Journal of Controlled Release 160 (2012) 570–576

Contents lists available at SciVerse ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Low-frequency ac electroporation shows strong frequency dependence and yields comparable transfection results to dc electroporation

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ARTICLE INFO

Article history:
Received 11 January 2012
Accepted 1 April 2012
Available online 10 April 2012

Keywords:
Gene delivery
Electroporation
Alternating current
Direct current
Frequency

ABSTRACT

Conventional electroporation has been conducted by employing short direct current (dc) pulses for delivery of macromolecules such as DNA into cells. The use of alternating current (ac) field for electroporation has mostly been explored in the frequency range of 10 kHz–1 MHz. Based on Schwan equation, it was thought that with low ac frequencies (10 Hz-10 kHz), the transmembrane potential does not vary with the frequency. In this report, we utilized a flow-through electroporation technique that employed continuous 10 Hz-10 kHz ac field (based on either sine waves or square waves) for electroporation of cells with defined duration and intensity. Our results reveal that electropermeabilization becomes weaker with increased frequency in this range. In contrast, transfection efficiency with DNA reaches its maximum at medium frequencies (100–1000 Hz) in the range. We postulate that the relationship between the transfection efficiency and the ac frequency is determined by combined effects from electrophoretic movement of DNA in the ac field, dependence of the DNA/membrane interaction on the ac frequency, and variation of transfection under different electropermeabilization intensities. The fact that ac electroporation in this frequency range yields high efficiency for transfection (up to ~71% for Chinese hamster ovary cells) and permeabilization suggests its potential for gene delivery.

1. Introduction

Electroporation has been demonstrated as an efficient physical tool for delivering genes into mammalian cells [1–4]. Electroporation dramatically and transiently increases the permeability of the cell membrane under the influence of an external electric field [5–7]. Macromolecules such as DNA are able to migrate into cells during the process. The vast majority of electroporation techniques are based on application of short intense pulses of direct current (dc) field (rectangular or exponential decay pulses). Similar dc-based approach has also been miniaturized on microfluidic platforms for cell electroporation or electrofusion [8–22]. In comparison, the reports on experimental work using alternating current (ac) field for electroporation have been scarce with even fewer papers presenting systematic investigations on gene delivery [20,23–26]. Electrofusion of red blood cells and electroporation of eukaryotic cell lines by lifted oscillating fields were conducted and a strong dependence on the field frequency was observed [23,24]. Successful fusion and gene delivery were observed when the field frequency was 40–100 kHz (with 10 kHz to 1 MHz studied). The authors concluded that electroporation based on an oscillating field had superior performance than conventional dc pulse electroporation due to less dramatic polarization and "sonicating effect". In a separate study, electroporation with bipolar square wave (60 kHz) pulses were compared with electroporation done by unipolar and single square pulses [25]. Transfection efficiency was markedly higher with bipolar pulses than with the other two wave forms due to symmetric permeabilization of cells under bipolar conditions. The cell viability after electroporation by either bipolar or unipolar pulses was substantially higher than by single square wave pulses. The authors mentioned that increasing the frequency to 1 MHz decreased the transfection efficiency. There was also a conflicting report that revealed no dependence of electropermeabilization on the ac frequency in the range of 20 kHz–160 kHz when a dye such as calcein was delivered [26].

Schwan equation was proposed to describe transmembrane potential $\Delta\psi_{membr}$ induced by an oscillating ac field [27,28] and has been used to analyze ac electroporation data in the frequency range

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doi:10.1016/j.jconrel.2012.04.006

of 10 kHz–1 MHz (with the assumptions of the cell being a sphere and the plasma membrane being purely dielectric).

$$\Delta\psi_{membr} = \frac{1.5aE\cos\theta}{\left[1+(\omega\tau)^2\right]^{1/2}} \quad (1)$$

where a is the radius of the cell, θ is the angle between the field line and a normal from the center of the cell to a point of interest on the cell membrane, ω is the angular frequency of the external field ($\omega = 2\pi f$ with f being the frequency of the ac field), $E = E^0\sin(\omega t+\phi)$ with E^0 being the amplitude of the external field, t being time and ϕ being the initial phase. The relaxation time $\tau = aC_{membr}\left(\rho_{int}+\frac{\rho_{ext}}{2}\right)$ where C_{membr} is the membrane capacitance per unit area, ρ_{int} and ρ_{ext} are the resistivity of the internal fluid and the external medium, respectively. The equation indicates that the transmembrane potential $\Delta\psi_{membr}$ in general decreases with higher ac frequency, given that the amplitude of the field is kept constant. Furthermore, based on the equation the decline of $\Delta\psi_{membr}$ with the frequency is only pronounced in the range of 10^4–10^7 Hz [28,29]. When the frequency is below 10 kHz, $\Delta\psi_{membr}$ effectively does not change with the frequency, due to the relatively small value of $(\omega\tau)^2$.

There has not been systematic experimental study of electropermeabilization and gene delivery by low-frequency (<10 kHz) ac field in the literature. Based on Eq. (1), low-frequency ac (<10 kHz) produces higher transmembrane potential than high-frequency ac (10 kHz–1 MHz). Furthermore, low-frequency ac (50–60 Hz) is widely used in domestic power systems and may provide drastically simple solution to power need by electroporation. Ac pulses for electroporation have conventionally been generated by triggering an ac generator using discrete dc pulses of several milliseconds or shorter. In this work, we generated electropermeabilization and DNA delivery using low-frequency sine-wave or square-wave ac field of 10 Hz-10 kHz by modulating a constant ac field across a microfluidic channel with alternating wide and narrow sections. Similar to flow-through electroporation based on dc voltage [30–32], the ac field intensity in the narrow section was sufficiently high to generate electroporation while the low field in the wide sections did not disrupt the membrane. Our results indicate that electropermeabilization becomes more intensive when the ac frequency decreases in the range of 10 Hz-10 kHz, arguing against the common belief that $\Delta\psi_{membr}$ does not vary with the ac frequency in this range. DNA transfection efficiency increases first with the ac frequency and reaches the maximum around 100–1000 Hz before decreasing with further increase in the frequency. The transfection efficiency by ac electroporation is up to ~71% for Chinese hamster ovary cells after first-round optimization and this result is very comparable to that of dc electroporation. We provide mechanistic discussion about the factors that affect the relationship between the transfection efficiency and the ac frequency. We believe that low-frequency ac field of 10 Hz-10 kHz offers promising potential for gene delivery.

2. Experimental

The experimental procedures are detailed in the Supplementary content.

3. Results

We applied a microfluidic flow-through electroporation design to create cell electroporation by ac field (sine wave or square wave) of various frequencies (10 Hz-10 kHz) with durations of milliseconds (1–3 ms). Flow-through electroporation based on constant dc voltage was applied to electroporate flowing cells in our previous works [30–32]. As shown in Fig. 1, in this study cells flowed through a microfluidic channel with wide and narrow sections while a constant ac field was established across the channel. Due to the difference in the cross-sectional area between wide and narrow sections, the ac

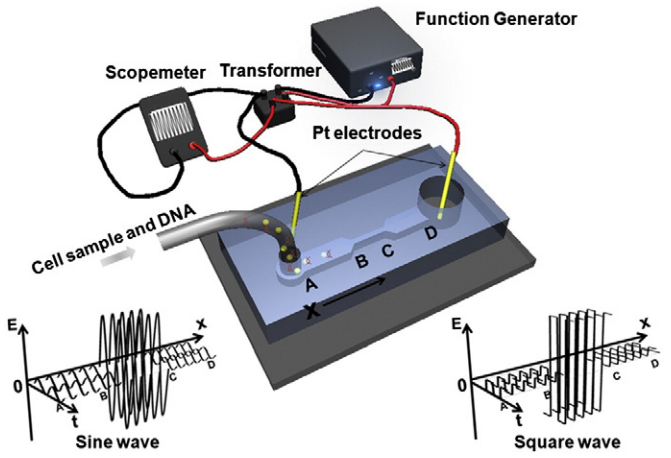

Fig. 1. The layout of the microfluidic flow-through electroporation device based on low-frequency ac field. Cells flow through the channel while a constant ac field is established across the channel. A microfluidic channel with geometric variation (700 μm wide in the wide sections, 70 μm wide in the narrow section, and 60 μm deep) was used to produce electroporation in the narrow section. The inset images show the field variation of ac voltage across the microfluidic channel and over the time in the cases of sine (left) and square (right) waves.

field intensity in a particular section is inversely proportional to its cross-sectional area (thus its width when the channel depth is uniform in the device). The ac field variation with time and along the channel length is indicated in the inset schematics of Fig. 1. In our design, the field intensity in the wide sections was roughly 1/10 of that in the narrow section (with the width of 700 μm for the wide sections and 70 μm for the narrow section). Thus the root mean square (rms) field intensity in the wide sections was substantially lower than the electroporation threshold and the electroporation occurred exclusively in the narrow section. The microfluidic device functioned as a resistor due to the very low capacitance and did not produce any alteration to the ac signal. All electroporation was conducted in an electroporation buffer with low ionic strength (8 mM Na_2HPO_4, 2 mM KH_2PO_4, and 250 mM sucrose, pH = 7.2, ρ = 6.25 Ω m). In here, for the convenience of reference, we refer to the residence time in the narrow section(s) (determined by the section length and cell velocity) as the electroporation duration (strictly speaking, the electroporation duration should be a fraction of the residence time during which the field intensity is higher than the electroporation threshold). The electroporation duration was determined by the dimensions of the narrow section(s) and the flow rate. It is worth noting that with very low frequencies (e.g. <100 Hz), the time period of the ac field (i.e. the time taken by the waveform to complete one cycle) is comparable to or even longer than the electroporation duration. As the result, an individual cell may experience only partial ac wave. The uniformity of the treatment at the single cell level may be affected in this circumstance. However, an electroporated cell population collectively still experiences full characteristics of the waveform due to the fact that cells continuously enter the narrow electroporation section at random initial phases for a period (several minutes) that is much longer than the ac time period.

The expansion in the cell size is a very distinct and visual phenomenon that can be readily observed during or after electroporation, as previously described by us and other groups [30,33–35]. As shown in Fig. 2a, we took a series of snap shots of the same cell flowing into the narrow section under sine-wave ac field of a certain frequency using phase contrast imaging. The cells had bright and well-defined boundaries before entering the narrow section. Upon entering the narrow section with the strong ac field (the rms field intensity at ~773 V/cm) with very low frequencies (27–379 Hz), the cells experienced pronounced increase in their diameters that was also associated with blurring of their outer boundaries. While similar phenomenon