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Interaction between cavitation microbubble and cell: A simulation of sonoporation using boundary element method (BEM)

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ABSTRACT

Sonoporation has been widely accepted as a significant tool for gene delivery as well as some bio-effects like hemolysis, bringing in high demands of looking into its underlying mechanism. A two-dimensional (2D) boundary element method (BEM) model was developed to investigate microbubble-cell interaction, especially the morphological and mechanical characteristics around the close-to-bubble point (CP) on cell membrane. Based on time evolution analysis of sonoporation, detailed information was extracted from the model for analysis, including volume expansion ratio of the bubble, areal expansion ratio of the cell, jet velocity and CP displacement. Parametric studies were carried out, revealing the influence of different ultrasound parameters (*i.e.*, driving frequency and acoustic pressure) and geometrical configurations (*i.e.*, bubble-cell distance and initial bubble radius). This model could become a powerful tool not only for understanding bubble-cell interactions, but also for optimizing the strategy of sonoporation, such that it could be safer and of higher efficiency for biological and medical studies especially in clinics.

1. Introduction

Encapsulated microbubbles with an insoluble gas core, benefiting from their higher echogenicity than those of the background tissues, were initially used in clinics as contrast agents for ultrasound diagnostic imaging [1,2]. In the past decades, an increasingly important role of microbubbles in therapeutic ultrasound has also been witnessed [3–5], in which bubbles could introduce decreased acoustic cavitation threshold and enhanced ultrasonic bio-effects, attracting much interests on targeted drug delivery, gene transfection, hemolysis as well as high intensity focused ultrasound (HIFU) [3,6–12].

Many existing studies have led to a consensus that the enhancement of ultrasonic bio-effects relies dominantly on microbubble-cell interaction, especially the so-called “sonoporation” phenomenon attributed by microbubble cavitation [13–21]. In sonoporation, bubbles sonicated by ultrasound pulses may grow, oscillate and collapse violently, resulting in short-lived, non-specific pores on cell membranes [22–28]. As was indicated by Lentacker et al., stable cavitation appears at low acoustic pressure, in which oscillation of microbubbles causes microstreaming, ‘pushing’ or ‘pulling’ the membrane of a neighboring cell; while inertial cavitation occurs under relatively high acoustic pressure, in which bubbles experience violent destruction after rapid expansion, generating strong shock waves or micro-jets [13]. The cell membrane could hence be broken during these interaction processes, forming reversible or irreversible micro-pores.

Originating from the promising applications of sonoporation, there emerges high demands in better understanding detailed dynamic process of this complicated procedure, which involves microbubble dynamics, fluid dynamics and responses of cells. Related experimental approaches could include fluorescent microscopy [20,28], monitoring of biological indicators such as the activity of calcium ions [14,19], as well as high-speed capturing of bubble/cell morphology [16,22], *etc*. Quantitative measurements of micro-jet behavior as well as shock wave emissions were realized in 2005 by Brujan et al., who then pointed out that jet formation and shock wave dynamics were the working mechanism of sonoporation [24]. As for theoretical descriptions and simulations, existing bubble dynamic models could only deal with symmetric bubble response. For example, in a theoretical analysis of

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coupling between a gas bubble and a rigid particle, areal expansion of the bubble was determined by its time dependent radius [29]. Two-dimensional (2D) and three-dimensional (3D) numerical models have also been developed to investigate asymmetric vibration of bubbles. Based on the prevalent finite element method (FEM) and boundary element method (BEM), various approaches have been proposed, aiming at studying the behavior of microbubbles situated close to different boundaries. In an early study of Shima et al., non-spherical deformation of a microbubble near a solid wall was reported, while micro-jet was predicted to direct toward the boundary [15]. But recent studies by Chen et al. revealed that, for a bubble localized in a vessel, cavitation induced micro-jets directed away from the vessel wall [16]. Therefore, the interaction between a microbubble and a physical boundary might be distinctively different depending on the property (*e.g.*, elasticity or viscoelasticity) of boundaries.

There have been very limited works on modelling the dynamic interaction between a cavitation bubble and a cell. Forbes and O'Brien, Yu and Chen respectively explained how shear stress due to microstreaming surrounding a bubble would influence cell membrane [30,31]. 2D hybrid models combing FEM and BEM algorithm were developed by Miao et al., studying the interaction between a microbubble and either a rigid/deformable sphere or a micro-vessel [26,27]. They found that, areal expansion of a red blood cell exposed to bubble expansion in response to a 1-MHz, 0.2-MPa ultrasonic pulse was well below the threshold of cell lysis. Their works generally focused on the entire deformation of cell membrane; while a comprehensive description of the sonoporation process should reflect spatially distributed mechanical and morphological characteristics of both the bubble and the cell. Since the major mechanical actions that a cavitation microbubble exerts on a cell's membrane are 'pushing', 'pulling' and shock from the jets, it is of greatest interest if spatial deformation and stress distribution could be examined locally around the membrane's CP point [26,28,32]. After all, formation of micro-porous structure happens exactly around this point. Up to date, there still lacks effective models capable of doing that.

The general purpose of this work is to present a delicate model incorporating the dynamic response of an interacting bubble-cell pair, with which the inherent mechanism of sonoporation could be better revealed. To achieve this purpose, BEM simulations were carried out to examine the temporal evolution of microbubble and cell behaviors, while their responses were investigated in detail by varying ultrasonic parameters as well as geometrical configurations.

2. Model and methods

2.1. Microbubble dynamics

To reduce the complexity of simulations, a free bubble instead of an encapsulated one was adopted. Although dynamic responses of the bubble could be extracted from BEM simulations, its initial state was determined from the well-known Rayleigh-Plesset (RP) equation. For a spherical microbubble sonicated in a flow field, when assuming its oscillating mode to be spherically symmetrical, its dynamic response could be described with [33]

$$\rho_l\left(R_b\ddot{R}_b+\frac{3}{2}\dot{R}_b^2\right)=p_{g,0}\left(\frac{R_{b,0}}{R_b}\right)^{3\kappa}+p_v-\frac{2\sigma}{R_b}-\frac{4\eta\dot{R}_b}{R_b}-p_0-p_{ac}(t), \tag{1}$$

where ρ_l was the density of the surrounding liquid, R_b was the radius of the bubble as a function of time t, with $R_{b,0}$ being its equilibrium value, $\dot{R}_b = dR_b/dt$ and $\ddot{R}_b = d^2R_b/dt^2$ being its first- and second-order time derivatives, respectively. $p_{g,0} = p_0\text{-}2\sigma/R_{b,0} + p_v$ was the initial pressure inside the bubble, while p_0 and p_v were the hydrostatic pressure of the surrounding liquid and the saturated vapor pressure of the gas, respectively. σ was the interfacial tension, κ is the polytropic exponent of the gas core, and η was the dilatational viscosity coefficient of the fluid. $p_{ac}(t)$ was the time-dependent pressure of the exerted ultrasonic field.

For a given free bubble, its initial motion could be estimated through the RP equation according to its initial radius $R_{b,0}$ and ambient pressure p_0. After sonication was turned on, time evolvement of symmetrical or asymmetrical bubble oscillation could be established through BEM simulations, depending on whether the cell was present at its neighborhood or not.

2.2. Description of a cell

For different cell lines, structures and components of cells could be significantly diversified. However, from the acoustical point of view, suspension cells could all be treated as liquid-filled, compressible spheres. Hence, the empirical Tait Equation was chosen as its equation of state,

$$p_c=(p_0+B)\left(\frac{\rho_c}{\rho_{c,0}}\right)^N-B, \tag{2}$$

where p_c was the pressure inside the cell, B and N were temperature related constants which could be validated from experiments. p_0 was the reference pressure inside the cell, identical to the ambient pressure outside the microbubble. ρ_c and $\rho_{c,0}$ were densities of the liquid inside the cell at p_c and p_0, respectively. Given the fact that volume fraction of water in each cell could be as high as 80%, it was reasonable to assume properties of the cell resembled those of water, i.e., B = 330.9 MPa, N = 7.15, $\rho_{c,0} = \rho_l$ = 1000 kg/m^3 [26].

For a cell of time dependent radius R_c and its equilibrium state value $R_{c,0}$, its surface area was denoted as $S_c = 4\pi{R_c}^2$ with its initial value $S_{c,0} = 4\pi{R_{c,0}}^2$. Elasticity of the cell membrane was evaluated with a surface tension T_c [26],

$$T_c=K\frac{S_c-S_{c,0}}{S_{c,0}}, \tag{3}$$

which increased linearly with the cell's areal expansion. In simulations, areal expansion modulus of the cell, K, was chosen as that of the red blood cell, *i.e.*, K = 0.5 N/m [34,35].

2.3. BEM modelling

An interacting pair of microbubble-cell was placed in a liquid environment, which was assumed to be an infinite flow field. At their equilibrium states (*i.e.*, before ultrasound was turned on), both the bubble and the cell were spherical in shape. As is illustrated in Fig. 1a, the initial distance between the two (from bottom of the cell to top of the bubble) was denoted as H_0.

BEM modelling was then achieved with a commercial code package 3DynalFS-BEM (DynaFlow Inc., Jessup, MD, USA), which was designed to simulate 3D fluid flows including highly nonlinear free surface dynamics. Benefiting from the axis-symmetrical configuration of the

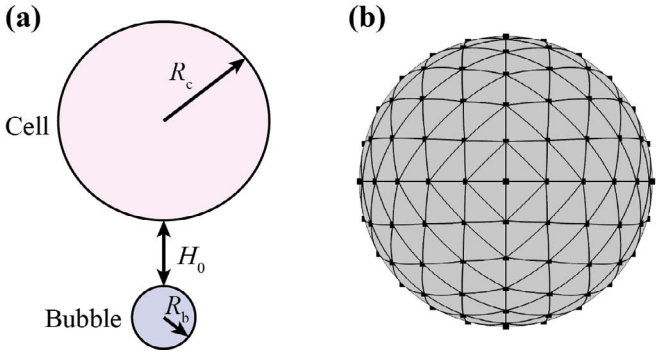

Fig. 1. (a) Cross-sectional view of the model configuration. (b) Discretization of the spherical bubble and cell, surface of each consisted of 250 nodes and 512 triangle units.