A Review of Ultrasonic Cavitation for Targeted Therapeutic Delivery

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1. Abstract

A core goal of drug development is to design an efficacious delivery apparatus that offers targeted, localized drug release with minimal side effects. Ultrasound mediated cavitation is the embodiment of this goal and represents an incredible opportunity for targeted therapeutic delivery. Microbubbles can be modified with antigens that signal the body to bring them to a desired tissue or system. These microbubbles can be loaded with drug molecules, proteins, or oligonucleotides. Microbubbles can also work independently of a therapeutic agent. Once microbubbles are present in the body and at the targeted site, ultrasound can induce cavitation. This results in the targeted uptake of desired therapeutic molecules.

Ultrasound mediated cavitation increases the porosity of tissue and can lower the total amount of a therapeutic agent needed to display efficacy in a system. Although this technology is still in development and will need more research to prove its safety in the body, ultrasound mediated cavitation, in conjunction with therapeutic loaded microbubbles, represents a promising new approach in targeted, localized drug delivery.

2. Background and Significance

Ultrasound is a type of sound wave that occurs at frequencies greater than 20,000 Hz. In the past, ultrasound has been primarily used as a diagnostic device due to its noninvasive nature and low risk of damage to tissue. Diagnostic ultrasound is usually around 2 to 15 MHz and prioritizes minimizing absorption (Paliwal et al., 2006). It is important to minimize absorption in a diagnostic setting so that the device can pick up backscattering sound and reconstruct what a tissue or system looks like.

The use of ultrasound clinically has slowly transitioned from a diagnostic device to more therapeutic applications. In the early days of ultrasounds therapeutic applications, the objective was to use the physical properties of sound to noninvasively manipulate cells and tissue. One of the early versions of therapeutic ultrasound was found in physical therapy. Physical therapists used therapeutic ultrasound (0.75 to 5.0 MHz) to cause thermal changes in tissue through prolonged exposure (Paliwal et al., 2006). As the field of therapeutic ultrasound gains more traction, researchers have begun investigating acoustic cavitation and its role in drug transport. This paper covers the use of ultrasound and microbubbles to facilitate targeted drug delivery.

The use of ultrasound and microbubbles to enhance drug delivery underscores two significant underlying trends in drug development: targeted drug delivery and localized drug delivery. Past models of drug release required the entire body be subjected to a drug or therapeutic agent for the treatment to reach a level of efficacy in a specific system or tissue. Currently, drug development revolves around using less of a drug or therapeutic and compensating for this decrease in drug by ensuring potency at the specific site, system, or tissue. Combining the principles of localized drug delivery and targeted drug delivery gives scientists the useful ability to lower the dosage of a drug needed without sacrificing the efficacy of the treatment.

3. Cavitation

To understand cavitation, it is important to understand that ultrasound's soundwaves physically interact with the tissue they pass through. These soundwaves can cause exposed materials to vibrate based upon their resonant capabilities. If a material is not very

compressible, the sound waves have a less pronounced effect on a material. However, if a material is very compressible, the soundwaves can place a high amount of compressive force on the material. In the case of microbubbles, the ultrasonic waves cause them to pinch and grow when the soundwave alternates between its positive and negative amplitudes. However, the end behavior of this oscillation depends upon what type of cavitation takes place: transient or stable (Apfel et al., 1982).

Transient cavitation is a very brief phenomenon in which ultrasound ruptures microbubbles by inducing such great change between the rarefaction and compression (the oscillatory patterns) that the sinusoidally varying pressure physically tears the microbubble apart (Leong et al., 2011). These transient cavitation events have a great effect on surrounding tissues due to the instantaneous release of energy at the collapse location. Shockwaves are produced with initial velocities as high as 10^3 m/s, pressure amplitudes can exceed 10^4 ATM, and temperature increases have been documented to reach 10^4 Kelvin (Paliwal et al., 2006). If a microbubble collapses near tissue, due to transient cavitation, it may generate a microjet that can disrupt membranes in a controlled fashion. The process of sonic induced cavitation increasing membrane permeability is referred to as sonoporation (Van Wamal et al., 2006). This increased permeability is important for drug development as it allows for a better uptake of macro molecules.

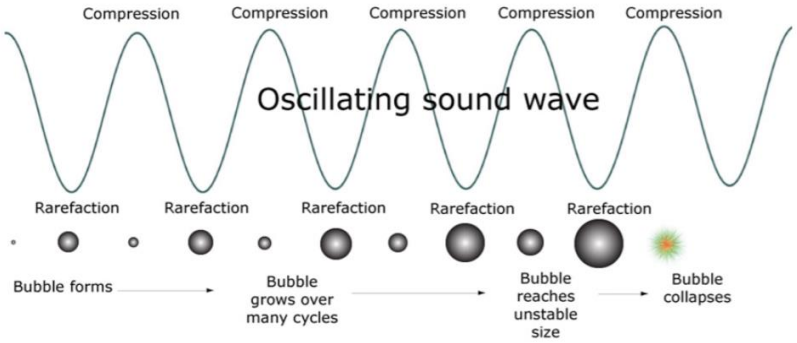

Figure 1: This image depicts how soundwaves impart physical change causing transient cavitation (Leong et al., 2011)

Stable cavitation is a more drawn out process where microbubbles continuously oscillate due to the pressure changes. In stable cavitation, microbubbles never exceed a degree of stress that might result in transient cavitation. Although the microbubbles do not rupture in stable cavitation, they can generate Reynolds stresses in the localized area. These pressure gradients can generate a cyclical fluid motion known as microstreaming (Nyborg, 1982). The process of microstreaming can result in the formation of shear forces in nearby tissues. These shear forces can provide a different means to accomplish sonoporation (Nyborg, 1982). Additionally, microstreaming generates currents that can help move drug molecules about a localized area increasing possibility for drug interactions or cellular uptake in the nearby environment.

It is important to note that sonoporation by stable cavitation is significantly less effective than sonoporation by transient cavitation (Paliwal et al., 2006). Consequently, most ultrasound-based therapies strive to induce transient cavitation in their microbubble-based drug delivery. Regardless of a treatment being chemotherapy, protein therapy, or gene therapy, ultrasound induced transient cavitation, or sonoporation, is key to localized, targeted drug delivery. Although most ultrasound based therapies are built around the same mechanics of sonoporation, the way each therapy uses microbubbles tends to vary.

4. Microbubbles

Microbubbles primarily emerged as contrast agents for ultrasound imaging. In this use, microbubbles were injected into a patient in order to better analyze the return signal from diagnostic ultrasound. Microbubbles make excellent contrast agents as they have a strikingly different impedance compared to surrounding tissue. As they became more widely studied,

researchers additionally recognized that microbubbles had unique properties that allowed them to serve as excellent carriers for targeted therapeutic delivery.

A microbubble has a diameter roughly the size of a red blood cell (~10µm diameter) (Sirsi et al., 2009). This allows its inherent deformation behavior and flow through the body to be accurately modeled by red blood cells (Sirsi et al., 2009). Microbubbles have a gas core stabilized by a shell made from lipids, polymers, or proteins. The gas core takes up most of the microbubbles volume while the shell acts as a barrier between the encapsulated gas and the surrounding environment. Since gas is a notably poor solvent for drug molecules and therapeutics, researchers tend to load a microbubble's therapeutic payload in the shell surrounding the gas core. As previously stated, shells can be made from different materials and the decision of what material to use usually stems from what level of shell thickness is desired. In order of increasing thickness: lipid shells (~3nm), protein shells (15-20 nm), and polymer shells (100-200 nm). Lipid shells are primarily held together by physical interactions: hydrophobicity and van der Waals interactions (Sirsi et al., 2009). Protein shells are composed of covalently cross-linked disulfide bonds. Lastly, polymer chains are covalently cross-linked, or they can be entangled to form a mesh-like material.

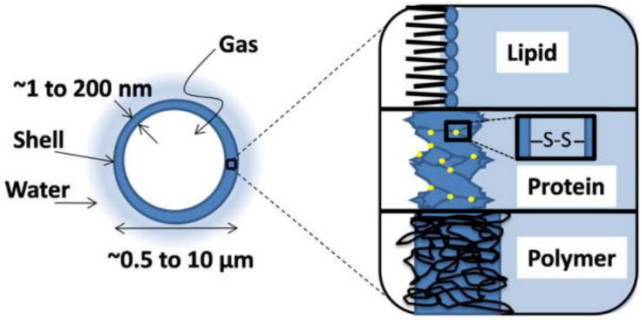

Figure 2: This image displays microbubble structural information and shell types (Sirsi et al., 2009)

When microbubbles were first being used in diagnostic applications, the primary gas inside of the acoustically responsive microbubble was air (Unnikrishnan et al., 2012). However, microbubbles with an air core had stability issues and were frequently too soluble for therapeutic drug delivery *in vivo*. To combat this, researchers began using fluorocarbons as the gas core and combined this with surface modifications (albumin, dextrose, or cationic materials) to the microbubbles in order to increase microbubble stability (Chen et al., 2000). In addition to increased stability and circulation, microbubbles have charged shells that allow scientists to bind drugs, proteins, and genetic material to them (Paliwal et al., 2006).

The core utility of microbubble-based drug delivery is its controllable, local release. Microbubbles can be ruptured with ultrasound through the process of transient cavitation at any desired location throughout the body. The targeted nature of microbubble release can be further tuned through the inclusion of tissue specific antigens on the surface of the microbubbles (Paliwal et al., 2006). These modifications allow microbubbles to be guided by the body to specific systems or tissues. After allowing time for the body to move microbubbles to the target location, they can then be ruptured with ultrasound. This allows microbubble mediated delivery to avoid many of the typical pitfalls of drug release such as systemic toxicity, clearance, and degradation.

In addition to being almost entirely released at a localized, target location, microbubble cavitation's intrinsic properties increase the uptake of therapeutic agents. Specifically, it was found that microbubble destruction through ultrasonic exposure resulted in ruptures in the bodies microvasculature (~< 7μm) (Skyba et al.,1998). This is consequential as capillary rupture due to sonoporation assists in allowing for a quick uptake and spread of whichever theraputic is loaded in the microbubbles shell.

5. Microbubble Loading Strategies

5.1 Co-administration

Co-administration refers to a loading strategy of loading the drug separately from the microbubbles. This methodology relies mostly on the physical effect of sonoporation. In co-administration, the therapeutic agents (chemotherapy related, protein related, or gene therapy related) are injected intravenously alongside microbubbles. The goal of co-administration is to supplement the baseline absorbtion rate of a drug with the presence of microbubble cavitation (Hernot et al., 2008). Outside of therapeutic compatibility concerns, the type of microbubble used in co-administration is not important. The objective is only for the injected microbubbles to ensure a low energy is needed for cavitation to occur (Birnbaum et al., 2000). Researchers want to encourage cavitation to occur in order to increase the porosity of a targeted area and, consequently, its uptake of any therapeutic agent that has been introduced to the body.

Co-administration does not shield the drug, protein, or genetic therapy from the body. Instead, the therapeutic is exposed to the body as it typically would be if injected intravenously. Thus, many therapeutics will require a treatment (coating, vector, plasmid etc.) in order to stay in the bloodstream long enough to reach the site and avoid clearance from the body. Since a drug or therapeutic agent is applied to the whole body in co-administration, sonoporation using co-administration sacrifices some of its ability to be considered a highly localized therapy. However, the application of sonoporation using co-administration does alter the uptake rates of specific regions of the body and can still be considered a semi-targeted therapy.

This conditional statement is not to put a damper on co-administration. When therapies using co-administration were tested with anti-cancer drugs, studies found that co-administration

lowered the drug dose necessary to achieve a therapeutic effect at the target site (Sonoda et al., 2007).

This same property was observed in gene therapy applications. Increasing cellular uptake of plasmids injected into the body is a key difficulty for gene therapy. Studies have found that sonoporation using co-administration increases permeability and interstitial delivery and that these factors correlate with increased gene transfer (Endoh et al., 2002).

5.2 Therapeutic-loaded

An important limitation with co-administration is that drug molecules or therapeutic agents will not always be next to a microbubble undergoing transient cavitation. If no drug or therapeutic agent is present and a microbubble undergoes ultrasonic induced transient cavitation, no benefit will be gained from the increase in porosity in the surrounding environment. To combat this issue, researchers load drug molecules and therapeutic agents onto the microbubble vehicle itself. This loading process is referred to as therapeutic-loaded delivery.

As its name suggests, therapeutic-loaded microbubbles are microbubbles that are carriers for drugs and genes. Typically, therapeutics are loaded into the microbubbles shell, but in rare instances, therapeutic agents can be kept inside the gas core.

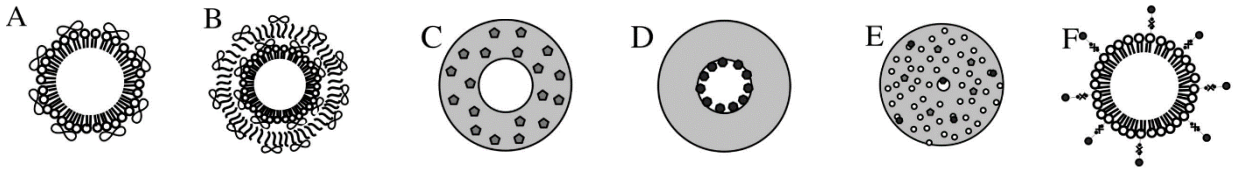

Figure 3: This image depicts loading methods for microbubbles and is analyzed in the next paragraph (Hernot et al., 2008)

The graphic above represents the main approaches researchers can take when loading microbubbles with drugs or genes. Option A displays how researchers can non-covalently bind DNA to the surface of cationic lipid microbubbles. Option B displays how a lipid microbubble can be sequentially coated with DNA and poly-L-lysine layers. Option C represents how a polymeric microbubble shell can be loaded with a hydrophobic drug. Option D shows a polymeric microbubble with the hydrophobic drug inside the internal cavity. Option E displays how a polymeric microbubble can be filled with a drug in an aqueous solution then lyophilized (freeze-dried) to leave numerous 'pockets' across the microbubbles volume. Option F illustrates how liposomes and nanoparticles can be attached to lipid microbubbles through biotin-avidin-biotin bridging (Hernot et al., 2008).

In order to get DNA on a microbubble shell, researchers mix the plasmid containing the DNA with the microbubbles before they are injected into the body. The DNA that is attached to the surface of the microbubbles is protected from the body's degradation pathways. After ultrasound induced cavitation occurs, the DNA is released by the microbubbles and will be up taken by nearby cells that now have increased porosity. Interestingly, this is a case where therapeutic-loaded trials do display an increase in cellular uptake when compared to co-administration of plasmids with microbubbles as separate entities (Hernot et al., 2008).

6. Targeted Chemotherapy

As stated above, polymer microbubbles can hold drug molecules within them (see type D from Section 5.2) and this behavior is especially valuable for chemotherapy applications. Researchers want chemotherapy to interact with as little healthy tissue as is possible. Thus,

enclosing the drug in a type D microbubble helps prevent the drug from interacting with the body until the polymeric microbubble is ruptured. When the microbubble is ruptured, the treatment is released only around cancerous tissue. The targeted nature of this treatment and its local delivery helps lower the amount of drug needed to maintain efficacy. Traditional chemotherapy is applied across the body and thus needs higher doses to arrive at the desired location at an efficacious level. A study found ultrasound-based activation of polymeric micelles containing anti-cancer treatment(doxorubicin) resulted in an increased uptake of the drug and suggested that ultrasound gated polymeric micelles may offer a new approach to targeted drug release for cancer treatment (Munshi et al., 1997).

7. Targeted Protein Therapy

Ultrasound can also be used to deliver proteins to difficult locations in the body to reach. For example, ultrasound in conjunction with microbubbles can be used for direct arterial cannulation (Mukherjee et al., 2000). A study found that vascular endothelial growth factor could see eight times the uptake in the heart with just ultrasound and thirteen times the uptake of vascular endothelial growth factor if ultrasound was used in conjunction with the microbubble perfluorocarbon exposed sonicated dextrose albumin (PESDA) (Mukherjee et al., 2000). This is relatively novel as it highlights the flexibility of ultrasound mediated cavitation treatments. In this example, researchers opted for co-administration by injecting PESDA and vascular endothelial growth factor as a mixture rather than encasing vascular endothelial growth factor in the shell of the PESDA microbubbles.

8. Targeted Gene Therapy

Targeted gene therapy is another application where ultrasound mediated cavitation offers an answer. An experiment found that PESDA microbubbles can bind with synthetic antisense oligonucleotides. This capacity to bind with antisense oligonucleotides is due to the bioactive albumin that is present on the microbubbles surface (Porter et al., 1996). Porter also found that ultrasound can release these bonds allowing for the antisense oligonucleotides to be up taken by the local cellular environment.

9. Limitations and Concerns

The largest concern surrounding sonoporation based therapies is safety. Cavitation can accurately be characterized as a double-edged sword. The line that must be walked with ultrasound treatments is how much energy can be put into a system before damage occurs. Controlled cavitation increases the permeability of cells by rupturing microbubbles present in the body. However, too much energy and uncontrolled cavitation can lead to the literal destruction of nearby tissue. Tissue death is problematic in general and damaging key vascular infrastructure can result in necrosis in the affected tissue (Paliwal et al., 2006).

In addition to localized tissue damage from cavitation, it is important to consider heat-based safety concerns. One of the early adoptions of therapeutic ultrasound was for heating tissue in physical therapy settings. Furthermore, it is well studied that high intensity focused ultrasound can reach temperatures needed to ablate tissue (Kennedy et al., 2005). Since much of the body's cellular machinery and numerous chemical reactions are incredibly sensitive to heat, it is important to ensure ultrasound is kept at an intensity level adequate for the human body. It is equally important that whichever drug is being used with ultrasound does not exhibit drastic changes or loss of function with temperature variations.

Although there is existing research on how to balance therapy effectiveness and cytotoxicity, many of these findings are system or tissue specific. This makes it difficult to extrapolate the proper conditions for a new tissue. It is possible that, in due time, most tissues tolerance for microbubble cavitation and heat sensitivity will be known. If this does come to pass, researchers will gain the ability to use ultrasound therapies on novel tissue types with more confidence.

A therapy specific limitation surrounds the targeted nature of microbubble-based drug, protein, or gene delivery. This paper has largely viewed therapeutic-loaded microbubbles as a hyper targeted and localized treatment. This is not without due cause. As previously stated, the microbubbles can be fixed with surface modifications that direct microbubbles to target tissues and the microbubbles only experience ultrasonic induced transient cavitation at a specific site in the body. However, the body is still a connected system. A drug released anywhere in the body will eventually pass into the bloodstream and then spread throughout the body. Even if the initial release was incredibly targeted and local to only one tissue, the drug release will still interact with the whole body given enough time.

A final limitation with ultrasound-based therapies is that they are in their infancy. Because of this, there is not a large degree of physical ultrasound devices or mainstream acceptance for ultrasound therapies. Eventually the field will benefit from an improved catalogue of devices that allow for a greater degree of control over inducing cavitation. Devices with different intensities, frequencies, beam width, and transducer size would offer a greater amount of flexibility for testing and improving ultrasound-based therapies (Paliwal et al., 2006). Overall, ultrasounds non-diagnostic capabilities are just being tapped, but it will take longer to change ultrasounds association from a diagnostic device to a therapeutic tool.

10. Conclusion

Overall, ultrasound induced transient cavitation of microbubbles offers a novel methodology for targeted, local drug release. The ability to increase uptake of therapeutic agents at targeted sites makes microbubble cavitation a compelling method for drug delivery. Ultrasound mediated cavitation offers the ability to deliver drug doses to a specific site all while minimizing the amount of actual drug needed in the body. This capacity for localized, targeted drug delivery cannot be overstated and offers serious potential in cancer treatment, protein delivery, and gene therapy. Due to its largely noninvasive nature and promising clinical trials, ultrasound mediated drug delivery is sure to become a more regular tool for drug delivery in the future. In its current condition, ultrasound induced transient cavitation would benefit from increased safety trials to ensure cavitation and temperature changes do not result in the destruction of non-targeted cells.

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