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# Fabrication of piezoelectric components for a tunable and efficient device for DNA delivery into mammalian cells

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ABSTRACT

We fabricated three piezoelectric components (PZT) that can produce ultrasonic waves with various generated power in order to improve the delivery of DNA molecule and polymer/DNA complexes into cells. Two cationic polymers (PEI and PDMAEMA) were interacted with DNA to form nano-scaled DNA/polymer complexes with/without the help of PZT devices. The application of PZT devices under optimal conditions helped to avoid cytotoxicity and greatly increased the transfection (DNA delivery) efficiency of these complexes in mammalian cells. The cytotoxicity and transfection efficiency were found to be correlated with the PZT-generated power, waveforms and duration of ultrasonic treatment. There was no observable cytotoxicity in our experimental models and, a maximum transfection efficiency 700% greater than that of polymer/DNA complexes without applying ultrasound was achieved. The transfection efficiency of plain polymer/DNA complexes (without PZT treatment) corresponded to a 630-fold increase in comparison to the naked DNA. The waveforms of generated ultrasound greatly influenced the transfection efficiency, while cytotoxicity was not significantly affected. This means that, for optimal DNA delivery, duration of the peak voltage ($V_{max}$/Div) also plays a role. In addition, the generated waves from PZT do not cause dissociation of polymer/DNA complexes or a change in the particle sizes of these complexes. In conclusion, these results suggest that the operation of PZT devices can be a tunable/safe way to greatly improve DNA delivery for gene therapy.



## 1. Introduction

Many human diseases, including metabolic disorders, hereditary diseases [1,2] and cancers [3–5], are gene-related and gene therapy opens new doors for curing such diseases. At present, the use of viruses for transfecting genes into mammalian cells is efficient [6], but concerns regarding cytotoxicity, the recombination of viruses and immune responses limit the use of such transfection agents for therapeutic applications. Non-viral carriers are easily synthesized and are considered relatively safe because of their low host immunogenicity. However, most non-viral carriers (e.g., polycations [7]) lack efficient cellular uptake and result in poor transfection efficiency. Efforts such as structural modification [8,9] and blending of polycations [10] have improved their transfection efficiency, but more has to be done to achieve better performance in gene delivery.

Mammalian cells exposed to ultrasound have been shown to have an increase in membrane permeability [11,12]. Therapeutic ultrasound is potentially useful because ultrasound energy can be transmitted through the body without damaging tissues and could be applied on a restricted area where the desired DNA is to be expressed. Piezoelectric components (PZT) have usually been used in the past as sensors for molecule detection [13,14] or transducers to generate ultrasound [15]. The use of ultrasound in a combination with cationic polymers could greatly improve transfection efficiency and holds great potential for therapy with site specificity [16,17]. However, cytotoxicity or irreversible damage *in vitro* and *in vivo* has been reported due to ultrasound-caused cavitation [18] or the acoustic pressure caused by a high power pulse [19].

In this study, we used three PZT with different frequencies to produce ultrasound with tailored power (watt) by adjusting voltages (volts). The resonant frequency of around 2 MHz (1.2–2.4 MHz in this case) was chosen because it is approved for clinical applications given that it can penetrate through soft tissues and have no influence on DNA integrity [20–22]. Up to this point, settings of ultrasound treatment (i.e., power (watt), frequency of elements and exposure time in using ultrasound to transfect cells) have been used differently in cell studies [23,24,18]. Also, the bioeffects of these setting parameters on transfection efficiency as well as cytotoxicity have not been systematically investigated yet. We used two cationic polymers, poly(ethylenimine) (PEI) and poly(2-dimethylamino-ethyl methacrylate) (PDMAEMA), to interact DNA into nano-scaled complexes before deployment of

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the PZT. The influence of ultrasound on the transfection efficiency and cytotoxicity of COS-7 cells was also evaluated. In addition, variables such as the generated power, waveforms of PZT and duration of ultrasonic treatment were correlated with the *in vitro* performance of polymer/DNA complexes. Then we examined optimal conditions for the synergistic effects of ultrasound and cationic polymers on DNA transfection efficiency.

## 2. Experimental

### 2.1. Materials

We purchased PEI (25 kDa) from Aldrich and a cell proliferation kit II (XTT) from Roche. Luria Broth (LB) medium was purchased from Sigma. The plasmid DNA, pCMVLacZ, containing a LacZ gene preceded by a nuclear localization signal under control of a CMV promoter, was purchased from Sanvertech (Heerhugowaad, The Netherlands). We synthesized PDMAEMA (Mw = 194 kDa) as previously described [25]. The PZT which consist of lead, zirconate and titanate were obtained from Sunnytec, Taiwan (A-PZT, 0.8 mm; B-PZT, 1.1 mm; C-PZT, 5.7 mm).

### 2.2. Preparation and characterization of polymer/DNA complexes

PEI and PDMAEMA (10 mg/mL) were dissolved in 20 mM HEPES buffer (pH 7.4). We added PEI and PDMAEMA to interact with DNA (pCMV-βgal) in the buffer solution at 1/1 and 3/1 weight ratios, respectively, to obtain nano-scaled polymer/DNA complexes. The polymer/DNA complexes were allowed to self-assemble in the buffer at room temperature for 30 min. The final DNA concentration in the PEI/DNA complexes and PDMAEMA/DNA complexes were 2.5 μg/mL and 5 μg/mL, respectively.

### 2.3. PZT device for the generation of ultrasound

The PZT ultrasonic generator was assembled in a circuit module (Fig. 1) and the internal resistance and capacitance, as well as the operation frequency, were measured using an impedance analyzer (Agilent 4294A). The average generated power of PZT elements cam thus be calculated by the following equation.

$$P_{av} = \frac{V \cdot I}{2}\cos\theta = \frac{1}{2}\frac{V^2}{|Z|}\cos\theta$$

where $P_{av}$ is the average generated power (watt) of the PZT elements supplied from a function generator, $V$ is the voltage (peak-to-peak) drop between the two electrodes of the PZT elements, $I$ is the current flowing through the PZT elements, $\theta$ is the phase difference between the voltage and current, and $Z$ is the impedance of the PZT equivalent circuit.

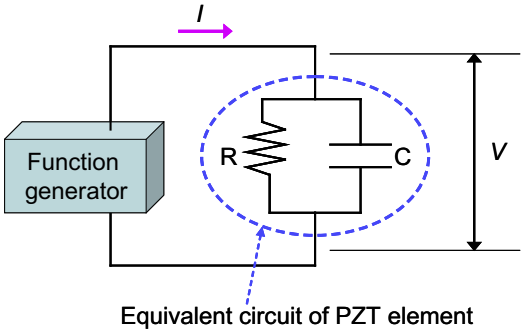


**Fig. 1.** The circuit module of the PZT elements. The generated power of PZT can be calculated by the following equation. $P = V^* I^* \mathrm{Cos}\theta = V^* (V^2/Z)^* \mathrm{Cos}\theta$ where $P$ is the generated power of the PZT elements supplied with a function generator at $V$ (volts); $I$ is the current flowing through the PZT elements, $\theta$ is the phase difference between the $V$ and $I$ in each PZT, and $Z$ is the impedance of the PZT equivalent circuit.

Ultrasound was generated by the PZT elements with a function generator (GRAN FG-101A). In this study, the ultrasound device consisted of three different thicknesses of PZT (*A*, 0.8 mm; *B*, 1.1 mm; *C*, 5.7 mm) which were operated at their harmonic resonance to generate ultrasonic waves with frequencies of 2.462 MHz, 1.726 MHz and 1.254 MHz, respectively (Table 1). By knowing the electric impedances (measured at the operating frequencies 2.462 MHz, 1.726 MHz and 1.254 MHz), the generated power of the PZT devices were calculated at the designated voltages (Table 1). The PZT devices were placed under a 24-well plate and separated with glycerol between the PZT and the plate for ultrasound conduction (Fig. 2).

### 2.4. Stability assay of polycations/ DNA complexes

The stability of polymer/DNA complexes after ultrasonic treatment was examined by agarose gel retardation and dynamic light scattering (DLS). Polymer/DNA complexes were treated with ultrasound for 30 and 60 min. In the agarose gel retardation assay, the polymer/DNA complexes, after ultrasonic treatment, were loaded into a 0.7% agarose gel in a Tris–Acetate-EDTA (TAE) buffer containing ethidium bromide (0.6 μg/mL) and performed at 100 V for 90 min. After electrophoresis, the band was visualized by UV irradiation. The hydrodynamic sizes of the polymer/DNA complexes, after ultrasonic treatment, were determined by dynamic light scattering (Nicomp 380 system, USA) at 25 °C using a 5-mW He–Ne laser ($\lambda$ = 633 nm) as the incident beam at a scattering angle of 90°.

### 2.5. XTT cell viability assay

The cytotoxicity of polymer/DNA complexes under the influence of ultrasonic treatment was evaluated in cell culture using the XTT assay [26]. COS-7 cells were cultured in complete DMEM at a density of $6.0 \times 10^4$ cells/well in a 24-well plate. The cells were incubated in a 5% $CO_2$ incubator for 24 h at 37 °C. Subsequently, the cells were incubated for an additional 1 h in 200 μL FBS-free DMEM containing polymer/DNA complexes together with ultrasonic treatment. Cells incubated in complete DMEM without ultrasonic treatment were used as a negative control. After 1 h, these cells were washed with 1200 μL PBS and replaced by complete DMEM. After 48 h of incubation, 300 μL of a XTT reagent was added to each well and incubated at 37 °C for 1 h. Results were expressed as the relative cell viability (%) with respect to control wells containing cells that were only treated with DMEM.

### 2.6. In vitro transfection protocol and ONPG Assay

In order to examine the expression of pCMV-lacZ gene after transfection, ONPG (o-nitrophenyl-beta-galactopyranoside) assay was conducted to measure the activity of produced beta-galactosi-

**Table 1**
Characteristics of PZT-generated power with tunable supplied voltage.

| Ultrasonic source | A-PZT | B-PZT | C-PZT |
|---|---|---|---|
| Operation frequency | 2.462 MHz | 1.726 MHz | 1.254 MHz |
| Impedance (*Z*) | 17.92 ohm | 24.10 ohm | 48.16 ohm |
| | $\Theta$ = 54.63° | $\Theta$ = 33.92° | $\Theta$ = 9.11° |
| Generated power @ (at supplied voltage) | 2.326 W@ 12 V | 2.482 W@ 12 V | 1.457 W@ 12 V |
| Fixed power @ (by adjusted voltage) | 1.457 W@ 9.4 V | 1.457 W@ 9.2 V | 1.457 W@ 12 V |