Supporting Information for:

A Small Library of DNA-Encapsulated Supramolecular Nanoparticles for Targeted Gene Delivery

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1. Experimental Section

1.1. General

Reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO) and used as received without further purification otherwise noted. Branched polyethylenimine (PEI, MW = 10 kD) was purchased from Polysciences Inc (Washington, PA). Polymers contain primary, secondary and tertiary amine groups in approximately 25/50/25 ratio. 1st-generation polyamidoamine dendrimer (PAMAM) with 1, 4-diaminobutane core and amine terminals in 20% wt methanol solution was purchased from Dendritic Nanotechnologies, Inc (Mount pleasant, MI). 1-Adamantanamine (Ad) hydrochloride and β-cyclodextrin (β-CD) were purchased from TCI America (San Francisco, CA). N-hydroxysuccinimide (SCM) and maleimido (MAL) hetero-functionalized polyethylene glycol (SCM-PEG-MAL, MW = 5 kD) was obtained from NANOCS Inc (New York, NY). Phosphate-buffered saline (PBS, 1X, pH 7.2 ± 0.05) for sample preparation, UltraPure™ 10X TBE buffer (including 1 M Tris, 0.9 M boric acid, and 0.01 M EDTA) for electrophoresis experiment and 1X TE buffer (including 10mM Tris-HCl containing 1mM EDTA) for DNA dilution were purchased from Invitrogen (Carlsbad, CA). 6-Mono-tosyl-β-cyclodextrin (6-OTs-β-CD) was prepared following the literature reported method. Octa-Ad-grafted polyamidoamine dendrimer (Ad-PAMAM), CD-grafted branched polyethylenimine (CD-PEI) and Ad-grafted polyethylene glycol (Ad-PEG) were prepared as the method we reported before. Dry CH2Cl2 was obtained by refluxing over CaH2 and freshly distilled before use. MCF7 breast cancer cell line, U87 brain cancer cell line and NIH 3T3 mouse fibroblast cell line were purchased from American Type Culture Collection. The Dulbecco's Modified Eagle Medium (DMEM), Earl's Modified Eagle's Medium (EMEM) growth medium, Opti-MEM reduced serum medium and Penicillin/streptomycin were obtained from Invitrogen (Carlsbad, CA). Fetal Bovine Serum (FBS) and EGFP-encoded plasmid DNA (pMAX EGFP®, 3.4 kb) were obtained from Lonza Walkersville Inc (Walkersville, MD). 4', 6-Diamidino-2-phenylindole (DAPI) was purchased from Invitrogen (Carlsbad,
CA). Arginine-glycine-aspartic-cystein (RGDC) peptide was purchased from GenScript Corp. (Piscataway, NJ).

$^1$H NMR spectra were recorded on a Bruker Avance 400 spectrometer in deuterated solvents. Mass spectra were acquired using an Applied Biosystems Voyager DE-STR MALDI-TOF mass spectrometer (Framingham, MA). Dynamic light scattering and zeta potentials of SNPs$\supset$DNA and RGD-SNPs$\supset$DNA were measured on Zetasizer Nano instrument (Malvern Instruments Ltd., United Kingdom). Transmission electron microscope (TEM) images were measured on Philips CM 120 electron microscope operating with an acceleration voltage of 120 kV. Ethidium bromide (EtBr) exclusion assay was performed on a spectrofluorometer (FluoroMax-3, Spex). Cell imaging and gene transfection studies were performed on a Nikon TE2000S inverted fluorescent microscope with a CCD camera (Photomatrix, Cascade II), X-Cite 120 Mercury lamp, automatic stage, and filters for three fluorescent channels (W1 (DAPI), W2 (EGFP and AO) and W3 (PI)).

1.2. Synthesis of RGD-PEG-Ad

Scheme S1. Synthesis of RGD-PEG-Ad targeting ligand

To a solution of 1-adamantanamine hydrochloride (0.94 mg, 5 μmol, 5.0 equiv.) in 1 mL CH$_2$Cl$_2$, triethylamine (0.60 mg,
5 µmol, 5.0 equiv.) and SCM-PEG-MAL (5 mg, 1.0 µmol, 1.0 equiv.) were added in sequence. The reaction mixture was stirred at room temperature for 2 h. After the reaction, the solvent was subsequently removed in vacuo, and the PBS buffer solution (1 mL) containing RGDC (2.25 mg, 5.0 µmol, 5.0 equiv.) was added to the reaction residue. The mixture was stirred for another 2 h at room temperature, followed by remove of insoluble 1-adamantanamine by filtration. The solution was then dialyzed with Slide-A-Lyzer® dialysis cassette (MWCO, 2 kD) against water overnight and lyophilized to give RGD-PEG-Ad (3.4 mg, 0.63 µmol), a white powder in 63% yield. ^1H NMR (400 MHz, DMSO-d6): δ 7.83-9.12 (br, protons on RGD), 3.42-3.54 (br, protons on PEG), 1.13-1.18 (br, protons on Ad). MS (MALDI-TOF, positive mode, DHB): the observed Mn for SCM-PEG-MAL was 5373.49; the Mn value of RGD-PEG-Ad based on the SCM-PEG-MAL was calculated as 5859.78 (M+H^+); found: 5859.33.

1.3. Synthesis of FITC-PEG-Ad

Scheme S2. Synthesis of FITC-PEG-Ad
To a solution of adamantane-1-thiol (0.84 mg, 5 μmol, 5.0 equiv.) in 1 mL CH₂Cl₂, SCM-PEG-MAL (5.00 mg, 1.0 μmol, 1.0 equiv.) was added slowly. The reaction mixture was stirred at room temperature for 2 h. Then ethylenediamine (0.60 mg, 10 μmol, 10.0 equiv.) was added to the mixture slowly. The mixture was stirred for another 2 h at room temperature. After the reaction, the solvent was subsequently removed in vacuo and the residue was dissolved in PBS buffer. The solution was then dialyzed with Slide-A-Lyzer® dialysis cassette (MWCO, 2 kD) against water overnight and lyophilized to give H₂N-PEG-Ad as a white powder. Then the lyophilized H₂N-PEG-Ad was slowly added to the PBS buffer solution (1 mL) containing fluorescein isothiocyanate (1.95 mg, 5.0 μmol, 5.0 equiv.). The mixture was stirred for another 4 h at room temperature under dark. Subsequently, the solution was dialyzed with Slide-A-Lyzer® dialysis cassette (MWCO, 2 kD) against water overnight and lyophilized to give FITC-PEG-Ad (3.65 mg, 0.73 μmol), an orange powder in 73% yield.

\(^{1}\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 7.23-9.12 (br, protons on FITC), 3.42-3.54 (br, protons on PEG), 1.13-1.18 (br, protons on Ad). MS (MALDI-TOF, positive mode, DHB): the observed Mn for SCM-PEG-MAL was 5373.49; the Mn value of FITC-PEG-Ad based on the SCM-PEG-MAL was calculated as 5939.78 (M+H\(^+\)); found: 5941.34.

2. Electrophoresis analysis

The DNA loading capacity of the cationic Ad-PAMAM/CD-PEI hydrogel for the anionic DNA was determined by electrophoresis analysis using 1.0% agarose gel in 1X TBE buffer. Experiments were run at 80 V for 90 min. About 50 ng/μL of DNA was mixed with different amounts of the mixture of cationic Ad-PAMAM/CD-PEI hydrogel to achieve the desired N/P ratio. The resulting solutions were incubated for 1 h prior to running the experiment. DNA was visualized under UV illumination by staining the gels with ethidium bromide (0.5 μg/mL) at room temperature (Figure S1).
**Figure S1.** Gel electrophoresis of the cationic Ad-PAMAM/CD-PEI hydrogel with anionic DNA at various nitrogen-phosphate (N/P) ratios from 0 to 19 as indicated. The two bands of plasmid DNA observed by electrophoresis represent the nicked circular and supercoiled forms of plasmid, respectively.

3. **Ethidium bromide exclusion assay**

The DNA loading capacity of cationic Ad-PAMAM/CD-PEI hydrogel for anionic DNA were further validated via ethidium bromide (EtBr) exclusion assay. A PBS solution (pH 7.2) containing the EtBr (400 ng/mL) and DNA (20 μg/mL) was incubated at room temperature for 10 min to form a stable complex (DNA$\rightarrow$EtBr). After adding various amounts of cationic Ad-PAMAM/CD-PEI hydrogel along with other controls (CD-PEI and the mixture of CD-PEI/Ad-PEG), the fluorescence intensity changes of DNA$\rightarrow$EtBr were measured using a spectrofluorometer (FluoroMax-3, Spex). The excitation and emission wavelengths were 510 and 590 nm, respectively. Slit width was 5 nm. The most significant fluorescence quenching of DNA$\rightarrow$EtBr was observed in the presence of cationic Ad-PAMAM/CD-PEI hydrogel. This result indicated that cationic Ad-PAMAM/CD-PEI hydrogel can condense the anionic plasmid DNA more efficiently than CD-PEI or the mixture of CD-PEI/Ad-PEG (Figure S2).
Figure S2. The fluorescence intensity changes of DNA→EtBr, composed of EtBr (400 ng/mL) and DNA (20 μg/mL) in PBS solution (1mL), were measured at 590 nm after adding various amounts of Ad-PAMAM/CD-PEI hydrogel along with other controls (CD-PEI and the mixture of CD-PEI/Ad-PEG).

4. Preparation of DNA-encapsulated SNPs→DNA and RGD-SNPs→DNA for DLS, TEM and zeta potential studies

4.1. Preparation of 100-nm DNA-encapsulated SNPs→DNA and RGD-SNPs→DNA

Scheme S3. Preparation of 100-0 SNPs→DNA, 100-1% RGD-SNPs→DNA, 100-5% RGD-SNPs→DNA and 100-10% RGD-SNPs→DNA.

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To a solution of CD-PEI (15 µg, 0.84 nmol) in 750 µL PBS solution (pH = 7.2), the mixture of Ad-PAMAM (1.2 µg, 0.42 nmol), Ad-PEG (21.0 µg, 4.2 nmol) and DNA (7.5 µg, 3.4 pmol) in 750 µL PBS solution was added slowly via a Hamilton syringe under vigorous stirring. The 100-0 SNPs\(\overset{\rightarrow}{\text{DNA}}\) was obtained after the mixture was incubated at room temperature for 20 min. The resulting solution was split into four aliquots, and three of them were subjected to the \textit{in situ} ligand exchange by adding PBS solutions (10 µL) containing 246 ng (0.042 nmol), 1230 ng (0.21 nmol) and 2460 ng (0.42 nmol) of RGD-PEG-Ad, respectively. Three mixtures were incubated for another 20 min at room temperature to obtain 100-1% RGD-SNPs\(\overset{\rightarrow}{\text{DNA}}\), 100-5% RGD-SNPs\(\overset{\rightarrow}{\text{DNA}}\) and 100-10% RGD-SNPs\(\overset{\rightarrow}{\text{DNA}}\), respectively.

4.2. Preparation of 300-nm DNA-encapsulated SNPs\(\overset{\rightarrow}{\text{DNA}}\) and RGD-SNPs\(\overset{\rightarrow}{\text{DNA}}\)

Scheme S4. Preparation of 300-0 SNPs\(\overset{\rightarrow}{\text{DNA}}\), 300-1% RGD-SNPs\(\overset{\rightarrow}{\text{DNA}}\), 300-5% RGD-SNPs\(\overset{\rightarrow}{\text{DNA}}\) and 300-10% RGD-SNPs\(\overset{\rightarrow}{\text{DNA}}\).
In a similar manner of preparing 100-nm DNA-encapsulated SNPs\textsubscript{DNA} and RGD-SNPs\textsubscript{DNA}, CD-PEI (15 µg, 0.84 nmol) Ad-PAMAM (2.4 µg, 0.84 nmol), Ad-PEG (21.0 µg, 4.2 nmol) and DNA (7.5 µg, 3.4 pmol) were mixed to prepare 300-0 SNPs\textsubscript{DNA}. Subsequently, 246 ng (0.042 nmol), 1230 ng (0.21 nmol) and 2460 ng (0.42 nmol) of RGD-PEG-Ad were used to obtain 300-1% RGD-SNPs\textsubscript{DNA}, 300-5% RGD-SNPs\textsubscript{DNA} and 300-10% RGD-SNPs\textsubscript{DNA}, respectively.

4.3. Preparation of 100-nm FITC-SNPs\textsubscript{DNA}

In a similar manner of preparing 100-nm DNA-encapsulated SNPs\textsubscript{DNA} and RGD-SNPs\textsubscript{DNA}, CD-PEI (15 µg, 0.84 nmol) Ad-PAMAM (1.2 µg, 0.42 nmol), Ad-PEG (21.0 µg, 4.2 nmol) and DNA (7.5 µg, 3.4 pmol) were mixed to prepare 100-0 SNPs\textsubscript{DNA}. Subsequently, 248 ng (0.042 nmol), 1240 ng (0.21 nmol) and 2480 ng (0.42 nmol) of FITC-PEG-Ad were used to obtain 100-1% FITC-SNPs\textsubscript{DNA}, 100-5% FITC-SNPs\textsubscript{DNA} and 100-10% FITC-SNPs\textsubscript{DNA}, respectively.

Scheme S5. Preparation of 100-0 SNPs\textsubscript{DNA}, 100-1% FITC-SNPs\textsubscript{DNA}, 100-5% FITC-SNPs\textsubscript{DNA} and 100-10% FITC-SNPs\textsubscript{DNA}. 
4.4. Preparation of 300-nm FITC-SNPs\(\rightarrow\)DNA

Scheme S6. Preparation of 300-0 SNPs\(\rightarrow\)DNA, 300-1% FITC-SNPs\(\rightarrow\)DNA, 300-5% FITC-SNPs\(\rightarrow\)DNA and 300-10% FITC-SNPs\(\rightarrow\)DNA.

In a similar manner of preparing 300-nm DNA-encapsulated SNPs\(\rightarrow\)DNA and RGD-SNPs\(\rightarrow\)DNA, CD-PEI (15 µg, 0.84 nmol) Ad-PAMAM (2.4 µg, 0.84 nmol), Ad-PEG (21.0 µg, 4.2 nmol), DNA (7.5 µg, 3.4 pmol) were mixed to prepare 300-0 SNPs\(\rightarrow\)DNA. Subsequently, 248 ng (0.042 nmol), 1240 ng (0.21 nmol) and 2480 ng (0.42 nmol) of FITC-PEG-Ad were used to obtain 300-1% FITC-SNPs\(\rightarrow\)DNA, 300-5% FITC-SNPs\(\rightarrow\)DNA and 300-10% FITC-SNPs\(\rightarrow\)DNA, respectively.

4.5. Quantification of target ligand coverage on RGD-SNPs\(\rightarrow\)DNA

In order to quantify the RGD target ligand coverage on the RGD-SNPs\(\rightarrow\)DNA, we synthesized a fluorophore (FITC) labeled molecule (FITC-PEG-Ad) as a RGD-PEG-Ad analogue. Different ratios of FITC-PEG-Ad, i.e., 1, 5, 10%
(based on Ad-PEG), were mixed with other building blocks and DNA to form the FITC covered SNPs\(_{\text{DNA}}\) (FITC-SNPs\(_{\text{DNA}}\)) with sizes of 100 and 300-nm. The free FITC-PEG-Ad in the mixture was separated by microcentrifugation (MWCO = 10 kD) with a speed of 11,000 rpm for 30 min, and the FITC-SNPs\(_{\text{DNA}}\) remained in the retentate. The amount of free FITC-PEG-Ad in the mixture and the amount of FITC-PEG-Ad on the FITC-SNPs\(_{\text{DNA}}\) were determined by UV/Vis spectroscopy (Figure S3). Furthermore, the saturated FITC-PEG-Ad coverage on SNPs\(_{\text{DNA}}\) was measured by mixing 100% FITC-PEG-Ad with other building blocks and DNA. The mixing ratios of 1, 5 and 10% FITC-PEG-Ad gave surface coverage as shown in the table below for 100 and 300-nm FITC-SNPs\(_{\text{DNA}}\) (Table S1).

![Figure S3](image)

**Figure S3** The amount of free FITC-PEG-Ad in the mixture and the amount of FITC-PEG-Ad on the (a) 100- and (b) 300-nm FITC-SNPs\(_{\text{DNA}}\) were determined by UV/Vis spectroscopy. Blue and red curves represented the amount of free and bonded FITC-PEG-Ad.
**Table S1** The surface coverage of FITC-SNPs\(\rightarrow\)DNA by mixing of 1, 5 and 10\% FITC-PEG-Ad with other building blocks and DNA were determined by UV/Vis spectroscopy.

<table>
<thead>
<tr>
<th>Mixing ratios of FITC-PEG-Ad (%)</th>
<th>1</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface coverage for 100-nm FITC-SNPs(\rightarrow)DNA (%)</td>
<td>0.8±0.2</td>
<td>2.9±0.5</td>
<td>6.7±0.8</td>
</tr>
<tr>
<td>Surface coverage for 300-nm FITC-SNPs(\rightarrow)DNA (%)</td>
<td>0.9±0.2</td>
<td>2.2±0.4</td>
<td>6.7±0.6</td>
</tr>
</tbody>
</table>

5. **Dynamic light scattering (DLS)**

DLS experiments were performed with a Zetasizer Nano instrument (Malvern Instruments Ltd., United Kingdom) equipped with a 10-mW helium-neon laser (\(\lambda = 632.8\) nm) and thermoelectric temperature controller. Measurements were taken at a 90\(^\circ\) scattering angle. The sizes and the standard derivations of assembled SNPs\(\rightarrow\)DNA and RGD-SNPs\(\rightarrow\)DNA were calculated by averaging the values of at least three measurements (Figure S3).

![DLS measurements](image)

**Figure S4.** Hydrodynamic sizes of 100 and 300-nm SNPs\(\rightarrow\)DNA and RGD-SNPs\(\rightarrow\)DNA in the PBS (pH 7.2) solution measured by DLS. The DLS curves of 100-0 SNPs\(\rightarrow\)DNA and 300-0 SNPs\(\rightarrow\)DNA were shown in (a) and (b), respectively.
6. Transmission electron microscope (TEM)

The morphology and sizes of SNPs\(\rightarrow\)DNA and RGD-SNPs\(\rightarrow\)DNA were directly examined using transmission electron microscope. The studies were carried out on a Philips CM 120 electron microscope, operating at an acceleration voltage of 120 kV. The TEM samples were prepared by drop-coating 2 \(\mu\)L of SNPs\(\rightarrow\)DNA or RGD-SNPs\(\rightarrow\)DNA solutions onto carbon-coated copper grids. Excess amounts of droplets were removed by filter papers after 45 s. Subsequently, the surface-deposited SNPs\(\rightarrow\)DNA or RGD-SNPs\(\rightarrow\)DNA were negatively stained with 2% uranyl acetate for 45 s before the TEM studies (Figure 1). The sizes of SNPs\(\rightarrow\)DNA observed by TEM were 62 ± 8 nm for 100-0 SNPs\(\rightarrow\)DNA and 210 ± 24 nm for 300-0 SNPs\(\rightarrow\)DNA. The reduced sizes obtained by TEM were attributed to the dehydration of SNPs\(\rightarrow\)DNA during the TEM sample preparation.3

7. Zeta potential measurements

Zeta potentials of SNPs\(\rightarrow\)DNA and RGD-SNPs\(\rightarrow\)DNA were determined by photon correlation spectroscopy using a Zetasizer Nano instrument, (Malvern Instruments, Malvern, Worcestershire, UK). The measurements were performed at 25°C with a detection angle of 90°, and the raw data were subsequently correlated to Z average mean size using a cumulative analysis by the Zetasizer software package (Figure S5).
Figure S5. Zeta potential variations of SNPs-DNA and RGD-SNPs-DNA in PBS buffer solution (pH 7.2). Zeta potentials of 100- and 300-nm SNPs without DNA encapsulation were summarized for comparison. Error bars were obtained from three independent measurements.

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8. Cell culture

3T3 and U87 cell lines were routinely maintained in DMEM containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA). MCF7 was cultured in EMEM containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin.

8.1. αvβ3 high-expressed 3T3 cells.

The αvβ3 high-expressed 3T3 cells were generated by passaging normal cultured 3T3 cells using scraping-collection. Cells that were detached via scraping resulted in high densities of αvβ3 integrins on the cell surface. These cells will be referred to as αvβ3 high-expressed 3T3 cells.
8.2. αβ3 low-expressed 3T3 cells.

The αβ3 low-expressed 3T3 cells were generated by passaging the normal cultured cells using 0.25% trypsin. Trypsin, an enzyme that acts to degrade protein, can be used to cleavage the integrins on the cell surface. Cells that were detached using 0.25% trypsin resulted in low densities of αβ3 integrins on the cell surface. These cells will be referred to as αβ3 low-expressed 3T3 cells.

9. Gene transfection studies

Cells (1x10⁴ cells / well) were plated in an 8-well chamber slides and allowed to adhere overnight. EGFP-encoded DNA was diluted in 1x TE buffer. The 100 and 300-nm SNPsDNA for the gene transfection studies were separately prepared. After slowly adding a PBS solution (pH = 7.2) of CD-PEI (600 nM) into a PBS solution containing Ad-PAMAM (300 nM for 100-nm SNPsDNA or 600 nM for 300-nm SNPsDNA), Ad-PEG (3 μM) and DNA (2.2 nM), the resulting mixture was incubated at room temperature for 20 min. The samples of each size of SNPsDNA were split into four aliquots, and three of them were subjected to RGD ligand exchanges by adding 30, 150 or 300 nM of Ad-PEG-RGD separately. The resulting RGD-SNPsDNA were incubated for another 20 min at room temperature. Each SNPsDNA or RGD-SNPsDNA (20 μL) was diluted with 200 μL Opti-MEM medium and, subsequently, transferred to each well. For the control groups, bare DNA (2.2 nM), the complex of CD-PEI (600 nM) with DNA (2.2 nM) and the complex of CD-PEI (600 nM)/Ad-PEG (3 μM) with DNA (2.2 nM) were prepared in PBS buffer (20 μL). Each control sample was diluted with 200 μL Opti-MEM medium and transferred to each well of an 8-well chamber slide (Lab-Tek®, Electron Microscopy Sciences, PA). RGD-jet-PEI was used as a standard transfection reagent and operated according to the protocol provided by the manufacturer. SNPsDNA and RGD-SNPsDNA along with controls were incubated with the cells for 4 hours, then removed by aspirating, and replaced with 400 μL/well of fresh regular
DMEM/EMEM culture medium. Cells were allowed to grow for 24 h at 37°C and 5% CO₂ and then fixed (4% paraformaldehyde for 15 min at room temperature), washed with PBS three times, stained with DAPI and a final PBS rinse prior to EGFP expression analysis by fluorescence microscope.

10. **Microscope settings, imaging processing and data analyzing**

The 8-well chamber slide was mounted onto a Nikon TE2000S inverted fluorescent microscope with a CCD camera (Photomatrix, Cascade II), X-Cite 120 Mercury lamp, automatic stage, and filters for three fluorescent channels (W1 (DAPI), W2 (EGFP and AO) and W3 (PI)). Following image acquisition, MetaMorph (Molecular Devices, Version 7.5.6.0) was used to quantify EGFP expressed cells. The Multi-Wavelength Cell Scoring module of the Metamorph software allows image analysis. A nuclei counting application in the module allows us to calculate the total cell number. In order to determine the gene transfection efficiency, the EGFP-expressed cell number was counted by the MetaMorph program that distinguishes the transfected cells from the non-transfected cells. The fluorescence intensity difference between the regular cultured cells and background around 200~300 is measured as a baseline. The cell fluorescence intensity difference between cells and background above 300 are recognized as the transfected cells. The gene transfection efficiency was obtained by the EGFP-expressed cell number divided by the total cell number.

11. **Cell viability assay**

To determine the number of dead and live cells after transfection, two fluorescent dyes were used. Propidium iodide (PI) is a fluorescent dye that cannot pass through intact cell membranes but readily passes through damaged membranes and binds with DNA. PI fluorescence was bright red when exposed to UV light. The presence of PI in a cell indicates that the cell membrane integrity has been compromised and that the cell is severely damaged. Red fluorescent cells are
judged non-viable. Acridine orange (AO) is a fluorescent dye that readily passes through all cell membranes and stains the cytoplasm and nucleus bright green when exposed to UV light. Green fluorescent cells are judged viable. All dyes were used in accordance with the manufacturer’s directions. First, we added 1 mL of 10× AO solution and 1 mL of 10× PI solution into 8 mL of PBS in a 15 mL tube to make the AO/PI working solution. Then, we added approximately 0.5 mL AO/PI working solution into each well. Using the fluorescence microscope, we took images and evaluated the viability of the cells transfected by SNPs DNA and RGD-SNPs DNA and the cells cultured in the normal medium. There were no significant differences in viability, which suggested that the toxicity of SNPs DNA and RGD-SNPs DNA were negligible for in vitro transfection studies (Figures S6).

![Figure S6](image)

**Figure S6.** (a) Cell viability of RGD-jet-PEI, SNPs DNA and RGD-SNPs DNA transfected 3T3 cells along with non-treated 3T3 cells were determined by cell viability assay after 48 h of transfection. Error bars were obtained from three independent experiments. (b) The fluorescence microscope image of 100-5% RGD-SNPs DNA transfected 3T3 cells after 48 h. The green and red fluorescence expressing cells were depicted as live and dead cells, respectively.
References


