After more than 20 years of development and a somewhat hesitant start, microbubbles have finally come of age as a contrast agent for ultrasound. In 2016 the United States was finally added to the more than 50 countries worldwide that have approved them for radiologic indications, with the Food and Drug Administration (FDA) announcing approval for their use for liver diagnosis in adults and children. Their exploitation has already opened many new areas for diagnostic ultrasound imaging and is becoming a central area for technologic advances of the modality. The bubbles themselves comprise small spheres of a gas with low solubility in blood—such as a perfluorocarbon—stabilized by a thin shell layer of a flexible, biocompatible material which is typically a lipid, although proteins and polymers are also used. The diameter of the resulting encapsulated bubble is about 3 to 5 µm, slightly smaller than a red blood cell (Fig. 3.1). Because of this size, bubbles provide a pure intravascular contrast agent (Fig. 3.2). A suspension of bubbles in water is injected into a peripheral vein in the arm or hand; a typical whole-body human dose ranges from 0.2 to 2 mL in volume and contains on the order of 10 million bubbles, roughly comparable to the number of red blood cells in a single milliliter of blood. The effect of a bolus injection is to increase the echo from blood by a factor of 500 to 1000. After about 5 minutes the gas from the bubbles has diffused into the blood and the very small mass of shell material is metabolized. By infusing the bubbles through a saline drip, a steady enhancement lasting up to 20 minutes can also be obtained.¹ Microbubble contrast agents make possible for the first time ultrasound imaging of organ and lesion perfusion...
in real time. This chapter aims to provide both a tutorial and a reference for the practical use of contrast agents for these indications.

### REQUIREMENTS AND TYPES

The principal requirements for an ultrasound contrast agent are that it should be easily introducible into the vascular system, be stable for the duration of the diagnostic examination, have low toxicity, and modify one or more acoustic properties of tissues that can be detected by ultrasound imaging. Although it is conceivable that applications will be found for ultrasound contrast agents that will justify their injection into arteries, the clinical context for contrast ultrasonography requires that these agents be capable of intravenous administration and intact passage through the heart and lungs. These are demanding specifications that have been met only in the past decade. The technology universally adopted is that of encapsulated bubbles of gas that are smaller than red blood cells and therefore capable of circulating freely in the pulmonary and systemic vasculature.

Contrast agents act by their presence in the vascular system, from where they are ultimately metabolized (“blood pool” agents), or by their selective uptake in tissue after a vascular phase. Of the properties of tissue that influence the ultrasound image, the most important are linear and nonlinear backscatter coefficient, attenuation, and acoustic propagation velocity. Most agents enhance the echo by increasing as much as possible the backscatter of the tissue that bears them, while increasing the attenuation.
in the tissue as little as possible, thus enhancing the echo from blood. More important, they change the nature of the echo from blood in a way that allows them to be imaged selectively in real time.

**Blood Pool Agents**

**Free Gas Bubbles**

Gramiak and Shah first used bubbles to enhance the blood echo in 1968. They injected agitated saline into the left ventricle during an echocardiographic examination and saw strong echoes within the lumen of the aorta. It was subsequently shown that these echoes originated from free bubbles of air that came out of solution either during agitation or at the catheter tip during injection. Agitated solutions of compounds such as indocyanine green and Renografin—already approved for intraarterial injection—were also used. The application of free gas as a contrast agent was confined to the heart, including evaluation of valvular insufficiency, intracardiac shunts, and cavity dimensions. The fundamental limitation of bubbles produced in this way is that they are large, so they are effectively filtered by the lungs, and unstable, so they go back into solution on the order of a second. Apart from occasional use in the echocardiography laboratory to identify shunts, free bubbles are rarely used as a contrast agent today.

**Encapsulated Air Bubbles**

To overcome the natural instability of free gas bubbles, various shell coatings were developed to create a more stable particle (Table 3.1). In 1980 Carroll and colleagues encapsulated nitrogen bubbles in gelatin and injected them into the femoral artery of rabbits with VX2 tumors in the thigh. Although echo enhancement of the tumor rim was identified, the large diameter of the coated bubbles (80 µm) precluded administration by an intravenous route. The challenge to produce a stable encapsulated microbubble of a comparable size to that of a red blood cell and that could survive passage through the heart and the pulmonary capillary network was first met by Feinstein and colleagues in 1984, who produced microbubbles by sonication of a solution of human serum albumin and showed that it could be detected in the left side of the heart after a peripheral venous injection. This agent was subsequently developed commercially as Albonex (Mallinckrodt Medical, Inc., St. Louis, MO).

Another approach to stabilizing an air bubble is to add a lipid shell upon dissolution of a dry powder. Levovist (Scher- ing AG, Berlin, Germany), is a dry mixture comprising 99.9% microcrystalline galactose microparticles and 0.1% palmitic acid. On dissolving in sterile water, the galactose disaggregates into microparticles, which provide an irregular surface for the adherence of microbubbles 3 to 4 µm in size. Stabilization of the microbubbles takes place as they become coated with palmitic acid, which separates the gas/liquid interface and slows their dissolution. The resulting microbubbles have a median bubble diameter of about 3 µm with the 97th percentile at approximately 6 µm and are sufficiently stable for transit through the pulmonary circuit. The agent is chemically related to its predecessor Echovist (Schering), a galactose agent that forms larger bubbles and that has been used principally for visualization of nonvascular ductal structures such as the fallopian tubes. Numerous early

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**TABLE 3.1 Regulatory and Marketing Status of Some Current Ultrasound Contrast Agents as of 2016**

<table>
<thead>
<tr>
<th>Name</th>
<th>Company/Developer</th>
<th>Composition (Shell/Gas)</th>
<th>REGULATORY/MARKETING STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definity</td>
<td>Lantheus Medical Imaging¹⁰</td>
<td>Lipid/perfluoropropane</td>
<td>Cardiology Indications: Approved in United States, Canada</td>
</tr>
<tr>
<td>SonoVue</td>
<td>Bracco¹¹</td>
<td>Phospholipid/sulfur hexafluoride</td>
<td>Radiology Indications: Approved in Canada, China, Australasia, Americas</td>
</tr>
<tr>
<td>Lumason</td>
<td></td>
<td></td>
<td>Approved in Europe, Union, Canada</td>
</tr>
<tr>
<td>Optison</td>
<td>GE Healthcare¹²</td>
<td>Sonicated albumin/octafluoropropane</td>
<td>Approved in Europe, Union, United States, Canada</td>
</tr>
<tr>
<td>Sonazoid</td>
<td>GE Healthcare and Daiichi Sankyo³</td>
<td>Lipid perfluorobutane</td>
<td>Clinical development</td>
</tr>
<tr>
<td>biSphere</td>
<td>Point Biomedical⁴</td>
<td>Polymer bilayer/air</td>
<td>Suspended development</td>
</tr>
<tr>
<td>Imagify</td>
<td>Acusphere⁵</td>
<td>Polymer/perfluorobutane</td>
<td>Clinical development</td>
</tr>
<tr>
<td>PESDA</td>
<td>Porter¹⁶</td>
<td>Sonicated albumin/perfluorocarbon</td>
<td>Not commercially developed</td>
</tr>
<tr>
<td>BR55</td>
<td>Bracco¹⁷</td>
<td>Lipopeptide VEGFR-2-targeted/perfluorocarbon</td>
<td>Suspended development</td>
</tr>
</tbody>
</table>

VEGFR, Vascular endothelial growth factor receptor.
studies with Levovist\textsuperscript{21,22} demonstrated its capacity to traverse the pulmonary bed in sufficient concentrations to enhance both color and spectral Doppler signals, as well as gray-scale examinations using nonlinear imaging modes such as pulse inversion imaging. Levovist remains approved for use in the European Union, Canada, Japan, and numerous other countries, although not the United States. Many clinical applications of intravenous contrast were pioneered using Levovist, which has now given way to the so-called “second-generation” agents and is no longer marketed.

**Second-Generation Agents**

Second-generation agents were designed both to increase backscatter enhancement and to last longer in the bloodstream by taking advantage of low-solubility gases such as perfluorocarbons. These heavier gases diffuse more slowly through the bubble shell and have much lower solubility in blood. Optison (GE Healthcare, Milwaukee, WI) (see Fig. 3.1A) is a perfluoropropane-filled albumin shell with a size distribution similar to that of its predecessor, Albunex. It is currently approved for cardiology indications in the European Union, the United States, and Canada. SonoVue (Bracco, Milan, Italy) uses sulfur hexafluoride in a phospholipid shell and is available for cardiology and radiology indications in the European Union, China, and a number of other countries; it is approved in the United States under the name Lumason. Definity (Lantheus Medical Imaging, North Billerica, MA) (see Fig. 3.1B) comprises a perfluoropropane microbubble coated with a flexible bilipid shell, which also showed improved stability and high enhancement at low doses.\textsuperscript{23} It is currently approved for cardiology and radiology indications in Canada, Australasia, and a number of Central and South American countries, and for cardiology indications in the United States. Finally, Sonazoid (Daiichi Sankyo, Tokyo, Japan and GE Healthcare, Milwaukee, WI) consists of a perfluorobutane bubble in a lipid shell\textsuperscript{24} and is currently approved for radiology indications in countries such as Japan and Korea. It should be noted that although these bubbles are very small, they are large when compared with the molecules and particles used as contrast agents for computed tomography (CT) and magnetic resonance imaging (MRI), which diffuse through the fenestrated endothelium of blood vessels into the interstitium. Thus x-ray and magnetic resonance contrast-enhanced images frequently show an “interstitial” or “parenchymal” phase of enhancement, which may be used to identify hyperpermeable vascular structures, such as those involved with tumor angiogenesis.\textsuperscript{25} Microbubbles, on the other hand, are of a size comparable to that of a red blood cell, so they go where a red blood cell goes (see Fig. 3.2) and, more important, do not go where a red blood cell does not go. They are clinical radiology’s first pure blood pool contrast agent.

**Selective Uptake Agents**

An ideal blood pool agent displays the same flow dynamics as blood itself, and is ultimately metabolized from the blood pool. Agents such as Definity, SonoVue, and Optison are generally not detected outside the vascular system, and therefore come close to this ideal. Contrast preparations can be made, however, that are capable of providing ultrasound enhancement during their metabolism as well as while in the blood pool. Colloidal suspensions of liquids droplets such as perfluorocyclobromide\textsuperscript{26} and microbubble agents with certain shell properties\textsuperscript{27,28} are taken up by the reticuloendothelial system, from where they ultimately are excreted. There they may provide contrast from within the liver parenchyma, demarking the distribution of Kupffer cells.\textsuperscript{29} Agents such as Levovist and Sonazoid provide “late-phase” enhancement in the parenchyma of the liver and spleen after having cleared from the vascular system,\textsuperscript{30} allowing detection of Kupffer cell–poor lesions such as cancers.\textsuperscript{31,32}

A typical dose of an ultrasound contrast agent is on the order of 10s of microliters of bubble suspension per kilogram of body weight, so a whole-body dose might be on the order of 0.1 to 1 mL. Fig. 3.3 shows the enhancement of the echo from systemic arterial blood after a peripheral venous injection of a second-generation agent. A first-pass peak occurs, followed by recirculation and washout as the agent is eliminated over the next few minutes. By infusing the bubbles through a saline drip or pump, a steady enhancement lasting up to 20 minutes can be obtained.\textsuperscript{1} The small amount of perfluorocarbon gas goes into solution in the blood and is ultimately excreted by the lungs and liver. The trace amount of shell material is reduced to biocompatible elements that, in the case of the commonly used agents, are already present in the blood.\textsuperscript{33}

**THE NEED FOR BUBBLE-SPECIFIC IMAGING**

One of the major diagnostic objectives in using an ultrasound contrast agent in a solid organ is to detect flow at the perfusion—that is, the arteriolar and capillary—level. The peak enhancement in Fig. 3.3 is more than 30 dB, corresponding to a 1000-fold increase in the power of the ultrasound echo from blood. Although this may seem impressive, it does not necessarily help ultrasound to image perfusion. The echoes from blood associated with such flow, in the hepatic sinusoids for example, exist in the midst of echoes from the surrounding solid structures of the liver parenchyma, echoes that are almost always stronger than even the contrast-enhanced blood echo. When they can be seen, blood vessels in a nonenhanced image have a low echo level, so an echo-enhancing agent actually lowers the contrast between blood and the surrounding tissue, making the lumen of the blood vessel less visible. Thus in order to be able to image flow in small vessels of the liver, a contrast agent is required that either enhances the blood echo to a level that is substantially higher than that of the surrounding tissue or can be used with a method for suppressing the echo from noncontrast-bearing structures. Bubble-specific imaging methods provide this capability.

**Bubble Behavior and Incident Pressure**

The key to understanding contrast-specific imaging modes—and the key to their successful clinical use—lies in the unique interaction between a microbubble contrast agent and the process that images it. Controlling and exploiting this interaction is central to all contrast-specific methods. Unlike tissue, microbubbles
scatter ultrasound in a manner dependent on the amplitude of the sound to which the imaging process exposes them. The results are three broad types of bubble behavior, with three broad types of resulting echoes (Table 3.2). The types of bubble behavior depend primarily on the intensity (more precisely, the peak negative pressure and frequency) of the incident sound field produced by the scanner. At low incident pressures (corresponding to low transmit power of the scanner), the agents produce linear backscatter enhancement, resulting in an augmentation of the echo from blood. This is the behavior originally envisaged by contrast agent manufacturers. As the transmit intensity control of the scanner is increased and the negative pressure incident on a bubble goes beyond about 50 to 100 kPa, which is still below the level used in most diagnostic scans, the contrast agent backscatter begins to show nonlinear characteristics, such as the emission of harmonics. It is the detection of these that forms the basis of contrast-specific imaging modes such as harmonic and pulse inversion imaging. Finally, as the peak pressure passes about 300 kPa (or 0.3 MPa) and approaches the level emitted by a typical ultrasound imaging system in conventional B-mode imaging, bubbles produce a strong but brief echo as they are disrupted by the ultrasound beam. This behavior forms the basis of the most common way of quantifying perfusion. It should be noted that in practice, because of the different sizes present in a realistic population of bubbles and the additional effect of frequency, the borders between these behaviors are not sharp. Nor will they be the same for different bubble types, the acoustic behavior of which is strongly dependent on the gas and shell properties.

To reiterate, three types of behavior of bubbles in an acoustic field have been identified and depend on the amplitude and frequency of the transmitted ultrasound beam. In practice, this exposure is best monitored by means of the mechanical index (MI) displayed by the scanner. At very low MI, the bubbles act as simple but powerful echo enhancers. This is most useful for spectral Doppler enhancement but is rarely used in the

### TABLE 3.2 Three Types of Acoustic Behavior of a Typical Perfluorocarbon, Lipid-Shelled Agent in an Ultrasound Field

<table>
<thead>
<tr>
<th>Peak Pressure (Approximate)</th>
<th>Mechanical Index (MI) at 2 MHz</th>
<th>Bubble Behavior</th>
<th>Acoustic Behavior</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100 kPa</td>
<td>&lt;0.07</td>
<td>Linear oscillation</td>
<td>Linear backscatter enhancement</td>
<td>Doppler signal enhancement</td>
</tr>
<tr>
<td>0.1-0.3 MPa</td>
<td>0.07-0.2</td>
<td>Nonlinear oscillation</td>
<td>Nonlinear backscatter</td>
<td>Real-time (low-MI) perfusion imaging</td>
</tr>
<tr>
<td>&gt;0.5 MPa</td>
<td>&gt;0.4</td>
<td>Disruption</td>
<td>Transient nonlinear echoes</td>
<td>Triggered perfusion or disruption-replenishment flow measurement</td>
</tr>
</tbody>
</table>
abdominal organs. At slightly higher intensities (the bottom of the range of those used diagnostically), the bubbles emit harmonics as they undergo nonlinear oscillation. These nonlinear echoes can be detected by contrast-specific imaging modes, which generally rely on trains of low-MI pulses modulated in phase and/or amplitude. Pulse inversion imaging is an example of such a method. Finally, at the higher intensity settings, comparable to those used in conventional scanning, the bubbles can be disrupted deliberately, creating a strong, transient echo. Detecting this echo with harmonic power Doppler is one of the most sensitive means available to image bubbles in very low concentrations, but it comes at the price of destroying the bubble. Because of the long replenishment periods of tissue flow, intermittent imaging using an “interval delay” in which the high-MI imaging is arrested becomes necessary. Disrupting bubbles offers a unique method for quantifying perfusion at the tissue level.

The Mechanical Index

For reasons unrelated to contrast imaging, ultrasound scanners marketed in the United States are required by the FDA to carry an on-screen label of the estimated normalized peak negative pressure to which tissue is exposed. Of course, this pressure changes according to the tissue through which the sound travels as well as the amplitude and geometry of the ultrasound beam: the higher the attenuation, the less the peak pressure in tissue will be. A scanner cannot “know” what tissue it is being used on, so the arrived-at definition of an index reflects the approximate exposure to ultrasound pressure at the focus of the beam in an average tissue. The mechanical index (MI) is defined as the peak rarefational (i.e., negative) pressure divided by the square root of the ultrasound frequency. This quantity is related to the amount of mechanical work that can be performed on a bubble during a single negative half-cycle of sound and is thought to give an indication of the propensity of the sound to cause cavitation in the medium. In clinical ultrasound systems, this index usually lies somewhere between 0.05 and 2.0. Although a single value is displayed for each image, in practice the actual MI varies throughout the image. In the absence of attenuation, the MI is maximal at the focus of the beam. Attenuation shifts this maximum toward the transducer. Furthermore, because it is a somewhat complex procedure to calculate the index, which is itself only an estimate of the actual quantity within the body, the indices displayed by different machines are not precisely comparable. Thus, for example, more bubble disruption might be observed at a displayed MI of 0.2 on one machine but at 0.3 with the same patient on another machine. For this reason, recommendations of machine settings for a specific examination are not transferable between manufacturers’ instruments. Nonetheless, the MI is the operator’s most important indication of the behavior of the contrast agent bubbles to be expected. For this reason, it is usually incorporated into the preset initial settings for a contrast imaging mode on the scanner and the output power adjustment brought to the operator’s front line of controls. A typical initial setting of output power in a contrast mode is as low as 1% to 2% of that used in conventional imaging.

The Mechanical Index (MI)

<table>
<thead>
<tr>
<th>The mechanical index (MI):</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Is defined as</td>
</tr>
<tr>
<td>[ \text{MI} = \frac{P_{\text{neg}}}{f} ]</td>
</tr>
<tr>
<td>where ( P_{\text{neg}} ) is the peak negative ultrasound pressure in MPa and ( f ) is the ultrasound frequency in MHz</td>
</tr>
<tr>
<td>- Reflects the normalized negative pressure to which a target (such as a bubble) is exposed in an ultrasound field</td>
</tr>
<tr>
<td>- Is defined for the focus of the ultrasound beam</td>
</tr>
<tr>
<td>- Varies with depth in the image (lessens with increasing depth)</td>
</tr>
<tr>
<td>- Varies with lateral location in the image (lessens at the sector edges)</td>
</tr>
<tr>
<td>- Is estimated differently in systems from different manufacturers</td>
</tr>
</tbody>
</table>

NONLINEAR ECHOES AND HARMONIC IMAGING

Two important pieces of evidence are presented by the behavior of bubbles in an acoustic field. First, the size of the echo enhancement shown in Fig. 3.3 is much larger than would be expected from such sparse scatterers of this size in blood. Second, investigations of the acoustic characteristics of early agents demonstrated peaks in the spectra of attenuation and scattering that are dependent on both ultrasound frequency and the size of the microbubbles. These important observations suggest that the bubbles resonate in an ultrasound field. As the ultrasound wave—which consists of alternating compressions and rarefactions—propagates over the bubbles, they experience a periodic change in their radius in sympathy with the oscillations of the incident sound. Like vibrations of a string of a musical instrument, these oscillations have a natural, or resonant, frequency of oscillation at which they will both absorb and scatter ultrasound with a peculiarly high efficiency. Considering the linear oscillation of a free bubble of air in water, we can use a simple theory to predict the resonant frequency of radial oscillation of a bubble 3 μm in diameter, the median diameter of a typical transpulmonary microbubble agent. As Fig. 3.4 shows, it is about 3 MHz, approximately the center frequency of ultrasound used in a typical abdominal scan. This extraordinary, and fortunate, coincidence explains why ultrasound contrast agents are so efficient and can be administered in such small quantities. It also predicts that bubbles undergoing resonant oscillation in an ultrasound field can be induced to nonlinear motion, the basis of harmonic imaging.

It has long been recognized that if bubbles are “driven” by an ultrasound field at sufficiently high acoustic pressures, the oscillatory excursions of the bubble reach a point at which the alternate expansions and contractions of the bubble’s size are not equal. Lord Rayleigh, the originator of the theoretical
understanding of sound on which ultrasound imaging is based, was first led in 1917 to investigate this by his curiosity over the creaking noises that his teakettle made as the water came to a boil. The consequence of such nonlinear motion is that the sound emitted by the bubble, and detected by the transducer, contains harmonics, just as the resonant strings of a musical instrument, depending on how they are bowed or plucked, will produce a timbre comprising overtones (the musical term for harmonics), exact octaves above the pitch of the fundamental note. The origin of this phenomenon is the asymmetry that begins to affect bubble oscillation as the amplitude becomes large. As a bubble is compressed by the ultrasound pressure wave, it becomes stiffer and hence resists further reduction in its radius. Conversely, in the rarefaction phase of the ultrasound pulse, the bubble becomes less stiff, and therefore enlarges much more (Fig. 3.5). Fig. 3.6 shows the frequency spectrum of an echo produced by a microbubble contrast agent after exposure to a 3-MHz burst of sound. The particular agent is Optison, though most microbubble agents behave in a similar way. Ultrasound frequency is on the horizontal axis, with the relative amplitude on the vertical axis. In addition to the fundamental echo at 3 MHz, a series of echoes occur at whole multiples of the transmit frequency, known as higher harmonics. Here, then, is one simple method to distinguish bubbles from tissue: excite them so as to produce harmonics, and detect these in preference to the fundamental echo from tissue. Key factors in the harmonic response of an agent are the incident pressure of the ultrasound field, the frequency, the size distribution of the bubbles, and the mechanical properties of the bubble capsule (a stiff capsule, for example, will dampen the oscillations and attenuate its nonlinear response).
Harmonic B-Mode Imaging

An imaging and Doppler method based on the phenomenon of harmonic emission, called "harmonic imaging," is widely available on modern ultrasound scanners. In harmonic mode, the system transmits normally at one frequency but is tuned to receive echoes preferentially at double that frequency, where the echoes from the bubbles lie. Typically, the transmit frequency lies between 1.5 and 3 MHz and the receive frequency is selected by means of a detection strategy (originally simply a radiofrequency bandpass filter whose center frequency is at the second harmonic), between 3 and 6 MHz. Harmonic imaging uses the same array transducers as conventional imaging and for most of today's ultrasound systems involves only software changes. Echoes from solid tissue, as well as red blood cells themselves, are suppressed. Real-time harmonic spectral Doppler and color Doppler modes have also been implemented in a number of commercially available systems. Clearly, an exceptional transducer bandwidth is needed to operate over such a large range of frequencies. Fortunately, much effort has been directed in recent years toward increasing the bandwidth of transducer arrays because of its important bearing on conventional imaging performance, so harmonic imaging modes do not require the additional expense of dedicated transducers.

Harmonic Spectral and Power Doppler Imaging

In harmonic images, the echo from tissue-mimicking material is reduced, but not eliminated, reversing the contrast between the agent and its surrounding environment (Fig. 3.7). The value of this effect is to increase the conspicuity of the agent when it is in blood vessels normally hidden by the strong echoes from tissue. In spectral Doppler, one would expect the suppression of the tissue echo to reduce the tissue motion or "thump" artifact that is familiar to all Doppler sonographers and limits the detection of flow in moving vessels. In vivo measurements from spectral Doppler show that the signal-to-noise ratio is improved by a combination of harmonic imaging and the contrast agent by as much as 35 dB. Applications of this method include detection of blood flow in small vessels surrounded by tissue that is moving, such as the branches of the coronary arteries; it remains a somewhat specialized technique.

In conventional color Doppler studies using a contrast agent, the increased echo signal does nothing to suppress the clutter "flash" from moving tissue, but instead adds to it a "blooming" artifact of flow signals as the receiver is overloaded with the enhanced echo from blood (Fig. 3.8). Harmonic power Doppler mode effectively overcomes this clutter problem by suppressing the signal from tissue, revealing better detail of small vessels. Combining the harmonic method with power Doppler produces an especially effective tool for the detection of flow in the small vessels of the organs of the abdomen, which may be moving with cardiac pulsation or respiration (Fig. 3.9). In a study in which flow imaged on contrast-enhanced power harmonic images was compared with histologically sized arterioles in the corresponding regions of the renal cortex, it was concluded that the method is capable of demonstrating flow in vessels of less than 40-µm diameter—about 10 times smaller than the corresponding imaging resolution limit, even as the organ is moving with normal cardiac pulsation.

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**FIG. 3.7 Demonstration of Pulse Inversion Imaging.** In vitro images of a vessel phantom containing stationary perfluorocarbon contrast agent surrounded by tissue equivalent material (Biogel and graphite). (A) Conventional image, mechanical index (MI) = 0.2. (B) Harmonic imaging, MI = 0.2, provides improved contrast between agent and tissue. (C) Pulse inversion imaging, MI = 0.2. By suppressing linear echoes from stationary tissue, pulse inversion imaging provides better contrast between agent and tissue than both conventional and harmonic imaging. (With permission from Becher H, Burns P. Handbook of contrast echocardiography: left ventricle function and myocardial perfusion. New York: Springer; 2000.)
respiration. Using this power mode method in the heart, perfusion can be imaged in the myocardium.\textsuperscript{44,45}

**Tissue Harmonic Imaging**

In second harmonic imaging, an ultrasound scanner transmits at one frequency and receives at double this frequency. The resulting improved detection of the microbubble echo is due to the peculiar behavior of a gas bubble in an ultrasound field. However, any source of a received signal at the harmonic frequency that does not come from the bubble will clearly reduce the efficacy of this method. Such unwanted signals can come from nonlinearities in the transducer or its associated electronics, and these must be tackled effectively in a good harmonic imaging system. However, tissue itself can produce harmonics that will be received by the transducer. They are developed as a wave propagates through tissue. Again, this is due to an asymmetry: this time, the fact that sound travels slightly faster through tissue during the compressional part of the cycle (when it is denser and hence stiffer) than during the rarefactual part. Although the effect is very small, it is sufficient to produce substantial harmonic components in the transmitted wave by the time it reaches deep tissue, so that when it is scattered by a linear target such as the myocardium, there is a harmonic component in the echo, which is detected by the scanner along with the harmonic echo from the bubble.\textsuperscript{46} This is the reason that solid tissue is not completely dark in a typical harmonic image. The effect is to
reduce the contrast between the bubble and tissue, rendering the problem of detecting perfusion in tissue more difficult.

Tissue harmonics, although a foe to contrast imaging, are not necessarily a bad thing. In fact, an image formed from tissue harmonics without the presence of contrast agents has so many properties that commend it over conventional imaging that it is used as the routine “default” mode in many scanner presets. Its advantages stem from the fact that tissue harmonics are developed as the beam penetrates tissue, in contrast to the conventional beam, which is generated at the transducer surface.⁴⁷,⁴⁸ Artifacts that accrue from the first few centimeters of tissue, such as reverberations, are reduced by using tissue harmonic imaging. Sidelobe and other low-level interference is also suppressed, making tissue harmonic imaging the routine modality of choice in many situations, especially when visualizing fluid-filled structures.⁴⁹ Nonetheless, for contrast studies, the tissue harmonic limits the visibility of bubbles within tissue and therefore can be considered an artifact. In considering how to reduce it, it is instructive to bear in mind differences between harmonic produced by tissue propagation and by bubble echoes. First, tissue harmonics require a high peak pressure, so are only evident at high MI. Use of low-MI contrast imaging, as is most often the case, leaves only the bubble harmonics. Second, harmonics from tissue at high MI are continuous and sustained, whereas those from bubbles at high MI are transient in nature as the bubble disrupts.

**Pulse Inversion Imaging**

There were problems with early harmonic imaging. First, the splitting of the bandwidth of the transducer had the effect of decreasing image resolution. Second, when the received echo is weak (which from bubbles is typically the case), the overlapping region between the transmit and receive frequencies becomes a larger portion of the entire received signal, so that contrast in the harmonic image is dependent on how strong the echo is from the bubbles. In practice, this forces use of a high MI in harmonic mode, which reduces contrast more because of the tissue harmonic.⁵⁰ It also results in the transient and irreversible disruption of the bubbles.⁵¹ As the bubbles enter the scan plane of a real-time ultrasound image, they provide an echo but then disappear. Thus vessels that lie within the scan plane are not visualized as having continuous walls in such a harmonic image, but instead have a punctate appearance (Fig. 3.10).

Pulse inversion imaging overcomes the conflict between the requirements of contrast and resolution in harmonic imaging and provides greater sensitivity, thus allowing low incident power, nondestructive, continuous imaging of microbubbles in an organ such as the liver. The method also relies on the asymmetric oscillation of an ultrasound bubble in an acoustic field, but detects “even” nonlinear components of the echo over the entire bandwidth of the transducer. In pulse inversion (also known as “phase inversion”) imaging, two pulses are sent in rapid succession into the tissue. The second pulse is a mirror image of the first (Fig. 3.11): that is, it has undergone a 180-degree phase change. The scanner detects the echo from these two successive pulses and forms their sum. For ordinary tissue, which behaves in a linear manner, the sum of two inverted pulses is simply zero. For an echo with nonlinear components, such as that from a bubble, the echoes produced from these two pulses will not be simple mirror images of each other, because of the asymmetric behavior of the bubble radius with time. The result is that the sum of these two echoes is not zero. Thus a signal is detected from a bubble but not from tissue. It can be shown mathematically that this summed echo contains the nonlinear “even” harmonic components of the signal, including the second harmonic.⁵² One
Contrast Agents for Ultrasound

Pulse Inversion is now the preferred method used by many systems for tissue harmonic imaging. Optimal pulse inversion contrast imaging is often, then, performed at low MI. The principle of pulse inversion is the basis of many commercially named imaging modes, such as coherent contrast imaging, ensemble harmonic imaging, and phase inversion imaging.

Pulse Inversion Doppler Imaging

In spite of the improvements offered by pulse inversion over harmonic imaging for suppressing stationary tissue, the method is somewhat sensitive to echoes from moving tissue. This is because tissue motion causes linear echoes to change slightly between pulses, so that they do not cancel perfectly. Furthermore, at high MI, nonlinear propagation also causes harmonic echoes to appear in pulse inversion images, even from linear scattering structures such as the liver parenchyma. Although tissue motion artifacts can be minimized by using a short pulse repetition interval, nonlinear tissue echoes can mask the echoes from bubbles, reducing the efficacy of microbubble contrast, especially when a high MI is used. Pulse inversion Doppler seeks to address these problems by means of a generalization of the pulse inversion method. This technique combines the nonlinear detection performance of pulse inversion imaging with the motion discrimination capabilities of power Doppler. Multiple transmit pulses of alternating polarity are used and Doppler signal processing techniques are applied to distinguish between bubble echoes and echoes from moving tissue and/or tissue harmonics, as desired by the operator. This method offers potential improvements in the agent-to-tissue contrast and signal-to-noise performance, although at the cost of a somewhat reduced frame rate. The most advantage of pulse inversion over the filter approach to detect harmonics from bubbles is that it no longer suffers from the restriction of bandwidth. The full frequency range of sound emitted from the transducer can be detected in this way, providing a full bandwidth—that is, a high-resolution image of the echoes from bubbles. Fig. 3.12 illustrates how pulse inversion imaging provides better suppression of linear echoes than harmonic imaging and is effective over the full bandwidth of the transducer, showing improvement of image resolution over harmonic mode. Because this detection method is a more efficient means of isolating the bubble echo, weaker echoes from bubbles insonated at low, nondestructive intensities can be detected. It should be noted, however, that as the MI increases, tissue harmonic renders the tissue brighter. Indeed, pulse inversion is now the preferred method used by many systems for tissue harmonic imaging. Optimal pulse inversion contrast imaging is often, then, performed at low MI. The principle of pulse inversion is the basis of many commercially named imaging modes, such as coherent contrast imaging, ensemble harmonic imaging, and phase inversion imaging.

![FIG. 3.11 Basic Principle of Pulse Inversion Imaging.](image)

A pulse of sound is transmitted into the body, and echoes are received from agent and tissue. A second pulse, which is an inverted copy of the first, is then transmitted in the same direction and the two resulting echoes are summed. Linear echoes from tissue are inverted copies of each other and cancel to zero. The microbubble echoes are distorted copies of each other, so the even nonlinear components of these echoes will reinforce each other when summed, producing a strong harmonic signal.

![FIG. 3.12 Pulse Inversion Image of a Hypervascular Liver Mass in the Arterial Phase, Made in Real Time at Low Mechanical Index.](image)

Note that the spatial resolution of this image is comparable to that of conventional imaging, reflecting the advantage of a broad-bandwidth contrast-specific image.
dramatic manifestation of this method’s ability to detect very weak harmonic echoes has been its demonstration of real-time perfusion imaging of the myocardium.\(^\text{34}\) By lowering the MI to 0.1 or less, bubbles undergo stable, nonlinear oscillation, emitting continuous harmonic signals. Because of the low MI, very few bubbles are disrupted, so that imaging can take place at real-time rates. Because sustained, stable nonlinear oscillation is required for this method, perfluorocarbon gas bubbles work best.

**Plane-Wave Contrast Imaging**

In practice, the low frame rate that line-by-line imaging forces on pulse inversion Doppler has precluded its inclusion as a clinical scanning mode in most systems. However, line-by-line beam-forming is not the only way to form an ultrasound image. It is possible to form an ultrasound image as is conventionally done by sweeping a focused beam in 100 consecutive directions, with a single, unfocused plan wave, which insonates the entire field of view with a single pulse. The echoes from each volume element of tissue are then received simultaneously on all transducer elements and processed to extract the echoes at each location in the field of view. The result is a single image, albeit of somewhat compromised quality, for a single ultrasound pulse. Thus imaging frame rates equal to the pulse repetition frequency of 5 kHz or more are possible. Although computationally demanding, the advantage of such “software beam-forming” methods are legion. One is that they provide an opportunity to send long ensembles of pulse for Doppler methods without affecting frame rate. Pulse inversion Doppler has been implemented in such systems with dramatic results, providing simultaneous perfusion, imaging, and color Doppler from a contrast injection at frame rates of 100 Hz or more.\(^\text{35}\)

**Amplitude and Phase Modulation Imaging**

On receiving the echoes from a pulse inversion sequence, the receiver combines them in a way that ensures that the mirrorlike echoes from tissues combine to zero. What is left is then some combination of the nonlinear components of the bubble echo. Changing (or modulating) the pulse from one transmission to the next by flipping its phase is only one of a large number of strategies that can be employed. For example, by changing the amplitude of the pulse in consecutive transmissions and amplifying the echoes to compensate for this, linear echoes can also be cancelled out. What is left are all components of the nonlinear echoes from bubbles.\(^\text{56}\) Precisely what nonlinear components are produced by a particular sequence of pulses can be determined mathematically and the contrast-specific imaging mode optimized for specific applications.\(^\text{57}\) Almost all diagnostic systems now use some form of multipulse modulation processing in their contrast-specific imaging modes, variously known by such names as power modulation pulse inversion (PMPI) or contrast pulse sequence (CPS). As long as the peak negative pressure is kept low (less than about 100 kPa) so that the bubble is not disrupted by the pulses, real-time imaging of perfusion can be achieved in many organs, including the myocardium, liver, kidney, skin, prostate, and breast, even in the presence of tissue motion. Because one performance criterion that improves detection of perfusion is complete suppression of background tissue, many contrast-specific images are quite black before the contrast agent is injected, making it very hard to scan the patient. Thus side-by-side imaging, in which a simultaneous, low-MI, fundamental image is seen alongside (or superimposed on) the contrast image, has become a preferred method for hunting small lesions or guiding interventional devices such as needles or ablation probes, the echoes of which are visible in the fundamental image but suppressed in the contrast image (Fig. 3.13). Although the technology for low-MI, real-time, bubble-specific imaging in commercial ultrasound systems has stabilized over the past few years, clinical applications are still experiencing expansion, especially in tumor imaging, which in turn presents new challenges for imaging methodology.

**Temporal Maximum Intensity Projection Imaging**

One clinically striking elaboration of contrast-specific imaging exploits the fact that it is sufficiently sensitive to detect and display echoes from individual bubbles in real time. By creating the equivalent of an open-shutter photograph, in which bright objects create tracks of their own motion, the bubbles can be made to trace the morphology of the microvessels that contain them. The result, known as temporal maximum intensity projection (or temporal MIP) imaging can produce a detailed picture of vascular morphology lasting a few seconds or the duration of a breath hold. Usually the maximum intensity projection process is initiated after a flash, which disrupts the bubbles within the scan plane (Fig. 3.14). As new bubbles wash into the plane, their tracks are traced in an image, which is integrated over a chosen period between 100 ms to a few seconds.\(^\text{58}\) These images can also provide dynamic information—for example, revealing whether the pattern of arterial enhancement of a liver lesion is centripetal or centrifugal.\(^\text{59}\)

**DISRUPTING BUBBLES: INTERMITTENT IMAGING**

As the incident pressure to which a resonating bubble is exposed increases, so its oscillation becomes more wild, with radius increasing in some bubbles by a factor of 5 or more during the rarefaction phase of the incident sound. Just as a good soprano can shatter a wine glass by singing at its resonant frequency, so a microbubble, if driven by higher amplitude ultrasound, will sustain irreversible disruption of its shell. A physical picture of precisely what happens to a disrupted bubble is only now emerging from high-speed video studies using cameras with frame rates up to 25 million pictures per second\(^\text{60,61}\) (Fig. 3.15). It seems certain, however, that the bubble shell disappears (not instantly, but over a period of time determined by the bubble composition) and releases free gas, which forms a highly effective acoustic scatterer, giving strong, nonlinear echoes for a brief period of time. This process was once incorrectly thought to constitute a release of energy, like a balloon bursting, and was wrongly termed “stimulated acoustic emission.” Its use is twofold. First, intermittent imaging represents a very sensitive way to detect a bubble.\(^\text{62}\)
CHAPTER 3  Contrast Agents for Ultrasound  65

FIG. 3.13 Side-by-Side Imaging Shows a Low–Mechanical Index (MI) Real-Time Conventional Image (Right) at the Same Time as the Low-MI Contrast-Specific Image (Left). This is particularly useful for characterizing small lesions and for guiding interventional devices. The contrast mode here combines phase and amplitude modulation.

However, because it results in its disruption, this process cannot be performed continuously. In fact, replenishment of bubbles in a typical microvascular bed takes about 5 to 10 seconds. The technique of imaging with high MI every few seconds to display perfusion is called “triggered” or “interval delay” imaging. Second, intermittent imaging by a fixed time interval can track the degree to which a region is replenished by bubbles between insonations. By demonstrating the flow rate of blood into the scan plane, intermittent imaging provides a unique method to measure tissue perfusion.

Triggered Imaging

It was discovered during the early days of harmonic imaging that by pressing the “freeze” button on a scanner for a few moments, and hence interrupting the acquisition of ultrasound images during a contrast study, it is possible to increase the effectiveness of a contrast agent. So dramatic is this effect that it was responsible for the first ultrasound images of myocardial perfusion using harmonic imaging. This is a consequence of the ability of the ultrasound field, if its peak pressure is sufficiently high, to disrupt a bubble’s shell and hence destroy it. As the bubble is disrupted, it releases energy, thus creating a strong, transient echo that is rich in harmonics. This process is sometimes incorrectly referred to as “stimulated acoustic emission.” The fact that this echo is transient in nature can be exploited for its detection. One simple method is to subtract from a disruption image a baseline image obtained either before or (more usefully) immediately after insonation. Such a method requires offline processing of stored ultrasound images, together with software that can align the ultrasound images before subtraction, and is useful only in rare circumstances.

Intermittent Harmonic Power Doppler

Power Doppler imaging was developed as a way to detect the movement of targets such as red blood cells in a vessel. It works by a simple, pulse-to-pulse subtraction method, in which two or more pulses are sent successively along each scan line of the image. Pairs of received echo trains are compared for each line: if they are identical, nothing is displayed, but if there is a change (owing to motion of the tissue between pulses), a color is displayed, the saturation of which is related to the amplitude of the echo that has changed. This method, although not designed for the detection of bubble disruption, is ideally suited for high-MI “disruption” imaging. The first pulse receives an echo from the bubble, and the second receives none, so the comparison yields a strong signal. In a sense, power Doppler may be thought of as a line-by-line subtraction procedure on the radiofrequency echo detected by the transducer. Interestingly, pulse inversion imaging—the most commonly used method at low MI—becomes equivalent to power Doppler if the MI is high and the bubble disrupted. Looking again at Fig. 3.11, one can easily see that if the echo from the second pulse is absent (because the bubble is gone), the sum of the two bubble echoes is the same as their difference, which is what is measured by power Doppler. The fact that the second transmitted pulse is inverted is immaterial for the bubble that has disappeared! Thus at high MI, power or pulse inversion Doppler becomes a sensitive way to detect bubbles, whether they are moving or not.

This method has been incorporated into modes specifically adapted to detect the distribution of bubbles taken up in the Kupffer cells in the postvascular phase of agents such as Levovist and Sonazoid. The transducer is slowly swept through the liver some minutes after the agent has left the vascular system; as it does so, the high-MI pulses disrupt the in situ bubbles and are detected in the image. Fig. 3.16 shows such an image, in which the defect in uptake represents the Kupffer-poor region of a cholangiocarcinoma. The preferred modes for this method are pulse inversion—which carries the attraction of high-resolution imaging but the disadvantage of a strong tissue harmonic background—or power Doppler modes known by such names as harmonic power angio or agent detection imaging (ADI).

Many systems offer a low-MI “monitor” mode that can be used to give a contrast-specific or fundamental image of the liver during the scan sweep, which, when blended with the high-MI contrast mode, can be helpful to keep the scan plane aligned in the region of interest.

Disruption-Replenishment Imaging

By disrupting bubbles and monitoring replenishment to a region of tissue, contrast ultrasound offers a unique, noninvasive, and validated method for the measurement of microvascular perfusion. In the disruption-replenishment method, microbubbles are infused at a steady rate until a steady enhancement is achieved throughout the vascular system. The bubbles are then disrupted by a high-MI flash, which clears them from the scan plane (Fig. 3.17). Immediately, new bubbles begin to wash in. The rate at which they do so is related to the local flow velocity and flow rate, which can be extracted from a physical model of the process. An important application for such measurement is in assessing the response of tumors and other organs to therapies that target the vasculature. In cancer therapy, a large number of new treatment strategies have been proposed that target the proliferating vasculature of a developing tumor, including drugs specifically designed to inhibit the angiogenic transformation itself. Such antiangiogenic or vascular-disrupting drugs have the effect of shutting down the tumor circulation and inhibiting further growth. They do not in themselves kill cancer cells, so the tumor often responds without shrinking in size—hence the need for a functional test to determine drug response.
Considerable experience accumulated to date suggests that dynamic contrast-enhanced ultrasound, with its advantage of high sensitivity, portability, and a pure intravascular tracer, is a strong candidate for this role; it has been incorporated into the current European guidelines for the clinical use of contrast agents.

**SAFETY CONSIDERATIONS AND REGULATORY STATUS**

Contrast ultrasound examinations expose patients to ultrasound in a way that is identical to that of a normal ultrasound examination. Yet the use of ultrasound pulses to disrupt bubbles that sit in microscopic vessels, and the knowledge that ultrasound and bubbles can be used deliberately to penetrate cell membranes for the purpose of drug delivery and other therapies, raise questions about the potential for hazard. When a bubble produces the brief echo that is associated with its disruption, it releases energy it has stored during its exposure to the ultrasound field. Can this energy damage the surrounding tissue? At higher exposure levels, ultrasound is known to produce bioeffects in tissue, the thresholds for which have been studied extensively. Do these thresholds change when bubbles are present in the vasculature? Whereas the safety of ultrasound contrast agents as drugs has been established to the satisfaction of the most stringent requirements of the regulating authorities in a number of countries, it is probably fair to say that there is much to be learned about the interaction between ultrasound and tissue when bubbles are present.

The most extreme of these interactions is known as inertial cavitation, which refers to the rapid formation, growth, and collapse of a gas cavity in fluid as a result of ultrasound exposure. It was studied extensively before the development of microbubble contrast agents. In fact, most of the mathematical models used to describe contrast microbubbles were originally developed to describe cavitation. When sound waves of sufficient intensity travel through a fluid, the rarefractional half-cycle of the sound wave can actually tear the fluid apart, creating spherical cavities within the fluid. The subsequent rapid collapse of these cavities during the compressional half-cycle of the sound wave can focus large amounts of energy into a very small volume, raising the temperature at the center of the collapse to thousands of degrees Kelvin, forming free radicals and even emitting electromagnetic radiation. The concern over potential cavitation-induced bioeffects in diagnostic ultrasound has led to many experimental studies, many of them assessing whether the presence of contrast microbubbles can act as cavitation seeds, potentiating bioeffects. This work has been reviewed by ter Haar and by the World Federation for Ultrasound in Medicine and Biology. Although it has been shown that adding contrast agents to blood decreases the threshold for cavitation and related bioeffects (e.g., hemolysis,
platelet lysis), no significant effects have been reported in conditions that are comparable to the bubble concentrations and ultrasound exposure of a low-MI diagnostic clinical examination. It nonetheless remains prudent to practice an extension of the ALARA (as low as reasonably achievable) exposure principle to contrast ultrasound. The contrast ultrasound examination should expose the patient to the lowest MI, the shortest total acoustic exposure time, the lowest contrast agent dose, and the highest ultrasound frequency consistent with obtaining adequate diagnostic information.

In the meantime, at least 8 million injections of microbubble contrast for clinical diagnosis have been performed worldwide; they are very well tolerated and have an excellent safety record. In fact, postmarketing surveillance has suggested that the predominant cause of severe adverse events is anaphylactic reaction, with an estimated rate of 1 per 7000 for both the perflutren microspheres approved for cardiac indications in the United States and the sulfur hexafluoride microspheres approved in Europe. This rate is comparable to that of most injectable analgesics and antibiotics and lower than that for other imaging contrast agents, such as those used in CT imaging. A 2006 study of more than 23,000 injections of a microbubble contrast agent for abdominal diagnosis in Europe showed no deaths and two serious adverse events, giving a serious adverse event rate of less than 1:10,000. Although FDA approval for radiologic indications has only just occurred in the United States, there is extensive experience with ultrasound contrast in echocardiography laboratories. In 2008 Kusnetzky and colleagues reviewed more than 18,671 hospitalized patients undergoing echocardiography in an acute setting in a single center and reported no effect on mortality from the use of contrast in this group. In 2008 Main and colleagues analyzed registry data from 4,300,966 patients who underwent transthoracic echocardiography at rest during hospitalization, of whom 58,254 were given the contrast agent Definity. Acute crude mortality was no different between groups, but multivariate analysis revealed that in patients undergoing echocardiography, those receiving the contrast agent were 24% less likely to die within 1 day than patients not receiving contrast. The FDA has since dropped its notice of caution when using microbubble agents in patients with severe cardiopulmonary compromise. From the accumulated knowledge of the considerable benefits of using microbubble contrast, combined with the very low incidence of adverse events associated with its administration, it is fair to conclude this technique is ready to play a major role in the practice of ultrasound diagnosis. We might speculate that in offering the 2016 approval for the use of microbubble contrast in the pediatric population (children were not part of the pivotal studies included in the submission dossier), the FDA is tacitly pointing to the additional benefit that may accrue from reducing the exposure of these patients to x-rays, as well as to CT and MRI contrast agents.

THE FUTURE
Development of microbubble technology is proceeding in two complementary areas: the instruments and the bubbles. In the first, the development of three-dimensional (3-D) and fast imaging for other applications will have a substantial impact on contrast studies. Ultrafast imaging using plane waves offers the opportunity to gain quantitative flow information from large vessels, using bubble-specific vector Doppler methods, at the same time as imaging perfusion. The separation of large vessel flow from tissue perfusion is a challenge to all contrast-enhanced modalities and is likely to be solved first by ultrasound. Although 3-D contrast images have been made using mechanically swept transducers, the slow acquisition rates mean that the dynamics of enhancement—a crucial diagnostic feature—are lost. Two-dimensional matrix arrays transducers, on the other hand, offer real-time volumetric contrast imaging. In one emerging application, they allow the response of a tumor. Considering the heterogeneity of tumor perfusion, the sampling of a single plane has been shown to be unreliable; real-time 3-D imaging solves this problem, allowing very large tumors to be evaluated rapidly (Fig. 3.18).

In contrast imaging, the potential for functional information yielded by the bubbles can be increased by active targeting to a specific cellular or molecular process. Thus a bubble attaches itself to the cells lining blood vessels (endothelial cells) that are involved in a disease process such as inflammation (in atherosclerosis) or proliferation (in cancer). This is achieved by attaching ligands to the surface of the lipid shell, such as a peptide and an antibody. Antibodies to factors such as vascular cell adhesion molecule (VCAM), a marker of inflammation, and vascular endothelial growth factor (VEGF) receptor 2, a marker of vascular proliferation, have already been shown to effectively make bubbles “stick” selectively to the endothelial surface (Fig. 3.19). This form of molecular imaging has potential applications in identifying the target and assessing the effectiveness of new therapies, with new agents under active clinical development. In addition, the bubbles are used as a potentiator of the therapy itself. Bubbles can concentrate and lower the threshold for thermal tissue damage in high-intensity focused ultrasound (HIFU) treatments. They can also have the effect of opening or penetrating the endothelial layer, even the blood–brain barrier, allowing drugs to pass through into a region of tissue selected by the ultrasound beam. The drugs can be circulating in the bloodstream, or incorporated into the bubbles themselves. In the latter case, plasmid DNA, which cannot survive in the blood, can be carried in the bubble shell and released by acoustic disruption. Oscillation of the free gas near the cell membrane allows the DNA to enter the cell. Both endothelial cells and myocytes have been successfully transfected in this potentially new form of gene therapy. Finally, the barrier of the endothelium itself can be overcome by injecting liquid nanodroplets—precursors of the gas contrast agents—allowing them to diffuse into the interstitium, and then using external acoustic energy to activate them into gas bodies, which are both targeted and detectable and can be used to enhance therapy.

The use of bubbles as molecular and cellular probes, their targeting as a means for detection as well as drug and gene delivery, and their application as focal potentiators for minimally invasive therapies are all applications in their infancy. The coming years are likely to see an unprecedented union of ultrasound
imaging with a unique series of injectable constructs that will propel an already versatile imaging modality to the forefront of the interface between diagnosis and therapy.

**CONCLUSION**

Microbubble contrast agents for ultrasound are safe, effective, and well tolerated by patients. They offer a clinically approved blood pool agent unique to radiological modalities. Unlike contrast agents for other modalities, microbubbles are physically modified by the process used to image them. Understanding the behavior of bubbles while exposed to an ultrasound imaging beam is the key to performing an effective contrast ultrasound examination. The appropriate choice of a contrast-specific imaging method is based on the behavior of the agent and the requirements of the examination. The MI is the major determinant of the response of contrast bubbles to ultrasound. Low-MI harmonic and multipulse imaging offer real-time, contrast-specific methods for perfusion imaging using perfluorocarbon agents. Future developments offer the intriguing prospect of molecular and cellular imaging, potentiated therapy, and drug and gene delivery, all with ultrasound and microbubbles.

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**FIG. 3.18** Real-Time, Four-Dimensional Tumor Contrast Imaging Performed With a 9000-Element Ultrasound Array Transducer at a Harmonic Range of 2 to 4 MHz. Twenty-five pseudo-parallel 2.1-mm-spaced planes in a patient with renal cell carcinoma (about $5 \times 5 \times 8$ cm) are imaged simultaneously with low–mechanical index contrast-specific imaging. (A) A single volume acquisition taken from a 10-Hz, 60-volume series after acoustic disruption of the microbubble agent during an intravenous infusion.

*Continued*
FIG. 3.19 Molecular Ultrasound Imaging of Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) With Targeted Microbubbles. Figure shows 40-MHz images of a MeWo subdermal tumor derived from human melanoma cells in a mouse after injection of VEGFR-2-targeted microbubbles. (A) Circulating bubbles have left the vascular system; those imaged are adhering to the receptor target. Microbubble-specific signal is shown as a green overlay. (B) After disruption flash, the bubble echoes disappear. The difference in bubble signal between these two images quantifies adhesion of the tracer to the target receptor. Scale units are millimeters. (Reproduced with permission from Rychak JJ, Graba J, Cheung AM, et al. Microultrasound molecular imaging of vascular endothelial growth factor receptor 2 in a mouse model of tumor angiogenesis. Mol Imaging. 2007;6(5):289-296.)
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