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Current Status and Prospects for Microbubbles in Ultrasound Theranostics

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Abstract

Encapsulated microbubbles have been developed over the past two decades to provide both improvements in imaging as well as new therapeutic applications. Microbubble contrast agents are used currently for clinical imaging where increased sensitivity to blood flow is required, such as echocardiography. These compressible spheres oscillate in an acoustic field, producing nonlinear responses which can be uniquely distinguished from surrounding tissue, resulting in substantial enhancements in imaging signal-to-noise ratio. Furthermore, with sufficient acoustic energy the oscillation of microbubbles can mediate localized biological effects in tissue including the enhancement of membrane permeability or increased thermal energy deposition. Structurally, microbubbles are comprised of two principal components – an encapsulating shell and an inner gas core. This configuration enables microbubbles to be loaded with drugs or genes for additional therapeutic effect. Application of sufficient ultrasound energy can release this payload, resulting in site-specific delivery. Extensive pre-clinical studies illustrate that combining microbubbles and ultrasound can result in enhanced drug delivery or gene expression at spatially selective sites. Thus, microbubbles can be used for imaging, for therapy, or for both simultaneously. In this sense, microbubbles combined with acoustics may be one of the most universal theranostic tools.

Introduction

The combination of ultrasound and microbubbles has several unique advantages unavailable to most other imaging modalities. Microbubbles provide inherently high contrast for ultrasound imaging – yet can also act as a potent mediator for ultrasound therapeutics. The physical interaction between microbubbles and acoustic energy allows modulation of local permeability of both the microvasculature and cell membranes. Microbubbles can be physically moved and concentrated using acoustic radiation force and can be fragmented using ultrasound energy. Additionally, they can enhance conversion of acoustic to thermal energy, promoting selective tissue ablation. Furthermore, the microbubble structure enables drug and gene loading, as well as the inclusion of ligands able to target to specific tissues and/or biomarkers. Few other combinations of technology enable such varied and potent diagnostic and therapeutic tools from the same platform.

Theranostics refers to the combination of diagnostic and therapeutic approaches, typically for assessing response to therapy. Although it is possible for an agent to be used simultaneously for imaging and therapy, by definition, theranostics is fundamentally the combination of a diagnostic test with a therapeutic approach. Thus, theranostics can involve either application of a diagnostic technique followed by a therapeutic, or vice versa.¹ Theranostic approaches are a key aspect in the drive toward personalized medicine, where the tailoring of decisions and treatment practices for individual patients is based on diagnostic information specifically from that patient. In this review, we illustrate how

microbubbles can be used for imaging, for therapy, or for both simultaneously. Because of this broad application, microbubbles combined with acoustics may be one of the most universal theranostic tools.

BACKGROUND

Advantages of Ultrasound

Ultrasound is already one of the most widespread diagnostic modalities, routinely used in cardiology and obstetrics, but also commonly used for imaging other soft tissues.² The utility of the ultrasound platform is further enabled by recent advances in electronics. Rapidly shrinking and more powerful application-specific integrated circuits have led to ultrasound being one of the most portable imaging modalities. Many ultrasound manufacturers now offer imaging systems which are the size of laptop computers, and some of the latest devices are only slightly larger than smart phones. In contrast, the sizes of other clinical imaging systems such as MRI, CT, SPECT, and PET are substantially larger and therefore remain less portable and accessible. Although ultrasound does not provide the large-region volumetric imaging that is provided by these other modalities, ultrasound image acquisition rates for small regions of interest exceed that of MRI and nuclear imaging technologies by several fold. This high-frame rate capability provides an advantage in therapeutic applications with real-time feedback, which can be cumbersome with other imaging techniques. Another advantage of ultrasound is the significant soft-tissue contrast obtained in ultrasound imaging, although tissue attenuation severely limits the resolution and contrast of ultrasound for deep tissue applications. Furthermore, ultrasound imaging delivers no ionizing radiation, and thus is considered one of the safest imaging modalities.³ Although the safety of ultrasound contrast agents still needs to be examined for many applications, initial evaluations of contrast ultrasound in cardiology suggest that adverse effects resulting from contrast use are very rare.⁴⁻⁶ Furthermore, ultrasound contrast may play an important role in patient populations where MRI and CT contrast agents are contraindicated (such as renally compromised patients).⁷ Along with the inherent safety and portability of ultrasound imaging, the equipment cost is significantly lower in comparison to other imaging modalities. With these advantages, the future role of the microbubble platform in both diagnostics and therapeutics is highly promising.

Advent of microbubbles for medical imaging

Ultrasound image formation relies on the reception and interpretation of acoustic reflections scattered by blood and tissue. However, the scattering components associated with blood are weak, and thus blood flow in tissues and small vessels is challenging to image. Gramiak and Shah are credited with the first publication of the contrast ultrasound technique as they described the application of bubbles produced by rapid intracardiac saline injections to enhance delineation of aortic blood flow.⁸ It was not until over two decades later, with the marketing of Albunex® (Mallinckrodt Medical, Inc., St. Louis, MO, USA), that microbubbles became commercially available for use as an ultrasound contrast agent.⁹

MICROBUBBLE COMPOSITION

Encapsulating shell

Initial studies of gas bubbles for acoustic enhancement utilized un-encapsulated microbubbles that were generated *in situ*. Due to the solubility of air in blood, un-encapsulated microbubbles dissolved in seconds,¹⁰ and could not traverse the pulmonary vasculature.^{11, 12} First generation contrast agents utilized a stabilizing albumin shell which improved circulation time substantially over un-encapsulated microbubbles. The shell reduces the rate at which the gaseous core diffuses into the surrounding media.¹³

Microbubbles are normally injected intravenously as suspensions meaning they are exposed to aqueous solutions during clinical use. As such, shells composed of amphiphilic molecules help ensure microbubbles can achieve thermodynamic stability with a hydrophobic gas core. Various compositions have since been used to provide an encapsulating shell, including proteins, lipids, and polymers. The commercially produced contrast agents Optison™ (Mallinckrodt, San Diego, CA, USA) and Definity® (DuPont Pharmaceuticals Co., North Billerica, MA, USA), which are currently the only two FDA-approved agents in the US still in production, utilize albumin and phospholipid encapsulation, respectively.¹⁴

The diversity of encapsulating material composition, thickness, stiffness, charge, and surface area enables tailoring of bubble design for custom applications.¹⁵ Shell material choice is important in microbubble formulation and design since the shell functions as a scaffold for ligand binding, prevents core gases from diffusing, and influences biocompatibility. Phospholipid shells are widely used because they are easily modifiable for ligand binding and targeting applications. The polar heads of these phospholipids are frequently the site of conjugation to make targeted microbubbles.¹⁶ Direct modifications of the microbubble shells have been used in specialized applications to explore novel methods of enhancing microbubble function.^{16–21} Polyethylene glycol (PEG) chains of varying length attached to the shell of the microbubble act as steric brushes, reducing microbubble aggregation and immune recognition while also providing an attachment point for bioconjugates.^{16, 22, 23}

Gas Core

The composition of the gas core also determines the stability of the microbubble. Commercial formulations of the inner gas core have included air (Albunex®), sulfur hexafluoride (Sonovue®, Bracco, SpA, Milan, Italy), or perfluorocarbons (Definity®, Lantheus Medical Imaging, North Billerica, MA, USA). The transition from air to high molecular weight fluorinated gas cores has improved microbubble stability. Gases with lower blood solubility and higher molecular weight take longer to diffuse across the microbubble shell allowing for longer persistence times.²⁴

Microbubble Size Control

Most lipid and protein encapsulated microbubbles are formed through either mechanical agitation or sonication to produce a suspension of encapsulated bubbles. These methods result in microbubble populations that are highly polydisperse. The acoustic response of microbubbles varies with respect to many parameters but the largest contributor is diameter.^{25, 26} Acoustic scattering cross section is a function of size, and microbubbles of decreasing diameter naturally oscillate at higher resonant frequencies. By tailoring microbubble size distribution, imaging sensitivity can be optimized.^{27–30} Microbubble size also plays a significant role in therapeutic effects, as size is proportional to drug loading, and smaller microbubbles require lower pressure magnitudes to cause shell rupture.³¹ Microbubble size is also related to cavitation magnitude and corresponding changes in blood brain barrier permeability during microbubble-enhanced focused ultrasound treatment.^{32, 33}

Microbubble preparations with custom/narrowed size distribution are generally obtained in one of four ways: microfluidics,^{34, 35} electrohydrodynamic atomization,³⁶ filtering,³⁷ or differential centrifugation²⁸, or In microfluidics and electrohydrodynamic atomization, microbubbles are produced with uniform diameters by precisely controlling the flow rates of the encapsulating (usually lipid) phase that sheaths around the inner (usually high molecular weight gas) phase. Microfluidics uses flow-focusing devices driven by pressure along with advantageous channel geometries to direct and control flow while electrohydrodynamic atomization utilizes electric fields to pull droplets from a fluid cone. In contrast to the previous two techniques, differential centrifugation and filtering do not create microbubbles

at a given size but instead refine a polydisperse population into sorted samples. Both methods are generally higher throughput than either microfluidic or electrohydrodynamic techniques. Some groups have utilized filtering to refine microbubble populations, although this method can be challenging due to the fact that most microbubbles are very susceptible to destruction at high shear rates or pressure changes,³⁸ and soft shelled microbubbles are deformable and thus may not be efficiently sorted by specific pore sizes. Centrifugal sorting is commonly used in microbubble size refinement since it can be performed on large batches of microbubbles using common laboratory equipment.

MICROBUBBLE BEHAVIOR

Acoustic response

The gas core of a microbubble has a compressibility that is several orders of magnitude greater than an equivalent volume of blood. It is this combination of high compressibility coupled with the low density of the core that provides a substantial impedance mismatch between microbubbles and surrounding blood or tissue, and thus makes microbubbles excellent ultrasound contrast agents.³⁹ When exposed to an ultrasound pulse, microbubbles oscillate in response to the acoustic pressure waves (Figure 1).^{10, 40} At low acoustic pressures, most microbubbles oscillate stably, scattering sound energy as they resonate. At high acoustic pressures, large cycles of expansion and contraction can result in instability that results in fragmentation of the shell and consequential microbubble destruction with diffusive loss of the gas core. Microbubble destruction can also result in the release of any incorporated payload, facilitating the use of microbubbles as a therapeutic agent.^{31, 41} Microbubble behavior depends on many factors, including acoustic frequency and pressure, bubble size, and physical properties of the shell and core. Environmental conditions such as hydrostatic pressure and dissolved gas saturation are also influencing factors. In general, low acoustic frequencies, high acoustic pressures, and smaller diameters increase the likelihood of microbubble destruction.^{31, 42}

The phenomenon of acoustic radiation force provides an additional method to increase the theranostic utility of microbubbles. Primary radiation force displaces bubbles along the direction of acoustic wave propagation. This force can physically displace microbubbles from the center of flow of a vessel and concentrate them against the endothelium.⁴³ Secondary radiation force can cause groups of microbubbles to aggregate together and is most significant when microbubbles are in close proximity (several bubble diameters) of one another.⁴⁴ Radiation force has been shown to both enhance the *in vivo* retention of molecularly targeted contrast agents,^{5, 45, 46} as well as increase the local delivery of drug-carrying microbubble vehicles.^{47–49}

In vivo behaviour

During a contrast imaging exam, a solution of microbubbles is injected through a peripheral vein. Typically, circulation half-life is fairly short, and microbubbles remain in circulation on the order of several minutes.⁵⁰ Gamma scintigraphy of commercially available Quantison™ (Quadrant Healthcare Ltd, Nottingham, UK) suggests the liver and spleen as the final destination of most microbubble shell components.⁵¹ These observations have been corroborated more recently by PET imaging with lipid shelled microbubbles.⁵² Inert gases from defunct microbubbles are rapidly cleared through exhalation from the lungs.

MICROBUBBLE ALTERNATIVES

Although microbubble research occupies the majority of publications on ultrasound contrast and therapeutic agents, several alternative agents exist that enable unique theranostic approaches. Several groups have explored echogenic liposomes (or acoustically-active

liposomes) which contain small pockets of gas and therefore can provide image contrast and/or can be triggered to release therapeutic contents by the influence of ultrasound.^{46, 53–55} Others have developed targeted perfluorocarbon nanodroplets that increase in echogenicity as they accumulate in sites of atherosclerosis and angiogenesis and can be made to carry therapeutic payloads.^{56, 57} Many recent studies have focused on phase change contrast agents (PCCAs), which combine the functional similarity of microbubbles with unique delivery and activation possibilities. PCCAs are encapsulated droplets with liquid perfluorocarbon cores capable of being triggered to the gaseous phase when stimulated with acoustic energy from an ultrasound transducer.⁵⁸ Once vaporized, PCCAs expand to form bubbles on the order of 5–6 times larger than the precursor droplets.⁵⁹ As a result, the advantages of each state (i.e. stability and small size in liquid state, echogenicity and ultrasound interaction in gas state) can be utilized efficiently. PCCAs have been produced by several groups with mean diameters both in the microscale and nanoscale for a variety of unique approaches not possible with standard microbubble agents. This includes microvascular occlusion, cavitation/thermal ablation enhancement, and both vascular and extravascular drug delivery and imaging. A full discussion on the nature, history, and design considerations of PCCAs are reviewed elsewhere.^{59–61}

DIAGNOSTIC APPLICATIONS

Imaging Approaches

There are two primary categories of methods for detecting microbubbles that enable contrast specific imaging. First, the oscillation of microbubbles in an acoustic field results in scattered broadband acoustic energy with a frequency range much greater than that produced by tissues. This signal is separated from tissue response via high or low pass filtering of the backscattered signal. Techniques include subharmonic imaging,^{62–64} the detection of microbubble signal at frequencies half of the imaging frequency; harmonic imaging,^{65, 66} the detection of microbubble signal at twice the imaging frequency; and superharmonic imaging,^{67, 68} the detection of higher harmonic multiples of the imaging frequency. A high-frequency contrast imaging technique called broadband or transient imaging is similar to harmonic and superharmonic imaging in that it involves low-frequency excitation and high-frequency detection of the broadband response from microbubbles well above that which is scattered from tissue.^{69–71} The isolation of the microbubble-specific signal enables visualization of contrast agents without the high background signal from tissue which could otherwise obscure the region of interest. The main advantage of these techniques is that they require only a single pulse of ultrasound for each line of sight to create image data and therefore are not susceptible to tissue motion artifacts and can operate at high frame rates. The second method of isolating microbubble signals relies on their nonlinear response to consecutive acoustic pulses, as opposed to linear responses from tissue. In order to separate microbubbles from tissue echoes, these nonlinear imaging strategies excite microbubbles with two or more pulses of varying amplitude or phase and then combine the resulting response across multiple pulses.^{72–75} Nonlinear components of the received signal, such as those from the echoes of microbubbles, will remain after the images are combined, while linear components such as echoes from most tissues, will be reduced.

Perfusion Imaging

Ultrasound contrast-enhanced imaging can be used to monitor tissue or organ perfusion rates by measuring the transit time of microbubbles into or out of tissue. This is accomplished through observation of a single contrast agent bolus, or by modulating contrast agent wash-in acoustically. The latter is achieved by sending a short, high intensity ultrasound pulse that fragments microbubbles in the field of view. After this clearance pulse, the returning microbubble signal wash-in rate is measured and can be correlated to microvascular flow.

This method is typically performed under constant infusion of contrast agents, and imaging is performed using low intensity contrast-specific ultrasound imaging in order to preserve the microbubble population during the measurement phase.^{76, 77} This method, often referred to as “destruction-reperfusion”, “flash-replenishment”, or “clearance-refill” imaging, can detect changes in very small flow rates below the tissue motion noise level that limits conventional color Doppler velocity estimates.⁷⁸ Use of perfusion imaging has been demonstrated to be effective in assessing blood flow in the kidneys^{79–81} and capillary beds of tumors,^{82–86} among others.

The ability of contrast ultrasound perfusion imaging to detect small changes in blood flow conditions, such as those found in capillary beds, suggests substantial potential for assessing response to therapeutic approaches that affect tissue blood flow (such as anti-angiogenic therapies – Figure 2).^{85, 87–92} Anti-angiogenic treatment of tumors attempts to decrease tumor viability by inhibiting vascular function and development in order to curb tumor growth and cause eventual ischemic and necrotic conditions within the tumor.⁹³ Initial studies focusing on anti-angiogenic therapy response in tumors using perfusion imaging have reported ease of use, low cost, and high spatial resolution.⁸⁵ Similarly, contrast ultrasound perfusion imaging has been utilized as an immediate means to assess clinical response to high intensity focused ultrasound ablation.^{94, 95}

Molecular Imaging

Molecular imaging enables contrast ultrasound to progress beyond anatomical imaging and provide information about changes in physiology on the molecular level.^{96–98} Molecularly targeted contrast agents are formulated by incorporation of targeting ligands, such as peptides, antibodies, or other adhesive molecules specific for disease biomarkers, into the contrast agent shell. Typical microbubble sizes are large enough to stay within the vascular lumen without extravasating to surrounding tissues, and thus are well equipped to target endothelial markers of disease. Upon injection, circulating targeted microbubbles adhere to endothelium presenting the appropriate receptor. After natural clearance of unbound circulating microbubbles, retained molecularly targeted agents can be imaged using nondestructive microbubble imaging methods, providing information as to extent and degree of biomarker expression. Since ultrasound cannot directly assess molecular changes, the assumption that targeted microbubble retention on diseased endothelium is proportional to biomarker expression is a crucial tenet to this method. Commonly imaged biomarkers include the $\alpha_v\beta_3$ integrin, associated with angiogenesis, which can be targeted through cyclic RGD peptides, as well as inflammatory markers such as ICAM-1 and VEGFR2, often targeted directly with microbubbles bearing specific antibodies. Intriguing applications of ultrasound molecular imaging include but are not limited to assessment of atherosclerosis predisposition or progression,⁹⁹ thrombus,¹⁰⁰ ischemic damage,^{101, 102} inflammatory diseases,¹⁰³ and tumor-related angiogenesis.^{104–106}

As any physical change in biological tissue is likely preceded by changes at the molecular level, the ability to assess molecular markers enables a method of predicting forthcoming biological response to treatment. Through the use of microbubbles targeted to angiogenesis markers, researchers have observed changes in biomarker expression in response to therapy (Figure 3).¹⁰⁷ More recently, ultrasound molecular imaging has been shown to classify tumors as non-responder or responder earlier than standard tumor size measurement techniques.^{87, 108}

Acoustic Angiography

When combined with an imaging mode that provides both high resolution and tissue signal rejection, microbubbles (inherently limited to the vascular space) can provide traces of

detailed vascular structure (Figure 4). Several studies reported in the literature over the last decade have utilized contrast ultrasound as a means of assessing vascular architecture with the goal of either determining tumor presence or tumor malignancy. Contrast-enhanced acoustic angiography has been demonstrated in multiple cancer types, including breast¹⁰⁹, liver^{110, 111}, and thyroid.¹¹² More recently, high-frequency acoustic angiography has enabled analysis of vessel tortuosity in a preclinical model that uses vessel morphology to distinguish between healthy and tumor tissue.¹¹³ While various segmentation approaches have been implemented to extract and categorize vascular features from image data, the diagnostic utility of acoustic angiography is encouraging. Contrast-enhanced acoustic angiography could be utilized to assess vascular changes in response to therapy, based on the current understanding that vascular abnormalities re-normalize in response to treatment.^{114–116}

THERAPEUTIC APPLICATIONS

The behavior of the oscillating bubble in an acoustic field lends itself not only to conventional imaging enhancement, but also to therapeutic applications which could be performed during the same sonography session. These applications range from slight physical modifications of the tissue itself – such as reversible changes in vascular or cellular permeability – to more aggressive disruption, such as clot fragmentation. The mechanical action of oscillating microbubbles also enhances conversion from acoustic to thermal energy, resulting in tissue ablation at lower acoustic energy than that required for similar effect without microbubbles. Other applications involve secondary interactions such as the local delivery and/or release of a therapeutic compound mediated by acoustically driven microbubbles. Guidance can be provided by ultrasound imaging,¹¹⁷ magnetic-resonance imaging,¹¹⁸ or other modalities to help direct the treatment to the desired location, or to monitor the therapeutic effects of microbubble mediated therapies.

Modulation of vascular and cellular permeability

The mechanical oscillations of acoustically stimulated microbubbles have been shown to increase local vascular permeability.^{119, 120} Effects range from mild transient changes in vascular permeability to gross vascular disruption and hemorrhage, depending on microbubble and acoustic parameters. Early *in vivo* studies demonstrated that insonified microbubbles were capable of small vessel rupture creating transcellular pathways for polymer microspheres (503 nm) and red blood cells (~7 μm) to extravasate.¹²¹ Extensive studies have shown that with appropriately chosen acoustic parameters, changes in vascular permeability due to insonified microbubbles can be mild and transient.^{122, 123} Thus, there is a great interest for using this approach to locally enhance extraluminal bioavailability of therapeutic materials to surrounding tissues. Studies have illustrated substantial potential for the application of acoustically excited microbubbles to locally enhance delivery to the brain,^{124, 125} tumors,¹²⁶ and other tissues.^{19, 127} This is of particular interest in the brain, where the blood-brain barrier makes extravascular drug delivery challenging. There are several mechanisms hypothesized to be responsible for this permeability modulation, including disruption of the endothelium due to mechanical stresses on the vessel wall,^{128, 129} endothelial disruption due to liquid jetting that occurs with bubble collapse,¹³⁰ and increased cellular transport (Figure 5).^{128, 131}

Similar to the effect on vascular permeability, acoustically activated microbubbles have also been shown to enhance the permeability of individual cell membranes. This phenomenon, termed “sonoporation”, can result in the internalization of compounds into a cell which otherwise would not cross the cellular membrane. The mechanisms causing sonoporation are still being studied; however, common mechanistic hypotheses include mechanical stresses, the formation of pores due to liquid jetting,¹³² or microstreaming,^{133, 134} as well as active

transport processes.¹³⁵ Sonoporation can be transient or irreversible, the latter often associated with cell death, depending on the acoustic parameters.^{122, 136, 137}

The magnitudes of changes in both vascular and endothelial permeability are related to ultrasound frequency, pressure, and microbubble parameters. In general, lower acoustic frequencies and increased pressures result in greater permeability. Identifying and characterizing which parameters can be used to modify vessel or cellular permeability, the mechanisms involved, and to what extent permeability can be modulated is an active area of study in microbubble acoustics.¹³⁸

Drug Delivery

Many anticancer drugs suffer from a low therapeutic index, where the systemic dose becomes toxic at only slightly higher doses than may be effective in treating the disease. Thus, any means to significantly increase drug delivery at the disease site while reducing dose to healthy tissue is of great clinical interest. One approach is to spatially distribute the drug concentration unevenly such that the local dose to the disease site is elevated while the systematic dose remains low. Microbubbles can behave as a targeted delivery system by loading them with drugs and selectively lysing (fragmenting) them in regions where the drug delivery is desired.^{139–141}

Examples of drug loading techniques include the incorporation of therapeutic agents into the microbubble shell,^{142, 143} the formulation of multi-layer vehicles containing drugs in a layer separate from the shell or gas core,^{144, 145} or conjugation of drug-loaded liposomes or nanoparticles directly to the microbubble surface (Figure 6).^{48, 49} Several drugs which have been evaluated for microbubble delivery to date include, but are not limited to, paclitaxel,^{146, 147} doxorubicin,^{144, 148} rapamycin,¹⁴³ and 10-hydroxycamptothecin.¹⁴⁹

Although there is a broad range of drug loading methods and capacities, several studies have shown that certain types of drug carrier vehicles retain the capability for substantial acoustic response, indicating that these vehicles could be fully capable of both acting as imaging agents as well as therapeutic carriers.^{150, 151}

Gene Delivery

While the application of ultrasound alone has increased transfection rates compared to systemic delivery during gene therapy, the presence of acoustically-stimulated microbubbles results in additional benefit. The results of several microbubble-mediated gene therapy studies indicate that gene transfection rates are highest when ultrasound, microbubbles, and genes are simultaneously delivered to the same target location.^{17, 152, 153, 127} Although the mechanisms for enhanced transfection are still being assessed, permeability changes in cellular membranes due to the oscillating microbubbles (as described above) likely play a role.¹³⁵ Because of the strongly anionic backbone of plasmids and other gene transfection agents, cationic microbubbles can be used to electrostatically attach genetic material to microbubble shells in a similar manner first developed for liposomes.¹⁵⁴ Positively charged microbubbles bound with genetic material can use lower systemic concentration of plasmids because they have higher transfection efficiencies compared to neutral microbubbles.¹⁵⁵ Cationic binding of genetic materials likely increases transfection rates due to the increased proximity between genetic material and cell membranes which promotes intracellular flow of the genetic material.¹⁵⁶ Thicker shelled microbubbles are an attractive option in targeted gene delivery since they are less likely to prematurely rupture, can load more genetic material, and can protect genetic material from nuclease activity if bound within the shell.^{157, 158}

Sonothrombolysis

Sonothrombolysis is the acoustically-mediated disruption of blood clots. The addition of microbubbles further enhances the thrombolytic effect,¹⁵⁹ both with and without the combination of thrombolytic agents. The rate of clot dissolution increases and clots degrade more fully when treated directly with ultrasound and microbubbles in comparison to administration of thrombolytic agents alone.^{160, 161} This technique has been explored for stroke,¹⁶² venous thrombosis,¹⁶³ and myocardial infarction.¹⁶⁴ Ultrasound-assisted thrombolysis is one of the truly combinational theranostic procedures since real-time ultrasound measurements of clot occlusion and therapeutic recanalization have been performed simultaneously with microbubbles and ultrasound.¹⁶⁴

Tissue Ablation enhancement

Acoustic ablation involves focusing high intensity ultrasound to heat and destroy abnormal tissue. The conversion of acoustic energy into thermal energy causes a rapid rise in temperature at the target site resulting in localized tissue destruction and necrosis. One of the current challenges of high intensity focus ultrasound (HIFU) ablation is to heat only target tissue without damaging healthy tissues. HIFU ablation transmits wide beams of low acoustic energy through tissue which is then focused into a tighter beam of high acoustic energy to be delivered at depth to the target site. Microbubbles can be advantageous for HIFU ablation because they can enhance the conversion of acoustic energy to thermal energy, therefore making this approach more efficient and reducing the likelihood of thermal damage to healthy tissues.^{165–169} Early *in vivo* studies examining the effect microbubbles have on ablation enhancement during HIFU was demonstrated in canines¹⁷⁰ and has been clinically evaluated in humans for uterine fibroid ablation.¹⁷¹

Current Challenges

Microbubbles are a unique platform that can unite established diagnostic ultrasound with relatively nascent therapeutic ultrasound. The largest challenges faced by microbubble technologies are government approval and physician acceptance. Microbubbles are still used clinically only for imaging applications. Even then, as an imaging agent – microbubbles are underutilized. Although microbubbles are used for several clinical imaging applications in Europe and Asia, FDA approved microbubble use in the United States is still limited only to enhancement of the left ventricle during echocardiography. A third major challenge involves industrial interest and support and production of ultrasound systems optimized for use with microbubbles, as well as in design and manufacture of the microbubbles themselves. Largely due to the lack of clinical use, industry interest in supporting microbubble imaging and therapeutic approaches on commercial systems has been a low priority. Similarly, there are only a handful of companies involved in the development and sales of microbubble products, likely due to the same reason.

Nevertheless, an active push by both academia and industry is currently underway to extend the use of microbubbles for imaging outside the heart as well as for therapeutic applications. Numerous studies, such as the ones discussed here, have demonstrated the potential uses of microbubbles beyond their current scope. Regulatory agency approval must be met both in the US and in other countries for both additional diagnostic applications, as well as any therapeutic applications of microbubbles, before their use will expand.

CONCLUSION

The microbubble platform is unique in that it presents a wide variety of strategies for ultrasound mediated diagnostics as well as therapeutics – thereby acting as a definitive theranostic agent. Because of the relatively low cost, portability, and safety of ultrasound,

these techniques have advantages for reduced healthcare costs, bedside support, repeated imaging or treatment studies, and access for rural or underserved populations. While there is extensive use of microbubbles in pre-clinical studies, clinical trials are still only in early stages for molecular imaging as well as for many therapeutic approaches. However, pre-clinical data are highly encouraging for the future use of microbubbles in theranostic medicine.

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Figure 1.

High-speed optical photography of acoustically excited microbubbles illustrating microbubble fragmentation as well as stable oscillation. A) Diameter vs. time streak photography showing a 3 micron bubble in response to 2 cycle insonation at ~1.5 MHz and 1200 kPa. The microbubble is observed to expand and contract substantially and then fragment B) Standard 2-D framing photography acquired simultaneously to the diameter vs. time image presented in A). C) 20 cycle insonation of a 3.5 micron bubble at ~3.5 MHz and 200 kPa, showing stable, linear, low-amplitude oscillation. (unpublished data, courtesy of Paul Dayton)

Figure 2.

Example of contrast enhanced destruction-reperfusion imaging in a rodent tumor xenograft model, A) prior to and B) 48 hrs post anti-angiogenic administration, illustrating reduction in tumoral blood flow in response to therapy. Grayscale indicates anatomical orientation, the colormap indicates blood flow, where red is slower flow and green is faster flow. (unpublished data, courtesy of Steven Feingold)

Figure 3.

Ultrasound molecular imaging used to evaluate the efficacy of an aurora kinase inhibitor in treating human pancreatic adenocarcinoma xenografts in a pre-clinical model. Data illustrate that molecular imaging indicates response to therapy prior to tumor size changes. A) Ultrasound images of a representative treated and a representative untreated tumor, which were each acquired before and after treatment. The green color overlay illustrates contrast agent targeted to $\alpha_v\beta_3$, an angiogenic biomarker. The brightness of the green image overlay is assumed to be correlated with the degree of molecular marker expression. B) 3-D ultrasound rendering of a treated pancreatic adenocarcinoma tumor on day 0. The green overlay represents the contrast agent targeting to $\alpha_v\beta_3$. A section is removed to illustrate the spatial variability of contrast targeting to $\alpha_v\beta_3$ biomarker expression. C) Percent increase or decrease in volumetric contrast targeting before and after therapy (Untreated - N=5, Treated - N=5). * $p < 0.05$ compared to untreated tumors on day 2. D) Percent increase or decrease in volume as measured by regions of interest from brightness mode ultrasound images taken at known distances across the tumor (Untreated - N=5, Treated - N=5). (unpublished data, provided by Jason Streeter, Gabriela Herrera, and J.J. Yeh)

Figure 4.

Two 3D images from the same sample volume acquired of a rat fibrosarcoma tumor model using traditional 30 MHz b-mode imaging (left) and contrast enhanced acoustic angiography (right). Scale bars below the images indicate 1 cm. Cartoon in the center illustrates the approximate location of the tumor denoted by the red circle (unpublished data, courtesy of Ryan Gessner)

Figure 5. Illustration of three hypothesized methods of how microbubbles may cause enhanced permeability. A) An ultrasound wave places a high pressure on the microbubble to compress it and is followed by low pressure that rapidly expands it creating several microhemorrhages due to mechanical stress. B) An oscillating microbubble stimulates a cell to increase transcellular transport from the lumen of the vessel to the basal membrane. C) A microbubble oscillates nonlinearly to the point of asymmetric collapse, producing a powerful microjet that breaches the endothelium.

Figure 6.

Schematic of commonly used drug attachment strategies in microbubble mediated drug delivery. A) Drugs can be dissolved in a secondary oil layer using a multi-layer microbubble construction. B) Therapeutic agents can be seeded within the thin encapsulating shell C) Nanoparticles or other therapeutics can be attached to the outside of the shell, such as tethered to PEG chains. (image courtesy of Paul Sheeran)