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Transfection of Cells in Suspension Using Sonoporation Method

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ABSTRACT

Sonoporation has an important role in biomedicine, drug delivery and biotechnology. Development of nonviral method would be a valuable alternative for gene therapy. Sonoporation represents nonviral transfection method. This method has been widely used to transfect genetic materials to living cells. Sonoporation creates opportunities for numerous transfections of cells in suspension. This paper describes a sonoporation method based on ultrasound and also presents relevant studies on the sonoporation demonstrating the use of ultrasound to produce transient pores in cell membrane which allows transfection to occur. The paper discusses the design of the sonoporation system based upon the ultrasonic wave and acoustic cavitation. Finally, the paper also presents applications of sonoporation in gene and cell transfection.

Keyword: Sonoporation, transfection of cells, gene

1. Introduction

Sonoporation method is a widely used in biological research. It is increasingly importance due to various biomedical applications involving the delivery of DNA into cells as a transfection technique. Transfection of cells in suspension has been demonstrated by several researchers [1-4] Transfection methods can be divided into two categories – viral and nonviral methods. Cell transfection is generally achieved by three different viral methods: chemical, viral vector and mechanical. For instance, chemically facilitated methods include calcium phosphate and DEAE-dextran. Vector mediated methods are liposome and retroviruses. Mechanical methods are described as follows; for example, particle bombardment (gene gun) and micro injection. Nonviral methods are represented by sonoporation, lipofection, and electroporation and particle bombardment. Viral methods such as using retroviruses and adenovirus as gene vehicles are reported. Viral vectors present some drawbacks such as a lack of site specificity represented. This paper presents the background and literature review of the sonoporation. The first aspect is to study permeability of the membrane via an applied ultrasound and to provide transfection to the cells.

2. Physical mechanisms of Sonoporation

Sonoporation is the use of ultrasound to produce transient pores in a cell membrane. Several groups of researcher [5-8] attempt to investigate a simple way to deliver DNA to cells

for genetic information. It has been shown that sonoporation is able to uptake DNA and permeabilization of plasma membrane. Sonoporation method illustrates that it is more efficient way to delivery DNA when this method compares to other methods such as electroporation, bombardment and microinjection. Permeability is theoretically described as a process very small opening pore formation in the plasma membrane. The primary mechanisms of sonoporation for the uptake of DNA are cavitation and heating. One theory is that the ultrasonic pressure waves induce cavitation bubbles near the cells that collapse, release energy and create transient pores in the cell membrane. Cavitation is induced by a nonthermal collaboration between gas and propagating pressure wave. Typically cavitation phenomena occur at low frequencies (1 – 3 MHz). The mechanical action of the cavitation bubbles is the ability to cause cell lysis and disintegration. This method allows foreign molecules to pass cell wall entrance into the cells. Ultrasound is formed a vibrating oscillating pressure wave. The fundamental frequency of ultrasound is same as audible sound, but frequencies (f*)* are higher than 20 KHz, which is above limits of human audibility. Another advantage of ultrasound that can achieve sonoporation without cavitation is the ultrasonic pressure wave from itself. The term ultrasound applies to sound waves at a high frequency. Most of the previous studies on sonoporation with microbubble have been carried out on cell transfection in suspension. The performance of sonoporation and the transfection efficiency depend upon ultrasound conditions: Ultrasound power output, pressure wave magnitude, frequency, duty cycle ambient temperatures (room temperature or 37 degrees celcius), plasmid concentrations, and initial cell populations. These parameters should be controlled for achieving optimal transfection of cell in suspension.

3. Acoustic cavitation

Cavitation is one of the key phenomena to enhance sonoporation in cell transfection. The cavitation phenomenon is generated by ultrasound. Most recent studies show that the cavitation is able to improve cell transfection. Cavitation is considered as a major mechanism for increasing membrane permeability of biological tissues and cells. The acoustic cavitation is involved to enhance sonoporation. However, the physical mechanisms of sonoporation are not completely understood. The different types of transducer are used to generate vibrations to propagate through the fluid medium in the chamber. Its applications produce pressure wave, and undergoes a compression and rarefaction cycles. Cavitation bubbles are formed and grow when the liquid reaches the significant state of tension. The bubbles formation occurs depend upon the number of acoustic cycle, and where the radius of each bubble varies about an equilibrium value. Therefore, liquid is unable to support sheer stress and is able to support compressive stresses during short periods of tension state. The pressure wave becomes a negative in the liquid during the process rarefaction portion of the cycle. Therefore, the pressure wave of acoustic wave can cause cavitation bubbles to form in the fluid medium, while the negative pressure declines to be below the vapor pressure of the fluid medium. The bubbles oscillate in an unstable manner and expand 2 to 3 times from their resonant size before the bubbles collapsing as shown in figure 1.

The bubble dynamics in the acoustic field is described by the Rayleigh-Plesset equation as equation (1):

$$R = \frac{d^2R}{dt^2} + \frac{3}{2}\left(\frac{dR}{dt}\right)^2 = \frac{1}{\rho}\left[P_i - P_\infty - \frac{2\sigma}{R} - \frac{4\mu}{R}\left(\frac{dR}{dt}\right)\right] (1)$$

Where R is the radius (m) of cavitation bubble, μ is the viscosity of the liquid medium (Ns/m^2), ρ is density of liquid medium, σ is the surface tension (N/m), P_i is the pressure inside the bubble (N/m^2) and P_∞ is the pressure in the liquid far from the bubble (N/m^2). This equation is used to calculate bubbles dynamic in liquid medium.

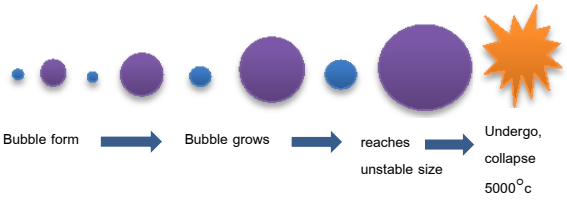

Figure 1 the process of an acoustic bubble [6]

4. Ultrasonic wave for sonoporation system

Ultrasonic wave for sonoporation system is a key role successfully cell transfection. Most researchers show the evidence using of the combination of ultrasonic wave, cavitations and plasmid DNA [6, 7]. The sonoporation system typically consists of a transducer, a power amplifier, a function generator, and a chamber as shown in figure 2. The function of a transducer is to generate an ultrasonic wave. Function generator is used to generate frequency and then amplitude of frequency is amplified using the power amplifier before sending to the transducer. The chamber is typically used to contain cells, plasmid DNA, and liquid medium. Ultrasonic wave is generated by transducer and its frequency should range from 1-3 MHz, in order to achieve high yield of transfection rate and cell permeability [4]. If sonoporation system is used to transfect cells, it is important to optimize the conditions for cell transfection without causing damage to the cells. Recently, Miller et al [2] investigated plasmid DNA (pEGFP-N1) transfer using enhanced sonoporation in monolayer cells (Epidemoid cell A434) in vitro using low power diagnostic transducer (1.5 MHz). Miller et al [3] also carried out an experiment to demonstrate of the plasmid DNA (pGL2) transfection by sonoporation of cultured mammalian cells (Chinese hamster ovary) in a rotating tube system. Their experiments used a fixed ultrasonic frequency (2.25 MHz).

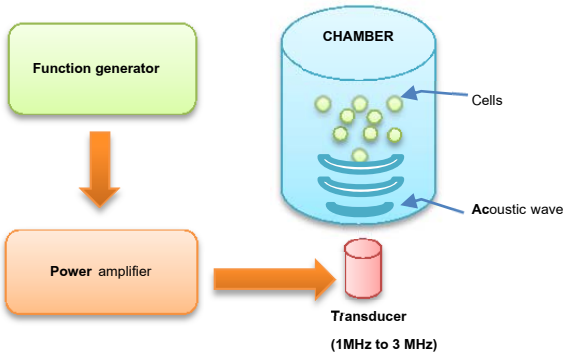

Figure 2 schematic of ultrasonic wave for sonoporation system

Lai et al. [10] studied the use of ultrasound for gene delivery in mammalian systems. This examination qualitatively studied the effect of cavitation induced by 1 MHz pulsed ultrasound wave. Miller et al [2, 20] carried out to demonstrate that mammalian cells were effectively transfected with plasmid DNA in vitro via ultrasound transmitted through the walls of cell culture flasks. This experiment shows that 1-MHz continuous wave can be used to evaluate transfection of cells with plasmid DNA. These researchers successfully showed the evidence of using the combination of plasmid DNA and cavitation to increase both the transfection rate and cell viability. Thus the

ultrasonic frequency from 1 to 3 MHz is appropriately used for the examination of the sonoporation system with cell transfection and plasmid DNA *in vitro*.

5. Gene and Single cells

Gene therapy is potentially beneficial modality that requires effective gene delivery into living cells. The common method for transferring gene into cells is *in vitro* which include sonoporation, electroporation and retrovirus and adenovirus. In order to study cell transfection, a variety of genes is widely used to examine in the process of transfection. Therefore, gene are important resources for cell transfection due to the rapid advances of gene take place in cell transfection and it is possible that therapeutic single cell can enhance cell permeability and deliver drug and nanoparticle. In the current study, the most commonly cell transfection is aimed to use gene to investigate cells permeability and cell viability. Several studies have shown that different types of gene such as the plasmids pCMV-LacZ, pGFPN-1 coding for β-galactosidase and gene fluorescence protein are used to examine cell viability and cell permeability [1,8]. In addition, plasmid DNA has been generally used in research of cancer, protein transfection and drug delivery. Single cell line has also been widely used for investigating cell transfection [1, 12, and 15]. For instance, HeLa cell and 3T3-MDEI cells are single cell lines and they are commonly used to examine cell transfection and viability. For example, HeLa cell and 3T3-MDEI were commonly used in the investigation of cell transfection with gene. HeLa cells represent one of the leading causes of female mortality around the world and proliferate abnormally rapidly even compared to other cancer cells.

6. Application of sonoporation in gene and cell transfection

As we know, there are very difficult to transfect cell because the cell membrane is hard and thick. Sonoporation is a novel method for gene transfer into cells. Figure 3 describes the gene transfer based on sonoporation mechanism. Sonoporation has been reported to mediate gene uptake in cells suspension, plant cells and pieces of tissues [8, 12, and 14]. Gene transfer by sonoporation employs the same simple procedure of non-viral vector. Gene transfection is the process of introducing gene into the cells. Gene transfection is very useful instrument for gene therapy (treatment of cancer cell and hereditary disease) and for creation of genetically modified plants.

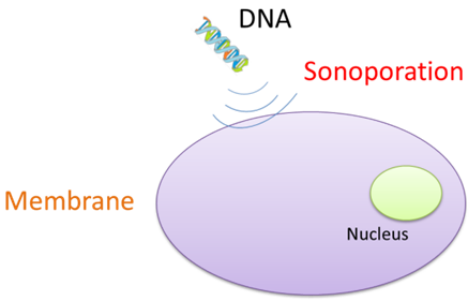

Figure 3 Gene transfer based on sonoporation mechanism

For example, Miller et al studied and examined plasmid DNA (pEGFP-N1) transfer by enhanced sonoporation in monolayer cells (Epidemoid cell A434) *in vitro*. Low power diagnostic ultrasound (1.5 MHz) was used to investigate gene transfer with the contrast agent Optison® [3, 4]. In addition, Chun-Yen Lai et al. [10] found that gene delivery could be introduced into mammalian cells using the ultrasound of 1 MHz pulsed ultrasound wave (pulse duration 1-10 cycles) and the contrast agent Levovist® on the delivery of short DNA-FITC molecule into HeLa cells. This study successfully

confirmed evidence of using the combination of gene delivery and cavitation to raise both the sonoporation efficiency and cell viability. This research obtained sonoporation transfection efficiency of ~28% and 62% cell viability was achieved. Moreover, Joersbo and Brunstedt [8] demonstrated that plasmid DNA could be introduced effectively into sugar beet and tobacco protoplasts using 20 KHz ultrasound at 0.5 W/cm^2 of acoustic power and exposure time from 500 to 900 ms. They found that the optimal transient expression was obtained when plasmid concentrations in the sonication medium are between 40 and 100 mg/l. The results achieved a high transfection (7 to 15 fold) and are higher than the transfection obtained by electroporation [8]. The following table summary applications of sonoporation conditions used in a range of studies and the efficiency results were obtained upon its conditions.

Table 1 Summary of applications sonoporation in gene and cell transfection

Type of Cells and Gene	Sonoporation conditions	Efficiency	Reference:
HeLa cells , pEGFP-N1	980 KHz, 100Vp-p (19.5 MPa) , 5-20 sec.	68.9% transfection efficiency and cell viability 77% at 10 sec.	Rodamporn, S., et al [1]
Neuronal cells	20 KHz,0.5-3 w/cm^2	20% transfection efficiency	Fischer, A.J., et al. [12]
HepG2 cells	0.8 MHz ,1w/cm^2,10% duty cycle	45% transfection efficiency	Guo, D.-P., et al [13]
Kidney, baby hamster kidney cells	1 MHz,20-30% duty cycle, 1-2 w/cm^2	44% increase compare with Therapeutic ultrasound	Duvshani-Eshet et al. [14]
3T3-MDEI cells	1 MHz, 0.5- 3w/cm^2,20% duty cycle probe under Meath well in water bath, 5-80 sec.	15% transfection efficiency increase in a dose-dependent manner.	Chen, Y.-C., et al [15]
DU145	500 KHz,6% duty cycle, 1-60 J/cm	Increase transfection by almost 100 fold in the absence of significant DNA damage.	Zarnitsyn, V.G. & M.R. Prausnitz [16]
Endothelial , Human umbilical vein endothelial (HUVE) ECV 304 cells	0.8 MHz, 1w/cm^2, 10 duty cycle	25% increase transfection rate versus with different treatments plasma DNA, Microbubbles	Dongping GUO, et al. [17]
HL60 and CHO-K1 cells	2.25-MHz continuous ultrasound ,0.4 MPa	19.0 ± 5.5% and 9.6 ± 4.2% (non-necrotic cells)	Miller Douglas and Chunyan Dou [2]
Myoblast,H2K	1MHz,20% duty cycle, 2-3 w/cm^{2,} 5 sec	Cell viability 83%	Liang, H.-D., et al. [18]

Table 1 shows that the most commonly used frequency sonoporation conditions around 1 MHz to be the most effective when used in uptake gene with different cells. In addition, ultrasonic power output can be used from 0.5 to 2w/cm^2. These parameters have been used *in vitro*. Sonoporation will be effective *in vitro*. In addition, the sonoporation conditions have been employed to improve the efficiency of cell transfection. For instance, Pepe, J. [22] reported that the comparison of the efficiency of cell transfection using electroporation and sonoporation. Transfection efficiency was obtained 2.73±0.21% using the sonoporation method; but the electroporation method produced transfection efficiency of 0.43±.06% for human peripheral blood mononuclear. The advantage of sonoporation mechanism introduces more safety for patients or cells such as less immunogenic and inflammatory side effect. Also sonoporation mechanism can be used to carry relatively large DNA sequences and so it is able to increase DNA transfection. Finally, the method can be used to focus on almost any location in the body (*in vivo*). On the other hand, the disadvantage of sonoporation can cause cell damaged depending upon ultrasound duty cycle and power output.

7. Conclusion

Sonoporation has been demonstrated to be a successful mechanism in cell transfection in vitro and in vivo. Sonoporation method gives high transfection efficiency. Many researchers achieved transfection gene uptake into different cell because Sonoporation method has more effective advantages in term of safely, less immunogenic, lack of potential contamination with human pathogens and inflammatory side effects. Recently, sonoporation and contrast agent have been examined to increase the yield of transfection efficiency in animal cells [2,19] and drug delivery. In addition, the transfection efficiency in different cells depend upon exposure duration time, ultrasonic power, intensity which play a key important role in transfection efficiency [12-18]. Therefore, the optimal conditions are also proposed to determine by trial depending upon the sonoporation system, different cells and size of gene. It can be concluded that this method is potentially useful capable of providing significant transfection with cell and gene impact on viability.

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