Ultrasound-Directed Drug Delivery

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REFERENCES

INTRODUCTION:

Until recently, biomedical imaging in vivo relied on fluorescent and light-controlled proteins (such as the Green Fluorescent Protein, GFP) to tag structures and make them visible to the human eye. However, these proteins are not practical in healthcare or even lab settings in vivo because of their minimal effect in deep tissues due to light scattering. Focused ultrasound (FUS) and MRI, however, can penetrate tissue much more effectively which gives scientists a minimally invasive imaging technique to study processes that cannot be observed in a petri dish (Piraner 2017). These include:

- Which genes do T-cells express after migrating into a tumor and recognizing a neoantigen?
- How does the activity of excitatory neurons in a certain part of the hippocampus relate to the development of seizures?

This project, however, focuses on the applications of ultrasound imaging and manipulation on drug delivery including a background on gas vesicles, an overview on ultrasound imaging, a discussion of ultrasound drug delivery and ultrasound neuromodulation, and where this science and technology could improve in the future.

GAS VESICLES:

Gas vesicles (GVs), air-filled protein sacs, were found in Halobacterium salinarum, a cyanobacterium that uses GVs to float and sink as needed. They are large (~45-250nm) with thin walls that are hydrophobic inside. Air enters through diffusion and the hydrophobic surface prevents water from entering. The major benefits of GVs are their larger size and that they are the same pressure inside as the surroundings so they are more stable.

Additionally, the GVs can be collapsed quickly by increasing the hydrostatic pressure of the system (FIGURE 1). When collapsed, the contrast produced by the GVs is reduced dramatically (FIGURE 2). Finally, experiments done in mice show that GVs can be used as a molecular reporter for ultrasound imaging in vivo (Shapiro 2014).

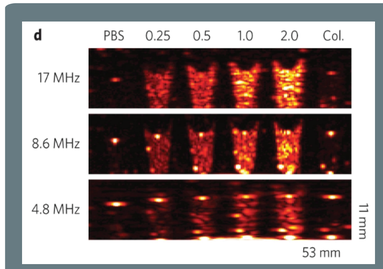


FIGURE 2: (Shapiro et al.)

d, Ultrasound images of a gel phantom containing PBS buffer, Ana gas vesicles at optical densities ranging from OD 0.25 to 2 (concentrations of 150 pM to 1.2 nM) or collapsed Ana gas vesicles (OD 2.0). Images were acquired at multiple frequencies, as indicated.

MECHANOCHEMICAL-GATED DRUG DELIVERY:

First, mechanochemical drug delivery is a process by which a therapeutic drug and gas vesicles are engineered into a bacterium. Then, using low-frequency focused ultrasound (FUS), the gas vesicles can be collapsed and restored at the frequency of the FUS (cavitation). Because gas vesicles are stable and inertial bubbles, they collapse and expand violently, which can lyse cells and release the drug (FIGURE 3). This process was initially explored using microbubbles but they need to be more stable to be practical, so GVs are perfect for the job. The bacteria are engineered with the drug and GVs and injected into the site of interest. Then, ultrasound imaging is used to locate it and potentially move it using FUS control over the GVs (thus reducing the room for error). Finally, FUS can cavitate the GVs and lyse the carrier or make holes in the membranes to release the drug (Szablowski 2019).

FIGURE 3:
(Szablowski et al.)

(F) GVs used as genetically encoded cavitation nuclei that can lyse the host cell and release a coexpressed payload.

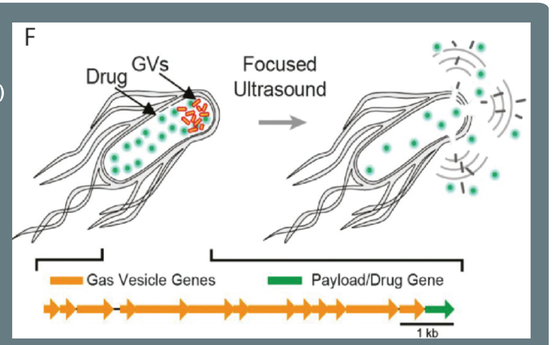
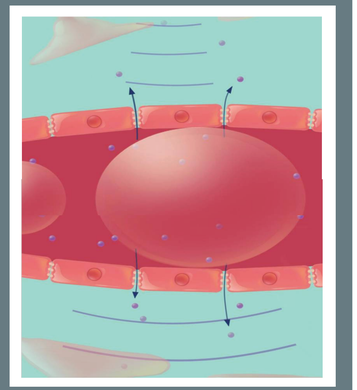


FIGURE 4:
(Burgess et al.)

Preformed microbubble contrast agent is injected intravenously and moves through the blood vessel. The microbubbles undergo stable cavitation and expand and contract when they travel through the low power ultrasound field. This causes the blood vessels to be mechanically stimulated and the BBB to be opened, allowing therapeutic agents temporarily to move into the brain.



OPENING BLOOD-BRAIN BARRIER (BBB):

Next, focused ultrasound (FUS) can be used to open the BBB. The BBB controls the exchanges between blood and interstitial fluid, not allowing large/polar molecules to pass through. This means that drugs must be smaller than 400 g/mol and lipid soluble, which means that 99% of small molecule and 100% of large molecule drugs cannot pass through, including antibodies, proteins, gene therapeutics, cells, and more. Current solutions to allow drugs to reach the brain are direct injection (risk of gross hemorrhage, infection), intrathecal injection into the cerebrospinal fluid (target concentration is unpredictable), and inducing permeability of the BBB using sugar alcohol, solvents, and vasodilators (unpredictable and untargeted). Using low-power FUS and intravenous delivery of microbubbles, the cavitation of the microbubbles increases gaps in the epithelial cells of the BBB, allowing drugs to pass through (FIGURE 4). Since this FUS is focused, there is less risk of transcranial heating. The main advantages of this method are that it is non-invasive (works through the skull), can be very targeted (chemotherapy for brain tumors), can be used for widespread drug delivery (amyloid antibodies for Alzheimer's), and the BBB closes again within 6 hours and has no long-term deficits (Burgess et al).

ULTRASOUND NEUROMODULATION:

Ultrasound has numerous direct applications in the brain. Currently, brain stimulation can be used to probe neurologic processes and can treat Alzheimer's Disease (AD), minimizing Essential Tremor, Parkinson's Disease, and more. However, current approaches are invasive, dangerous, or ineffective. Local injection of pharmacologic agents is effective but has significant adverse risk. Optogenetics involves very invasive genetic manipulation. Finally, deep brain stimulation (DBS) is ineffective for patients without AD.

Ultrasound, however, is non-invasive (FIGURE 5) and can have thermal/mechanical effects on the brain using acoustic pressure waves. Importantly, it can be focused to locations deep within the brain. The sound waves reach the target without disturbing the tissue along the way.

High-intensity focused ultrasound (HIFU) can destroy the tissue that it is focused on to reduce tremors even shrink brain tumors. Ultrasound can also have excitatory and suppressive effects on the central nervous system to treat chronic pain and anxiety related to PTSD (Blackmore 2019).

THE FUTURE OF FOCUSED ULTRASOUND:

In the future, combining neuromodulation with gas vesicles (GVs) could lead to increased focus/specificity in brain targeting with ultrasound and could remove the need for surgical access through the skull, which is still necessary for low-power ultrasound. New technology, such as more accessible/precise ultrasound, can make focused ultrasound (FUS) safer and further reduce the adverse effects of these treatments. Also, combining big data/AI with biomedical engineering can increase the reliability, efficiency, and accuracy of brain mapping and FUS.

For cancer treatment, there is early research on genetically encoding microbubbles into tumors cavitating them to destroy the tumor structure, which could be improved with GVs. Not only is FUS a tool for drug delivery, but as an imaging medium, it can further the study of cancers, proteinopathies, and other still mysterious diseases.

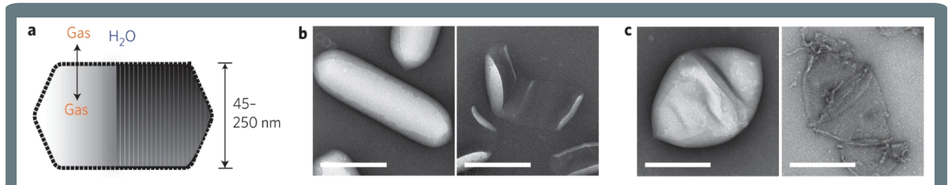


FIGURE 1: (Shapiro et al.)

a, Diagram of a gas vesicle: a hollow gas nanocompartment (solid shading) surrounded by a gas-permeable protein shell (ribbed shading). b, TEM images of intact (left) and hydrostatically collapsed (right) Ana gas vesicles. c, TEM images of intact (left) and collapsed (right) Halo gas vesicles. All scale bars, 200 nm.

THERMO-ACTUATED DRUG DELIVERY:

Focused ultrasound (FUS) - controllable bacteria can be used primarily to deliver chemotherapy drugs to tumors. Immune cells cannot reach tumors, but some other bacteria grow in tumors and can reach the tumor. These bacteria can be engineered to carry therapeutic payloads. However, injecting microbes without control over the dissemination of the therapies is a safety concern, mainly because they can reach and damage stem cell areas. To control when the payload is released, scientists can use chemical inducers (not localized), light (good precision, low penetration), radiation (risk of damage to host immune cells), or temperature. Temperature is a good choice because it can provide good spatiotemporal control and is not harmful. After engineering temperature-dependent repressors to control the expression of therapies, a brief FUS stimulus can increase the temperature in a localized area and provide lasting therapeutic output (Abedi 2022).

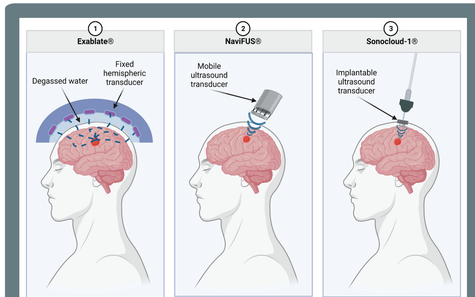


FIGURE 5:
(Rao et al.)

Posisble setups for ultrasound neuromodulation or FUS treatment of brain tumors or manipulation of BBB.