

論文の内容の要旨

論文題目 Experimental Study for High Sonoporation Efficiency
(ソノポレーションの高効率化に向けた実験的研究)
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Ultrasound, especially with the appearance of contrast agents, is recently developed as a noninvasive and nonviral targeting delivery method. It's gaining more and more popularity due to non-invasiveness and spatial and temporal control over delivery. Ultrasonic waves can increase cell membrane permeability temporally by inducing transient holes, termed as sonoporation, in the phospholipids bilayer and thus allow the transfer of large molecule into the cell. Thus, in targeted drug delivery system (DDS) and gene delivery research, ultrasound-mediated method is gaining more and more attention. Typical applications include cancer cells killing, anti-cancer drug or gene delivery, blood-brain barrier (BBB) treating and so on. A major issue with sonoporation related research is that the detailed mechanism is yet to be fully elucidated. Acoustic cavitation is nowadays generally believed to play a very important role, which can be significantly enhanced by utilizing ultrasound contrast agents (UCA) since these added microbubbles can serve as cavitation nuclei. But even with UCA an obvious deficiency of ultrasound mediated delivery method is the low sonoporation efficiency.

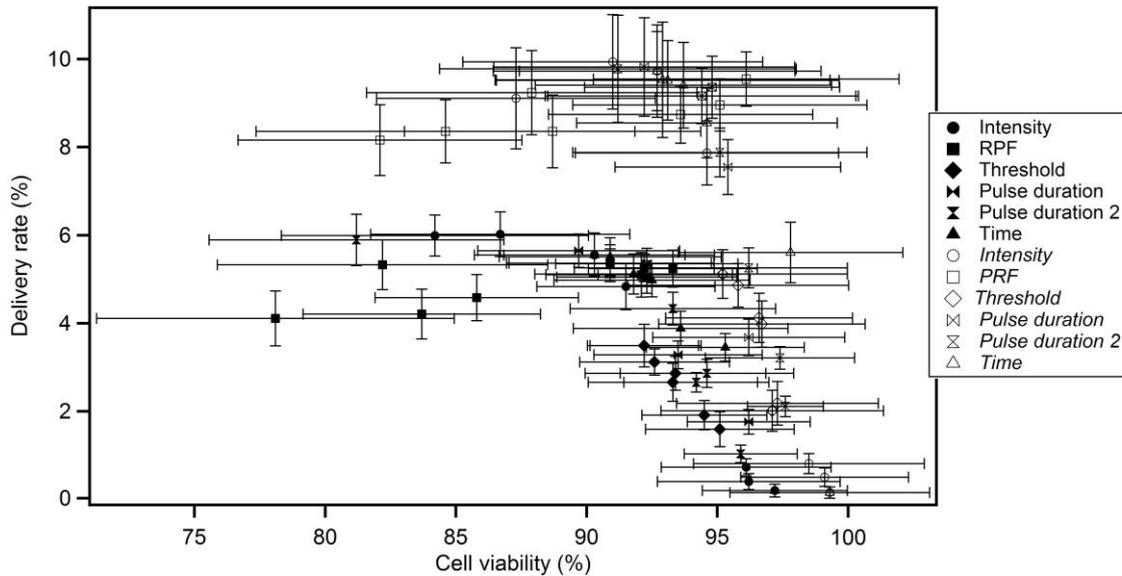
Various parameters are involved in sonoporation, which makes this issue more complicated. Studies have been carried out but lack of detailed analysis is a major problem. The acoustical mechanisms underpinning sonoporation include effects associated with stable microbubble oscillation such as microstreaming, and microbubble disruption by inertial cavitation leading to the generation of shock waves and microjets. Both oscillation and collapse may come from the shelled agents but may also come from the cavities generated in the liquid, which adds complexity to this problem. To obtain detailed understanding and overcome the low efficiency shortage, parameters that influence sonoporation efficiency were studied independently from two sides. One is bubble behavior, including concentration change during irradiation and cavitation noise, which are emitted during bubble oscillating and collapse; the other one is cell behavior, including sonoporation rate and cell viability. Parameters, including intensity (0 to 1.2

MPa), irradiation time (0 to 60 seconds) and burst settings (pulse repetition frequency, 0 to 50 kHz; pulse duration 0 to 0.5 ms) were then tested independently. Methods of improving sonoporation efficiency were then summarized from parametric influence.

The results of bubble behavior are very briefly listed as following. Shelled bubble oscillations appear at low intensities and dominant bubble behavior under 0.3 MPa. Between 0.3 MPa and 0.4 MPa, oscillation and collapse of shelled bubbles coexist. From 0.4 MPa, collapse of shelled bubble dominates and collapse becomes quicker as intensity and pulse duration increase. There is a threshold intensity of 0.34 MPa, where microbubble behavior changes from oscillation dominant to collapse dominant. For shelled bubbles, pulse duration is leading influencing factor rather than rest time when pulse duration is longer than 10 μ s. Pulse duration and PRF also influence cavity behavior. With longer pulse duration, the oscillation of cavity becomes more nonlinear. At high PRFs oscillations of cavities are stronger. Collapses of cavities are obvious at high pulse durations and low PRFs.

The results of cell behavior are briefly listed as following. Sonoporation efficiency increases but cell viability decreases when intensity increases. The difference in increasing between 0.3 MPa and 0.4 MPa is large. The increase of efficiency plateaus from 0.8 MPa but cell viability keeps decreasing. Sonoporation efficiency increases but cell viability decreases when pulse duration increases. As pulse duration increases, sonoporation efficiency increases but cell viability decreases. The cell viability of 500 μ s case is very low. The difference in sonoporation efficiency among different PRF values is small. The cell viability at 50 Hz, 20 kHz, and 50 kHz is lower than other cases. The sonoporation efficiency reaches more than 60% of its final value at 60 s during first 20 seconds. After 20 seconds, the increasing in efficiency is small. But such small increase is more obvious with an unstable emission level.

A summary of all the experiments done on cells is shown in the following figure, including both sonoporation efficiency and cell viability.



The relationship between cavitation behavior and sonoporation efficiency is quite complicated and is briefly listed as following. Weak oscillation of shelled bubbles can cause sonoporation but is very ineffective; stable oscillation of cavity does not sonoporate cells; collapse of both shelled bubbles and cavities are effective in sonoprating cells while the first one is more effective. The self-sealing time of cells is around 10 seconds. With such understanding, one continuing work is refilling of microbubbles. Applying high sonoporation efficiency suggestions to *in vivo* cases can be further work.