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Ultrasound Contrast Materials in Cardiovascular Medicine: from Perfusion Assessment to Molecular Imaging

Alexander L Klibanov

University of Virginia Division of Cardiovascular Medicine (Department of Medicine),
Cardiovascular Research Center, Cardiovascular Imaging Center, University of Virginia School of
Medicine, Charlottesville VA

Abstract

Ultrasound imaging is widely used in cardiovascular diagnostics. Contrast agents expand the range of tasks that ultrasound can perform. In the clinic in US, endocardial border delineation and left ventricle opacification have been an approved indication for more than a decade. However, myocardial perfusion contrast ultrasound studies are still at the clinical trials stage. Blood pool contrast and perfusion in other tissues might be an easier indication to achieve: general blood pool ultrasound contrast is in wider use in Europe, Canada, Japan, and China. Targeted (molecular) contrast microbubbles will be the next generation of ultrasound imaging probes, capable of specific delineation of the areas of disease by adherence to molecular targets. The shell of targeted microbubbles (currently in the preclinical research and early stage clinical trials) is decorated with the ligands (antibodies, peptides or mimetics, hormones, carbohydrates) that ensure firm binding to the molecular markers of disease.

Keywords

ultrasound contrast; microbubbles; perfusion; molecular imaging; inflammation; ischemia-reperfusion injury; P-selectin; E-selectin; VCAM-1

1. INTRODUCTION

Ultrasound imaging is widely applied in cardiovascular medicine, especially as a tool for rapid diagnostics in case of urgent decision-making conditions. Ultrasound imaging systems are now easily available and affordable worldwide. They have always been portable; hand-held and pocket equipment versions have now become a convenient option. Ultrasound provides real-time anatomic and functional (e.g., wall motion abnormality) information that could be applied for diagnostics and image-guided interventions. Ultrasound imaging is usually performed by a transducer probe placed on the skin above the organ or tissue of interest. For higher image quality the probe can be inserted in the body (e.g., intra-cavity – such as trans-esophageal, for high-resolution heart imaging, or intravascular, thin catheter-based, for high-frequency observation of stent placement and vascular plaque monitoring with spatial resolution as low as tens of micrometers).

Doppler ultrasound has been used for decades to assess blood flow – but that technique is limited to large vessels. Ultrasound imaging benefits from the development of contrast agents, as is common for all other imaging modalities. Such agents, based on gas-filled microspheres, were proposed decades ago [1,2], but are only now starting to gain wider

clinical acceptance. Blood pool agents represent the first [3,4] and second [5–7] generations of ultrasound contrast. Targeted microbubbles that provide contrast for ultrasound molecular imaging have already reached clinical trials stage [8]. This review will focus on the design, logic and potential translational use of the injectable microbubble materials for contrast ultrasound imaging.

2. DESIGN OF MICROBUBBLES FOR INTRAVASCULAR CONTRAST ULTRASOUND

The concept of ultrasound contrast is based on the difference between compressibility of water (extremely low) and gas (very high). When ultrasound (it is a pressure wave) travels through the uniform biological tissue (which consists of water to a significant extent and is almost incompressible), it propagates with minimal loss of energy. When an ultrasound pulse reaches a microbubble in a tissue (e.g., in the bloodstream), its gas core compresses and expands in accordance with the respective positive or negative acoustic pressure [9]. As a result, the excursion of the gas-liquid interface results in the generation of a secondary ultrasound wave: each bubble becomes a small point source “speaker”, which effectively scatters ultrasound. Luckily, resonance frequency of micrometer-size gas bubbles is the same as the frequency applied for medical ultrasound imaging. Therefore ultrasound imaging can detect intravascular microbubbles that have size smaller than red blood cells and can freely circulate in the bloodstream without being trapped in the blood capillaries.

Expansion and compression of the gas void is crucial for generation of acoustic backscatter signal from microbubbles. This implies the need for a very thin (several nm) shell, either a simple lipid or surfactant monolayer [3] or a thin coat of denatured albumin [4] which could be easily expanded or compressed even by the moderate acoustic pressure (ultrasound Mechanical Index (MI) < 0.1 – 0.2 , depending on the frequency), without destroying the bubbles, so that repeated real-time imaging at tens of frames per second can be performed. An alternative approach, where the microbubbles are subjected to ultrasound with higher acoustic pressure, still lower than the approved maximum of the transmit pressure, results in a destruction of microbubbles within a single imaging frame. Backscatter generated during microbubble destruction is extremely intense and nonlinear, so it can be easily detected even by regular B-mode ultrasound imaging [10]. In some of the ultrasound contrast agent designs, e.g., those entrapped in a thick polymer shell [11], destruction is required for bubble imaging: the shell has to crack to ensure efficient vibration of the gas-liquid interface. This scenario is only implemented at higher acoustic pressures (MI > 0.6), and imaging acoustic pressure threshold depends on the thickness of the polymer shell surrounding the gas core.

Some of the clinical ultrasound imaging systems (e.g., Sequoia 512 with CPS) are outfitted with low power contrast agent detection capabilities for some of the available probes (Figure 1). These contrast-specific presets enhance the signal from ultrasound contrast agents and efficiently suppress the background signal from the tissue itself (Figure 2); fusion frames of contrast and grey-scale images can be generated to combine anatomy and microbubble data. Most effective microbubble detection routines apply multi-pulse techniques, which combine phase inversion with power modulation of transmitted ultrasound [12]. Sensitivity of contrast detection is excellent, with the ability to observe individual microbubble particles of ultrasound contrast, in real time, without destruction, many cm deep in the tissues [13]. Contrast microbubbles typically are ~ 1 – $3 \mu\text{m}$ in diameter, and mass of each particle is ~ 0.1 – 1 pg . A typical dose of these microbubbles does not exceed several billion particles in case of intravenous administration in humans. Therefore, very small amount of material, sub-mg dose in humans, is sufficient to provide successful ultrasound contrast. It is several orders of magnitude less than the required dose of X-ray or MRI contrast. This is a significant characteristic, which is especially important for the design of contrast agents useful for

molecular imaging, which may gain clinical use in the future. However, the initial success for contrast ultrasound practical application in the clinic was achieved with the non-targeted, blood pool contrast agents.

3. PERFUSION STUDIES WITH CONTRAST ULTRASOUND

In the initial early-stage ultrasound contrast experiments, microbubbles were manufactured from air immediately before injection and did not have any stabilizer shell; these early generation air particles were extremely unstable. They were not able to pass through the capillaries and could not be used as blood pool imaging agents following intravenous administration, only intra-arterial. Next, air-filled bubbles with a stabilizer shell were introduced [4,3]. These agents were able to provide transpulmonary blood pool contrast useful for cardiac imaging: after intravenous injection, microbubble contrast signal from the blood in the left ventricle was obtained. Circulation lifetime of these microbubbles in the bloodstream was rather short (seconds), so the second generation of microbubbles was brought to clinical practice, which contained biocompatible fluorinated gases with low solubility in blood [5–7]. Attempts to expand the indication of second generation microbubble agents from the approved “endocardial border delineation and left ventricle opacification” to myocardial perfusion brought a variety of clinical trials, large and small scale [14,15], but are yet to gain an approved indication status. In part this is due to the fact that heart is a difficult organ to image: the heart is located rather deep beneath the skin surface, and tissue (e.g., fat) attenuation of ultrasound signal may be significant. Ultrasound propagation is hindered by the ribcage, and by the fact that high concentration of contrast microbubbles present in the heart chambers during the perfusion study in the immediate proximity to the heart muscle will attenuate ultrasound. To make myocardial perfusion imaging even more complicated, heart muscle is in constant 3D motion, both in a cardiac cycle, and a displacement due to breathing. A robust approach to myocardial perfusion by ultrasound contrast imaging was suggested very early by Wei and Kaul [16], where imaging of circulating microbubbles was performed as intermittent high power destructive frames, synchronized with the heart cycle (e.g., images taken at end-systole). The method used in that study was to perform destruction-replenishment imaging at the next contraction, then missing one heartbeat, missing two heartbeats, then three, four, etc, so that replenishment kinetics of microbubbles in the blood perfused through the interrogated area could be deduced. Destruction of microbubbles can provide superb acoustic signal for contrast detection even for polymer-coated bubbles [17]. Synchronization with heart cycle can provide alignment of the interrogated tissue for all the frames in the sequence, so that the blood replenishment rate could be computed for every pixel of the imaged tissue.

In other organs, where tissue motion is not a significant issue, destruction-replenishment perfusion studies can be performed in real time, where an intense ultrasound pulse quickly destroys the bubbles in the interrogated area first (e.g., Figure 1, right frame), and is then followed by real-time (up to ~20–50 Hz) non-destructive low-power imaging of microbubble contrast (Supplement Video 1). This technique allows observation of the influx of microbubbles into the vasculature of interest. Modern lowpower ultrasound detection schemes provide tracking of bubble trajectories and thus obtaining the maps of the feeding vasculature [18]. This approach is also useful for kidney perfusion assessment [19].

Real-time high sensitivity non-destructive ultrasound contrast imaging may be quite useful for assessment of low-perfusion vasculature. One important example is imaging of vasa vasorum within the active atherosclerotic plaque in the carotid arteries [20]. High sensitivity and high resolution nondestructive contrast ultrasound imaging capability is necessary to perform vasa vasorum contrast imaging: vasculature in the active plaque has very low density, therefore the movement of individual microbubbles can provide information

whether the plaque has active vasculature. Imaging is complicated by the fact that the large artery lumen in an immediate proximity to the plaque is filled with microbubbles, which would cause ultrasound attenuation. Consequently, intraplaque vessels can be imaged effectively only in the plaque located between the skin (and transducer face) and the artery lumen; monitoring the status of a distal plaque may be complicated. Even more complicated will be to assess the plaque status in the coronary arteries. Most likely, intravascular contrast ultrasound capability will be required for the latter [21]. Possibly, many of these issues could be resolved by the use of targeted microbubble imaging, where targeting ligands against a vascular marker of disease is immobilized on the microbubble shell [22].

4. ULTRASOUND MOLECULAR IMAGING WITH ULTRASOUND CONTRAST

4a. Targeted ultrasound imaging: general principles

Molecular imaging with ultrasound contrast is performed in a manner similar to other imaging modalities (e.g., radiolabeled ligands or fluorescent probes). The shells of microbubbles are decorated with a targeting ligand directed towards a molecular marker of disease. Following intravenous administration, targeted microbubbles circulate with the blood flow, and during this period they have a chance to adhere to the zones of the vasculature that overexpress the target receptor. Due to microbubble size (μm range) we do not expect contrast agent extravasation. Therefore, to achieve microbubble adhesion, target receptors have to be located within the vascular bed, e.g., on vascular endothelium. Within 5–15 minutes the recirculating non-bound microbubbles are cleared from the bloodstream almost completely (mostly by collapse, via gas loss from the core; gas is removed from the body by exhalation, via the lungs). After this dwell period, molecular ultrasound imaging can be performed by detecting the bubbles retained by the target receptor molecules in the region of interest (Figure 3 cartoon of the concept; also see Figure 4, left panel as an example of targeted microbubbles in vitro). To confirm binding specificity, and to verify that circulating microbubbles do not contribute to the signal, acoustic power of the imaging system can be increased to destroy the adherent (target-bound) bubbles, and imaging repeated to observe the contrast from the few circulating microbubbles and/or occasional tissue speckle signals (Figure 1, right panel).

4b. Microbubble design: specific formulations

Microbubble formulation strategy relies generally on three options. First, contrast agent microparticles (gas-filled microbubbles surrounded by a thin shell) can be generated ahead of time and packaged in the vials for extended storage [22,13]. The shell should be thin, typically, monomolecular, to allow successful vibration of the bubble in the ultrasound pressure field. At the same time, the shell should be sturdy enough to withstand long-term storage in the aqueous media, without a significant loss of a gas core, for several years of refrigerated storage in sealed vials under the headspace of the same gas as is inside microbubbles. This can be achieved by using a denatured protein shell (albumin) as in Alunex and Optison microbubbles, or by using a phospholipid monolayer decorated with an additional PEG polymer brush coat, so that microbubbles would not fuse when they form a dense “cake” at the top of the aqueous phase in the vial upon storage. Microbubble cake needs to be dissociated into individual bubble aqueous dispersion by gentle mixing, e.g., by hand rotation, immediately before administration.

Second approach is based on the dry powder matrix precursor, which is water-soluble and can be reconstituted with the aqueous medium immediately before use. The solid formulation is exceptionally stable on storage: all the components are in the dry state, lacking any water, so there is almost no chance of microbubble fusion, ligand denaturation or a hydrolytic/chemical degradation. The vial with the dry matrix is sealed, in most cases

with a non-reactive (e.g., perfluorinated) gas atmosphere inside. A soluble precursor often is PEG [6,23] or carbohydrate [3]. When water is added to the vial that contains dry matrix with embedded lipid or polymer shell components, the matrix is quickly penetrated by the aqueous phase and dissolved, leaving behind the pockets of gas phase that form bubbles surrounded by the insoluble stabilizer shell in the aqueous phase, ready for injection. Several non-targeted ultrasound contrast formulations are prepared this way, such as Sonovue [6] or Imagent [24], as well as targeted formulations, such as BR55 [23] or Sonazoid [25]. This formulation implies the use of expensive lyophilizers or spray drying equipment.

Third approach is based on an amalgamation principle, where a sealed 2 ml vial that contains gas and sterile aqueous liquid phase with a stabilizer lipid mixture is placed in the holder arm of a small desktop apparatus (a slightly modified version of a dental amalgamator), and subjected to high-shear mixing. Vibration occurs with an amplitude of ~2cm and ~4000 rpm, for less than a minute. It results in the formation of gas microbubbles stabilized with a lipid shell dispersed in the aqueous phase [7]. Manufacturing process is easy and quick (Figure 5 and Supplement Video 2): bubbles can be prepared by the patient bedside, in the doctor's office or in the hospital pharmacy, to be used within hours.

4c. Microbubble shell and gas core selection: imaging considerations

The microbubble shell is a crucial component of the particle design. If it is too thick and non-compliant polymer (e.g., polylactide [11]), the only way to obtain efficient acoustic response is to crack it with high acoustic power (see Section 2) – but that will lead to bubble destruction and inability to repeat imaging of the same area and obtain targeted contrast signal. For thin (several nm) shells there is another limiting factor: gas exchange can happen quickly through a thin membrane. Air-filled microbubbles lose their gas, and thus the ultrasound contrast capability, quite quickly after intravenous administration. This happens within seconds if oxygen-only breathing gas is applied (nitrogen is extracted from the bubbles into nitrogen-depleted blood) [26]. Perfluorocarbon bubbles circulate for much longer period (minutes) because of low solubility of these gases in water or blood, even if the shell is quite thin and acoustic response without bubble destruction is possible.

4d. Ligand attachment to the microbubble shell. Choice of ligands for efficient targeting

Molecular ultrasound imaging succeeds only when ligand-coated microbubbles attach to the target receptor and are retained on the target surface for the duration of the imaging exam (Figure 3). For this scenario to take place, multiple ligand-receptor binding events need to occur on the contact zone between the bubble and target surface; otherwise, the interaction may be too weak to retain the bubble in place in the significant shear created by the blood flow. The higher the ligand density, the better the resistance of adherent microbubbles to the shear flow [27]. But the main issue is not with the efficacy of retention of adherent microbubbles: to dislodge the bubbles already bound and retained on the target surface, very fast flow is needed, well in excess of physiological conditions [28]. The main problem is the low efficacy of initial microbubble adhesion in the faster flow conditions. As microbubbles pass by the target surface with the flow of blood quite quickly, the ligand on the bubble shell has very limited time to bind to the surface receptor (for m/s flow speed, estimated contact time for ligand and target molecules can be as short as nanoseconds). Therefore, many antibodies, which are selected by overall affinity but not the rate of association with the target receptor, are unable to achieve effective targeting in fast flow [28]. The simplest approach is to increase the ligand surface density on the microbubble exterior, which may not be possible for antibodies (typically, only $\sim 10^5$ molecules per microbubble), but is quite feasible for smaller peptide or peptide mimetic ligand molecules [23]. Nature addresses this problem by combining rapidly adhering ligands, such as sialyl Lewis X, with extended molecular structures, such as PSGL-1 or L-selectin, and micron-size extensions on the

surface of leukocytes. Same approaches can be applied for the microbubble ligand attachment. Polymeric sialyl Lewis X (or Lewis A) oligosaccharide derivatives [29,30] or short glycosulfopeptides [31] provide efficient microbubble targeting in fast flow, as rapidly binding selectin ligands. An extended PEG spacer arm, ~20–30 nm long, provides improvement of microbubble targeting efficacy [32,33]. Microscopic structures (folds) on the surface of the lipid monolayer shell ensure more efficient adhesion of microbubbles to the target [34]. A combination of several of those approaches may be useful for targeting enhancement, as well as a combination of two [35,36] or more ligands on the bubble surface.

5. MOLECULAR ULTRASOUND IMAGING: APPLICATIONS

5a. Targeted microbubbles in inflammation imaging

Targeted microbubble imaging of inflammatory conditions may rely on two scenarios: 1) imaging of activated leukocytes and 2) imaging of the markers of activated vascular endothelium. The first approach is quite simple, and it is somewhat surprising that it has not been reported in the clinical setting so far. It relies on the ability of leukocytes to actively bind apoptotic cells and particles and internalize them via phagocytosis, due to phosphatidylserine recognition [37]. When microbubbles are prepared as mimics of apoptotic cells, by simply incorporating phosphatidylserine in their shell, they adhere to phagocytic cells quickly and efficiently [38,39]. Bubbles bind mostly to Kupffer cells in the liver and to spleen macrophages, but in case of significant presence of activated neutrophils adherent to the vasculature in the inflamed tissue, then phosphatidylserine microbubbles would specifically adhere to neutrophils and mark the inflamed area for targeted imaging. Control bubbles that lack phosphatidylserine do not demonstrate this effective adherence. This approach has been successful for detecting neutrophils within the inflamed vasculature in a variety of inflammation models [39,40].

The second approach, more complicated in terms of contrast agent design and formulation, relies on the direct molecular ultrasound imaging of the specific biomarkers on the surface of activated vascular endothelium. A set of molecules upregulated on the surface of vascular endothelium in response to inflammation is relatively narrow as reported at this point: ICAM-1 [41], VCAM-1 [42], P-selectin [43] or E-selectin [44,45]. Initially, for preclinical proof-of-principle studies, monoclonal antibodies against these receptors were successfully applied [43,41]. These ligands offer excellent binding affinity to the target receptors, and outstanding specificity, with low nonspecific background in normal tissues. A significant disadvantage of these molecules is their large size (typical molecular mass of an IgG is ~150 KDa, with only a small fragment useful for targeting) and origin (mostly mouse or rat, foreign proteins immunogenic to a human). Humanized or fully human antibodies are gradually gaining ground as injectable therapeutic and diagnostic agents, as are novel methods of their discovery [46]. Preparation and design of shorter fragments (such as nanobodies [47] or affibodies [48]) may also be an option. However, translation of targeted microbubbles to clinical practice will most likely occur first with even smaller molecules, such as peptides, peptide mimetics, or oligosaccharide (see Section 4d).

Ischemia-reperfusion injury is an important target subset of inflammatory imaging: when the blood flow to certain tissue is transiently restricted and eventually restored, significant inflammatory response of the affected tissue takes place. Vascular endothelium in these tissues overexpresses P- and E-selectin and other inflammatory markers. Therefore, targeted bubbles can efficiently bind to endothelium in these areas, so that delineation of the areas of disease can be achieved [44]. Obviously, a prerequisite for the success of targeted microbubble contrast imaging is the existence of blood flow: in case of no-reflow [49], if the

vasculature is severely damaged, microbubbles will not have a chance to enter the affected tissue.

A “milder” case of ischemia-reperfusion injury is represented as “ischemic memory”: a transient ischemic event is resolved, so by the time the diagnosis needs to be performed, perfusion is normal. However, transient ischemia still leads to upregulation of the inflammatory markers on the endothelium, therefore, molecular ultrasound imaging of the risk area can be performed with microbubbles targeted to the activated endothelium markers [50,44].

Inflammation is implicated in one other condition that may require monitoring: transplant rejection. Microbubbles might play a useful role in that setting as well: ICAM-1-targeted microbubbles successfully visualized tissue rejection in a heterotopic transplant model in rats [51], so contrast ultrasound molecular imaging may serve as a non-invasive tool to alert of the need for an aggressive intervention.

5b. Molecular Ultrasound Imaging of Thrombi or clot components

It is generally assumed that clot imaging by ultrasound may not require contrast or targeting: a large dark mass of the clot in a vessel is often detectable by non-contrast ultrasound, with the aid of Doppler. Contrast in the bloodstream may aid in the delineation of the clot contours. However, detection of small clot on the surface of active plaque, or imaging of activated platelets adherent on the vessel wall might not be feasible without targeting. Molecular ultrasound imaging can be useful in these situations. Microbubbles can be outfitted with antibodies against GPIIb/IIIa [52] or RGD-peptides [53] to achieve clot targeting. Alternatively, P-selectin expressed on the surface of activated platelets can be targeted [30]. In the latter approach, application of rapidly adhering targeting ligand Sialyl Lewis A for microbubble coating ensures efficient targeting in the conditions of very fast flow, with the ultimate goal to mark arterial vascular injuries and possibly detection of vulnerable plaque. Targeting fibrin in the clot (e.g., with anti-fibrin antibodies) might also be feasible [54].

5c. Atherosclerotic plaque imaging: targeting vascular inflammation markers

It is well established that atherosclerotic plaque development, and especially plaque vulnerability, are directly related to inflammation. Markers of endothelium activation, such as VCAM-1, are known to be widely expressed in the plaque and may boost leukocyte accumulation in the plaque. Therefore, they may serve as receptors for microbubble targeting [55]. Targeting of microbubbles to inflammatory markers (e.g., VCAM-1) with antibodies is therefore possible and has been demonstrated in murine models, such as apoE $-/-$ mice on high-cholesterol diet [42,56]. In this (rather acute) model, all the vasculature of the animal is essentially inflamed and VCAM-1 is overexpressed systemically just ~2–3 months from the initiation of high-cholesterol diet, so microbubble adhesion is observed in many areas, not specifically in the atherosclerotic plaque lesions in aorta or carotid arteries (Figure 6, right panel). Next step is to investigate if selective detection of plaque and especially distinguishing active plaque will be possible by molecular ultrasound imaging in the setting of human atherosclerotic disease.

5d. Imaging of bacterial biofilms

Bacterial biofilms in the infective endocarditis setting are mostly caused by streptococci or staphylococci. These bacteria (often resistant to antibiotic treatment) can form embedded structures with fibrin-platelet complexes and deposit on heart valves. These complexes are easily accessible from the bloodstream, therefore microbubble targeting of bacteria for targeted imaging of biofilm location, by targeting surface markers of bacteria, is feasible

[57]. Even more useful, a combination of microbubbles and ultrasound treatment may aid antibacterial therapy [58].

APPROACHES TO IMAGE-GUIDED THERAPY IN THE VASCULAR SETTING

In the presence of microbubbles the acoustic energy brought by the ultrasound waves can be deposited locally in the immediate proximity to the microbubble. This energy deposition can be applied for a wide range of image-guided interventions, from assisting transfection [59–61] to siRNA delivery [62] to removal of bacterial biofilms on the surface of implanted devices [58] to accelerated thrombolysis [63]. The latter approach has already reached clinical trial stage [64], where it can reduce the dose of a thrombolytic agent needed for stroke therapy, or, in some instances, provide clot dissolution without the administration of an exceptionally expensive thrombolytic enzyme. Ultrasound-assisted thrombolysis could minimize the risk of hemorrhage associated with the current clot dissolution therapy that requires a significant dose of tissue plasminogen activator. All these procedures can be performed under ultrasound guidance, with ultrasound probes that combine low-power contrast ultrasound imaging with high acoustic pressure therapeutic transducers. Alternatively, MRI-guided focused ultrasound equipment is gaining popularity and interest, due to the low frequency (sub- MHz) ultrasound penetration capabilities and an excellent imaging quality of MRI, especially in the transcranial setting, e.g., in accelerated drug delivery across blood brain barrier [65].

CONCLUSION

Microbubble contrast agents are on a verge of wider expansion into the cardiovascular ultrasound imaging arena. Initial successful approvals for endocardial border delineation and left ventricle opacification took place more than a decade ago in US and Europe. However, those accomplishments were not yet followed by more complex milestones, such as myocardial perfusion indication approval. In Europe, China, Canada and Japan contrast ultrasound imaging is quite popular and approved for general radiology use (mostly for liver imaging). In US no such clinical indication is approved yet. Use of microbubble contrast targeted towards the molecular markers of disease will expand the diagnostic capabilities of ultrasound to the area of molecular imaging; this will be especially useful in the urgent diagnostics cardiovascular environment and beyond, as well as in image-guided biopsy and therapy setting. Focused ultrasound in combination with microbubble agents might also find applications in targeted drug and gene delivery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Dilute microbubble dispersion imaging before and after destructive ultrasound.

**MI 0.2 nondestructive imaging:
detection of individual
microbubbles, ~1-3 μm
diameter, <1pg mass**

**following 2 seconds of MI 1.9
ultrasound: most of the insonated
bubbles destroyed quickly**

Fig. 1.

Ultrasound contrast imaging of individual microbubbles at high dilution, suspended in normal saline. Left panel: imaging at Mechanical Index (MI) 0.2 (nondestructive, low power). Right panel: imaging following 2 second exposure to MI 1.9 leads to destruction of microbubbles within the beam elevation. Imaging performed on Siemens Sequoia with 15L8 probe, 7 MHz, CPS mode, 2 cm depth, 1 cm focus

Greyscale B-mode ultrasound vs. tissue suppression by a multipulse phase inversion power modulation.

1 cm

Greyscale B-mode imaging of a tissue sample in a water tank.

1 cm

CPS contrast mode: almost complete suppression of tissue signal; no microbubbles present in the tissue.

Fig. 2.

Ultrasound imaging of a tissue immersed in a water tank. Left panel: anatomy imaging (B-mode grey-scale). Right panel: CPS contrast mode suppresses the signal from the tissue lacking microbubbles in the bloodstream – thus improving the signal/noise ratio for contrast ultrasound imaging. Imaging performed on Siemens Sequoia with 15L8 probe, 7 MHz, 2 cm depth, 1 cm focus, MI 0.2

Ligand-carrying microbubbles targeted to molecular markers of disease on vascular endothelium

Fig. 3.

Targeted microbubble: an ultrasound molecular imaging agent. Typical microbubble size: 1–3 μm . Coating with a monomolecular shell (e.g., $\sim 2\text{nm}$ thick lipid monolayer) and a grafted brush of PEG polymer, decorated with a targeting ligand. Microbubble will selectively attach to the target surfaces that carry as little as 10 target molecules per μm^2

Microbubbles on a target surface: imaging before and after destructive ultrasound.

Fig. 4.

Ultrasound imaging of microbubbles attached to the Petri dish surface (~0.5 cm patch). Imaging performed in a saline tank, non-destructively, at MI 0.2 (left panel) and following destructive ultrasound treatment (MI 1.9), right panel, leading to almost complete destruction of microbubbles in the patch (confirmed by microscopy). Imaging performed on Siemens Sequoia with 15L8 probe, 7 MHz, CPS mode, 2 cm depth, 1 cm focus

Aqueous lipid mixture with perfluorocarbon gas headspace: before and after microbubble preparation

Fig. 5. Microbubble preparation by amalgamation. Left panel: aqueous lipid mixture under perfluorocarbon atmosphere in a sealed vial. Right panel: aqueous dispersion of perfluorocarbon microbubbles ($>10^9$ particles per ml) following 45 seconds of amalgamator treatment at 4000 rpm. Microbubbles are stable for many hours after preparation

Contrast ultrasound imaging with inflammation-targeted microbubbles in a murine model: 10 min after iv injection

**normal C57BL/6 mice, control diet,
minimal retention of microbubbles
in the vascular bed**

**atherosclerosis model,
apoE^{-/-} mice on a Western diet
diffuse retention of microbubbles
throughout the inflamed vasculature**

Fig. 6.

Targeted imaging of microbubbles: visualization of inflammation in a murine atherosclerosis model. Left panel: inflammation-targeted microbubbles do not accumulate in the carotid vasculature of control mice (bright specular reflections on the bottom of the image represent non-tissue response). Right panel: significant accumulation of targeted microbubbles is observed in the vasculature of apoE^{-/-} mice on a Western diet. Imaging performed 10 min after intravenous bolus of 10^7 bubbles, with Siemens Sequoia apparatus (15L8 probe, 7 MHz, CPS mode, image frame 1.5 cm tall)