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Understanding ultrasound induced sonoporation: Definitions and underlying mechanisms☆

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ABSTRACT

In the past two decades, research has underlined the potential of ultrasound and microbubbles to enhance drug delivery. However, there is less consensus on the biophysical and biological mechanisms leading to this enhanced delivery. Sonoporation, i.e. the formation of temporary pores in the cell membrane, as well as enhanced endocytosis is reported. Because of the variety of ultrasound settings used and corresponding microbubble behavior, a clear overview is missing. Therefore, in this review, the mechanisms contributing to sonoporation are categorized according to three ultrasound settings: i) low intensity ultrasound leading to stable cavitation of microbubbles, ii) high intensity ultrasound leading to inertial cavitation with microbubble collapse, and iii) ultrasound application in the absence of microbubbles. Using low intensity ultrasound, the endocytotic uptake of several drugs could be stimulated, while short but intense ultrasound pulses can be applied to induce pore formation and the direct cytoplasmic uptake of drugs. Ultrasound intensities may be adapted to create pore sizes correlating with drug size. Small molecules are able to diffuse passively through small pores created by low intensity ultrasound treatment. However, delivery of larger drugs such as nanoparticles and gene complexes, will require higher ultrasound intensities in order to allow direct cytoplasmic entry.

Contents

Abbreviations: AFM, Atomic Force Microscopy; FITC, Fluorescein isothiocyanate; IC, Inertial Cavitation; PI, Propidium Iodide; PNP, Peak Negative Pressure; PRF, Pulse Repetition Frequency; ROS, Reactive Oxygen Species; SEM, Scanning Electron Microscopy; TEM, Transmission Electron Microscopy; TMC, transmembrane current.

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1. Introduction

Starting from the mid 90's several papers were published showing that ultrasound can facilitate the transport of membrane impermeable compounds into living cells. This includes several reports showing the ultrasound induced uptake of low molecular weight drugs, genetic drugs (pDNA, siRNA, mRNA), peptides and proteins [1–13]. In general, the uptake of these drugs or model-drugs is attributed to ultrasound mediated transient permeabilization of the cell membrane.

The first studies on ultrasound induced cell permeabilization introduced the term "sonoporation" to describe the temporal cell membrane openings that can arise after ultrasound exposure [12,14,15]. Several research papers investigating sonoporation reported the use of microbubbles to amplify the biophysical effects of ultrasound. These microbubbles are gas-filled structures stabilized by a lipid, protein or polymer shell and some of them have been clinically approved as ultrasound contrast agents [16,17]. Due to their gas-filled, and hence compressible core they can respond to the ultrasound pressure waves. This process of alternate growing and shrinking is called cavitation and can be divided into (i) stable cavitation, mainly occurring at lower ultrasound intensities and (ii) inertial cavitation, occurring at higher ultrasound intensities (Fig. 1). The latter cavitation event may finally lead to microbubble implosion which will result in much stronger biophysical effects.

Although several in depth reports have been published, it remains extremely difficult to quantitatively characterize the effects of different physiologic processes contributing to ultrasound induced drug uptake. This is mainly due to the plethora of different ultrasound settings and methods used to study sonoporation. For this reason we have defined the following three main ultrasound conditions: i) low intensity ultrasound leading to stable cavitation of microbubbles, ii) high intensity ultrasound leading to inertial cavitation with bubble collapse, and iii) ultrasound application in the absence of microbubbles. In this review, we give an overview of the state-of-the-art knowledge on ultrasound induced biophysical effects for each condition and the related physiological reactions of the sonicated tissue. It is important to note that the interaction of ultrasound with tissue can induce (i) mechanical effects, (ii) chemical effects and (iii) thermal effects, depending on the ultrasound setting, which in turn can lead to several bio-effects. We will limit the scope of this review to the mechanical and chemical aspects of ultrasound induced drug delivery. However, it cannot be ruled out that thermal mechanisms are contributing as well. In this regard, it is indeed important to mention that any temperature increase, provoked by ultrasound exposure, could change the physicochemical state of the cell membranes and could render them more sensitive to membrane deformation. Besides the sonoporation mechanisms, recent literature suggests that other mechanisms like endocytosis might be involved as well in ultrasound triggered drug delivery. Therefore, we focused in the last paragraph on recent contributions to elucidate the role of endocytosis in ultrasound triggered drug delivery.

To the best of our knowledge, this is the first extensive review categorizing and discussing the different cellular mechanisms which have been reported to contribute to ultrasound enhanced drug internalization. We believe that the understanding of sonoporation mechanisms and their relation to different biophysical processes are crucial steps to optimize and fully explore ultrasound induced drug delivery.

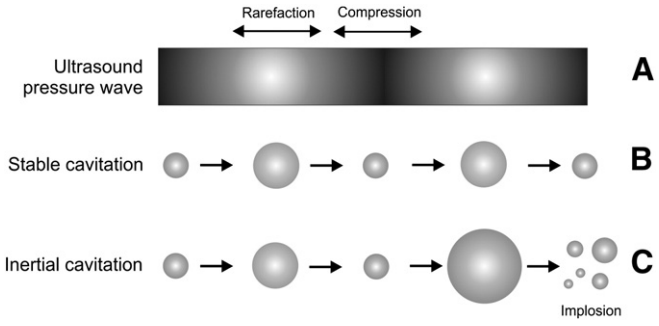

Fig. 1. Stable and inertial cavitation. (A) Schematic representation of an acoustic pressure wave. (B) and (C) show, respectively, stable and inertial cavitation of microbubbles. Adapted from reference [118] with permission from the Royal Society of Chemistry.

2. Mechanisms contributing to ultrasound induced sonoporation

2.1. Cell membrane permeabilization by stably cavitating microbubbles

2.1.1. Biophysical aspects of stable cavitation

At very low acoustic pressures, microbubbles oscillate in a symmetrical, linear way. This means that their expansion and compression is inversely proportional to the local ultrasound pressure [18]. At higher ultrasound intensities, microbubbles behave non-linearly with a lengthening of the expansion phase of the microbubbles, as the microbubbles are more resistant to compression than to expansion [16,19]. This phenomenon is also known as stable cavitation or non-inertial cavitation. During stable cavitation of the microbubble, there is gas influx (during expansion) and gas efflux (during compression). In the case of symmetrical oscillations, the netto gas influx over one expansion/compression cycle is zero. However, when the expansion phase extends, there is a net gas influx into the microbubble. For this reason, the microbubble grows until it reaches its resonant size, whereupon it demonstrates stable, low amplitude oscillation (Fig. 1). Such stable oscillations create a liquid flow around the microbubbles, the so-called microstreams [20] (Fig. 2). When these oscillating microbubbles are in close vicinity of cells, these cells will experience shear stress. The level of shear stress is largely dependent on the ultrasound parameters and can, according to simulations, range between 100 Pa and 1000 Pa [21]. The shear stress related to micro streaming is relatively high compared to the shear stress associated with blood flow (0.1–4 Pa) [22]. Consequently, these US induced elevated shear stress levels may induce a large spectrum of biological effects [23,24].

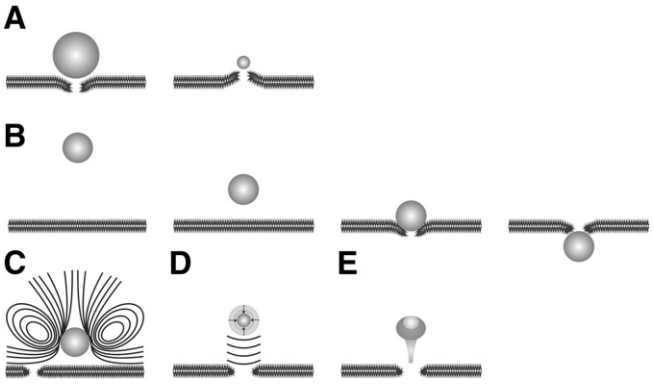

Fig. 2. Biophysical effects of stably and inertial cavitating microbubbles. (A), (B) and (C) refer to biophysical effects caused by stable cavitation, while (D) and (E) depict effects of inertial cavitation. (A) Pushing (left) and pulling (right) effects during the expansion and compression phase, respectively, of a stably oscillating microbubble, thereby disturbing the membrane integrity. (B) Acoustic radiation force causes microbubble displacement and compresses the microbubble against the cell membrane resulting in membrane disruption. The microbubble may even be pushed through the lipid bilayer to enter the cell. (C) Stable oscillation of a microbubble creates microstreamings in the surrounding fluid, which exert mechanical stress on the cell membrane, causing pore formation. (D) Shock waves produced by microbubble collapse generate high stresses on cell membranes, which results in membrane disruption. (E) When a microbubble collapses near a surface, the collapse is asymmetrical, leading to the formation of a liquid jet towards the surface. This microjet punctures the cell membrane, thereby creating a pore. Adapted from reference [46] with permission from Elsevier.