Size effect on transfection and cytotoxicity of nanoscale plasmid DNA/polyethyleneimine complexes for aerosol gene delivery

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Experimental Procedure

Aerosol fabrication: Figure S1 presents a diagram of fabrication of the pDNA/PEI complexes in the aerosol state. Different masses of PEI (408727, Sigma-Aldrich, USA), and a fixed mass (60.23 µg) of pDNA were dissolved in 10 mL of deionized water. The pDNA and PEI were gently mixed by pipetting up and down 5-8 times. Complexes with different pDNA/PEI ratios were incubated for 20 min at room temperature. The pDNA/PEI complexes were finally obtained by collision atomization of the aforementioned PEI pDNA solutions. Briefly the atomized droplets then passed through a cool-walled diffusion dryer operating at -16 °C of wall temperature (performed through a cylindrical peltier cooler) to drive water from the droplets. The condition for complete evaporation was estimated by considering the time required for the evaporation of the droplets and comparing it with the appropriate residence time in the diffusion dryer. The characteristic time to saturate the gas with vapor from the evaporating droplets, $\tau$, is given via the equation,

$$\tau = \frac{1}{2\pi D_d \delta_v C(D_d)}$$  \hspace{1cm} (S1)

where $D_d$ is the diameter of the droplet, $\delta_v$ is the diffusivity of the vapor, and $C(D_d)$ is the droplet number concentration. The $D_d$ value can be estimated via the equation,

$$D_d = \left( \frac{\rho_s w + \rho_p (1 - w)}{\rho_s w} D_p^3 \right)^{1/3}$$  \hspace{1cm} (S2)

where $\rho_s$ and $\rho_p$ are the densities of the solvent and the solid particle, respectively, and $w$ is the weight fraction of solid in liquid.
Aerosol fabrication of pDNA/PEI complexes in this work.

**Cell lines:** Human alveolar epithelial (A549) cells were purchased from the American type culture collection (ATCC). The cell lines were grown in a minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS) at 37 °C in a 5% CO_{2} humidified atmosphere.

**In vitro transfection:** For *in vitro* transfection, A549 cells were seeded in 24-well plates 24 h prior to transfection with a density of 10^5 cells per well. pDNA/PEI complexes corresponding to 1 μg of pCLuc per well were pipetted onto the cells in a serum free medium. Aerosol fabricated complexes were dissolved to a final concentration of 20 μg mL^{-1} and 50 μL applied per well. After 4 h incubation, the transfection medium was replaced with MEM containing 10% FCS supplemented with antibiotics (1% penicillin/streptomycin). After 24 h post-transfection, luciferase activity was measured in a relative light unit (RLU) using a luminometer (TD-20/20, Promega, USA).
In vitro cytotoxicity: Cytotoxicity of the pDNA/PEI complexes was evaluated on A549 cells with different mass ratios between the PEI and the pDNA. Cells were seeded 24 h prior to the experiment with a density of $2.5 \times 10^4$ cells per well into 96-well plates. After 4 h, the transfected mixture was replaced with the medium. And after another 20 h, cell viability was measured using an ATP luminescence detection assay system (ATPlite, PerkinElmer, USA).
FTIR spectra of aerosol fabricated pDNA/PEI complexes. Gel retardation assay of pDNA/PEI complexes including pDNA is also displayed (inset).
TABLE SI. A summary of the size distributions of aerosol fabricated pDNA/PEI complexes including pDNA, PEI I and PEI II cases.

<table>
<thead>
<tr>
<th>Case</th>
<th>GMD (nm)</th>
<th>GSD (−)</th>
<th>TNC (× 10^6 particles cm^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pDNA</td>
<td>38.7</td>
<td>1.62</td>
<td>2.01</td>
</tr>
<tr>
<td>PEI I</td>
<td>51.1</td>
<td>1.63</td>
<td>1.35</td>
</tr>
<tr>
<td>pDNA/PEI I</td>
<td>48.9</td>
<td>1.64</td>
<td>2.03</td>
</tr>
<tr>
<td>PEI II</td>
<td>120.7</td>
<td>1.94</td>
<td>5.80</td>
</tr>
<tr>
<td>pDNA/PEI II</td>
<td>122.7</td>
<td>1.93</td>
<td>5.85</td>
</tr>
</tbody>
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