The powerful microbubble: from bench to bedside, from intravascular indicator to therapeutic delivery system, and beyond

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Feinstein, Steven B. The powerful microbubble: from bench to bedside, from intravascular indicator to therapeutic deliver system, and beyond. Am J Physiol Heart Circ Physiol 287: H450–H457, 2004; 10.1152/ajpheart.00134.2004.—This review discusses the development, current applications, and therapeutic potential of ultrasound contrast agents. Microbubbles containing gases act as true, intravascular indicators, permitting a noninvasive, quantitative analysis of the spatial and temporal heterogeneity of blood flow and volumes within the microvasculature. These shelled microbubbles are near-perfect reflectors of acoustic ultrasound energy and, when injected intravenously into the bloodstream, reflect ultrasound waves within the capillaries without disrupting the local environment. Accordingly, microbubble ultrasound contrast agents are clinically useful in enhancing ultrasound images and improving the accuracy of diagnoses. More recently, ultrasound contrast agents have been used to directly visualize the vasa vasorum and neovascularization of atherosclerotic carotid artery plaques, thus suggesting a new paradigm for diagnosis and treatment of atherosclerosis. Future applications of these microscopic agents include the deliver of site-specific therapy to targeted organs in the body. Medical therapies may use these microbubbles as carriers for newer therapeutic options.

MICROBUBBLES CONTAINING GASES are near-perfect reflectors of acoustic ultrasound energy. Liquid suspensions of stable, gas-filled microbubbles may be used to enhance ultrasound images because, when they are injected into the bloodstream, they reflect ultrasound waves as they traverse the smallest human blood vessels, the capillaries, without disrupting the local environment (Fig. 1) (23). Accordingly, microbubble ultrasound contrast agents are clinically useful in enhancing ultrasound images and improving the accuracy of diagnoses. More recently, ultrasound contrast agents have been used to visualize the carotid artery vasa vasorum and neovascularization of atherosclerotic plaques, suggesting a new paradigm for analyzing the development of atherosclerosis and presenting new possibilities for the development of treatment options. In addition, medications or genes may be attached to an ultrasound contrast agent and subsequently used to deliver site-specific therapy to targeted organs in the body. This editorial will discuss the development, current applications, and therapeutic potential of ultrasound contrast agents.

BACKGROUND

It is unlikely that Lord Rayleigh (73, 86) could have anticipated that the calculations he developed related to the wave reflection and spheres would, one day, serve as a scientific basis for analyzing human microvascular physiology. However, based in part, on the works of Rayleigh, Ishimaru (33), Morse and Ingard (73), Powsner and Feinstein in 1986 (85) devised an in vitro experiment to quantitatively predict the reflected acoustic energy emanating from a group of random, micron-sized scatters (microbubbles). These investigators concluded that acoustic scattering of microbubbles could be predictably determined and ultimately used to a quantitative determination of microvascular blood flow and blood volume in patients (Fig. 2).

Microbubbles of air are ubiquitous within living organisms; however, it is uncommon for microbubbles to persist for any significant length of time due to powerful external compressive forces that act on the surface of the microbubble. Naturally occurring microscopic gases found within living systems are generally stabilized with organic matter, thus creating a film or shell. When tissues are radiated with ultrasound energy, the presence of a microbubble substantially alters acoustic reflectivity. A common source of microbubble production is from a process called cavitation as described by Willard in 1953 (115): “Cavitation is a subject of importance in such diverse fields as medicine, hydraulics, water-borne transportation and signaling, industrial cleaning of precision parts, and in the materials processing industries, as well as in the basic sciences” (p. 669).

Cavitation, an energy-dependent process, results from the “shearing” of matter (liquid) altering the surface tension and disrupting the cohesive properties of the medium. Microbubbles produced from cavitation include processes that involve electrical, thermal, mechanical (hydraulic), or acoustic energy. In the marine environment, turbulence from a ship’s propeller creates microbubbles by hydrodynamic cavitation, which results in erosion of the propeller blades, decreased efficiency, and creates a “target” for sonar detection. As a practical matter, the presence of cavitation microubbles within the human body is generally thought to be undesirable; however, cavitation microbubbles have been associated with prosthetic cardiac valves (91) and high-speed catheter-based infu-
sions (e.g., diagnostic angiography). A predictive unit termed the Thomal number was devised to avoid catheter-tipped induced cavitation microbubbles in patients undergoing arteriography procedures (53, 119).

**MICROBUBBLE ACOUSTICS**

Conventionally, the spatial resolution of an ultrasound field is approximately one quarter the wavelength of transducer frequency (i.e., a 7.4-MHz transducer would have an axial resolution of \( \frac{1}{4} \) mm). However, when imaging air-filled microbubbles, the nonlinear factors (harmonics) impact the amplitude of the scattering properties. These acoustic factors include 1) medium properties: surface tension, viscosity, thermal transfers, and liquid and gas vapor pressures; 2) gas properties: molecular weight and gas solubility; 3) shell properties: elastic modulus, thickness, and damping effects; 4) ultrasound transceiver: high-speed digital interfaces (16, 84, 85), harmonics (70, 71, 90), and ultra- and subharmonics (10).

**HISTORICAL IMPORTANCE OF DETECTING AND QUANTIFYING MICROBUBBLES IN LIVING SYSTEMS**

Contrary to the prevailing belief that micrometer-sized microbubble acoustic interference patterns are beneath the “radar” screen of the clinical transceivers, Mackay and Robissow in 1978 (62) described the interference patterns generated from individual 5-μm-diameter microbubbles. Their work was instrumental in predicting and monitoring decompression illness in deep-sea diving and, later, in extravehicular space travel. Traditionally, the earliest detection of decompression relied upon audible Doppler signals described as “chirping” to predict the presence of microbubbles in the bloodstream signifying decompression illness. A model to study decompression illness in primates was proposed by Sandler in 1986. In his research proposal, concentrations of sonicated albumin microbubbles were to be peripherally injected while using an external Doppler detection device. These studies were designed to develop a “look-up” table to predict the onset of decompression illness in astronauts.

**EARLY CLINICAL OBSERVATIONS**

Gramiak and Shah in 1968 (28) and Feigenbaum et al. in 1970 (19) observed ultrasound contrast effects on M-mode echocardiography recordings following the use of agitated indocyanine green in the catheterization laboratory. Subsequently, considerable academic research interests focused on efforts to characterize the ultrasound contrast effects [Bove et al. (11), Meltzer et al. (65–67), Ophir et al. (79), Kort and Kronzon (52), Kremkau et al. (53), and Ziskin et al. (119)]. Bove et al. (11), using in vitro studies, generated microbubbles from hydrodynamic cavitation and detected these bubbles using conventional ultrasound systems. In 1982, after these investigative in vitro studies, Armstrong et al. (2) and Tei et al. (99, 100) independently reported on the use of microbubbles for quantitation of myocardial perfusion. Additionally, DeMaria et al. in 1984 (17) used videodensitometry to noninvasively assess cardiac output with microbubbles. These initial in vitro and in vivo experimental studies, along with the development of commercial ultrasound contrast agents, presaged an imaging technology capable of providing real-time, microvascular physiology.

**DEVELOPMENT OF ULTRASONIC CONTRAST AGENTS**

The development of small, relatively stable microbubbles capable of unhindered transpulmonary passage was reported by Bommer et al. in 1979 (8, 9), Reale et al. (87), Meltzer et al. in 1980 (65, 66), and Ten Cate et al. in 1984 (102). And in the...
early 1980’s, Feinstein et al. (24) created small, stable microbubbles using a novel method termed sonication to generate “by-product” microbubbles of cavitation (115). The first direct, in vivo measurement of the microbubbles within the intact mammalian capillaries was published in 1984 (23) (see Fig. 1) and subsequently confirmed by Cheirif et al. in 1987 (13). Subsequently, numerous ultrasound contrast agents have been developed with several approved by the FDA. In the early 1990’s, Echovist, one of the first commercial ultrasound contrast agents, was approved in Europe for enhancement of the right heart structures. After the results of a multicenter trial in 1990 (21), Albunex became the first FDA-approved, commercial ultrasound contrast agent in the United States in 1994.

In 2003–2004, there are numerous companies with a variety of ultrasound contrast agents that are either approved or in clinical trials awaiting approval. Table 1, modified from Becher and Burns (7), lists several current ultrasound contrast agents.

### QUANTITATION OF MICROVASCULAR BLOOD FLOW AND VOLUME

The indicator-dilution theory by Mierer (64) and Zierler (118) states that intravascular indicators follow these principles: First, the agent must exhibit a quantifiable signal response when radiated by ultrasound energy. With regard to microbubbles, rigorous in vitro and in vivo experimental data established that a quantifiable (monotonic) acoustic response exists between the microbubbles (indicators) and the ultrasound transducer (external receiver).

Second, indicators conserve mass. The earliest microbubbles, however, exhibited a loss of indicator (bubble destruction) induced by the acoustic energy. With the subsequent development of the harmonic imaging systems, substantially lower acoustic energy was required for imaging of the microbubbles, thus reducing the loss of the indicator.

Finally, an intravascular indicator should not alter the underlying microvascular physiology. Because many of the earlier agents were hyperosmolar, a hyperemic response ensued after intravascular injections (51). With improvements in the ultrasound contrast agents and harmonic imaging the limitations are no longer relevant. Wienick et al. in 1993 (114) provided an in depth discussion of the early technical limitations of performing quantitative myocardial perfusion imaging with the first-generation microbubbles. Once the microbubbles had been shown to conform to the indicator dilution principles, analyses of tissue perfusion were possible by mathematically substituting the calibrated signal response for the indicator concentration within the vasculature.

### MICROBUBBLES AS PHYSIOLOGICAL INDICATORS OF MICROVASCULAR BLOOD FLOW AND BLOOD VOLUME

In 1987, Segil (92–94) first reported on the quantitation of microvascular blood volume using contrast echo techniques (20). These studies used two experimental conditions: intracoronary infusions of adenosine and normocapnia hypoxia, to distinguish the covarying parameters of microvascular blood volume and microvascular blood flow. The results demonstrated a comparable increase in arteriolar blood flow as measured by radiolabeled microspheres and microbubbles; however, a discordance was noted in the microvascular blood volumes. This “apparent” discrepancy became the subject of intense scientific scrutiny over the next few years. First, Jayaweera et al. (39) confirmed that the ultrasound contrast agent (air-filled albumin microspheres) exhibited microvascular transit properties similar to those of the red blood cells and not plasma (26). Subsequently, in vivo indicator functions of the microbubbles were validated using independent measurement techniques developed by Crystal et al. (14a). These studies assessed the microvascular hematocrit using chromium-labeled \((^{51}\text{CrO}_4\)^–\) red blood cells, the microvascular blood volume using \(^{55}\text{FeCl}_3\) labeled siderophilin, and radiolabeled microspheres \((15 \pm 5 \mu m)\) to measure perfusion. Kaul and associates \((40–46, 111, 112)\) and Porter et al. \((81–83)\) provided additional seminal, experimental validation results. The use of radiolabeled microspheres \([>15 \mu m (108)]\) resulted in indicator “trapping” at the precapillary level, thus precluding assessment of dynamic changes in the postcapillary blood volume. Microbubbles appear to be ideal intravascular indicators for assessment of the dynamic spatial and temporal heterogeneity of tissue microvasculature [Sestier et al. (95)].

### INITIAL CLINICAL APPLICATIONS OF CONTRAST ECHOCARDIOGRAPHY

On the basis of recommendations of the American Society of Echocardiography in 2000, the current clinical indications for the use of ultrasound contrast agents in 2000 includes opacification of the left heart structures \((75)\). The indication for assessment of myocardial perfusion awaits FDA approval.

### CLINICAL STUDIES OF MYOCARDIAL PERFUSION USING CONTRAST ECHO

Monaghan et al. in 1988 \((70, 71)\) were the first to identify a unique “acoustic signature” based on a frequency shift of back-scattered ultrasound from intra-arterially injected in patients undergoing a cardiac catheterization. Absorption or attenuation of the signal did not account for this “frequency

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**Table 1. Current ultrasound contrast agents**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Company</th>
<th>Design Characteristics</th>
<th>Approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1-700</td>
<td>Acusphere</td>
<td>Polymer/perfluorocarbon</td>
<td>No</td>
</tr>
<tr>
<td>Imavist</td>
<td>Alliance/Photogen</td>
<td>Surfactant/perfluorohexane-air</td>
<td>USA</td>
</tr>
<tr>
<td>SonoVue</td>
<td>Bracco</td>
<td>Phospholipid/sulphur</td>
<td>Europe</td>
</tr>
<tr>
<td>Definity</td>
<td>Bristol-Meyers-Squibb</td>
<td>Liposome/perfluoropropane</td>
<td>USA</td>
</tr>
<tr>
<td>Albunex</td>
<td>Molecular Biosystems</td>
<td>Albumin/air</td>
<td>USA</td>
</tr>
<tr>
<td>Optison</td>
<td>Molecular Biosystems/Amersham</td>
<td>Perfurther protein-type A perfuoropropane</td>
<td>USA, Europe</td>
</tr>
<tr>
<td>Bisphere</td>
<td>Point Biomedical</td>
<td>Albumin/polymer bilayer air</td>
<td>No</td>
</tr>
<tr>
<td>Echovist</td>
<td>Schering</td>
<td>Galatose particulate</td>
<td>Europe</td>
</tr>
<tr>
<td>Levovist</td>
<td>Schering</td>
<td>Lipid/air</td>
<td>Europe, Japan</td>
</tr>
</tbody>
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shift” from the myocardial tissue. This novel work ultimately lead to the development of harmonic ultrasound imaging capitalizing on harmonic properties of microbubbles within the myocardium. Curiously, the observed microbubble harmonics led to the realization that myocardial tissue exhibits an intrinsic “harmonic” signal (although tissue harmonics produce substantially less amplitude and frequency shift compared with the intravascular microbubbles).

_Preliminary experimental data_. Excellent preclinical experimental data have been produced over the years by such researchers as Armstrong et al. (2), Tei et al. (100), Kaul et al. (46), and Ten Cate et al. (102). These experimental data established the physiological basis from which future clinical perfusion imaging would be developed.

_Initial clinical studies_. The initial clinical studies using contrast echocardiography techniques for assessing myocardial perfusion have been described by Smith et al. (97), Feinstein et al. (22), Moore et al. (72), Monaghan et al. (69–71), Ten Cate et al. (101) (72), Cheirif et al. (14), and Goldman and Mindich (27). To identify myocardial perfusion patterns with contrast echo, these studies used direct intracoronary artery injections of sonicated Renografin-76. With the advent of more stable ultrasound contrast agents capable of traversing the lung capillaries and with the implementation of harmonic imaging, real-time myocardial perfusion was achieved. More recent clinical studies of myocardial perfusion with contrast echocardiography using corroborative imaging modalities have been reported by Dawson et al. (15), Cheirif et al. (12, 14), Porter et al. (81, 82), Spotnitz and Kaul (98), and Aronson et al. (4).

_Myocardial perfusion and microvascular “no-reflow”_. Clinically, the no-reflow phenomenon refers to reversible spasm of the epicardial vessels seen during coronary angiography (80). At the microcirculatory level, no-reflow refers to a myriad of cellular events leading to cell death (50). The initial event may be swelling of the myocardial cells due to lactate build up, hydrolysis of high-energy phosphate bonds, and eventual endothelial cell “blistering,” mitochondrial calcium influx, and endothelial damage. On the basis of early experimental data using contrast echo techniques, contrast within the microvasculature indicated a functional microvasculature. As a corollary, if the microvasculature revealed a contrast defect, microvascular viability did not exist. Ito et al. in 1992 (37) first used myocardial contrast echocardiography (MCE) techniques to identify microvascular no-reflow phenomena in patients undergoing cardiac catheterization after an acute myocardial infarction. Ito et al. and subsequently Kenner et al. in 1995 (48) found that ~25–30% of the left ventricular myocardial regions subtended by the “culprit” coronary artery revealed residual microvascular no reflow. These data were of interest because the culprit coronary artery had been restored to Thrombolysis in Myocardial Infarction grade III status and revealed a discrepancy between the patency of the coronary anatomy and the microvascular perfusion. In follow-up studies, Ito et al. concluded that tissue viability as assessed by contrast echo techniques predicted clinical outcomes [i.e., chronic heart failure, recurrent angina, or death (35, 36)]. In addition, Ikawara et al. (38) have shown that the microvascular no-reflow observed by MCE corresponded to an impaired flow reserve, as demonstrated by Doppler flow wire. The predictive value of using contrast echo to define tissue viability based on collateral vessels post myocardial infarction was reported by Sabia et al. in 1992 (88, 89). In their study, MCE techniques were used to prospectively assess the clinical utility of performing therapeutic coronary artery interventions (PCI) in patients that had a prior myocardial infarction. Subsequently, Agati et al. (1), using MCE, showed that larger areas of microvascular no-reflow defects were correlated with the use of thrombolysis therapy versus direct coronary intervention in patients studied at the time of an acute myocardial infarction. These studies reveal that contrast echo techniques define myocardial tissue viability and, as such, may be used to predict clinical outcomes.

_Reversal of the no-reflow phenotype_. With regard to pharmacological intervention for the treatment of experimentally induced microvascular no-reflow, Nayler and colleagues in 1988 (18, 76) reported that the influx of calcium into the mitochondria was a terminal cellular event. Hickle et al. (32) described the first use of intracoronary calcium channel blockers to reverse microvascular no-reflow using contrast echo techniques in patients after an acute myocardial infarction. In the future, it may be possible to initially use contrast echo techniques to diagnose the extent of the tissue damage and subsequently provide an assessment of therapeutic efficacy (i.e., PCI, thrombolysis, pharmacological agents).

**CLINICAL NONCARDIAC IMAGING**

With the availability of commercial contrast agents and the concomitant use of harmonic ultrasound imaging systems, contrast ultrasound has been used for organ imaging of liver tumors (117), renal perfusion (3, 5, 55, 110), breast tumors (47), prostate cancer (30), and venous thrombi (104).

**REAL-TIME IMAGING OF CAROTID ARTERY LUMEN, PLAQUE MORPHOLOGY, CAROTID INTIMA-MEDIA THICKNESS, AND Atherosclerotic PLAQUE NEOVASCULARIZATION**

The use of ultrasound contrast agents when used to enhance the lumen of the carotid artery permit a clearer visualization of the intima-media thickness and plaque luminal morphology (Fig. 3) (61). Most recently (2003), contrast ultrasound has been used to study the neovascularization within human carotid atherosclerotic plaques (Figs. 4) (77). MRI studies have provided corroborative data confirming the noninvasive visualization of carotid plaque neovascularization (49). On the basis of these data, the role of the vasa vasorum in the genesis of atherosclerosis appears to be undergoing a paradigm shift. That is, it appears that the neovascularization within the atherosclerotic plaque originate from the adventitial surfaces of the vessels as apposed to the lumen surfaces. The pathology reports of Barger et al. (6) and Kumamoto et al. (54) support the findings of the current in vivo contrast ultrasound imaging data. Furthermore, these clinical observations were described in experimental studies of the vasa vasorum reported by Heistad et al. (31), Wilson et al. (116), and Moulton (74). Recently, Gutterman (29) discussed therapeutic approaches of atherosclerosis utilizing pericardial infusions of angiogenesis agents that would impact the vasa vasorum and affect change of the neovascularization within the atherosclerotic plaques. Moreover, the presence of neovascular vessels within the intima correlate with obstructive clinical events (heart attack and stroke in patients) (68). And, as described by Folkman in 1971 (25) and
Isner et al. in 1995 (34), plaque angiogenesis appears to provide a link between atherosclerosis and cancer, such that local factors (tissue ischemic and hypoxic factors) appear to stimulate a variety of growth factors (i.e., VEGF and platelet-derived growth factor), ultimately leading to neovascularization and tumor growth. With the use of ultrasound contrast agents as local drug-delivery systems, it may be possible to directly “target” the coronary and carotid vasa vasorum and deliver antiangiogenesis medications or genes.

FUTURE OF DIAGNOSTIC AND THERAPEUTIC CONTRAST ULTRASOUND

The words of Daniel Burnham can be used to describe the future of contrast ultrasound, when he said “Make no little plans; they have no magic to stir men’s blood” (Columbian Exposition, 1893).

Ten Cate recognized that microbubbles exposed to substantial acoustic energy lost their “contrast effect” presumably due to disruption and therefore might be harnessed to deliver site-specific drug therapies (personal communication). Additional interesting work using acoustics to deliver drugs was reported in 1984 when Negishi et al. (78) used heat generated from externally applied ultrasound energy to release drugs from a matrix (“acousticochemical action”). And Ishihara in 1988 (33) used large-amplitude ultrasound resonance frequencies to release drugs from microcapsules (“drug carriers”). Historically, ultrasound energy was used to deliver drugs across the skin surfaces (sonoporation). However, the intracellular delivery of drugs/genes is more efficient when using microbubbles as cavitation sources.

Tachibana described that the therapeutic use of bubbles in the treatment of cancer dates back to about 1972. Subse-
quently, researchers have explored numerous new applications for unique properties of these microbubbles including use in cellular targeting of inflammation, gene delivery, and localizing cellular inflammation: targeting of cellular inflammation (58–60, 109), liposomal drug delivery (105–107), targeted therapies (56, 57, 103, 113), and myocardial gene delivery systems (96).

These site-specific, ultrasound-directed therapies utilize microbubbles as acoustic “microjets” resulting in a microgene gunshot permitting macromolecules to effectively cross the vascular endothelial borders and enter the cytoplasm.

The microbubble as a diagnostic imaging agent provides clinicians a unique opportunity to noninvasively image both the “macrovascular” anatomy (i.e., heart, aorta, and carotid arteries) and, importantly, the “microvascular” physiology. In the future, treatment plans may involve the use of microbubbles for ultrasound-directed, site-specific therapies (drug/gene) providing individualized treatment plans for patients. These diagnostic and therapeutic delivery systems can be performed at bedside and with minimal patient discomfort. The consummate use of these advanced diagnostic and therapeutic ultrasound systems will provide physicians with an unparalleled ability to diagnose and manage disease processes, thus providing for our patients the parameters necessary for improved patient care.

REFERENCES

12. Cheirif J, Desir RM, Bolli R, Mahmalian JJ, Zoghbi WA, Verani MS, and Quinones MA. Relation of perfusion defects observed with myocardial contrast echocardiography to the severity of coronary steno-


