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Jetting does not cause sonoporation

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Abstract

Ultrasound contrast agents consist of encapsulated bubbles in the micrometer size range. At low acoustic amplitudes these microbubbles pulsate linearly, but at high amplitudes they demonstrate highly nonlinear, destructive behaviour. Cellular drug uptake and lysis are increased under sonication, and even more so when a contrast agent is present, owing to the formation of transient porosities in the cell membrane (sonoporation). An overview is given of the physical mechanisms of microbubble behaviour. There are two hypotheses for explaining the sonoporation phenomenon, the first being bubble oscillations near a cell membrane, the second being bubble jetting through the cell membrane. Based on modelling, photography, and cellular uptake measurements, it is concluded that bubble jetting behaviour is unlikely to be the dominant sonoporation mechanism.

1 Introduction

It has been proven by numerous groups, that the cellular uptake of drugs and genes is increased, when the region of interest is under sonication, and even more so when a contrast agent is present. This increased uptake has been attributed to the formation of transient porosities in the cell membrane, which are big enough for the transport of drugs into the cell. The transient permeabilisation and resealing of a cell membrane is called sonoporation. The sonoporation-induced cellular uptake of markers with molecular weights between 10 kDa and 3 MDa has been reported in several studies. Ultrasound-induced cavitation facilitated cellular uptake of macromolecules with diameters up to 56 nm [1]. Even solid spheres with a 100-nm diameter have been successfully delivered aided by sonoporation, implying that drug size is not a limiting factor for intracellular delivery [2]. However, the pore opening times can be so short, that, if the drug is to be effectively internalised, it should be released close to the cell membrane when poration occurs [3]. There are two hypotheses for explaining the sonoporation phenomenon, the first being microbubble oscillations near a cell membrane, the second being microbubble jetting through the cell membrane. Without the presence of an agent, it has been assumed, that sonoporation is caused by bubbles, which have been generated in the transducer focus as a result of inertial cavitation.

2 Theory

The spherically symmetric oscillating behaviour of ultrasound contrast agent microbubbles under low-amplitude sonication has been described with models based on the Rayleigh-Plesset equation, modified for the presence of an encapsulating shell. To give an indication of the vast amount of existing models: Qin et al. defined 16 separate dynamic bubble model classes [4]. The reason for the high number of existing models is the fact that most physical properties of encapsulated microbubbles cannot actually be measured, so that pseudo-material properties have to be chosen when predicting ultrasound contrast agent microbubble behaviour. Examples of such pseudo-material properties are shell elasticity parameters and shell friction parameters.

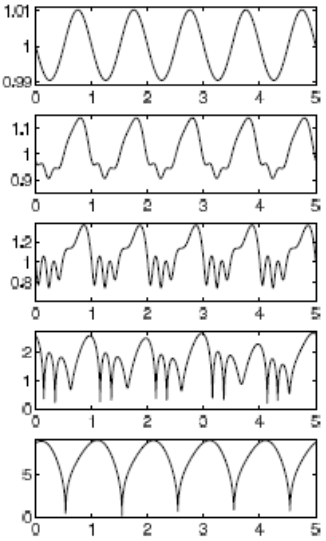

Figure 1: Simulated normalised radius as a function of cycle number of microbubbles with a 4.5 µm equilibrium diameter, subjected to a 0.5 MHz driving signal with acoustic amplitudes corresponding to (*top–bottom*) MI=0.01, 0.10, 0.18, 0.35, and 0.80, similar to the sonoporation experiments in [2].

At low-amplitude driving pressures, ultrasound contrast agent microbubbles oscillate linearly, but at high-amplitude driving pressures, they oscillate nonlinearly. **Figure 1** demonstrates the oscillation behaviour of a contrast microbubble slightly bigger than resonant size, subjected to continuous sine pressure waves with low, moder-

ate, and high amplitudes. It oscillates linearly at MI=0.01. With increasing driving amplitude, asymmetries in radial excursion and expansion time rise. At MI=0.8, both bubbles expand to a factor of the initial size, followed by a rapid collapse for the smaller bubble. The bubble demonstrates collapses at MI=0.18 and higher. Other types of nonlinear behaviour than asymmetric oscillations have been stated in **Table 1**. Of influence on the occurrence of these phenomena are a) the ultrasonic parameters: transmit frequency, acoustic amplitude, pulse length, pulse repetition rate and transmit phase; b) the ultrasound contrast agent composition: the composition of the shell, the bubble sizes, the size distribution and the gas; c) the physical properties of the medium: viscosity, surface tension, saturation. If a bubble with a negligible shell collapses near a free or a solid boundary, the retardation of the liquid near the boundary may cause a bubble asymmetry. This asymmetry causes differences in acceleration on the bubble surface. During further collapse, a funnel-shaped jet may protrude through the bubble, shooting liquid to the boundary. Such jets have been observed in high-speed observations of ultrasound contrast agent microbubbles. Empirical relations exist between the collapsing bubble radius, the jet length, and the pressure at the tip of jets. It has been speculated, whether microbubble jetting can be applied for ultrasound-guided drug delivery.

3 What causes sonoporation?

It was demonstrated that moderate microbubble oscillations are sufficient to achieve rupture of lipid membranes, in a regimen in which the bubble dynamics can be accurately controlled [5]. However, it was also computed that the pressure at the tip of the jet through a contrast microbubble is high enough to rupture any human cell membrane [6]. Several high-speed image sequences reveal jetting through cells [7], which, however, might be attributed to a solid substratum beneath the cell culture. Even in a controlled experimental environment, contrast jetting is quite rare. Other sequences demonstrate more subtle movements of the cell membrane as a result of microbubble oscillations, in combination with an improved cellular uptake. Karshafian *et al*. found, that contrast microbubbles sonicated near a cell at any acoustic pressure used cause large pores (300–500 nm), whereas microbubbles sonicated at high acoustic pressures cause smaller pores (20–500 nm), too, in more cells [2]. Pores on the order of 10–100 nm were observed with two different methods by [3], with pores opening lasting only milliseconds to seconds. The two contrast agents used in [2] have mean diameters 1.1–3.3 and 2.0–4.5 µm, respectively. The upper limit of these diameters has been modelled and shown in **Figure 1**. A conservative model had been chosen, and a conservative estimate of the shell stiffness had been used. Still, using the empirical jetting relations, the maximal expansions computed would be high enough to create pores of more than 1 µm. In order to create pores of 20 nm by jetting, the contrast microbubble diameter at the verge of collapse should be approximately 0.2 µm. For the agents chosen, this is a highly unrealistic value. These findings indicate that microbubble jetting behaviour does not play an important role in sonoporation. The influence of microbubble disruption, *i.e.*, fragmentation or sonic cracking, on sonoporation will have to be further investigated.

Phenomenon	Schematic representation	Microbubble classification	Acoustic regime
Translation		I, II, III, IV	L, M, H
Fragmentation		I, II	L, M, H
Coalescence		I, II	L, M, H
Jetting		I, II	H
Clustering		II, III	L, M, H
Cracking		II, III, IV	L, M, H

Table 1: Nonlinear phenomena and their occurrence regimes. Microbubble shell classes: I) free or released gas; II) thin shells <10 nm; III) thick shells <500 nm; IV) very thick shells >500 nm. Acoustic regimes: low (L) for MI<0.3; medium (M) for 0.3<MI<0.7; high (H) for MI>0.7.

4 Literature

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