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REVIEW ARTICLE

Piotr Wawryka, Aleksander Kiełbik, Gracjan Iwanek

Faculty of Medicine, Wroclaw Medical University, 5, J. Mikulicza-Radeckiego Str., 50-345 Wroclaw, Poland

Microbubble based sonoporation — from the basics into clinical implications

Corresponding author:

Piotr Wawryka, Faculty of Medicine, Wroclaw Medical University, 5, J. Mikulicza-Radeckiego Str., 50-345 Wroclaw, Poland, e-mail: piotr95wawryka@gmail.com

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ABSTRACT

Sonoporation is a rapidly developing novel technique serving for drug delivery and non-viral gene therapy. It is based on the interaction between microbubbles located in the surrounding of a cell and its membrane. The interaction is obtained by excitation of microbubbles with ultrasounds. This leads to reversible cell membrane poration. Depending on the intensity of ultrasounds, structure of microbubbles used in an experiment and different environmental factors, microbubbles can interact in two manners. First, in lower ultrasound intensities, stable cavitation – regular microbubbles oscillations due to changes in the environment pressure. Microbubbles have to be very close to a cell membrane, therefore, they are usually targeted to an antigen located on the cell membrane by antibodies. Consequently, microbubbles push and pull on the cell membrane and create microstreaming around it causing its disruption. Second, inertial cavitation, where in contrary to the previous one, oscillations cause rapid collapse of microbubbles, which creates shock waves and microjets for the same purpose. No matter in which manner prorated, cells tend to reseal their disrupted cell membrane. Ca2+ ions play a crucial role in the process as well as endo exocytosis. Sonoporation has proved to be an effective modality against different diseases, including variety of cancer types in of both laboratory and clinical studies.

Key words: sonoporation, microbubble, nanobubbles, inertial cavitation, gene therapy

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Introduction

Ultrasound is a type of sound that is beyond our hearing capabilities (> 20 kHz). Though, it has proven to be useful imaging tool from the very beginning as a sonar device developed before second World War, through its introduction to medical imaging in 1942 by Dr Karl Dussik and his brother, until present, modern techniques such as 3D imagining – nowadays widely used in gynaecology [1]. Different studies revealed diverse implications of ultrasounds in medicine: ultrasonic knife [2], targeted thermic cancer cells destruction by high-intensity focused ultrasound (HIFU) [3], lithotripsy [4], thrombolysis [5], hemostatics [6], blood-brain barrier disruption [7], sonodynamic therapy [8] and finally drug and nucleic acid facilitated delivery – sonoporation [9]. Sonoporation is a process of temporal permeabilization of a cell membrane caused by ultrasounds. A vast number of studies indicate that sonoporation phenomena are favored if done in combination with microbubbles (1–10 μm radius) [10–13] or by their nano analogue - nanobubbles (around 50nm radius) [14]. They are encapsulated gases, primarily used as contrast agents in diagnostics. Their outer shell usually consists of denatured albumin, surfactants, phospholipids or polymers and the core of air, perfluorocarbons or sulphur hexafluoride. They are rather unstable, therefore, their existence lasts several minutes after injection [15]. Outer shell composition defines stability of microbubbles (Fig. 1). Softshell is easy to excite but also more likely to rapture under small pressure variations caused by ultrasounds, whereas, hard shell is more resistant to oscillations, thus more difficult to excite [10]. Nanobubbles have significant advantage over microbubbles in in vivo studies by being able to escape blood vessels and reach the tumour site more effectively [16]. Antibodies bound to the surface of a microbubble may also enhance specific accumulation [17].

Although the topic is widely described in publications, the exact quantitative contribution of each biological process involved in ultrasound-induced drug uptake is still unknown. It is also due to variety of different methodologies and settings used in the studies. In this review we will focus on those experiments conducted in the ambience of microbubbles and nanobubbles since they are more prospective than those with ultrasounds only.

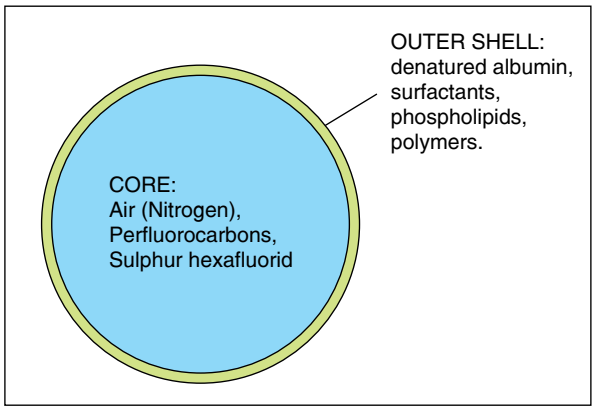

Figure 1. A microbubble simplified structure

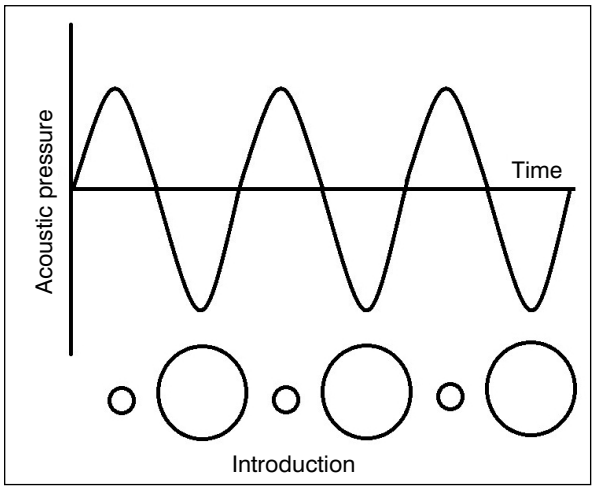

Figure 2. Stable cavitation of a microbubble

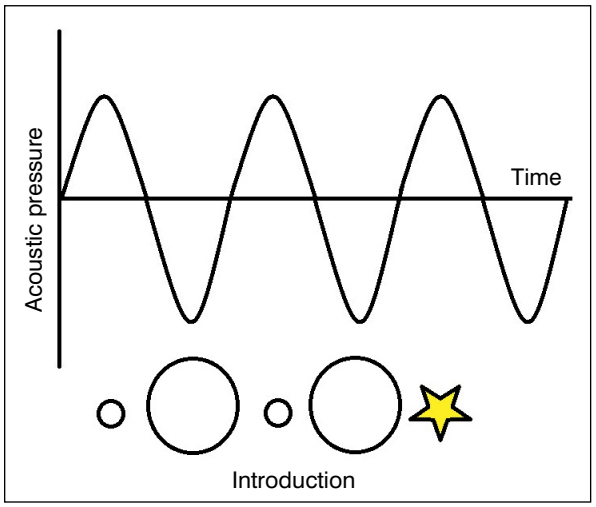

Figure 3. Inertial cavitation of a microbubble

However, it is worth mentioning that ultrasounds on their own are capable of improving drug and nucleic acids uptake [18]. Two essential types of interaction between ultrasounds and microbubbles can be distinguished. First, with low-intensity ultrasounds, microbubbles must be close to the cell membrane, stable cavitation forces have influence on permeabilization of a cell membrane. The second type, high-intensity ultrasound that leads to inertial cavitation with bubble collapse as a major factor of a cell membrane increased permeabilization. It is easier to carry out since the distance between the microbubbles and cells doesn't need to be as close as in stable cavitation sonoporation.

Low-intensity ultrasounds

In low-intensity ultrasounds stable cavitation or non-inertial cavitation occurs. Microbubbles oscillate in regular pattern (Fig. 2). They expand in lower ultrasound intensities and compress in higher ultrasound pressure [19]. This oscillation creates movement of liquid around the microbubbles that are called microstreaming [20]. The fluid flow implies shear stress on surfaces of the nearby cell membranes. It is one of the factors of stimulated uptake of particles observed during sonoporation experiments [21, 22]. Additionally microbubbles located near the cell membrane push and pull on it. This mechanical stress also disturbs cells membrane integrity. Moreover, it was also proven that microbubbles can sometimes enter into a cell by the push movements [23]. Kooiman et al. have proven that cell targeted microbubbles were lowering ultrasound intensities required for cell membrane poration. They used biotinylated anti-human CD31 antibody that was conjugated to the biotinylated microbubbles via avidin-biotin bridging [24]. Since that time scientist discovered that sonoporation can benefit from this modality. The idea behind it is that specific attachment of molecular probes on the outer shell of microbubbles leads to their accumulation at a specific site. Potential ligands might be antibodies, peptides, and polysaccharides. Monoclonal antibodies or, least common, other ligands that recognize antigens expressed and located the targeted tissue can be incorporated into or conjugated to the microbubble surface. To link higher number of ligand-receptor pairs chemical spacers are used. The most common is polyethylene glycol (PEG). It keeps the ligand away from the microbubble outer shell. Monoclonal antibodies are usually tagged with biotin. Furthermore, adhesion to molecules such as avidin may then form a bridge between a surface expressing these antigens and biotinylated microbubbles (Fig. 4) [25].

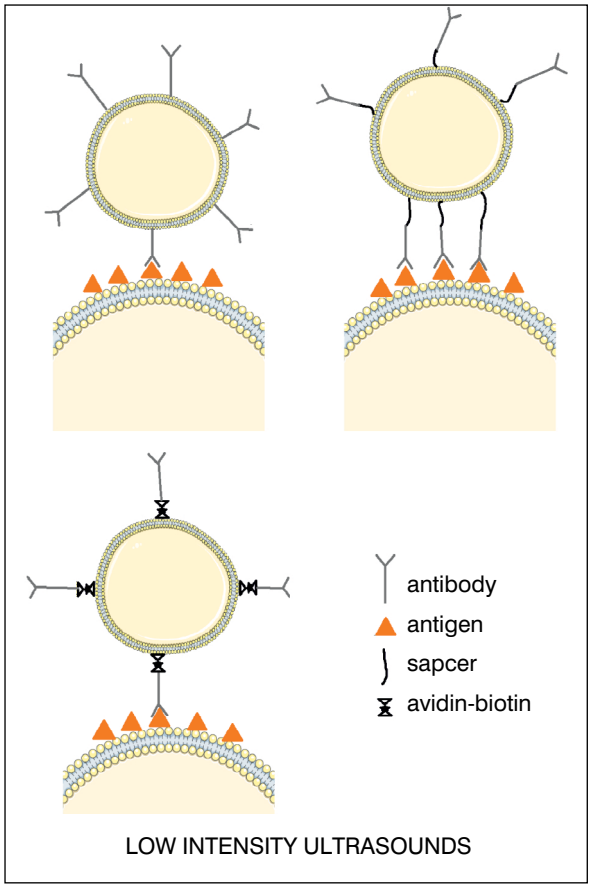

Figure 4. Targeted microbubbles

High intensity ultrasounds

At certain, high-intensity ultrasounds reach the threshold of microbubbles and induces their cavitation. After significant volume expansion follows compression leading to a rapid collapse of microbubbles (Fig.3). The process is called inertial cavitation and has some severe physical and chemical consequences [26, 27]. First of all, extremely high temperature is generated in the centre of a microbubble core. From this phenomena benefits mentioned before high intensity focused ultrasound (HIFU) [28]. Moreover, free radicals and photons are created. Photons are responsible for so-called sonoluminescence effect, which is said to be one of triggering factors in sonodynamic therapy (SDT). SDT is based on three inseparable compounds: a sonosensitive drug, ultrasound, and molecular oxygen. On their own they have no influence or very low influence on human tissue but together they create reactive oxygen species (ROS). Thus, SDT is vividly developing novel cancer therapy using also microbubbles [29]. High pressure that arises in the center of the core is often followed by shock waves. Those shock waves, if reach cells have two effects. First of all, observed in in vivo studies by high-speed optical techniques - detachment of cells from the substrate; and second of all, more important for sonoporation, the temporary deformation and disruption in cell membrane which leads to higher drug uptake. Another crucial effect of inertial cavitation are fluid microjets. They occur when a microbubble collapses aspherically near a cell. Fluid from the surrounding is pushed targeting a cell [26, 30].

The repair process of a sonoporated membrane

Since ultrasound induced poration is temporary process, it is quintessential to have an insight into how do the pores reseal. Without sufficient resealing, intracellular content would escape and extracellular ions influx would be highly toxic to sonoporated cells and other healthy cells in their surroundings. Healing pathway of a cell depends on pore size, which is commonly measured at the single-cell level in real-time using the voltage-clamp techniques, electron microscopy imaging or measured by its biological effect on the cells. The pore size depends on presence of microbubbles, type of a gas in the core of microbubble, duration of ultrasound exposure, microbubble suspension concentrations and ultrasound intensities - the higher the ultrasound intensity the larger pore diameter. Smaller pores (< 0.2 μm) are sufficient for transport of small molecules such as doxorubicin. They are mostly patched in subsecond time scale through self-sealing. The influx of extracellular Ca2+ ions has major influence on the process as well as on resealing bigger pores. Bigger pores allow to enter larger particles, for example whole nanoparticles. They are repaired by cell exocytosis, which is hypothesized to lower membrane tension and additionally, intracellular vesicles are recruited to create a mendable to patch disrupted cell membrane [31–34].

Sonoporation studies

There are two strategies of drug delivery in sonoporation. First is based solely on cavitation movements of microbubbles that lead to a cell membrane disruption triggering drug uptake. In the second strategy a drug is loaded into microbubbles. In this case inertial cavitation is needed to destroy microbubbles structure and release the drug into the sonoporated cells [35].

Secondarily, sonopotarion may be used as a non-viral vector in gene therapy. Although standard and the most efficient technique is virus vector, immunogenicity and cytotoxicity stand as a major drawback in its broader clinical use. Sonoporation is much safer in this context and has a lower cost of utilization in comparison with expensive in implementation viral-mediated gene therapy [36].

Some examples of sonoporation studies with different cancer types are as follow:

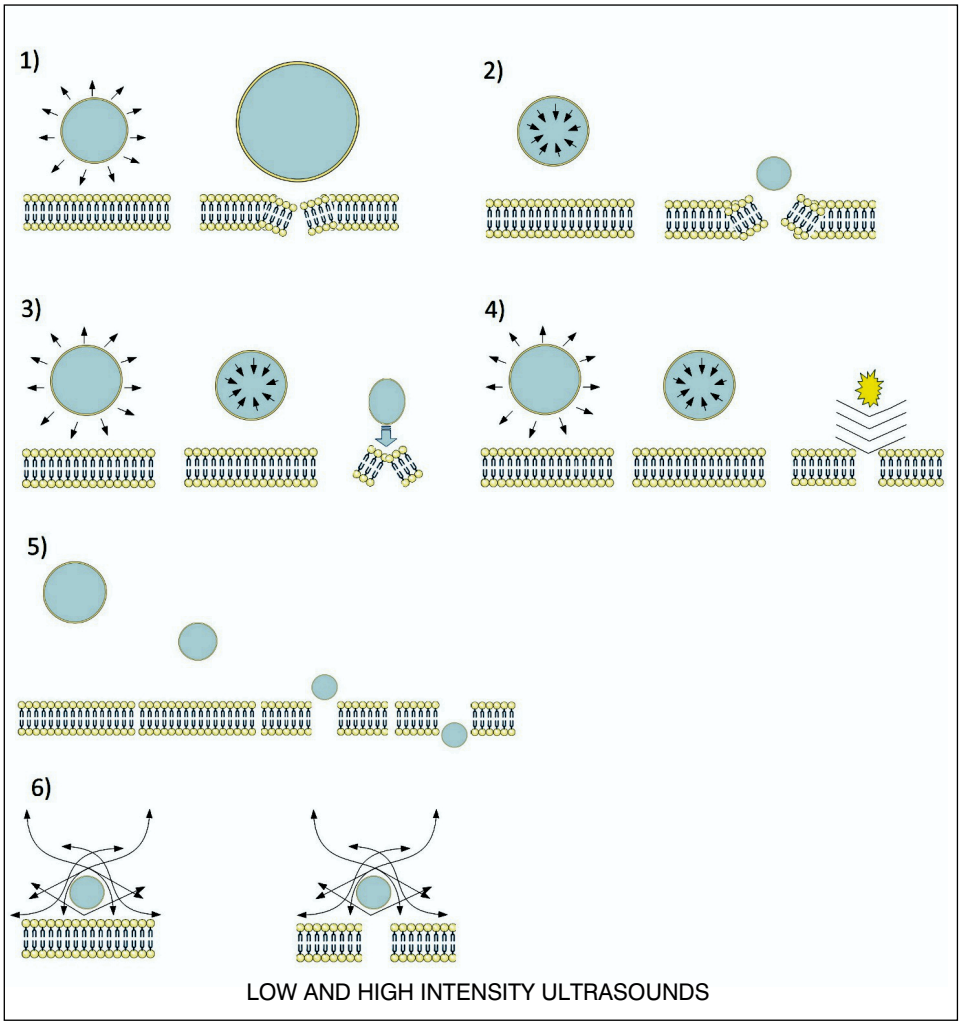

Figure 5. 1) Push movement **2)** pull movement **3)** microjetting **4)** shock wave created by inertial cavitation **5)** translation of a microbubble **6)** microstreaming

Pancreatic cancer

Dimcevski et al. documented clinical trial with use of sonoporation in the treatment of inoperable pancreatic cancer. Group of ten patients has been treated with Gemcitabine intravenously and then subsequent induction of sonoporation (reached by ultrasound scanner and intravenous administration of SonoVue®). Results showed better Gemcitabine tolerance in patients with combined treatment (Gemcitabine and sonoporation), prolonged median survival time and in 5 out of 10 patients the tumour diameter diminished from first to last cycle. The trial ended up with conclusion that combined treatment may improve the chemotherapy efficiency [37].

Liver cancer

Work published by Rinaldi et al. showed possibilities of apoptotic pathway redirection of silenced apoptotic genes (TRIAL and p53) in liver cancer cells (HepG2). They used Sonovue® as a delivery system with the result of exogenous expression of the pro-apoptotic gene TRIAL and p53 [38].

Squamous cell carcinoma

Hirabayashi et al. used epidermal growth factor receptor-targeted sonoporation delivery system to deliver bleomycin directly to a squamous cell carcinoma model (mouse tumour xenograft model) obtaining specific binding of EGFR-MBs to Ca9-22 cells that resulted in smaller tumour growth compared with the control group [39].

Prostate Cancer

Sarkar et al. combined viral gene transfer with sonoporation effect in the therapy of prostate cancer. Sonoporation has been used to transfer the cancer terminator virus payload to cancerous and inflamed tissue leading to oncolysis resulting in higher efficiency comparing to the control group [40].

Breast cancer

Rizitelli et al. used sonoporation to release Doxorubicin from liposomes under the MRI monitoring in breast cancer mouse model. In compare to control group, this procedure led to higher intratumor drug concentration that subsequently led to the complete regression of lesion. The protocol uses Doxorubicin, liposomes and Gadoteridol which are substances approved for human use what gives good clinical perspectives [41].

Cooperative study by Awad et al. proved higher efficiency of sonoporative targeting liposomes as against liposomes without sonical targeting. It led to higher drug uptake by breast cancer cell lines (MDA-MB-231 and MCF-7) after low-frequency ultrasound exposition [42].

Melanoma

Chandrashekhar Prasad and Rinti Banerjee developed curcumin and topotecan co-encapsulated nanoconjugates Cur_Tpt_NC with ultrasound contrast property. They co-delivered mentioned drugs with spatiotemporal control by ultrasound pulses to melanoma tumor on mice model reaching 3.5 times reduction of tumour growth in comparison to unexposed mice and 14.8 time reduction in comparison to the group treated with physical mixture of this drugs [43].

Glioma

Wenbin Cai et al. synthesized nanobubbles carrying siRNA. Then under sonic targeting they improve siRNA transfection to glioma cells resulting in glioma growth inhibition creating possibilities of noninvasive glioma treatment. They work also showed better therapeutic effects on mice models with sonic exposure comparing mice without sonic exposure [44].

Other studies on the implication of sonoporation phenomena include thrombolysis. Ebben et al. elaborated protocol for a phase II single-arm trial for peripheral arterial occlusions. They highlighted safety of the procedure and reduction of major hemorrhagic complications by lowering thrombolytic drug dosage [45]. Whereas Zhu et al. did a clinical study on intra-clot microbubble-enhanced ultrasound thrombolysis for deep vein thrombosis. An average thrombolysis time was almost two times shorter and urokinase dosage was diminished with no complications whatsoever [46]. Targeted nanobubbles with ultrasound were also successfully used in the treatment of Alzheimer disease [47] and Parkinson's disease by ultrasound mediated plasmid delivery by DNA-loaded MBs complexes [48].

Conclusions

Ultrasound technology is already well established in clinical practice, thus, sonoporation, if examined enough, is very likely to enter into common use. Further development of the technique is strongly dependent on broadening our knowledge about standardization of conditions in experiments. Laboratory studies laid the foundations for future clinical applications. Dynamic advance in synthesis of targeted microbubbles and nanobubbles will facilitate obtaining more efficient sonoporation effect with less toxicity from drugs to a patient. Nevertheless, more laboratory studies need to be performed in order to proceed with advanced clinical studies.

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