Sonoporation: why microbubbles create pores

Michiel Postema, Spiros Kotopoulis, Anthony Delalande, Odd Helge Gilja

Sonoporation: why microbubbles create pores

Michiel Postema[1], Spiros Kotopoulis[1,2], Anthony Delalande[1,3], Odd Helge Gilja[2,3]

1. Department of Physics and Technology, University of Bergen, Bergen, Norway
2. National Center for Ultrasound in Gastroenterology, Department of Medicine, Haukeland University Hospital, Bergen, Norway
3. Institute of Medicine, University of Bergen, Bergen, Norway

Correspondence to: Prof. Dr. Michiel Postema, Department of Physics and Technology, University of Bergen, Allégaten 55, 5007 Bergen, Norway. michiel.postema@ift.uib.no Telephone: +47-555-82880 Fax: +47-555-89440

Invited editorial: Sonoporation: why microbubbles create pores

Ultrasound contrast agents are commonly added to the blood stream in ultrasonic imaging: contrast-enhanced ultrasound (CEUS). They consist of microscopically small bubbles (microbubbles) encapsulated by elastic shells. The most common shell materials are phospholipids. During an ultrasound cycle microbubbles oscillate, *i.e*., they expand and subsequently contract. Depending on their elastic properties, on the local conditions, and on the acoustic settings, they move in the direction of the sound field, coalesce with other microbubbles, fragment, jet, cluster, release their contents, and dissolve in the surrounding liquid.[1] The diverse behaviour of encapsulated microbubbles in different acoustic regimes has triggered the idea to use them as ultrasound-controlled vehicles to facilitate the delivery of therapeutic agents to a site of interest. Such a noninvasive, localised, side-effect-free method would revolutionise drug delivery as we know it.

Cellular uptake of drugs and deoxyribonucleic acid (DNA) is increased when the region of interest is under sonication, and even more so when an ultrasound contrast agent is present.[2] This increased uptake has been attributed to the formation of transient porosities in the cell membrane that have diameters up to 0.1 μm, *i.e*., big enough for the transport of drugs into the cell. The pores reseal themselves within one minute. The ultrasound-assisted transient permeabilisation of a cell membrane is called sonoporation. Understanding the physics underlying sonoporation is of uttermost importance for the development of ultrasound-activated therapeutic agents.

There are five non-exclusive hypotheses for explaining the sonoporation phenomenon from a physics point of view.[3] These have been summarised in Figure 1. It is noted that fragmenting microbubbles cannot create pores in cells, since fragmentation costs energy.

(a) Push

During its expansion phase, a microbubble might touch a cell membrane surface. The pushing motion would then cause the cell membrane to be locally disrupted. The pushing mechanism would occur within half an ultrasound cycle. However, under typical sonication conditions, microbubble excursion amplitudes are low. Also, typically, microbubbles are much more flexible than cells. Our current understanding of microbubble physics does not support the push mechanism if the microbubble is not attached. However, if the microbubble is already attached to the cell membrane whilst expanding, pushing under influence of radiation forces might cause the membrane to rupture. The latter mechanism might occur over multiple cycles.

(b) Pull

During the contraction phase of an oscillating microbubble, the plasma filling the void left by the contracting bubble might pull the cell membrane towards the microbubble. This pulling motion would then cause the cell membrane to be locally disrupted. The pulling mechanism would occur within half an ultrasound cycle. However, under typical sonication conditions, microbubble contractions do not result in an inertial collapse. Moreover, the replacive mass of a contracting microbubble is much lower than the fluid supposedly causing the pull. Therefore, this mechanism is less plausible than the push mechanism in non-attached conditions. However, if the microbubble is attached to the cell membrane, a similar mechanism might occur as described under (a), where pulling under the influence of radiation forces might cause the membrane to rupture.

(c) Jetting

Jetting is the asymmetric collapse of a bubble, creating a funnel-shaped protrusion through the bubble that is directed towards a boundary. This spectacular phenomenon has actually been optically observed on a microscopic scale through cells.[4] The jetting phenomenon occurs within half an ultrasound cycle. However, jetting exclusively occurs when using high acoustic amplitudes, at the upper end of clinical settings. Also, there has not been any proof yet of cell survival after jetting. Based on empiric studies that relate jet length to pore size, we excluded the role of jetting as a dominant mechanism involved in sonoporation.[5]

(d) Streaming

If a microbubble is fixed to a membrane, the fluid streaming around the oscillating bubbles creates enough shear to rupture the membrane.[6] Here, long pulse lengths are required to set the fluid in motion. However, low acoustic amplitudes should suffice to create the rupturing shear of a typical cell membrane. Existing research has proven the feasibility of the streaming mechanism in bound conditions, so this mechanism should work for targeted contrast agents that attach to cells. Further research will show whether streaming around weakly-attached microbubbles creates enough shear for cell membranes to rupture.

(e) Translation

Owing to radiation forces, lipid-encapsulated microbubbles may translate through cell membranes or channels in the cell membrane. The microbubble may loose part of its shell whilst passing through the cell membrane. As a result, the gas will dissolve after entry. Figure 2 shows a graphic representation of the translation mechanism.
In case of therapeutic loading, the load would be delivered directly into the target cell. This mechanism requires long pulse lengths and low acoustic amplitudes. Very recently, fluorescence-coated ultrasound contrast agent microbubbles were observed to move into HeLa cells at a low mechanical index (MI) of 0.15, using high-speed video footage under confocal microscopy.[7] The microbubbles dissolved after entering the cells. The cells survived the sonoporation treatment, confirming the transient character of sonoporation.

All five of these mechanisms require the presence of a microbubble in the vicinity of the cell. Yet, increased drug uptake has also been reported without the use of any ultrasound contrast agent, but merely an ultrasound field itself. Despite speculations about the role of inertial cavitation in the latter, clinical ultrasound scanners do not generate fields strong enough to create such spontaneous microbubbles in blood.

Recently, it was found that (bilayer) cell membranes themselves act as cavitation nuclei.[8] Even at modest acoustic pressures, cell membranes might rupture due to the building up of gas cavities inside the bilayer. This important finding would explain the sonoporation in the absence of ultrasound contrast agent microbubbles. Moreover, it explains why bubbles are attracted to cells that are not in the travel path of the ultrasound wave. However, given the low MI at which cells attract microbubbles, more study is required regarding the safe use of ultrasound contrast agents in long-pulse-length imaging.

In conclusion, in current clinical settings, four non-exclusive mechanisms are plausible for the physical explanation for sonoporation with the aid of ultrasound contrast agent microbubbles.

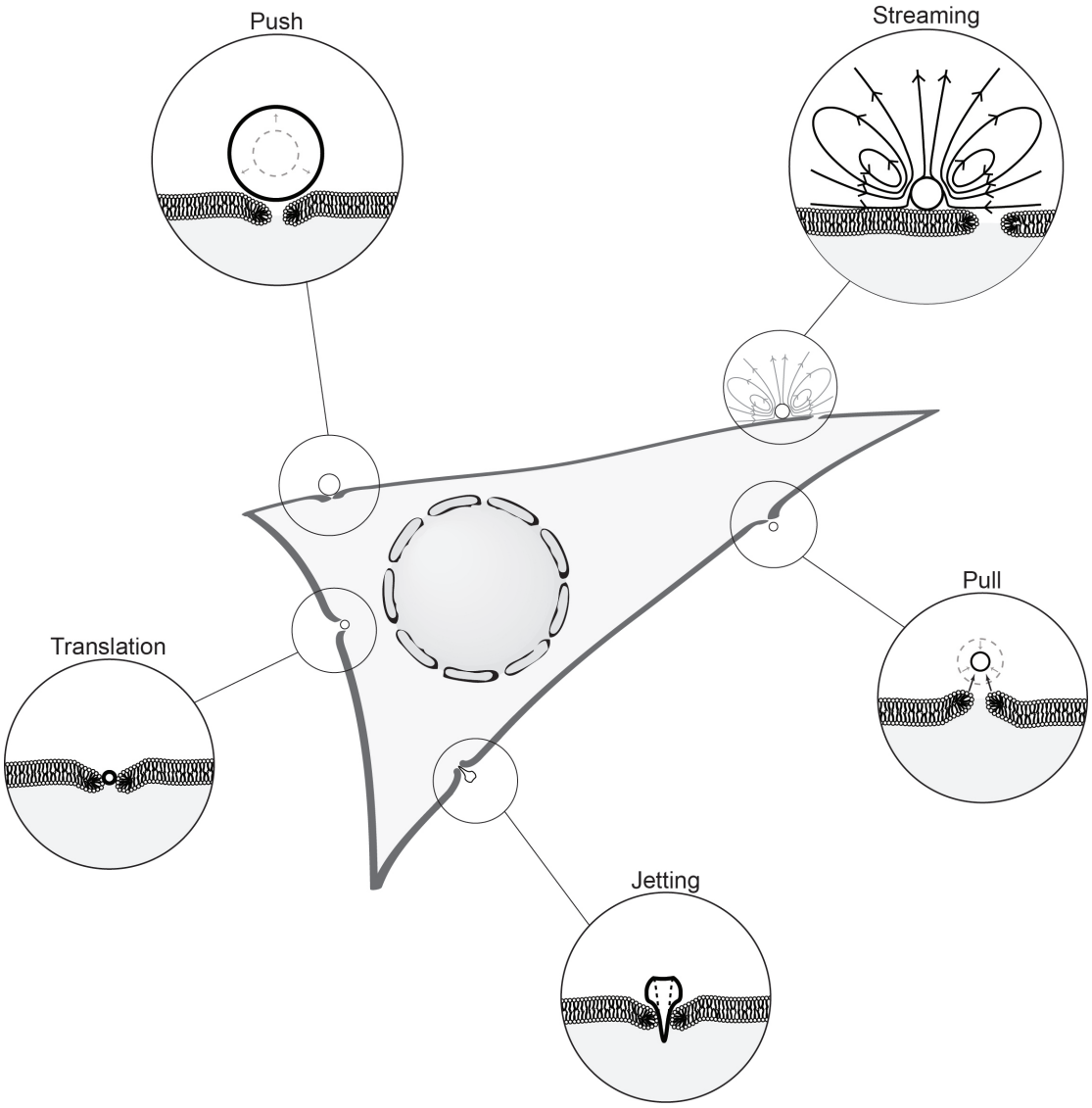

Figure 1: Schematic representation of the five physical mechanisms supposedly involved in sonoporation.

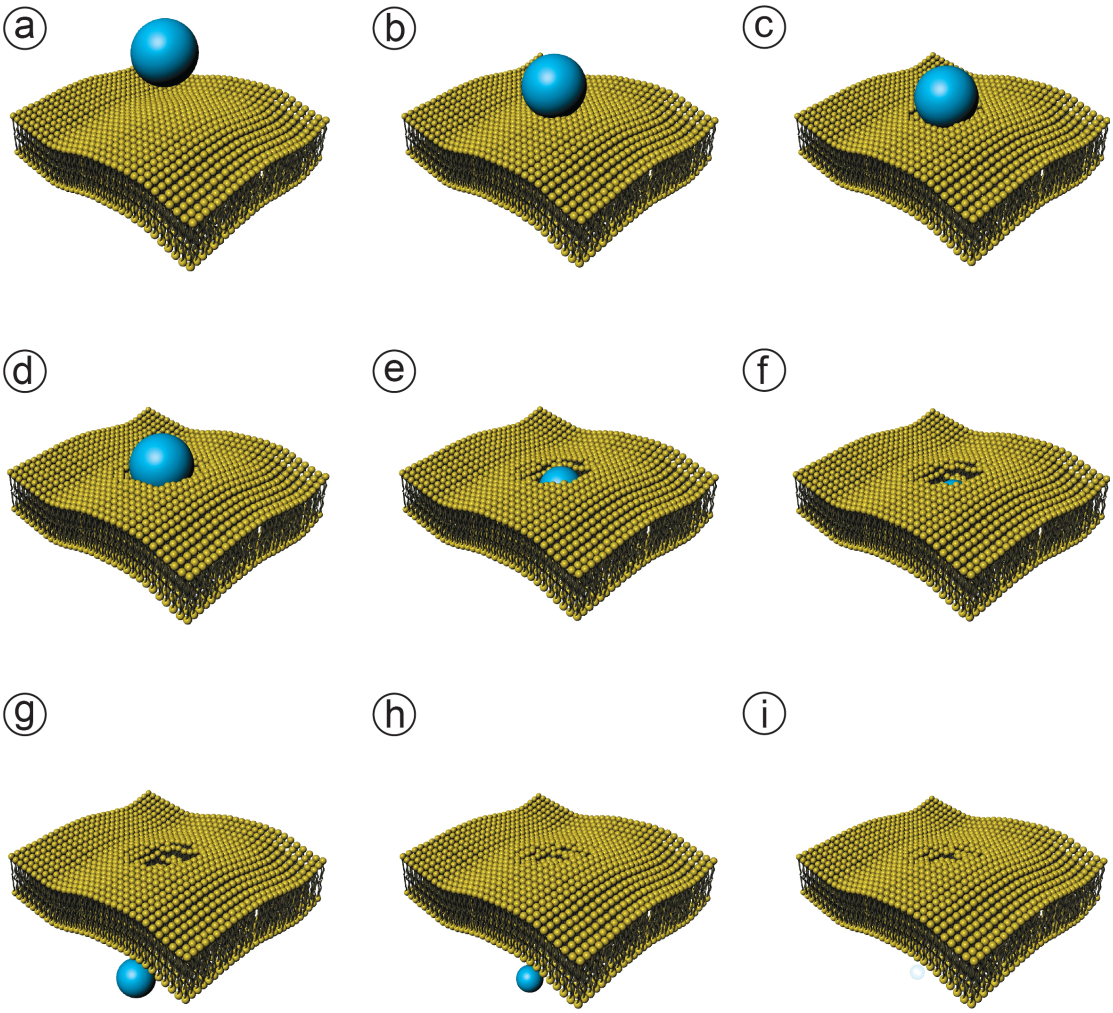

Figure 2: Schematic representation of a lipid-encapsulated microbubble translating into a cell owing to radiation forces (frames a-g) and subsequently dissolving (frames h-i).

[1] *Postema M, Gilja OH*. Contrast-enhanced and targeted ultrasound. World Journal of Gastroenterology 2011; 17: 28-41.
[2] *Postema M, Gilja OH*. Ultrasound-directed drug delivery. Curr Pharm Biotechnol 2007; 8: 355-361.
[3] *Postema M, Gilja OH, van Wamel A*. CEUS and sonoporation. In: Postema M. Fundamentals of Medical Ultrasonics. London: Spon press 2011 (ISBN 978-0-415-56353-6) 205-217.
[4] *Prentice P, Cuschieri A, Dholakia K, Prausnitz M, Campbell P*. Membrane disruption by optically controlled microbubble cavitation. Nature Phys 2005; 1: 107-110.
[5] *Postema M, Gilja OH*. Jetting does not cause sonoporation. Biomedizinische Technik 2010; 55(S1): 19-20.
[6] *Marmottant P, Hilgenfeldt S*. Controlled vesicle deformation and lysis by single oscillating bubbles. Nature 2003; 423: 153-156.
[7] *Delalande A, Kotopoulis S, Rovers T, Pichon C, Postema M*. Sonoporation at a low mechanical index. Bubble Science, Engineering and Technology 2011; 3: 3-11.
[8] *Krasovitski B, Frenkel V, Shoham S, Kimmel E*. Intramembrane cavitation as a unifying mechanism for ultrasound-induced bioeffects. Proc Nat Acad Sci 2011; 108: 3258-3263.