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Ultrasound contrast agents pushing drug delivery: high speed optical observations

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Ultrasound-activated-microbubbles can cause transient non-lethal permeabilization of cells. Studies of influx of cell membrane impermeable-dye, genetic materials, and nanoparticles have confirmed that the action of ultrasound-microbubbles on the cell membrane is to alter both the endothelial cell and vascular permeability. The permeabilizating actions of ultrasound contrast agent on cells have been optically recorded using a microscope and a high-speed camera. Micro-mechanical forces generated by the oscillating microbubble may also be used to locally 'push' the drugs into the cells and tissues. Ultrasound-activated-microbubbles are able to transiently increase the vascular-endothelial-layer permeability. These features may be used for future ultrasound-guided drug delivery systems. Next to increasing the drugs internalization, microbubbles can also be used as drug/particle carrier. Drug loaded contrast agents can be intentionally ruptured by diagnostic ultrasound. Using microbubbles to carry drugs to targets sites and rupturing the microbubbles by localized ultrasound energy, will result in a high local concentration of drugs.

1 Introduction

Ultrasound contrast agents consist of liquid containing encapsulated gas microbubbles. The size varies typically from 0.5 to 6 µm. De gas core can be air or a high molecular weight gas like perfluorobutane. A thin shell, that prevents the gas core from dissolving quickly, encapsulates the gas core and can consist of a phospholipids monolayer, albumin or polymers (Fig1).

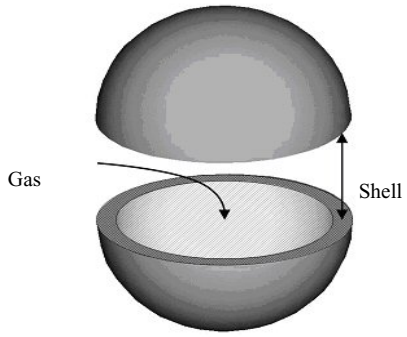

Fig. 1. Schematic representation of an ultrasound contrast microbubble. Adapted from Emmer et al. [1]

2 Microbubble-cell interaction

2.1 Brandaris recording

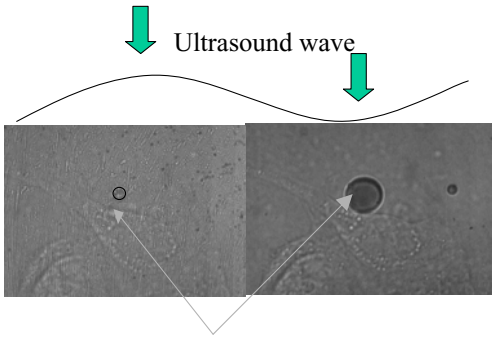

Fig. 2. Oscillating microbubble in an ultrasound field pushing against an endothelial cell. In the first frame the ultrasound field is at its maximum pressure and in the second frame the ultrasound is at its minimal pressure.

Oscillation microbubbles nearby cells have been optically recorded using a microscope and a high-speed camera [2].

This camera is able to record the MHz oscillation microbubbles and therefore the interaction between oscillation microbubbles and cells (fig 2). The microbubble oscillation causes displacement of the endothelial cell membrane. A pushing as well as a pulling displacement can be generated by the oscillating microbubbles. To what extent of cell deformation occurs is depended on the microbubble excursion and the distance of the microbubble to the cell. When in contact with cells, the total amplitude of smaller microbubbles are relatively higher (~2.5 times D_0) than those of bigger microbubbles (~2 times D_0) (fig.3A). Furthermore, smaller microbubbles (<3µm) expand more than they compress. Bigger microbubbles (>3µm) tend to expand as much more than they compress. The ratio between expansion and compression is for smaller microbubbles >1 and for microbubbles with a diameter of 3 or more the expansion compression ratio is around 1 (fig 3B). Without causing cell death, a maximum outward displacement of 7.5% of the cell size and inward displacement of 15% of the cell size could be measured.

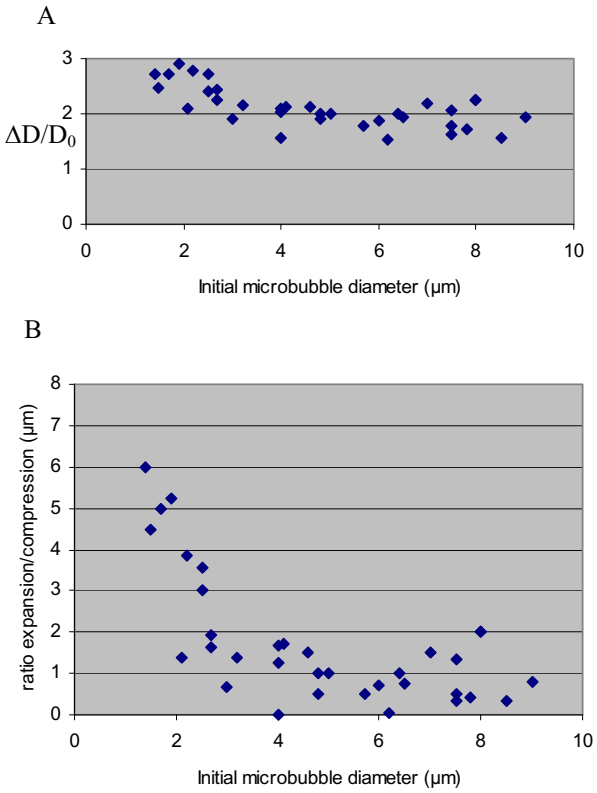

Fig. 3. Microbubbles insonified at 1 MHz, 500 kPa and in contact with endothelial cells. A). The relative amplitudes ($\Delta D/D_0$) versus the initial diameter. B). Ratio of expansion/compression as function of the initial diameter.

We believe that cell deformation is also depended on the elasticity (flexibility) of the cell, there are limitations to the extent to which ultrasound triggered contrast agent can induced. Because an endothelial cell can withstand compression better than elongation, the elongational phase may act the main component that determines pore formation. With this in mind, it would be obvious that bigger microbubbles have a bigger impact in the endothelial cells. However, this has still to be confirmed.

2.2 Pore formation

In earlier studies we found that during microbubbles oscillation, cells are open for small compounds like propidium iodide [2]. These findings are consistent with the idea that poration of the cell membrane occurs [3]. Poration is a transition of hydrophobic to hydrophilic pores. This transition creates cylindrical pores when a rotation of the polar heads brings a hydrophilic surface to the pore (Fig. 4).

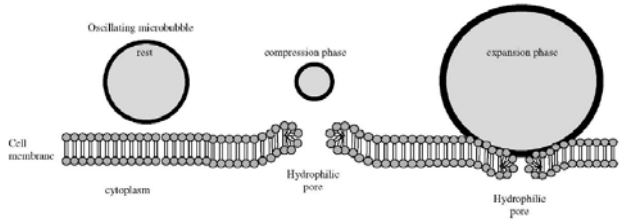

Fig 4. Proposed model of the oscillating microbubble enforced pore formation in the cell membrane. The pushing and pulling behavior of the microbubble causes rupture of the cell membrane creating a hydrophilic pore allowing trans-membrane flux of fluid and macromolecules.

Application of ultrasonic field pulses in combination with the presence of microbubbles to adherent cells generates a critical transmembrane 'shear' force, which leads to formation of pores once the membrane breakdown force is exceeded. Rapidly oscillating microbubbles generates a fluid flow over the cell surface. This flow is termed microstreaming and is probably responsible for the disruption of cell membrane by tearing the lipid bilayer membrane open [5]. However, pore formation is not the only mechanism through which enhanced drug-uptake can be reached using ultrasound contrast agents. The micro-mechanical forces can also act as external stimulus to encourage cells to actively internalize the drugs. For bigger particles, like plasmid DNA, (receptor mediated) endocytosis is probably the dominating mechanism [6,7].

2.3 Permeabilization Endothelial layer

Brandaris recordings also revealed that microbubbles can wiggle themselves in between endothelial cells (Fig 5). Ultrasound-activated microbubbles are able to transiently increase the endothelial layer permeability resulting in a local increased vessel wall permeability [8]. This is very useful for drugs that target the vascular smooth muscle cells and even vascular tissues beyond that.

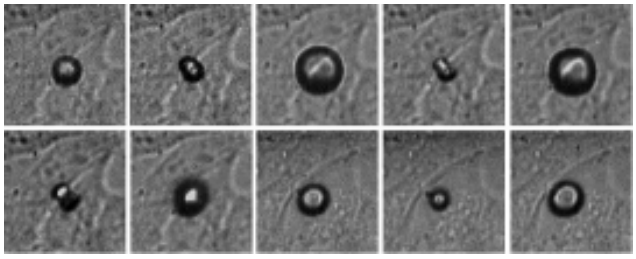

Figure 5. Ten selected frame of a microscopic ultra-fast Brandaris recording (10 million frame per second) of an oscillating microbubble pushing endothelial cells apart [8].

3 Ultrasound triggered drug release

Next to increasing the drugs internalisation, encapsulated gas microbubbles can also be used as drug/particle carrier. We and other groups have shown ultrasound triggered drug release from microbubbles carrying drugs [9,10]. After intravenous injection they disperse in the blood circulation penetrating even into the smallest capillaries. Echo contrast agents can be intentionally ruptured by diagnostic ultrasound. Using microbubbles to carry drugs to targets sites and rupturing the microbubbles by localized ultrasound energy, results in a high local concentration of drugs and a local therapeutic effect [11].

4 Conclusion

Although a lot of studies have been showing ultrasound contrast local drug delivery, every type of ultrasound contrast agent and target cells will require specified ultrasound settings. By adjusting the ultrasound contrast agents and the ultrasound setting, this system is suitable to carry all types of drugs to many different targets sites resulting in a personalized non-invasive therapy [12,13]. Microbubble oscillation and rupture behaviour is among others, dependent on the microbubble size, ultrasound frequency, shell composition, and the surrounding liquid and tissue.

Acknowledgments

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