Ultrasonics Sonochemistry 21 (2014) 113–120

Contents lists available at SciVerse ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

A transfer efficiency model for ultrasound mediated drug/gene transferring into cells

Hao Yu [a], Liang Xu [b], Siping Chen [c,*]

[a] Biomedical Engineering Department, Shenzhen Polytechnic, Shenzhen, China
[b] Shenzhen Institute for Drug Control, Shenzhen 518060, China
[c] Biomedical Engineering Department, Shenzhen University, Shenzhen, China

ARTICLE INFO

Article history:
Received 20 December 2012
Received in revised form 12 June 2013
Accepted 18 June 2013
Available online 2 July 2013

Keywords:
Ultrasound
Microbubble
Cells
Transfer efficiency
Sonoporation

ABSTRACT

Ultrasound is a very promising technology to mediated drug/gene transferring into cells. However the relations between cell experimental conditions and results have been still unknown. It seriously impeded the development of the technology. In the article, a transfer efficiency model for ultrasound mediated drug/gene transferring into cells in stable cavitation was constructed. To testify the model, the numerical results were compared with the cell experimental data from six literatures in the entirely different experimental conditions. The numerical results fit the cell experimental data well. Despite simplifications and limitations, the model for the first time established the relationship between the cell experimental results about transfer efficiency and the conditions including ultrasound, microbubble and cells in stable cavitation.

1. Introduction

Medical ultrasound is a mature diagnosing technology which has been widely used throughout the world. Nowadays there are so many literatures about ultrasound meditating drug/gene into cells, for the reason that ultrasound has the capability of transferring drug/gene into cells in desired area at desired time non-invasively. Concluded by Newman et al. [1], ultrasound exposure in the presence of microbubbles increases the drug/gene transferring efficiency into cells by the formation of short-lived pores on cell membrane lasting a few seconds which also called 'sonoporation'. The membrane-impermeant drug or gene is transferred passively into cells though the transient pores on cell membrane.

However, there are several important problems seriously impeding the researches on ultrasound mediating drug/gene delivery. The relationship between the experimental parameters and transferring effects is still unknown; the cell experimental conditions are chosen by experiences; and the experimental data can not be compared with each other despite of the similar phenomena. Those are the important reasons why the ultrasound technology can not be further developed for clinical applications. To solving the problems above, a transfer efficiency model was constructed in the article.

In inertial cavitation, microbubble expands and contracts rapidly and then forcibly collapses, accompanied by shock wave and microjet formation. Shock wave and microjet have been suggested to generate sonopores on cell membrane causing drug/gene delivery into cell [2,3]. In fact, it is not an absolute requirement to induce inertial cavitation to achieve sonoporation. In stable cavitation, pulsating microbubble generates acoustic microstreaming and then exerts local shear forces on cell membrane when it closes enough to the cell. It was found that sonoporation also can be caused as the shear forces enough to rupture the cell membrane [4,5]. To simply the problems, only the sonoporation in stable cavitation will be analyzed in the model. In addition, the numerical results were compared with the cell experimental data in several literatures to testify the model.

2. Theory

In a low-intensity acoustic field, the microbubble pulsating nearby a cell can generate microstreaming above the cell membrane and then exert a long period of shear stress on the cell membrane [6,7]. If the microstreaming-shear stress is enough for the cell membrane rupture, the sonopore will be produced by which the intercellular drug/gene is transferred passively into the cell. Therefore to construct the theoretical model of transfer efficiency, first the microstreaming-shear stress exerted on cell membrane, which is generated by a microbubble pulsating near cell, is theoretically analyzed; next, it can be estimated that how far away the

* Corresponding author.
E-mail address: Chensiping@szu.edu.cn (S. Chen).

1350-4177/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ultsonch.2013.06.015

microbubble from the cell will produce sonoporation, if the threshold shear stress for cell membrane rupture is known; finally, if the microbubble's distribution is known, the sonoporation possibility of one cell, namely the percent of transferred cells, can be calculated. The detailed analyzing is as flows.

2.1. A modified microstreaming-shear stress solution

To calculate the microstreaming-shear stress on cell membrane generated by microbubble pulsating near cell, Nyborg's theory [8] was adopted which allows someone to calculate the acoustically-induced steady vortical flow near a fluid–solid interface. Rooney JA [9], Lewin PA [10] and Wu J [11,12] etc. all adopted the theory to estimate the shear stress exerted on cell membrane, however it only can calculate the case of a hemisphere microbubble rest on the cell surface by the theory. For our purpose, here we modified the theory to calculate the microstreaming generated by a microbubble pulsating in the vicinity of a cell.

Because the diameter of cell is 10–100 μm generally [13] much more than the diameter of microbubble about a 1–5 μm, and the Young's modulus of cell membrane is about hundreds or thousands Pa [14,15] while the Young's modulus of phospholipids microbubble is about MPa [16]. Therefore we made some assumptions, including: (1) the distance between microbubble center and cell membrane is fixed as microbubble pulsating; (2) the microbubble pulsation is not affected by the ambient fluid or by the cell membrane, which means the microbubble shell is always spherical motion; (3) as the interaction result of the pressure given by pulsating microbubble with the resistant from cell, the small region S on cell membrane near the pulsating microbubble is supposed to be almost flat. Here, the microstreaming-shear stress generated by microbubble pulsating near cell is shown as Fig. 1.

The irrotational flow velocity distribution in the near-region $\sum$ of the point P is vector U_a, whose amplitude is U. Because of the radial nature of U_a, its components are:

$$\begin{cases} u_{a0} = U\sin\theta; \\ v_{a0} = 0; \\ w_{a0} = U\cos\theta \end{cases} \tag{1.1}$$

For zero-divergence condition of the incompressible flow, the first derivatives of U_a components with respect to x, y and z are:

$$\begin{cases} \frac{\partial u_{a0}}{\partial x}; \\ \frac{\partial v_{a0}}{\partial y} = 0; \\ \frac{\partial w_{a0}}{\partial z} = -\frac{\partial u_{a0}}{\partial x} \end{cases} \tag{1.2}$$

The general streaming solution by Nyborg [8] to be:

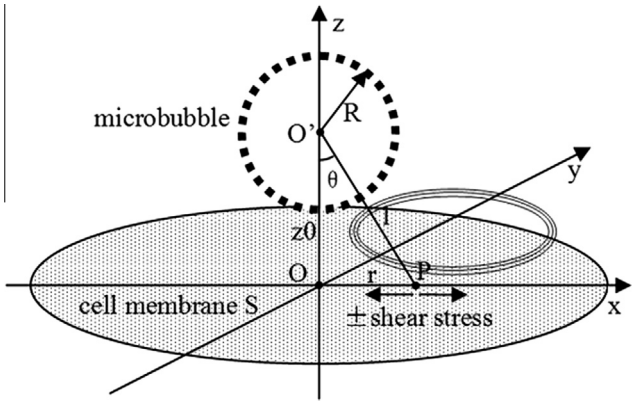

Fig. 1. The schematic of microstreaming-shear stress generated by a pulsating microbubble in vicinity of the cell membrane.

$$u_2 = \frac{1}{\omega}\left(\left(u_{a0}\frac{\partial u_{a0}}{\partial x} + v_{a0}\frac{\partial u_{a0}}{\partial y}\right)u_\alpha + u_{a0}\left(\frac{\partial w_{a0}}{\partial z}u_\beta + (\nabla\cdot U_{a0})u_\gamma - \frac{\partial h_x h_y}{\partial z}w_{a0}u_\delta\right)\right) \tag{1.3}$$

$$\begin{cases} u_\alpha = \frac{1}{4}e^{-2n} + e^{-n}\sin n - \frac{1}{4} \\ u_\beta = \frac{1}{2}ne^{-n}(\cos n - \sin n) - e^{-n}\sin n - \frac{1}{2}e^{-n}\cos n + \frac{1}{2} \\ u_\gamma = \frac{1}{2}e^{-n}(\cos n + \sin n) - \frac{1}{2} \\ u_\delta = \frac{1}{2}(e^{-n}\cos n - 1) \end{cases}$$

Here u_2 is steady vortical flow velocity. the $\nabla\cdot U_{a0}$, the divergence of flow vector U_{a0}, almost equal to zero when do not take into account the energy loss caused by friction. The space derivatives $h_x = ds/dx$ and $h_y = ds/dy$. It is obviously that h_x and h_y are just equal to 1, and the derivative of $h_x h_y$ with respect to z is zero, when the small region S is supposed to almost flat. n is defined as z/δ. z is the vertical distance inward from the point P, and $\delta = (\mu/\pi\rho f)$, which is an important parameter with physical significance called boundary layer thickness. Here, μ is the ambient fluid dynamic viscosity, ρ is the ambient fluid density and f is acoustic frequency.

From above, we can simplify the streaming solution in our situation as:

$$u_2 = \frac{1}{\omega}\left(u_{a0}\frac{\partial u_{a0}}{\partial x}(u_\alpha - u_\beta)\right) \tag{1.4}$$

The shear stress S_P on point P associated with the streaming u_2 gradient is:

$$S_P = \mu\frac{du_2}{dz}\Big|_{z=0} = \frac{1}{4}\rho\delta u_{a0}\frac{\partial u_{a0}}{\partial x} \tag{1.5}$$

In our situation, the radial velocity vector U_{a0}'s amplitude U can be simplified as:

$$U = \frac{2\pi R^2\dot{R}}{2\pi l^2} = \frac{R^2\dot{R}}{l^2} \tag{1.6}$$

Therefore we can get:

$$u_{a0} = U\sin\theta = \frac{R^2\dot{R}}{l^2}\sin\theta;$$

$$\frac{\partial u_{a0}}{\partial x} = \frac{dU}{dR} = \frac{\sin\theta}{l^2}\left(2R\dot{R} + R^2\frac{\ddot{R}}{\dot{R}}\right);$$

$$\textit{and then } S_P = \frac{1}{4}\rho\delta u_{a0}\frac{\partial u_{a0}}{\partial x} = \frac{1}{4}\rho\delta\frac{\sin^2\theta}{l^4}(2R^3\dot{R}^2 + R^4\ddot{R}) \tag{1.7}$$

Where R, $\dot{R}$, and $\ddot{R}$ represent the radius, radial velocity, and radial acceleration of the microbubble shell.

For the S_P solution, it is obviously that S_P is associated with r which is the distance between the point P and origin O. Here, we only consider the maximum microstreaming-shear stress on the cell membrane. S_P will be the maximum value when $dS_P/dr = 0$, therefore we can get:

$$S_P = \frac{1}{27}\rho\delta\frac{1}{z_0^4}(2R^3\dot{R}^2 + R^4\ddot{R}),\ \text{when } r = \frac{1}{\sqrt{2}}z_0 \tag{1.8}$$

From Fig. 1, z_0 is the distance from microbubble center to the cell membrane. By the shear stress solution 1.8 above, the maximum microstreaming-shear stress exerted on the cell membrane can be calculated, which is generated by microbubble pulsating in the immediate vicinity of, or attached to, the cell membrane.

In the model, Marmottant model is adopted to calculate the shell movement of microbubbles in large amplitude pulsating [17,18] as: