adopted in DNA form through whole experiment. The sequence of it is following; 5'-CCG TGT CTG GGG CCG ACC GGC GCA TTG GGT ACG TTG TTG C-3'

BMP and cytochrome c aptamer are conjugated with Bissulfosuccinimidyl Suberate (BS3) (S5799, sigma, USA) who can make amine-amine bond using two separated amine groups. The BMPs-Cyt *c* complexes were concentrated by a magnetic bar to remove the excess aptamer and washed several times with washing buffer.

After binding of aptamer, due to aptamer's negative charge, BMP-aptamer complexes are more easily dispersed than BMP-only state. These complexes are stored in PBS, 4°C.

Cell culture and Transfection of BMPs

HeLa cells, human cervical cancer, were cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo Scientific, USA) containing 10 % (v/v) fetal bovine serum (FBS, Thermo Scientific, USA) and 3 % (v/v) penicillin/streptomycin (Thermo Scientific, USA) at 37 °C in a humidified atmosphere containing 5 % CO_2. After initial incubation for 2 h, the cells were treated with BMPs and then incubated overnight for sufficient time to transfect. The BMP treated cells were trypsinized and collected by centrifuge at 1000 rpm for 5 min. The cells were resuspended in medium and BMPs transfected HeLa cells were separated from non-transfected HeLa using a magnet. Then the BMPs transfected HeLa cells and control HeLa cells were seeded and cultured separately.
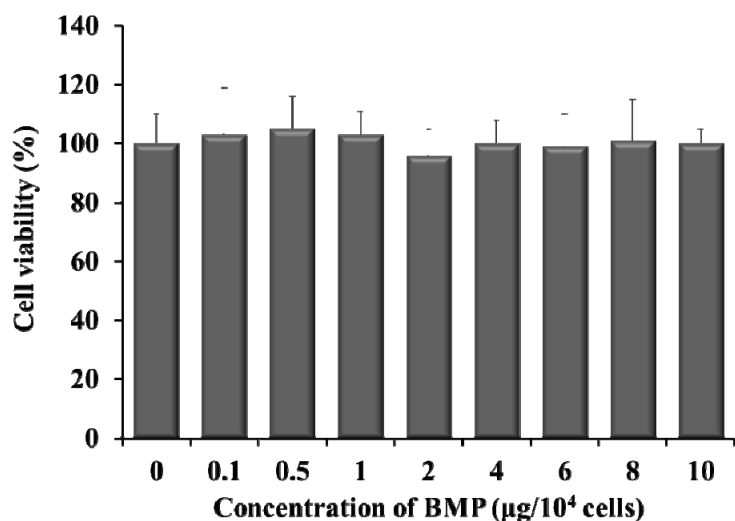
Fig. S1 Cytotoxicity of BMPs at various concentrations was insignificant.

MTS assay

The MTS assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, Promega, USA) was used to evaluate the cytotoxicity of the BMPs and viability of cells in several condition. For cytotoxicity test, HeLa cells were seeded on 96-well plate (10^3 cells/well) with 100 µl of the culture medium and incubated for 24 h to stabilize the cells. The BMPs were added at concentrations ranging from 2 µg to 15 µg per 10^4 cells. Every 24 h up to 72 h after treating with BMPs, 20 µl of CellTiter 96® AQueous One Solution Reagent (Promega, USA) was added into each well. Then the plate was incubated at 37 °C for 1–4 h in a humidified 5 % CO_2 atmosphere and absorbance was recorded at 490 nm with an absorbance reader (Tecan, Switzerland). To assess viability of the cells, Hela and BMP-HeLa cells were seeded on 96-well plate (10^3 cells/well) with 100 µl of the culture medium. After initial incubation about 2 h, the cells were exposed to SMF up to 48 h, and then the MTS assay was performed as same manner with cytotoxicity test. All of the assays were repeated twice at least and 4 wells for one group were investigated in each experiment. The absorbance of control cells was normalized to 100 % of viability in every assay.

Transmission Electron Microscopy (TEM)

Intracellular uptake of BMPs was directly confirmed by TEM. The TEM measurement was carried out on the HeLa cells cultured overnight after the treatment with the BMPs. At first, the cells immersed in the modified

Karnovsky's fixative solution (2 % paraformaldehyde and 2 % glutaraldehyde in 0.05 M sodium cacodylate buffer) for 4 h at 4 °C. They were washed three times for 10 min with 0.05 M sodium cacodylate buffer and post-fixed with 1 % osmium tetroxide in 0.05 M sodium cacodylate buffer for 2 h at 4 °C. The post-fixed cells were briefly washed twice with distilled water, and stained with 0.5 % uranyl acetate for 30 min at 4 °C. The stained cells were dehydrated in a graded ethanol series (30, 50, 70, 80, and 90 %) and three times in 100 % ethanol each for 10 min. Then, the sample were treated with 100 % propylene oxide twice for 10 min and embedded in Spurr's resin. Ultrathin sections were made by a diamond knife with an ultramicrotome (MT-X; RMC, Tucson, AZ). They were mounted on copper grids and the sections were examined with a transmission electron microscope (TEM, JEM-1010, JEOL, Japan).
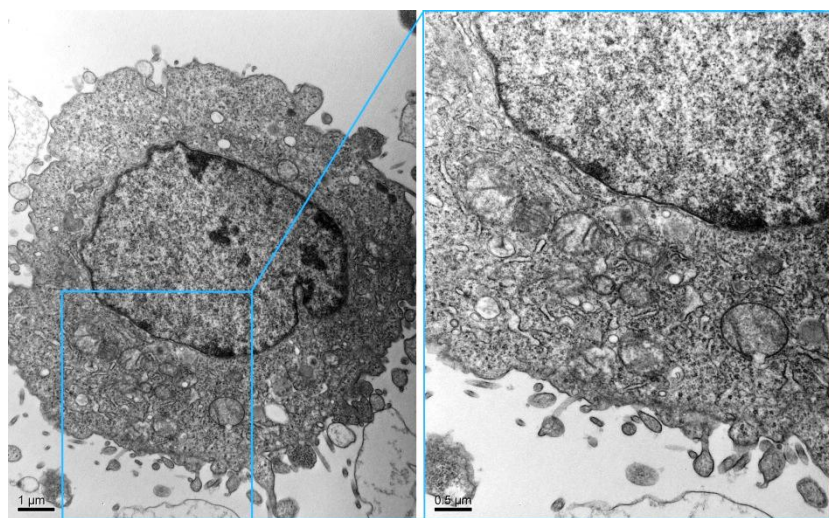

Fig S2. Transmission electron microscopy image of control HeLa cell without BMPs

Confocal Laser Scanning Microscope

Each treated cells were placed in 8-wells of chambered slide (Lab-Tek™, Nunc, Denmark). Mitotracker red CMXRos (Invitrogen, USA) was used for mitochondria staining; it is introduced to DMEM of 25 mM, for 45 min. Each well was washed briefly with PBS twice. After fixing with 3.7% of paraformaldehyde in PBS for 15 min, the mixture of 1% bovine serum albumin and 0.3% of triton X-100 was used for permeabilization of cell membrane for 20 min. After 3 times of washing with PBS, DAPI (Invitrogen, USA) was diluted to 1:1000 in PBS, and then introduced to each well for 15 min to view cell nucleus in blue color under confocal microscope.

Samples are treated with one drop of faramount mounting medium (Dako, Japan), and all slides were covered with cover glass and viewed by confocal laser scanning microscope (Carl Zeiss LSM710, Germany).

TUNEL Assay

Terminal deoxynucleotidyl transferase dUTP nick end labeling assay (TUNEL assay, promega, USA) was performed for confirmation of apoptosis. HeLa cells were seeded onto 35φ dish after transfection, cultured for 24 hours. Each sample was treated with 4% paraformaldehyde for 25 min, wash twice with PBS. For permeabilization, each dish was immersed with 0.2% Triton X-100 in PBS for 5min. After washing 3 times in PBS, 100 μl of equilibration buffer was added and 100μl of TdT solution also introduced to the whole sample for labeling. Each section was covered by coverslip to ensure even distribution of the mixture. All of samples were incubated in humidified chamber for 60 min, and then the reaction was stopped by immersing 2X SSC solution and blocked by 0.3% hydrogen peroxide. Streptavidin HRP which was diluted 1:500 in PBS was introduced to the sample for binding the end of DNA, when it reacts with DAB makes brown color. Whole sample were visualized by inverted microscopy, in glycerol.
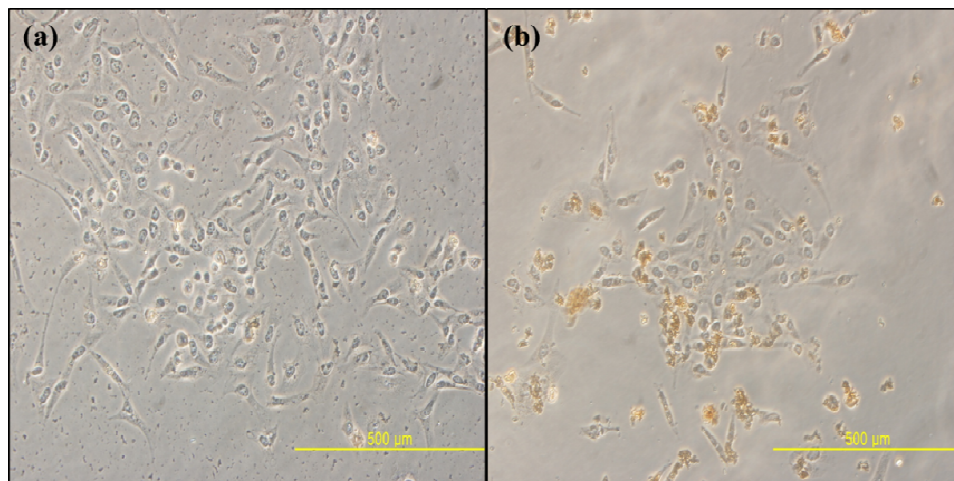

Fig. S3. TUNEL assay. (a) Control HeLa cells, (b) Mitochondria-targeted HeLa cells, with BMP-Cyt *c* aptamer complex exposed to the magnetic field

Magnetic Force on a BMP

To understand physical effect of the internalization of BMPs with SMF exposure on a cell, magnetic force on a BMP was estimated using a magnetofection model. When the BMP is trasnfected into a cell and exposed to an

SMF externally at the bottom of the cell, the environment is similar with magnetofection. Cytoplasm could be substituted for the transport fluid in the magnetofection model [1]. The magnetic force on a BMP is modeled using the effective dipole moment method in which a magnetic particle is replaced by an equivalent point dipole with a moment $m_{p,\text{eff}}$ [1, 2]. The force on the dipole (and hence on the particle) is given by

$$\mathbf{F}_m = \mu_f \mathbf{m}_{p,\text{eff}} \cdot \nabla \mathbf{H}_a$$

Where μ_f is the permeability of transport fluid, $m_{p,\text{eff}}$ is the effective dipole moment of the particle, and H_a is the externally applied magnetic field intensity at the center of a particle, where the equivalent point dipole is located. The $m_{p,\text{eff}}$ is depends on H_a as following equation.

$$\mathbf{m}_{p,\text{eff}} = V_p f(H_a) \mathbf{H}_a$$

where
$$f(H_a) = \begin{cases} \dfrac{3(\chi_p - \chi_f)}{(\chi_p - \chi_f) + 3} & \text{if } H_a < \left(\dfrac{(\chi_p - \chi_f) + 3}{3\chi_p} \right) M_{\text{sp}} \\ \dfrac{M_{\text{sp}}}{H_a} & \text{if } H_a \geq \left(\dfrac{(\chi_p - \chi_f) + 3}{3\chi_p} \right) M_{\text{sp}} \end{cases}$$

We assume that the cytoplasm is nonmagnetic ($\chi_f = 0$) with a viscosity and density equal to that of water. The BMP consists of Fe_3O_4 and have a density $\rho_p = 5170$ kg/m^3 and saturation magnetization $M_{\text{sp}} = 4.52$ x 10^5 A/m. When, $\chi_p \gg 1$

$$f(H_a) = \begin{cases} 3 & \text{if } H_a < M_{\text{sp}}/3 \\ \dfrac{M_{\text{sp}}}{H_a} & \text{if } H_a \geq M_{\text{sp}}/3 \end{cases}$$

In our SMF exposure condition, employing a rectangular neodymiumiron boron (NdFeB) magnet (L x W x T, 50x25 x15 mm), 1-D distribution of H_a is described as following equation which is provided from the international magnetic solutions (IMS).

$$H_a = \frac{M_s}{\pi} \left[\left[\tan^{-1} \frac{WL}{2x(4x^2 + W^2 + L^2)^{1/2}} \right] - \tan^{-1} \left[\frac{WL}{2(x+T)[4(x+T)^2 + W^2 + L^2]^{1/2}} \right] \right] H_a$$

$$= \frac{M_s}{\pi} \left[\tan^{-1} \frac{WL}{2x(4x^2 + W^2 + L^2)^{1/2}} - \tan^{-1} \left[\frac{WL}{2(x+T)[4(x+T)^2 + W^2 + L^2]^{1/2}} \right] \right]$$

The Flux density H_a is calculated along the center axis at a distance x from the magnet and $M_s = 9.09$ x 10^5 A/m. When the distance from center of the magnet $x < 0.01$ m, $H_a \geq M_{\text{sp}}/3$.

Finally, the magnetic force on a BMP could be described as following.

$$\mathbf{F}_m(x) = \mu_f V_p \left(\frac{M_{\text{sp}}}{H_a} \right) \mathbf{H}_a \cdot \nabla \mathbf{H}_a = \mu_f V_p \left(\frac{M_{\text{sp}}}{H_a} \right) H_a \frac{dH_a}{dx} = \mu_f V_p M_{\text{sp}} \frac{dH_a}{dx}$$

We assumed the distance x is 1 mm which is the thickness of the culture dish we used. The BMPs internalized cells attached right on the dish and ignored the height of a cell. Based on our experiment condition, the magnetic

force on a BMP was 0.59 fN toward the magnet.

μ_f : permeability of transport fluid $\approx \mu_0$

$m_{p,\text{eff}}$: effective dipole moment of the particle

H_a : externally applied magnetic field intensity at the center of the particle

V_p : volume of a magnetic nanoparticle, 6.54×10^{-23} m^3

r_p : radius of a magnetic nanoparticle, 25 nm

χ_p : magnetic susceptibility of the particle

χ_f : magnetic susceptibility of the fluid

M_{sp} : saturation magnetization of the particle

M_{s} : saturation magnetization of the magnet

Reference

[1] Furlani EP, Ng KC. Nanoscale magnetic biotransport with application to magnetofection. Physical Review E. 2008;77(6):061914.

[2] Furlani E, Ng K. Analytical model of magnetic nanoparticle transport and capture in the microvasculature. Physical Review E. 2006;73(6):061919.