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Review

# Cell experimental studies on sonoporation: State of the art and remaining problems

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ABSTRACT

Sonoporation is the formation of transient pores on cell membrane by ultrasound exposure. Sonoporation can be applied to the increasing delivery of drug or gene into cells. However, there are some problems encountered in sonoporation studies. The mechanisms to produce sonoporation are very complicated; there are too many experimental parameters affecting the sonoporation results; and there are many bio-effects accompanied with sonoporation. In the article, the cell experimental studies on sonoporation were sorted, including experimental methods, mechanisms to produce sonoporation, correlations between sonoporation experimental parameters and results, and bioeffects accompanied with sonoporation. We'd like to make the concepts about sonoporation clearer. The sonoporation technology may be a promising auxiliary technology to promote drug or gene therapy in the future.



**Contents**



## 1. Introduction

In the past decade, drug and gene delivery systems have attracted much special attention. It was mainly concerned about materials and construction of the delivery systems and the strategies for drug delivery. For examples, the mesoporous materials [1] and their nanoarchitectonics [2], layer-by-layer materials and their self-assemble strategy [3,4], and lipid-based and polymer-based drug delivery vehicles [5,6] have been developed greatly for drug loading, targeting and controlled release.

Furthermore, the stimuli-responsive drug delivery systems to trigger the release of drug or gene molecules under certain stimuli have became another important field of research. A number of physical methods, including ultrasound, mechanical, optical, magnetic, and electric methods, have been used as exogenous stimuli; and some chemical compounds and biomolecules, such as gases, salt, enzymes, glucose, antibodies, lectin, and DNA, have been used as endogenous stimuli to trigger drug delivery and gene transfer [7].

Ultrasound, as one non-invasive stimulus among them, can control drug or gene release from microbubbles and that released drug or gene can be taken up by the cells. Drug or gene can be bond to microbubbles by electrostatic interactions or avidin-biotin linkage, or enveloped in microbubbles [8]. However, it is not a necessary requirement to load drug or gene onto microbubbles; most commonly drug or gene has been added into cell solution separately with microbubbles. Microbubbles can act as the carrying vehicle but, more

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important, microbubbles act as the medium controlled by ultrasound to interact with the cells. Ultrasound-mediated microbubbles create temporary pores on cell membrane to allow permeabilization of the cell membrane and transferring drug or gene into cells. The cell membrane alteration is transient, leaving drug or gene trapped inside the cell after ultrasound exposure. In Newman C.M. et al. [9], they concluded that the mechanics of promoting drug or gene transfer into cells was the formation of short-lived pores on cell membrane lasting a few seconds by ultrasound-mediated microbubble, which also called 'sonoporation'.

At first, there were many studies on ultrasound-mediated drug or gene delivery into cells [10,11]. And then, Bao S. et al. [12] and Greenleaf W.J. et al. [13] have shown the enhanced transfer efficiency of genes into cells with the sonoporation technique. Because sonoporation, unlike other methods of auxiliary technologies, has the capability of promoting drug or gene transfer into cells with the possibility of restricting its effects to desired area and in desired time [14], the studies of sonoporation have been developed rapidly in over ten years. As analyzed above, the studies on sonoporation were mainly focused on the transfer efficiency of drug or gene into the cells and the microbubble's interactions with the cells. However, there are some problems encountered in sonoporation studies. The mechanisms to produce sonoporation are very complicated; there are too many experimental parameters affecting the sonoporation results; and there are many bio-effects accompanied with sonoporation. In our article, to rearrange the progress in cell experimental studies of sonoporation, experimental methods of sonoporation, possible mechanisms to produce sonoporation, correlations between sonoporation results and experimental parameters, and bioeffects accompanied with sonoporation were mainly illustrated.

## 2. Experimental methods for sonoporation

To find the direct evidence of sonoporation, Tachibana K. et al. sonicated the HL-60 cells by ultrasound in the presence of microbubbles. After that, the cells were fixed and the structure of cell surface was studied with the scanning electron microscope (SEM). They first directly observed the sonopores on HL-60 cells in 1999 [15], as seen in Fig. 2.1. Prentice P. et al. [16] directly measured the resultant sonopore by atomic force microscope (AFM). Ross J.P. et al. [17] studied the cell's morphology by optical microscope and AFM, and found that the sonicated cells were somewhat irregular in shape and less uniform in size, and Qin P. et al. [18] found similar results by SEM and differential interference contrast microscopy (DICM). After ultrasound exposure, the sonicated cells can be observed directly with SEM, AFM and Optical microscope. If it needs to be observed in real time, high speed camera connected with long

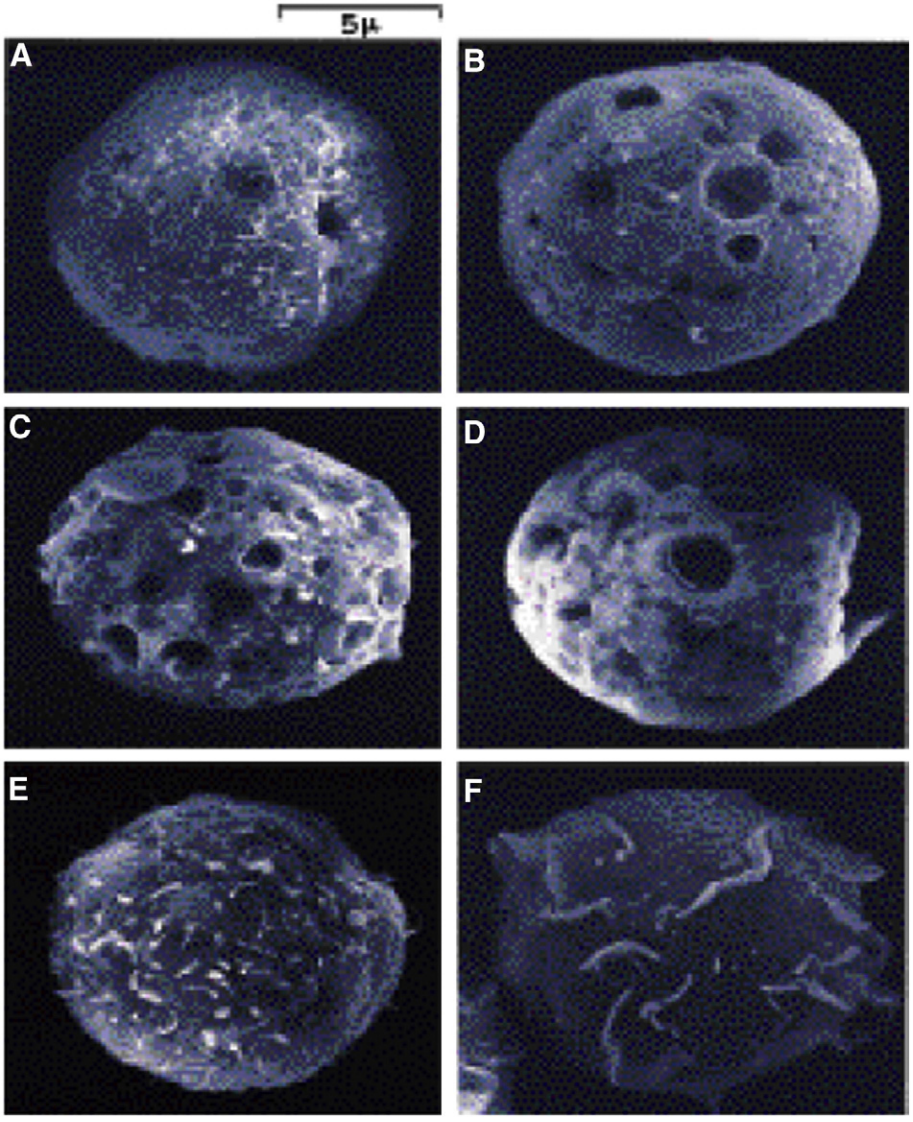


**Fig. 2.1.** Scanning electron microscopic images of HL-60 cells. Irradiated with ultrasound in the presence of MC540 (A to D), untreated intact cells (E), cells irradiated with ultrasound alone (F). Referenced from [15].