Enhanced Gene Delivery Efficiency in Resonant Acoustic Fields

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INTRODUCTION

To achieve the goal of enhancing retroviral transduction with a scalable process, an ultrasonic mediated device was developed. Previously, ultrasound has been applied in the field of gene delivery either using shock waves or in association with microbubbles to create temporary pores on cell membrane [1]. This study, instead, presents the use of resonant acoustic fields (RAF) to overcome the obstacle of cell-DNA vector co-location and investigates its feasibility in augmenting the retroviral transduction and nonviral transfection.

The ability of RAF to drive cells to the sound pressure nodes in non-cavitating, MHz-frequency standing wave fields has been widely studied in recent years [2]. In a suspension of small particles (diameter less than wavelength) subjected to an ultrasound-induced standing wave field, the fate of particles is mainly affected by the primary radiation force and the microstreaming drag. The radiation force shows a cubic dependence on the particle’s diameter [3]. The microstreaming drag force exerted on a particle is linearly proportional to its size [4]. This suggests that larger particles are mainly influenced by the radiation force, whereas the importance of microstreaming drag increases with decreasing particle size. The primary radiation forces drive the suspended cells to the local potential energy minima within the pressure nodal planes, and to give concentration regions that appear as columns of clumps striated at half-wavelength intervals in the direction of ultrasonic waves [5]. However, 100 nanometer-sized DNA vectors do not agglomerate on the nodal planes because the primary radiation force exerted on retroviruses is weaker than the microstreaming drag force, which leads DNA carriers to circulate in the zones between nodal planes. In this study, we hypothesized that when DNA vectors are dragged by microstreaming and close to the region where the cell clusters are pre-formed, they will use the cell bands as the nucleating sites to attach onto. As a result, the probability that encounter opportunity between target cells and DNA vectors is thereby augmented.
EXPERIMENTAL STUDIES

Effect of RAF exposure time on retroviral transduction

The cells used were human erythroleukemia K562 cells (ATCC), which were cultured in a spinner culture flask. A total of 7.0×10^5 cells in the exponential growth phase were collected from the flask. After centrifuging, K562 cells were re-suspended in 70 ml of VSV-G pseudotyped retroviral media (DMEM supplemented with 10% FBS, titers determined for all treatments were in the range of 5×10^5 CFU/ ml). Five milliliters of the cell suspension (5×10^6 cells plus retroviruses) was aliquoted into each of 14 glass chambers, and to half of them 8 μg/ml Polybrene (Sigma) was added. Each glass chamber was then loaded on the transducer smeared with glycerin as coupling agent, and RAF exposure was applied for 0, 1, 3, 5, 7, 9, and 25 min, respectively. For all chambers, bands of high cell concentration were identified at separations of 750 μm. After RAF exposure, cells in each set were transferred into T-flasks and incubated at 37°C for an additional 48 h. Retroviral transduction rates were then measured by detecting eGFP expression using a fluorometric microplate reader (SpectraMax M2 microplate reader, Molecular Devices Corp) with 472-nm excitation and 512-nm emission, respectively.

After additional 48-h incubation, the images of transgene expression were taken by fluorescent microscopy and the mean transduction efficiencies for each time point were roughly estimated from ten. More quantitatively, the intensities of eGFP expression in K562 cells were detected by a fluorometric microplate reader including Polybrene effect. For both methods, the resultant retroviral transduction efficiency increased with exposure duration, peaking at 5 min. For 5-min RAF exposure, the eGFP level increased approximately 3.3-fold as measured by fluorescent microscopy (Figure 1) and 4-fold as detected by fluorescence spectrophotometry (Figure 2) in comparison to one without ultrasonic treatment. In addition, Polybrene adding enhanced the transduction rate about 1.5-fold at 5-min RAF exposure as shown in Figure 2.

### Retroviral transduction using various cell concentrations

Acoustic chambers containing fixed retroviral titer (5×10^6 CFU/mL) and various cell concentrations of 10^5, 10^6, and 10^7 cells/mL respectively were exposed to 1-MHz and 25-Vpp RAF for 5 min. All the experimental operations were performed in the presence of 8 μg/mL Polybrene. After RAF treatment, the operating procedures for three sets were the same as mentioned above within 48 hr. Before quantitatively determining the retroviral transduction efficiency by detecting eGFP expression, three samples were transferred to 12-well culture dishes with the same cell concentration of 5×10^5 cells/mL. The relative fluorescence intensity of eGFP expressed in infected K562 cells was measured by a fluorometric microplate reader after 48 hr of incubation.

As shown in Figure 3, by fixing the titer of retrovirus at 5×10^6 CFU/mL and varying cell concentration, the transduction rate following 5-min ultrasonic exposure was augmented when the cell concentration was increased. With two orders of magnitude increase of cell concentration (from 10^5 to 10^7 cells/mL), the retroviral transduction efficiency revealed approximately 4-fold increments (From 1.64 ± 0.22 RFUs to 6.03 ± 0.25 RFUs) in eGFP transgene expression.

### Nonviral transfection under RAF exposure

After 5-min RAF exposure, cells were cultivated at 37°C for additional 48 h, followed by measuring transfection efficiency based on eGFP transgene expression using fluorescent microscopy and fluorometry. As measured by fluorescent microscopy shown in Figure 4, transfection efficiency with RAF exposure is obviously higher than the one without RAF treatment. The mean percentages of transfection were further calculated according to ten photomicrographic images, and obtained 5% and 57% respectively. Besides fluorescent microscopy, the intensity of eGFP expression in the transfected K562 cells were detected by using a fluorometric microplate reader after additional 48-h incubation, and plotted as Figure 5. The level of eGFP intensity increased approximately 10-fold in comparison to the one without RAF exposure, which was consistent with the analysis from fluorescent microscopy (about 11-fold).

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**Figure 1.** Transgene expression and transduction under different RAF exposure time. Photomicrographical images of K562 cells under inverted light and fluorescent microscopy. These images were represented the mean transduction rates for each group that was one of 10 individual images from triplicate experiments. Note that all sets were added with 8 μg/mL Polybrene. Bar=15 μm
Figure 2 Effect of RAF exposure time on the efficiency of retroviral transduction. Cells were transduced with retroviruses in the absence (□) or in the presence of 8 µg/mL Polybrene (■). RAF exposure was applied for 0, 1, 3, 5, 7, 9, and 20 min, respectively. The retroviral transduction rates were then measured by detecting eGFP expression after 48-h incubation at 37°C. Error bars are the S.E.M. relative fluorescence units (RFUs) obtained from three independent experiments.

Figure 3 Effect of cell concentration on the efficiency of retroviral transduction. Three sets with different cell concentrations: 10⁵, 10⁶, and 10⁷ cells/mL, were exposed to RAF at 1 MHz and 25 Vp-p for 5 min, respectively. The media of all groups were added with 8 µg/mL Polybrene and contained fixed retroviral titer (5×10⁶ CFU/mL). The relative fluorescence intensity of eGFP expressed in infected K562 cells was measured by a fluorometric microplate reader after 48-h incubation. Error bars are the means. S.E.M. relative fluorescence units (RFUs) obtained from three independent experiments.

Figure 4 Photomicrographic images of K562 cells with eGFP expression. K562 cells were transfected with PEI/DNA complexes in the absence (A-a) or presence (B-b) of RAF exposure performed at 1 MHz and 25 Vp-p for 5 min. After additional 48-h incubation at 37°C, images of cells were taken by bright-field (A & B) and fluorescent microscopy (a & b), respectively. The corresponding percentages of eGFP-expressing cells detected by fluorescent microscopy were 4.5% (A-a) and 51.4% (B-b). Scale bar = 15 µm.

Figure 5 Effect of RAF exposure on the nonviral transfection efficiency. K562 cells were transfected with PEI/DNA complexes in the absence or presence of RAF exposure performed at 1 MHz and 25 Vp-p output voltage amplitude for 5 min. The corresponding values of relative fluorescence units (RFUs) for three groups were 0, 1.62, and 15.37, respectively. Error bars are the S.E.M. of RFUs obtained from three independent experiments.
REFERENCES