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Theoretical study of microbubble dynamics in sonoporation

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ABSTRACT

Sonoporation is a promising technology for promoting the transfer of drug or gene into cells using ultrasound-mediated microbubbles that transiently break up the cell membrane. In this article, a model is established to analyze the dynamics of ultrasound-mediated microbubble near the cell membrane, which may be especially useful for understanding the mechanisms of sonoporation. In the model, the velocity potential of fluid on the microbubble surface and on the cell membrane is obtained by the unsteady Bernoulli equations, and it is solved by using the boundary integral equations. By numerically analyzing the model, the typical microbubble dynamics near the cell membrane are enumerated, which may be mainly governed by mechanical index. The model also established the connections among the parameters of ultrasound exposure, microbubble characteristics, and cell membrane properties in sonoporation.

1. Introduction

It has been demonstrated that ultrasound assisted with microbubbles could open cell membranes, deliver gene or macromolecular drug into cell nuclei and make affected cells transfected through acoustic cavitation. The ultrasound technique associated with this process is called "sonoporation" [1]. Sonoporation has been considered as a transient and reversible phenomenon that can ensure relatively good cell viability [2]. Sonoporation has obvious advantages such as non-invasive and local release, thus it can be a promising assistive technology for future therapy.

Several studies on sonoporation have been carried out *in vitro* and *in vivo* [3–5], and have shown complicated mechanisms contributing to sonoporation. Lentacker et al. [6] categorized the underlying mechanisms according to the microbubble dynamics: in stable cavitation, it can be the direct push and pull action upon cell membrane by the oscillating microbubble attached to cell membrane, and shear stress exerted on cell membrane by microstreaming generated by the nearby oscillating microbubble; in inertial cavitation, this can be the microjet produced by aspherical collapse of the microbubble near cell membrane boundary, and high stresses on cell membrane generated by shock wave due to collapse of microbubble. All of them can break up cell membrane and cause sonoporation. It suggests that the microbubble dynamics play an important role in sonoporation. Hence an appropriate numerical model of the microbubble dynamics can be of great value in sonoporation research, which is what we try to establish in this article.

A number of theoretical models have been proposed for microbubble dynamics. Most of them are based on the Rayleigh–Plesset equation which describes the growth and collapse of a spherical gas bubble. And most of them depict a system including three layers: the gas zone inside the microbubble, the encapsulating shell layer and the surrounding liquid zone. The main difference of existing theoretical models lies in the description of the shell. Some take it as a simple viscous Newtonian liquid layer [7], while others take it as viscoelastic solid layer [8] or rheological layer [9,10]. Tu et al. [11] used light scattering and Buchner Santos et al. [12] used atomic force microscopy to study the shell parameters used in conjunction with one microbubble dynamics model. Both of them achieved the results in agreement with previously published data.

All these models developed above that describe microbubble dynamics in ultrasound field implicitly assume the independent microbubble in an unbounded fluid: however, in sonoporation, the cell membrane nearby will affect the dynamics of microbubble. It has long been known that mechanical interactions between a cavitation bubble and a nearby boundary can induce bubble translation and jetting, where the direction of bubble translation and

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jetting depends on the properties of the boundary [13,14]. Similar studies on the dynamics of microbubble insonified in medical ultrasound applications in vessel have been carried out recently. Qiu et al. [15] analyzed the dynamic behavior of a microbubble within a rigid microtube; Miao et al. [16] simulated the response of an acoustically excited bubble centered within a deformable vessel tube; Hosseinkhah et al. [17] developed a numerical model of a coupled system including bubble, blood and viscoelastic microvessels. However, dynamics of microbubble sonication inside a vessel can be much different than that of inside a cell experimental chamber in *in vitro* experiments or inside an interstitial space in *in vivo* experiments. The surrounding liquid of microbubble in these studies is blood, and its mechanical properties are rather different than that of the interstitial fluid or the cell culture fluid in sonoporation. The blood vessel tubes used in these studies differ from tissue cell membrane in mechanical properties and geometries. Moreover, the finite element method (FEM) is used to construct the model within the vessel limited zone in these studies, which has difficulties when dealing with open boundary in sonoporation.

Therefore, for the first time, we present a boundary element method (BEM) model designed to specifically capture the microbubble dynamics in sonoporation. The typical microbubble's dynamics in sonoporation have been listed in the article. And important factors on microbubble dynamics in sonoporation have been analyzed, such as acoustic frequency, acoustic pressure, characteristics of cell membrane and the distance from microbubble to cell membrane. Some rules of microbubble dynamics in sonoporation were found, which may be useful for optimizing experimental parameters in cell experiments.

2. Model of microbubble dynamics near the cell membrane

2.1. Modeling assumptions

In sonoporation, the sonicated microbubble driven by ultrasound oscillates near the cell membrane to cause the deformation of the cell membrane, and in the meantime the dynamics of microbubble are affected by the cell membrane nearby. To establish the model of microbubble dynamics in sonoporation, here and thereafter, we suppose: (1) The cell membrane is the only boundary near the microbubble and other boundaries are far away from the microbubble, which means the microbubble is in semi-open field. (2) In contrast to the 1–5 μm diameter of microbubble, cell appears to be large enough. Therefore the microbubble is symmetrical about axis perpendicular to the cell membrane wherever the microbubble stays. (3) Whether the cell is floating or adherent, the cell membrane is a viscoelastic interface with fixed extremities and without thickness, and the cell volume is unchangeable. (4) For the reason that the acoustic impedance and viscosity of interstitial fluid or cell culture fluid are very similar to that of water, the flow characteristics of both intercellular fluid and intracellular fluid are Newtonian. (5) The elastic contact problem of the microbubble pressed onto the cell membrane is not included in the model. In this section, the appropriate boundary conditions at the interfaces will be established. Considering the simulation results can be compared with the cell experimental results in future, all following equations were in dimensional form.

2.2. Microbubble

Here and thereafter, the extracellular fluid surround the microbubble is fluid 1; the intracellular fluid is fluid 2; the microbubble shell interface is interface m; and the cell membrane interface is interface c, as seen in Fig. 1. At initial time t_0 of the exposure, microbubble is assumed to be in equilibrium with an initial radius R_0. The fluid pressure at the microbubble interface m, p_m, is assumed to be equal to the sum of uniform adiabatic pressure of the microbubble interior, surface tension, and viscosity throughout the simulation. The unsteady Bernoulli equation as applied to the microbubble surface leads to

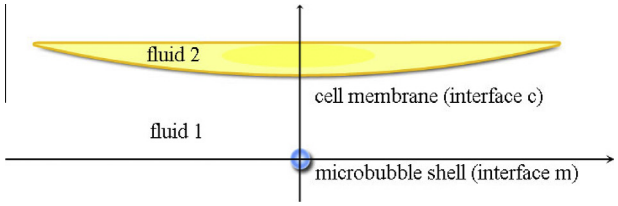

Fig. 1. Schematic representation of the oscillating microbubble near the cell membrane.

$$\rho\frac{D\Phi_m}{Dt}=p_0+p_{ac}-p_{g0}\left(\frac{V_0}{V}\right)^{\gamma}+\frac{2\sigma_m}{R_m}+\frac{4\mu}{R_m}|\vec{v}_m|+\frac{4\kappa_m}{R_m^2}|\vec{v}_m|+\frac{1}{2}\rho|\vec{v}_m|^2 \tag{1.1}$$

$$\sigma_m=\begin{cases}0; & \text{if } R_m\le R_{buckling}\\ 2\chi_m\left(\frac{R_m{}^2}{R_{buckling}^2}-1\right); & \text{if } R_{buckling}\le R_m\le R_{break\text{-}up}\end{cases} \tag{1.2}$$

In Eq. (1.1), the subscript "m" refers to the microbubble surface m. ρ is the fluid 1 density, Φ_m is the velocity potential of fluid 1 on microbubble surface, R_m is the transient radius of the microbubble, $\vec{v}_m$ is the velocity vector of microbubble shell with || being the length of a vector, p_0 is the initial fluid pressure, p_{ac} is the driving acoustic pressure as $p\sin(2\pi ft)$ (where p is the acoustic pressure and f is the acoustic frequency), p_{g0} is the initial pressure of the inert gas in microbubble. The polytropic exponent of the inert gas in microbubble is termed γ. The microbubble volume is termed V, and the initial volume V_0 corresponds to the initial radius R_0. In Eq. (1.1), the surface tension σ_m term and the shell viscosity term is referenced from Marmottant model which is applied for large amplitude oscillations of encapsulated microbubbles [18,19]. Here, μ (Pa s) is the fluid 1 viscosity, κ_m (N/m s) is the surface dilatational viscosity from the microbubble shell. The effective surface tension σ_m is defined as Eq. (1.2), with χ_m (N/m) as elastic modulus of microbubble shell, $R_{buckling}$ as the buckling radius of the microbubble below which the surface buckles, and $R_{break\text{-}up}$ as the upper limit radius which is given by the maximum surface tension. The situation after microbubble broken isn't included in the model. Also, the effects of gravity are ignored considering that the microbubble is small enough. If microbubble is in equilibrium at initial time t_0, p_0 is the same as p_{g0}, and $R_{buckling}$ is microbubble's initial radius R_0.

2.3. Cell membrane

Fluids 1 and 2 are supposed to be inviscid and incompressible, since they are modeled as Newtonian fluid. Velocity potential Φ can be introduced in both fluids, which relate to the velocity vectors as $\vec{v}_1=\nabla\Phi_1$ and $\vec{v}_2=\nabla\Phi_2$. Therefore, the Laplace equation $\Delta\Phi_1=0$and$\Delta\Phi_2=0$ can be applied in both fluids. Here, and thereafter, subscript "1" refers to fluid 1 and subscript "2" refers to fluid 2.

The cell membrane separates fluids 1 and 2. It has elastic properties and will vibrate with microbubble oscillating nearby. The dynamics of fluids 1 and 2 can be related mathematically via two boundary conditions at the cell membrane interface, one is normal velocities and the other is pressure difference across the cell