Supporting Information

Functionalized Spiky Particles for Intracellular Biomolecular Delivery

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**Experimental Method**

Fabrication of spiky particle: In the first step, 40 ml NaOH aqueous solution (5 M) containing 1 g TiO$_2$ (Degussa P25) powders was mixed and stirred for 30 min. The solution was placed into a Teflon-lined stainless steel autoclave. The autoclave was heated at 120 °C for 24 h for hydrothermal reaction. White precipitates were produced after reaction, which was Na-titanate one dimensional nanostructure bundles. They were collected by centrifugation, and washed with DI water for at least 3 times, and then dried at 60 °C in vacuum. In the second step, 0.1 g Na-titanate powders were mixed with 19 ml of 1 M NaOH aqueous solution and 1.25 mL H$_2$O$_2$ (30%) solution. The mixture solution was vigorously stirred and treated with sonication for 30 min, and was then placed into Teflon-lined stainless steel autoclave for hydrothermal reaction at 150 °C for 6 h. Precipitate product was generated and collected by centrifugation. The product was washed with DI water for at least 3 times, and then dried at 60 °C in vacuum. Next, the products were agitated with 0.05 M HNO$_3$ solution, and then washed with DI water for at least 5 times. The products were dried and calcinated at 400 °C for 1 h to form TiO$_2$ spiky particles.

To select well-dispersed spiky particles, the fabricated spiky particles were re-suspended in sterile water and sonicated for 30 min. Particles were collected by centrifugation at 5000 rpm for 2 min. 9 ml sterile water was placed in a 15 ml centrifuge tube, and 1 ml particle solution was added on top of the water surface. The spiky particles gradually sank to the bottom at different rates dependent on their sizes and dispersion states. The better-dispersed and smaller particles tend to sank slowly, staying in the upper water. After 30 min particle sediment, 4 ml upper solution which contained better-dispersed spiky particles was collected.

Plain particle preparation: Plain particle preparation: 2 μm TiO$_2$ plain particles (the actual size was found to be 1.6 ± 0.1 μm by SEM) were ordered from Microspheres-nanosperes Inc.

Fabrication of rough particle: Spiky particles were sonicated (Bandelin Sonorex) overnight to remove the nanospikes from particles. The nanospikes-particles mixture
solution was centrifuged at 5000 rpm for 2 min. The particles were collected, and washed with sterile water for at least 5 times.

Particle storage: solutions containing microparticles were sterilized by autoclaving, and stored at 4 °C.

SEM imaging: Particles were drop-casted on Si wafer and dried, and sputter-coated with Au-Pd. To preserve particle dispersity for SEM imaging, the solution containing particles could also be deposited on track-etched membrane to filter away the water immediately. The samples were then dried and sputter-coated with Au-Pd. Particle samples were imaged with SEM (Zeiss SUPRA 60).

TEM imaging: Particles were suspended in 100% ethanol, and embedded in plastic and sectioned. The samples were imaged with TEM (FEI Tecnai).

Optical microscopy imaging: Particle solution of certain amount was placed in a well of 96 well-pate. After 6 h, the particles were imaged with optical microscopy. For determining particle doses, the number of particles per area was counted.

Cell viability assay: For cell viability experiments, Hela cells (ATCC), RAW 264.7 cells (ATCC) or 3T3-L1 (ATCC) were pre-cultured for 24 h, and then incubated with spiky, plain or rough particles at different doses. The dose of particle is 0.02-0.16 particles/μm² for Hela cell viability experiment, and 0.01-0.08 particles/μm² for RAW and 3T3 cell viability experiments. Cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% antibiotic at 37 °C and 5% CO₂. For experimental comparison, TiO₂ nanoparticles (Sigma-Aldrich) and ZnO nanoparticles (Sigma-Aldrich) were applied to cells. For live/dead assay, live cells were stained with calcein AM (Thermo Fisher Scientific), dead cells were stained with ethidium homodimer (Thermo Fisher Scientific), and cell nucleus were stained with Hoechst (Thermo Fisher Scientific). The results were recorded with fluorescence microscopy (Mshot and Zeiss Axio).

Hela cell proliferation assay: GFP-expressing Hela cells (Cell Biolabs, Inc) were pre-cultured for 12 h. Spiky, plain or rough particles of indicated doses were treated to the cells. The fluorescent cells were monitored at 12 h, 48 h, and 72 h with fluorescence
microscopy (Mshot).

SEM studies of cell-particle interface: Cells were pre-cultured on glass slide for 24 h, then spiky, plain or rough particles of 0.08 particles/μm² were incubated with the cells for 24 h. The cell samples were fixed with 2% glutaraldehyde solution for 5 min, and then washed with ethanol of increasing concentrations (0%, 30%, 50%, 70%, 80%, 90%, 95%, and 100% x3). The samples were treated with critical point drying (Samdri), and sputter-coated with Au-Pd for SEM imaging.

Confocal fluorescence microscopy studies of cell-particle interface: Spiky, plain and rough particles were conjugated with (3-aminopropyl)triethoxysilane (APTES, Sigma-Aldrich) by dispersing the particles in ethanol containing 5% APTES, heated at 60 °C for 3 h. The particles were repeatedly washed with ethanol and water, and then incubated with 1 mg/ml Alexa Fluor 660 NHS Ester (Succinimidyl Ester, Thermo Fisher Scientific) for 30 min. The dye-labeled microparticles were then washed and collected.

Hela, RAW or 3T3-L1 cells were pre-cultured in glass-bottom plate for 24 h. Dye-conjugated microparticles were added to the cells and incubated for indicated time periods (e.g. 3 h, 6 h or 24 h). For cell cytosol staining, the cells were stained with calcein AM. For cell membrane staining, the cells were pre-treated with CellLight Early Plasma membrane-GFP BacMam (Thermo Fisher Scientific) for 24 hours before particle applications. For actin staining, cells treated with particles were fixed with paraformaldehyde and then rinsed, and the actin was stained with ActinGreen 488 ReadyProbes Reagent (Thermo Fisher Scientific). For endosome staining, the BMMs were pre-treated with CellLight Early Endosomes-GFP BacMam for 24 hours before particle applications. The cell samples were imaged with confocal fluorescence microscope (Olympus FV-10i, Zeiss LSM or Leica TCS SP8). The images were reconstructed with Zen lite for orthographic view and 3D view. For particle uptake statistical analysis, the confocal fluorescence images were analyzed. In each cell, the green fluorescence volume (indicating cell) and the red fluorescence volume (indicating particles) were measured.
Particle coating with PEI: Microparticles were firstly coupled with 3-glycidoxypropyl trimethoxysilane (3-GPS, Sigma-Aldrich) by mixing microparticles with 5% GPS in toluene solution, and then heated at 70 °C for 24 h. After reaction, the particles were centrifuged and washed with toluene, acetone and ethanol for multiple times. The microparticles were then mixed with polyethylenimine (PEI, MW ~ 800 Da, Sigma-Aldrich) in carbonate buffer (pH 9.5) with the weight ratio of particles/PEI to be 1:5. After 24 h, the microparticles were washed with 1 M NaCl solution and DI water for at least 5 times. The unreacted epoxy groups were blocked by incubating the microparticles with 1 M ethanolamine solution for 6 h. The particles were then washed again with 1 M NaCl solution and DI water.

Intracellular delivery of fluorescent siRNA: PEI-functionalized microparticles including spiky, plain or rough particles were incubated with 20 μM siRNA conjugated with cyanine 5 (Sigma-Aldrich) overnight. The siRNA-complexed microparticles were centrifuged and washed with PBS once. The particles were then suspended in cell medium and added to cells. Cells were pre-cultured in glass-bottom plate for 24 h. After 6 h particle treatments, the cells were stained with calcein AM, and imaged with confocal fluorescence microscopy. For fluorescent siRNA endocytosis imaging, Hela cells were pre-cultured with or without non-fluorescent spiky particles for 6 h. Cell medium containing 4 μM fluorescent siRNA was added to cells. After another 6 h, the cells were imaged.

Transfection assay of GFP-targeting siRNA: PEI-functionalized microparticles including spiky, plain or rough particles were incubated with 20 μM GFP-targeting siRNA or 20 μM negative siRNA whose sequence was scrambled. After incubation overnight, the siRNA-complexed microparticles were supplemented with cell medium and added to GFP-expressing Hela cells. Cells were pre-cultured in 96-well plate for 24 h. For control groups, 0.4 nmol siRNA was added to the cells in 96-well plate. Spiky particles were applied with the dose of 0.16 particles/μm². To compete with spiky particles, plain and rough particles were applied with extra doses of 0.32 particles/μm² to ensure the total particle doses and total used DNA was higher than the spiky particle
group. After 48 h particle treatments, the cell nucleus were stained with Hoechst for cell number counting, and the cells were imaged with fluorescence microscopy. For GFP ELISA assay, after 48 h particle treatments, the cells were lysed. The cell lysis solution were separated from cell debris and microparticles via centrifugation. GFP in lysis solution was extracted and analyzed with GFP ELISA kit (Abcam) according to the standard protocol. The GFP expressing level of each group was divided by the corresponding cell number of the group, and then normalized to the control group.

Determination of DNA binding to microparticles: PEI-functionalized microparticles including spiky, plain or rough particles were incubated with 0.05 μg/μl GFP-encoded DNA plasmid overnight. The microparticles were centrifuged, and the upper solution was withdrawn for measuring the remaining DNA concentration with Nanodrop.

DNA plasmid transfection assay: PEI-functionalized microparticles including spiky, plain or rough particles were incubated with 0.05 μg/μl GFP-encoded DNA plasmid overnight. The DNA-complexed microparticles were supplemented with cell medium and added to cells. Cells were pre-cultured in 96-well plate for 24 h. For control groups, 1 μg DNA plasmid was added to the cells in 96-well plate. Spiky particles were applied with the dose of 0.16 particles/μm² for the DNA transfection experiment on Hela cells, and 0.08 particles/μm² for the DNA transfection experiments on RAW and 3T3 cells. To compete with spiky particles, plain and rough particles were applied with extra doses (2-folds higher) of 0.32 particles/μm² on Hela cells and 0.16 particles/μm² on RAW and 3T3 cells, and to ensure the total particle doses and total used DNA was higher than the spiky particle group. After 48 h particle treatments, the Hela cells or 3T3 cells were stained with calcein red for cell number counting, while the RAW cells were stained with Hoechst for cell number counting, considering the convenience of cell counting visually. The transfection results were imaged with fluorescence microscopy to count the percentage of GFP-expressing cells. No unexpected or unusually high safety hazards were encountered.

Statistical analysis: t-test was used to analyze difference between two groups, and
one way ANOVA was used among multiple groups. p value was calculated by PRISM software (GraphPad) and difference was regarded significant if p < 0.05.

Supplemental information

S1. Supplemental information of delivery mechanism.

The engulfment and uptake of particles are relied on the active phagocytosis or endocytosis mechanism, while the delivery of biomolecules into cytosol is more likely a mixed process of direct penetration and endosomal escape.

The spiky particles were engulfed and internalized by the cells through phagocytosis or endocytosis mechanism. During this process, the nanospikes on PEI-functionalized particle surface might induce some permeation or portion on cell membrane. Some biomolecules bound on particles surface may thus release into the cell cytosol. In this process, the phagocytosis or endocytosis mechanism is still necessary for the spiky particles to initiate the engulfment of spiky particles. If the phagocytosis or endocytosis mechanism were blocked, it would compromise the engulfment of spiky particles, which is a critical step for generating necessary force and stress on cell membrane to induce membrane penetration. In addition, after the initial particle engulfment, the particles were gradually internalized and finally entrapped in the endosome as other conventional particles. Some other biomolecules bound on these particles might be eventually released into cytosol through conventional endosomal escape.

![Figure S1-1. Illustration of the spiky particles uptake process. (a-b) The spiky particles were initially getting contact with the cell membrane, and the gradually engulfed. (c) During the engulfment process, the nanospikes penetrated cell membrane, and some biomolecules bound on particles surface released into the cytosol. (d-e) After the initial particle engulfment, the particles were gradually internalized and finally](image-url)
entrapped in the endosome as other conventional particles. Some other biomolecules bound on these particles might be eventually released into cytosol through conventional endosomal escape.

S2. Supplemental information of Hela cells viability upon particle treatments.

**Figure S2-1.** Fluorescence and optical images of Hela cell viabilities upon incubation without particles (control) or with spiky particles at different particle doses for 48 h. Green: live cells; Red: dead cells; Blue: cell nucleus. Scale bar: 400 μm.
**Figure S2-2.** Fluorescence and optical images of Hela cell viabilities upon incubation with plain particles at different particle doses for 48 h. Green: live cells; Red: dead cells; Blue: cell nucleus. Scale bar: 400 μm.

|   | Calcein (Live) | Ethidium Bromide (Death) | Hoechst (Nucleus) | Optical Image | Merged |
|---|----------------|--------------------------|-------------------|--------------|--------|
| Plain, 48 h | ![Image](image1) | ![Image](image2) | ![Image](image3) | ![Image](image4) | ![Image](image5) |
| 0.16 particles/μm² | ![Image](image6) | ![Image](image7) | ![Image](image8) | ![Image](image9) | ![Image](image10) |
| 0.08 particles/μm² | ![Image](image11) | ![Image](image12) | ![Image](image13) | ![Image](image14) | ![Image](image15) |
| 0.04 particles/μm² | ![Image](image16) | ![Image](image17) | ![Image](image18) | ![Image](image19) | ![Image](image20) |
| 0.02 particles/μm² | ![Image](image21) | ![Image](image22) | ![Image](image23) | ![Image](image24) | ![Image](image25) |

**Figure S2-3.** Fluorescence and optical images of Hela cell viabilities upon incubation with rough particles at different particle doses for 48 h. Green: live cells; Red: dead cells; Blue: cell nucleus. Scale bar: 400 μm.

|   | Calcein (Live) | Ethidium Bromide (Death) | Hoechst (Nucleus) | Optical Image | Merged |
|---|----------------|--------------------------|-------------------|--------------|--------|
| Rough, 48 h | ![Image](image26) | ![Image](image27) | ![Image](image28) | ![Image](image29) | ![Image](image30) |
| 0.16 particles/μm² | ![Image](image31) | ![Image](image32) | ![Image](image33) | ![Image](image34) | ![Image](image35) |
| 0.08 particles/μm² | ![Image](image36) | ![Image](image37) | ![Image](image38) | ![Image](image39) | ![Image](image40) |
| 0.04 particles/μm² | ![Image](image41) | ![Image](image42) | ![Image](image43) | ![Image](image44) | ![Image](image45) |
| 0.02 particles/μm² | ![Image](image46) | ![Image](image47) | ![Image](image48) | ![Image](image49) | ![Image](image50) |
Figure S2-4. Fluorescence and optical images of Hela cell viabilities upon incubation with TiO$_2$ nanoparticles at different particle doses and ZnO nanoparticles for 48 h. Green: live cells; Red: dead cells; Blue: cell nucleus. Scale bar: 400 μm.

Figure S2-5. Fluorescence and optical images of Hela cell viabilities upon incubation without particles (control) or with spiky particles for 96 h. Green: live cells; Red: dead cells; Blue: cell nucleus. Scale bar: 400 μm.
Figure S2-6. Fluorescence and optical images of Hela cell proliferation upon incubation without particles (control) or with spiky particles of different doses for 72 h. The cell proliferation profiles of GFP-expressing Hela cells were monitored for 72 h with fluorescence microscopy. Green: GFP-expressing Hela cells. Scale bar: 400 μm.

Figure S2-7. Fluorescence and optical images of Hela cell proliferation upon incubation with plain or rough particles of 0.16 particles/μm² for 72 h. The cell proliferation profiles of GFP-expressing Hela cells were monitored for 72 h with fluorescence microscopy. Green: GFP-expressing Hela cells. Scale bar: 400 μm.
S3. Supplemental information of interface between particles and Hela cells.

**Figure S3-1.** Interfaces between Hela cells and microparticles (spiky, plain and rough particles) were studied via confocal fluorescence microscopy. Hela cells were incubated with microparticles for 3 h. Cell cytosol was stained with calcein AM (green fluorescence). Microparticles were stained with red fluorescence. The confocal fluorescence images were re-constructed with both orthographic view and 3D view for visualizing the cell-particle interface.

**Figure S3-2.** Interfaces between Hela cells and microparticles (spiky, plain and rough particles) were studied via confocal fluorescence microscopy. Hela cells were incubated with microparticles for 6 h. Cell cytosol was stained with calcein AM (green fluorescence). Microparticles were stained with red fluorescence. The confocal fluorescence images were re-constructed with both orthographic view and 3D view for visualizing the cell-particle interface.
Figure S3-3. Interfaces between Hela cells and microparticles (spiky, plain and rough particles) were studied via confocal fluorescence microscopy. Hela cells were incubated with microparticles for 24 h. Cell cytosol was stained with calcein AM (green fluorescence). Microparticles were stained with red fluorescence. The confocal fluorescence images were re-constructed with both orthographic view and 3D view for visualizing the cell-particle interface.

Figure S3-4. Interface between Hela cells’ membrane and spiky particles was studied via confocal fluorescence microscopy. Hela cells were incubated with spiky particles for 24 h. The cell membrane of Hela cell was specifically labeled with green fluorescent protein (GFP) by transfecting the cells with BacMam 2.0 product. Microparticles were stained with red fluorescence. The confocal fluorescence images were re-constructed with both orthographic view and 3D view for visualizing the cell-particle interface.
Figure S3-5. Interfaces between Hela cells’ actin network and microparticles (spiky, plain and rough particles) were studied via confocal fluorescence microscopy. Hela cells were incubated with microparticles for 24 h. Hela cells were fixed and stained with phalloidin conjugated with Alexa Fluor 488 (green fluorescent dye) on their actin network. Microparticles were stained with red fluorescence. The confocal fluorescence images were re-constructed with both orthographic view and 3D view for visualizing the cell-particle interface.

Figure S3-6. Interfaces between Hela cells’ endosome and microparticles (spiky, plain and rough particles) were studied via confocal fluorescence microscopy. Hela cells were incubated with microparticles for 24 h. Endosomes were specifically labeled with GFP by using BacMam 2.0 product that consists of a fusion construct of Rab5a and emGFP to transfec the cells. Microparticles were stained with red fluorescence. The confocal fluorescence images were re-constructed with both orthographic view and 3D view for visualizing the cell-particle interface.
Figure S3-7. Interfaces between Hela cells’ endosome and microparticles (spiky, plain and rough particles) were studied via confocal fluorescence microscopy. The co-localization of endosome marker and particles were analyzed. The endosome was labeled with green fluorescence, and microparticles were labeled with red fluorescence. The fluorescence area in the images was outlined, as indicated in rows of the “fluorescent area analysis”. The green fluorescent area and red fluorescent area were overlapped. The cell boundary was outlined with blue lines. The percentage of red fluorescence that was not overlapped with green fluorescence were analyzed. The results found that >99% spiky particles and plain particles were overlapped with endosome, and >90% rough particles were overlapped with endosome. Scale bar: 10 μm.

S4. Supplemental information of microparticles-mediated gene delivery into Hela cells.
**Figure S4-1.** The weight ratio of PEI on spiky particles were analyzed with thermogravimetric analysis (TA thermal analyzer A50). The weight ratio of PEI on spiky particles were found to be 2.82±0.03%.

**Figure S4-2.** The PEI-functionalized spiky particles did not induce significant cytotoxicity to the cells. Cell viability was higher than 95% after 48 h incubation with. The dose of the microparticles was 0.16 particles/μm². Scale bar=400 μm.

**Figure S4-3.** Hela cells were incubated with fluorescent siRNA (red)-complexed microparticles (spiky, plain and rough particles). Confocal fluorescence microscopy images showing fluorescent dye distribution within the cells. Cell cytosol was stained with calcein AM (green fluorescence). The cell border was not indicated with dash-lines for clear visualization of the fluorescence distribution. Scale bar: 10 μm.
**Figure S4-4.** Quantification of DNA binding to PEI-functionalized microparticles; (N=4).

**Figure S4-5.** GFP-expressing Hela cells were incubated without GFP-targeting siRNA for 48 h as control group. Scale bar: 400 μm.

**Figure S4-6.** GFP-expressing Hela cells were transfected with lipofectamine conjugated with GFP-targeting siRNA or negative siRNA for 48 h. Scale bar: 400 μm.
Figure S4-7. The SEM images of spiky particles before-, after-PEI functionalization, and Spiky/PEI conjugated with DNA plasmid (GFP DNA plasmid).

Figure S4-8. Hela cells were treated with DNA plasmid but without complexing with microparticles for 48 h. This is employed as control group of DNA transfection. Fluorescence images showed the GFP transfection results. Cells were stained with calcein red for cell number counting. Scale bar: 400 μm.

Figure S4-9. Hela cells were treated with DNA plasmid complexing with commercial lipofectamine. This is employed as positive control group of DNA transfection. Fluorescence images showed the GFP transfection results. Cells nucleus were stained with hoechst for cell number counting. Scale bar: 400 μm.

S4-10. Discussion of the gene transfection efficiency.

Based on this result, the PEI-functionalized spiky particles indeed absorbed more RNA or DNA due to their larger surface area. The spiky particles exhibited much more
pronounced delivery efficiency that was likely due to two combinational effects: the larger surface area effects, and the penetration effects.

In the RNA and DNA transfection experiments, we intentionally increased the application doses of control groups, the plain particles and rough particles, by 2-folds, to ensure the total particle doses and total used RNA or DNA amount was higher than the spiky particle group. PEI-functionalized spiky particles were applied with the dose of 0.16 particles/μm$^2$, while PEI-functionalized plain and rough particles were applied with extra doses of 0.32 particles/μm$^2$.

The amount of RNA/particles in spiky particles group was <2-folds higher than the plain particles and rough particles groups. From the siRNA transfection results, the plain and rough particles group produced 15.9±8.1% and 7.3±9.8% knockdown compared to the blank control group, while the spiky particles group produced 64.3±7.9% knockdown. The knockdown effects of spiky group was >4-folds higher than either plain or the rough group, which was much more pronounced than the larger surface area effects (<2 folds) would produce. Similarly, the spiky group produced DNA transfection efficiency of 12.7±4.0%, which was >6-folds higher than either plain or the rough group. These results suggested there is additional delivery effects than the larger surface area effects.

In addition, in the delivery assay of siRNA conjugated with red fluorescent dye, our results found that the PEI-functionalized spiky particles induced fluorescence delivery into cytosol with uniform cytosolic distribution of fluorescence. This result supports the hypothesis that the fluorescent siRNA was directly delivered into the cytosol, which was consistent with previous reported that delivery through cell penetration by vertical nanowire array would resulted in uniform cytosolic distribution of fluorescent dye (Reference: Nano Lett., 2012, 12 (8), pp 3881–3886).
S5. Supplemental information of RAW cells viability upon particle treatments.

Figure S5-1. Fluorescence images of RAW cell viabilities upon incubation without particles (control) or with spiky particles at different particle doses for 48 h. Green: live cells; Red: dead cells; Blue: cell nucleus. Scale bar: 400 μm.
Figure S5-2. Fluorescence images of RAW cell viabilities upon incubation with plain particles at different particle doses for 48 h. Green: live cells; Red: dead cells; Blue: cell nucleus. Scale bar: 400 μm.
Figure S5-3. Fluorescence images of RAW cell viabilities upon incubation with rough particles at different particle doses for 48 h. Green: live cells; Red: dead cells; Blue: cell nucleus. Scale bar: 400 μm.

S6. Supplemental information of interface between particles and RAW cells.

Figure S6-1. Interfaces between RAW cells and spiky particles were studied via confocal fluorescence microscopy. RAW cells were incubated with microparticles for 24 h. Cell cytosol was stained with calcein AM (green fluorescence). Microparticles were stained with red fluorescence. The confocal fluorescence images were reconstructed with both orthographic view and 3D view for visualizing the cell-particle interface.
S7. Supplemental information of microparticles-mediated gene delivery into RAW cells.

**Figure S7-1.** RAW cells were incubated with fluorescent siRNA (red)-complexed microparticles (spiky, plain and rough particles). Confocal fluorescence microscopy images showing fluorescent dye distribution within the cells. Cell cytosol was stained with calcein AM (green fluorescence). The cell border was not indicated with dash-lines for clear visualization of the fluorescence distribution. Scale bar: 10 μm.

**Figure S7-2.** RAW cells were treated with DNA plasmid but without complexing with microparticles for 48 h. This is employed as control group of DNA transfection. Fluorescence images showed the GFP transfection results. Cell nucleus were stained with hoechst for cell number counting. Scale bar: 400 μm.

**Figure S7-3.** RAW cells were treated with DNA plasmid complexing with commercial lipofectamine. This is employed as positive control group of DNA
transfection. Fluorescence images showed the GFP transfection results. Cells nucleus were stained with hoechst for cell number counting. Scale bar: 400 μm.

S8. Supplemental information of 3T3-L1 cells viability upon particle treatments.
**Figure S8-1.** Fluorescence images of 3T3-L1 cell viabilities upon incubation with spiky, plain and rough particles at different particle doses for 48 h. Green: live cells; Red: dead cells; Blue: cell nucleus. Scale bar: 400 μm.

**S9. Supplemental information of interface between particles and 3T3-L1 cells.**

![Confocal fluorescence microscopy images](image)

**Figure S9-1.** Interfaces between 3T3-L1 cells and spiky, plain and rough particles were studied via confocal fluorescence microscopy. 3T3-L1 cells were incubated with microparticles for 24 h. Cell cytosol was stained with calcein AM (green fluorescence). Microparticles were stained with red fluorescence. The confocal fluorescence images were re-constructed with both orthographic view and 3D view for visualizing the cell-particle interface.

**S10. Supplemental information of microparticles-mediated gene delivery into 3T3-L1 cells.**

![GFP Transfection and Calcein Red](image)

**Figure S10-1.** 3T3-L1 cells were treated with DNA plasmid but without complexing with microparticles for 48 h. This is employed as control group of DNA transfection. Fluorescence images showed the GFP transfection results. Cells were stained with calcein red for cell number counting. Scale bar: 400 μm.
Figure S10-2. 3T3-L1 cells were treated with DNA plasmid complexing with commercial lipofectamine. This is employed as positive control group of DNA transfection. Fluorescence images showed the GFP transfection results. Cells were stained with calcein red for cell number counting. Scale bar: 400 μm.

S11. Supplemental information of the spiky delivery efficiency.

S11-1. Discussion of the delivery efficiency.

The delivery efficiency would depend on many aspects. First, the cell phagocytic ability would determine whether the cell could generate sufficient force on the nanospike to induce stress on cell membrane. Second, whether nanospikes could penetrate cell membrane would depend on the critical rupture tension of the cell membrane. Third, it would depend on the release rate of bound biomolecule from the particles. If the biomolecules could not be sufficiently released, they would not enter the cellular cytosol even when the nanospikes penetrated cell membrane. Forth, it is likely that the cell membrane penetration was not a permanent event. The cell membrane is likely to reseal after the nanospikes penetrated it. The cell membrane resealing aspect would increase the complexity of drug delivery, but this is likely since it has been observed on many other cell membrane poration techniques such as electroporation or optoporation. The bound biomolecule need to be released in a right time window when the cell membrane was penetrated.

S11-2. Improving particle design.

The particle geometry played important role on modulate cellular uptake and delivery efficiency. Currently the spiky particles were fabricated with TiO$_2$ materials. Although it did not induce cytotoxicity to the cells, it is more favorable to use
biodegradable materials, such as organic polymer to fabricate spiky particles. However, at the current state, it is still challenging to fabricate stiff nanospikes on particles using polymer materials. The particle size would determine the particle engulfment and internalization rates. To optimize particle uptake, the particle size should be ideally between 50-200 nm (Reference: PNAS, 2008, 105, 11613-11618; Appl. Phys. Lett. 2010, 96, 033704), as these particle sizes were found to be most effective for cellular endocytosis. However, due to the presence of nanospikes, the spiky particles fabricated by our method tend to be at the size of 1 µm. Future exploration of fabricating spiky particles with smaller size may facilitate cellular uptake and increase delivery efficiency. In addition, reducing the diameter of nanospikes would produce higher stress on cell membrane, facilitating cell membrane penetration. Increasing the length of nanospikes would also facilitate cell membrane penetration. Our results showed that rough particles with reduced nanospikes length by sonication produced lower biomolecular efficiency than the spiky particles with longer nanospikes.

S11-3. Improving the delivery efficiency.

There are couple of ways that might potentially facilitate biomolecular delivery by spiky particles. First, the understanding of cell membrane penetration mechanism by 1D nanowires or nanospikes is essential for improving delivery efficiency. According to the cell membrane resealing phenomenon observed on other portion technique, cell membrane penetration by 1D nanospikes might not be a permanent event. If the cell membrane resealed after the initial penetration by nanospikes, the bound biomolecule need to be released in a right time window so that it could get into the cytosol through the membrane rupturing. Second, the biomolecular delivery efficiency could be further improved by reducing the particle sizes. Smaller particle size is favorable for particle engulfment and internalization. Particle size between 50-200 nm (Reference: PNAS, 2008, 105, 11613-11618; Appl. Phys. Lett. 2010, 96, 033704) would be ideal for particle endocytosis, yet the current fabrication technique have difficulty to produce particle size smaller than 1 µm. Second, reducing the diameter of nanospikes would facilitate cell membrane penetration, since it produced higher stress on cell membrane.
Third, surface functionalization of spiky particles with cationic polymer or other surface coating could facilitate particle uptake and enhance cell membrane rupture. The surface coating also played a critical role on absorbing and releasing biomolecules to deliver. A well-tuned surface coating could improve the loading efficiency of biomolecules on particles, and allowed readily release of biomolecules into cytosol.