**Ultrasound Microbubbles: A New Vista In Drug Delivery And Medical Imaging**

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**ABSTRACT**

Gas filled microbubbles are well known as ultrasound contrast agents for medical ultrasound imaging and for noninvasive delivery of drugs and genes to different tissues. The microbubbles have an average size less than that of red blood cells, so they are capable of penetrating even into the small blood capillaries and releasing drug and genes under the action of ultrasound field after reaching the specific area of interest. Recently, targeting ligands are attached to the surface of the microbubbles, which have been widely used in cardiovascular system and tumor diagnosis and therapy. The use of microbubbles in treatments is more important than their diagnostic uses. Microbubbles today have different characteristics that allow them to be used as targeting materials, including various sizes and the types of gases, and shell materials. Microbubbles can aid drug delivery in themselves (by acting as “cavitation nuclei”) and as agents to carry drugs for site-specific treatment. This review focuses on the characteristics of the microbubbles that give diagnostic and therapeutic properties, some important aspects of ultrasound parameters that are known to influence microbubble-mediated drug delivery.

Key Words: Drug delivery, gene therapy, microbubbles, parameters, ultrasound contrast agents.

**INTRODUCTION**

Ultrasound waves have a frequency above the audible range of humans, which is from 20Hz to 20kHz, therefore, ultrasound are sound waves with frequencies greater than 20kHz. Diagnostic ultrasound typically operates in the frequency range of 1-10MHz. Ultrasound imaging technique is widely using technique. The diagnostic applications of ultrasound imaging have been enormously popular because ultrasound is non-invasive, safest, fastest and less expensive method of scanning for many types of medical diagnosis. Compared with techniques such as magnetic resonance imaging, however image quality is often inferior, and methods for improving image contrast are highly desirable. Ultrasound imaging has developed into a very successful modalit in clinical diagnosis because it can provide real-time images of soft tissue structures and blood flow without ionizing radiation.

Medical ultrasound is now a well-established technique for clinical diagnostics and will continue to play an important role in the foreseeable future. Ultrasound images however do not have a very sharp contrast and sometimes the area being imaged is buried and shod by tissues. This problem can be resolved in part by using ultrasound contrast agents when imaging. The utility and scope of ultrasound is enhanced by the use of contrast agents (CA), which reveal underlying anatomical and physiological features that would otherwise be difficult to detect.

Gas bubbles have an added advantage when being considered as ultrasound contrast agent because they can act as harmonic oscillators and resonate when insonated at their resonant frequency. Resonance increases the backscatter cross section, it is more in case of microbubbles compare to regular gas bubbles. Figure 1 depicts the production of ultrasound after administration microbubbles.

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Figure 1. Production of ultrasonic waves after administration

Microbubbles have become well established over the past 20-30 years as the most effective type of contrast agent particles (CAP) available for ultrasound radiography. Microbubbles are miniature gas bubbles of less than 50µ (micron) diameter in water. The microbubbles which mostly contain oxygen or air can remain suspended in water for an extended period. Gradually, the gas within the microbubbles dissolves in to the water and the bubbles disappear. It is known that the effectiveness of microbubble CAPs (Contrast Agent Particles), is due to their dynamic response to the application of an ultrasonic field. Because of their compressibility they undergo volumetric oscillation (pulsation) and consequently scatter much more energy than rigid spheres of the same size would do. There is, in addition a fortuitous coincidence between the size of CAP able to pass through human capillaries (<8µm) and that which is resonant at the frequencies typically used in ultrasonic imaging (1-10MHz). The amplitude of CAP oscillations (and hence the scattering effect) is thus maximized. Depending on the intensity of applied acoustic field and the characteristics of the CAP, oscillations may be linear or non-linear and the scattered signal may thus conation both fundamental and harmonic components. The latter are potentially very useful s they can enable echoes from CAPs to be distinguished from those due to tissue. The mechanism of release of agents from microbubbles is depicted in Figure 2.
It is well known that air or gas microbubbles suspended in a liquid are exceptionally efficient ultrasound reflectors for echography. Microbubbles are useful as ultrasonic contrast agents. For example, injecting suspensions of gas microbubbles (0.5-10µm in diameter) in a carrier liquid into the bloodstream will strongly reinforce ultrasonic echography imaging, thus aiding in the visualization of internal organs for the detection of cardiovascular and other diseases. Coated microbubbles have the advantage of being stable in the body for a significant period of time, as the shells serve to protect the microbubbles from diffusion into the bloodstream. There has been a considerable degree of excitement in diagnostic ultrasound imaging with the introduction of these echo contrast agents. Second generation microbubbles contain perfluorocarbon gas rather than air, which result a longer life span of the contrast agents with in the circulatory system. This permits longer window time for the echographers to observe patients, echo contrast agent microbubbles are intentionally ruptured by diagnostic and therapeutic ultrasound. Microbubbles have a high degree of echogenicity, which is the ability of an object to reflect the ultrasound waves. The echogenicity difference between the gas in the microbubbles and the soft tissue surroundings of the body is immense. Thus, ultrasonic imaging using micro-bubble contrast agents enhances the ultrasound backscatter or reflection of the ultrasound waves to produce a unique sonogram with increased contrast. This reduces the time available for contrast imaging. The shell material also affects micro-bubble mechanical elasticity. The more elastic the material, the more acoustic energy it can withstand before bursting.

Currently, microbubble shells are composed of albumin, galactose, lipid, or polymers. The gas core is the most important part of the ultrasound contrast micro-bubble because it determines the echogenicity. When gas bubbles are caught in an ultrasonic frequency field, they compress, oscillate and reflect a characteristic echotis that generates the strong and unique sonogram in contrast-enhanced ultrasound. Gas cores can be composed of air or heavy gases like perfluorocarbon or nitrogen. Heavy gases are less water-soluble so they are less likely to leak out from the micro-bubble to impair echogenicity. Therefore, micro-bubbles with heavy gas cores are likely to last longer in circulation. There are two forms of contrast-enhanced ultrasound (CEUS). They are, untargeted (used in the clinic today) and targeted (under preclinical development). The two methods slightly differ from each other.

Table 1: Comparison of various echo contrast agent

<table>
<thead>
<tr>
<th>Agent</th>
<th>Bubble size mean (µm)</th>
<th>Gas</th>
<th>Shell composition</th>
<th>Behaviour</th>
<th>Microbubbles destruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albunex</td>
<td>4.5</td>
<td>Air</td>
<td>Albumin</td>
<td>Intravascular tracer</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Levovist</td>
<td>2-3</td>
<td>Air</td>
<td>None-bubble adhere to galactose microparticles</td>
<td>Intravascular tracer</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Echogen</td>
<td>2.5</td>
<td>Perfluoropentane</td>
<td>Stabilized surfactant</td>
<td>Deposit agent</td>
<td>Resistant</td>
</tr>
<tr>
<td>Sonoven</td>
<td>4.7</td>
<td>Perfluoropentane</td>
<td>Anionically charged surfactant</td>
<td>Deposit agent</td>
<td>Resistant</td>
</tr>
<tr>
<td>Opison</td>
<td>1.5</td>
<td>Perfluoropentane</td>
<td>Albumin</td>
<td>Intravascular tracer</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Definity</td>
<td>5</td>
<td>Perfluoropentane</td>
<td>Phospholipid</td>
<td>Intravascular tracer</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Imagent</td>
<td>3.5</td>
<td>Perfluoropentane</td>
<td>Perfluoropentane</td>
<td>Intravascular tracer</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Sonovue</td>
<td>2.5</td>
<td>Sulfur hexafluoride</td>
<td>Phospholipids</td>
<td>Intravascular tracer</td>
<td>Sensitive</td>
</tr>
<tr>
<td>PB 127</td>
<td>4.0</td>
<td>Nitrogen</td>
<td>Biodegradable polymer bilayer</td>
<td>Intravascular tracer</td>
<td>Designable</td>
</tr>
<tr>
<td>NC100100</td>
<td>3.4</td>
<td>Perfluorocarbon</td>
<td>Synthetic polymer</td>
<td>Intravascular tracer</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>

Targeted CEUS (Targeted microbubbles)

Targeted CEUS works in a similar fashion, with a few alterations. Microbubbles targeted with ligand that bind certain molecular markers that are expressed by the area of imaging interest are still injected systemically in a small bolus. Microbubbles theoretically travel through the circulatory system, eventually finding their respective targets and binding specifically. Ultrasound waves can then be directed on the area of interest. If a sufficient number of microbubbles have bound in the area, their compressible gas cores oscillate in response to the high frequency sonic energy field. The targeted microbubbles also reflect a unique echo that stands in stark contrast to the surrounding tissue due to the orders of magnitude mismatch between microbubble and tissue echogenicity. The ultrasound system converts the strong echogenicity into a contrast-enhanced image of the area of interest, thus allowing the clinician to distinguish blood from surrounding tissues.

How microbubbles work

Microbubbles work by resonating in an ultrasound beam, rapidly contracting and expanding in response to the pressure changes of the sound wave. By a fortunate coincidence, they vibrate particularly strongly at high frequencies used for diagnostic ultrasound imaging. This makes them several thousand times more reflective than normal body tissues. In this way they enhance both grey scale images and flow mediated Doppler signals. As well as being useful in itself, the resonance that microbubbles produce has several special properties that can be exploited to improve diagnoses. Just as with a musical instrument, multiple
harmonic signals or overtones are produced. Ultrasound scanners can be tuned to "listen" to these harmonics, producing strong preferential imaging of the microbubbles in an image. The selective excitation produced can also destroy microbubbles relatively easily, an effect that can be useful both in imaging and in emerging therapeutic applications. The mechanisms by which ultrasound facilitates the delivery of drugs and genes depends upon the therapeutic agent, the microbubble characteristics, the target tissue and the nature of ultrasound energy. The presence of microbubbles in the insonified field reduces the peak negative pressure needed to enhance drug delivery with ultrasound. This occurs because the microbubbles act as nuclei for cavitation, decreasing the threshold of ultrasound energy necessary to cause this phenomenon. The results of optical and acoustical studies have suggested the following mechanisms for microbubble destruction by ultrasound; it is depicted in Figure 3.

1- Gradual diffusion of gas at low acoustic power.
2- Formation of a shell defect with diffusion of gas.
3- Immediate expulsion of the microbubble shell at high acoustic power.
4- Dispersion of the microbubble into several smaller bubbles.

Figure 3: Destruction of microbubbles by ultrasound resulting in increased membrane permeability by shear stress, temperature rise and activation of reactive oxygen species. Drug delivery from microbubbles is by a) transient hole induced by shear stress b) increase in membrane fluidity c) endocytosis of microbubbles d) fusion of the microbubble membrane with the cell membrane.

Cavitation of the bubbles is characterized by rapid destruction of contrast agents due to a hydrodynamic instability excited during large amplitude oscillations, and is directly dependent on the transmission pressure.[15,16]

Preparation approaches of encapsulated microbubbles
Unlike conventional contrast agents a site-directed contrast agent is designed to specifically enhance the contrast of a pathologic tissue that would otherwise be difficult to distinguish from surrounding normal tissue.[17] The ability to identify pathologic sites in patients who do not have localizing symptoms is a key step in designing methods of administering the appropriate medical treatment. Ultrasound contrast agents can be designed to be disease-stage specific and even be developed in to drug delivery systems. Agents that could perform in a dual role as both target-specific contrast agent and drug delivery vehicle would be of significant advantage to the clinician. Contrast agents could be used as a vehicle to deliver macromolecular gene constructs for gene therapy treatment. Various techniques are using to incorporate drugs in microbubbles. These microbubbles undergo cavitation and release the drug locally into the tissues. Drug delivery can be monitored with ultrasound as the drug carrier themselves are in essence contrast agent. For targeted microbubble preparation, the microbubbles are attached to the ligand that may be mono clonal antibody, carbohydrate ligand. The agents can be incorporated in different ways in microbubbles.

Applications

Diagnostic applications
Microbubbles increase the intensity of Doppler signals from blood for several minutes after their injection and this effect can be prolonged by infusing them.[14] They can thus rescue or improve an undiagnostic Doppler examination by raising the intensity of weak signals to a detectable level. For example, they can improve detection of flow in the intracranial arteries by transcranial Doppler in adults, where the skull greatly attenuates the ultrasound signal.[19]

Another use is in detecting flow in smaller vessels, such as in the circulation of malignant tumours.[20] Figure 5 depicts the identification of cancer cells by ultrasound microbubbles. Several studies have confirmed that these techniques show high sensitivity and specificity compared with the established methods of X-ray micturating cystourethrography and salpingography (both of which involve ionising radiation) and could replace them in some situations.[21]

Figure 5. Schematic of a microbubble used for cancer
Passive targeting and active targeting of microbubbles are two possible approaches for targeted imaging. Passive targeting depends on the properties of the shell and size of bubbles. They target by accumulation in vasculature, within phagocyte cells, reticul-endothelial, lymphatic systems. Active targeting relies on variety of adhesive ligands. They target on ligands of inflammatory markers and thrombus, other factors.

a) Imaging the liver
This is the most promising clinical application of microbubbles in radiology. Some, but not all, microbubbles are taken up by the liver and spleen. The precise mechanism is unclear, but the reticulo-endothelial system is probably involved. This liver phase lasts about 30 minutes with the licensed agent Levovist[22] and several hours with some new agents in clinical trials. During this phase the liver is particularly well seen with microbubble-specific imaging modes such as harmonic imaging. The main practical importance is that many focal liver lesions, particularly metastases and hepatocellular carcinoma, appear as defects and their visibility is greatly increased with microbubbles.

b) Imaging the heart
Microbubbles can enhance Doppler flow signals in cardiac ultrasonography,
and this can be useful in several situations, such as detecting valvular stenoses. Microbubble contrast agents highlight the left ventricular cavity and make the blood-tissue boundary much clearer, which helps in assessing regional abnormalities in wall motion, estimating ejection fraction, and detecting left ventricular thrombus. Evaluating left ventricular function is key to the management of many cardiac conditions. Real time perfusion imaging in stress echocardiography may offer a potent tool for assessing both resting and inducible ischaemia. The method can be extended further by applying intermittent high power pulses to destroy most of the microbubbles in a scan plane and then watching refilling: the rate at which this occurs is a measure of microcirculatory flow speed. [23]

Therapeutic applications
The use of microbubbles in treatments is more important than their diagnostic uses. Microbubbles today have different characteristics that allow them to be used as targeting materials, including various sizes and the types of gases, and shell materials. Microbubbles can aid drug delivery in themselves (by acting as “cavitation nuclei”) and as agents to carry drugs for site-specific treatment. So they can be used as targets in various diseases like inflammation, thrombus, lymph nodes, cancer, angiogenesis, and antherosclerosis with various ligands such as ICAM-1, TNF-alpha, and antibodies P-selection, Arg-Gly-Asp, GPIIb/IIIa.

Their most exciting application is in the emerging area of gene therapy, where delivery of genetic material to a chosen site is difficult. Ultrasound can potentiate drug delivery by creating transient non-letal perforations in cell membranes to aid ingress of large molecules and particles into the cells (“sonoporating”). In general this requires high acoustic power, substantially beyond that permitted for imaging, but the power needed is greatly reduced when microbubbles are present. This is because microbubbles lower the amount of energy necessary for cavitation, the process in which extreme oscillations induced by ultrasound pulses lead to microbubble collapse. Cavitation of microbubbles in capillary beds also increases capillary permeability, which improves local access of the released therapeutic agent.

The clinical use of viral vectors for gene therapy is limited because viral proteins elicit an immune response within the target tissue and have been shown to cause an intense inflammatory activation of endothelial cells. [27]

On the other hand, the nonviral delivery of vehicles, such as plasmids and antisense oligonucleotides, has been associated with a lower transfection efficiency and transient expression of the gene product. Bao et al described the use of ultrasound and albumin-coated microbubbles to enhance the transfection of luciferase reporter plasmid in cultured hamster cells. [28] Yiya Qiu et al introduced beta-Gal gene into HeLa-S3 cells successfully by ultrasound exposure. Levovist enhanced the beta-Gal gene transfection efficiency more than 6-fold. They also studied two commonly used microbubbles of Albunex and Optison in gene transfer to VSCMs, the transfection efficiencies are significantly higher than that of no microbubble and it is similar to Levovist. [29]

A recent study showed that transfection of a reporter gene in a mouse heart model as increased 10 fold using microbubbles loaded with an adenovirus gene vector. [30] Delivery to a specific site can be aided by incorporating ligand on the membrane of microbubble. For example incorporation of a surface ligand that binds to the FPIIb/IIIa receptors on activated platelets allows microbubbles to bind to thrombus and deliver thrombolytic agents.[31]

Many therapeutics (high molecular weight-compounds like chemotherapeutics, viral vectors, siRNA, mRNA, pDNA and monoclonal antibodies) should be useful in stroke therapy; they cannot be used due to the impermeability of the Blood-Brain-Barrier (BBB). The barrier permeability of these compounds improved by using ultrasound and microbubbles. The viral vectors for gene therapy (Adeno-associated virus; AAV) and monoclonal antibodies for the treatment of tPA-toxicity are coupled, either as such or packed into a lipoplex on microbubbles using a covalent linkage (Biotin-Avidin), it is shown in the Figure 8.

Figure 8. Microbubble having legends on its surface

General applications
i. Due to their large surface area volume ratio, micro bubbles can penetrate deeply into a surface for effective cleaning. This cleaning effect of micro bubbles is used in cleaning the inside of vegetables such as cabbage and radish sprout, as well as maintenance of freshness with vegetables. ii. Micro bubbles can penetrate deeply into skin for a good scrub without the need for any shampoo or soap. The baths with microbubbles are especially helpful for pets which have skin allergies to pet shampoos.

CONCLUSION
The application of microbubbles with ultrasound is a widely dispersed technology for medical imaging and for drug/ DNA delivery. Various new microbubble contrast agents present a new era of ultrasound for future application in diagnosis. Microbubbles having conjugated antibodies or binding ligands on their surface are suitable for target drug delivery in cancer therapy and gene delivery. An alteration in chemical composition of microbubbles is required to permit prolonged circulation of them and to prevent their nonspecific removal from the blood pool by the reticulo endothelial system.

REFERENCE: