

## Educational Forum

# Recent update in ultrasound contrast agents

Poonam Vohra<sup>1\*</sup>, Kusum Jasuja<sup>2</sup>

<sup>1</sup>Department of Radiodiagnosis, PGIMER & Dr. R. M. L. Hospital, New Delhi, India

<sup>2</sup>Department of Microbiology, BRD Medical college, Gorakhpur, UP, India

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**\*Correspondence:**

Dr. Poonam Vohra

E-mail: pvohra03@hotmail.com

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### ABSTRACT

Contrast-enhanced ultrasound (CEUS) is the application of ultrasound contrast medium to traditional medical sonography. Ultrasound contrast agents rely on the different ways in which sound waves are reflected from interfaces between substances. This may be the surface of a small air bubble or a more complex structure. Commercially available contrast media are gas-filled microbubbles that are administered intravenously to the systemic circulation. Microbubbles have a high degree of echogenicity (the ability of an object to reflect ultrasound waves). There is a great difference in echogenicity between the gas in the microbubbles and the soft tissue surroundings of the body. Thus, ultrasonic imaging using microbubble contrast agents enhances the ultrasound backscatter, (reflection) of the ultrasound waves, to produce a sonogram with increased contrast due to the high echogenicity difference. CEUS can be used to image blood perfusion in organs, measure blood flow rate in the heart and other organs, and for other applications.

**Keywords:** Ultrasound contrast agents, Microbubbles

## INTRODUCTION

Contrast-enhanced ultrasound (CEUS) involves the administration of intravenous contrast agents containing microbubbles of perfluorocarbon or nitrogen gas. The bubbles greatly affect ultrasound backscatter and increase vascular contrast in a similar manner to intravenous contrast agents used in computed tomography (CT) and magnetic resonance imaging (MRI).

CEUS has the advantage over contrast-enhanced MRI and CT in patients with contraindications such as renal failure or contrast allergy. CEUS also allows for dynamic and repeat examinations. Microbubbles are not filtered in the lungs since they are equivalent in size to red blood cells. Microbubbles are different than the agitated saline used in echocardiographic "bubble studies".

### *Non-targeted CEUS*

Non targeted contrast enhanced ultrasound is a more common method used for dynamic evaluation of the vascularity of a target lesion, most commonly in the liver or kidney and also used to measure organ perfusion, which can be useful in diagnosing diffuse processes (e.g. cirrhosis).

### *Targeted CEUS*

Contrast agents designed to bind to specific molecules, which are then targeted at tissues expressing that substance. Microbubble contrast agents for ultrasound (US) have gained increasing interest in recent years, and CEUS is a rapidly evolving field with applications now extending far beyond the initial improvements achieved

in Doppler US. This has been achieved as a result of the safe profile and the increased stability of microbubbles persisting in the bloodstream for several minutes, and also by the availability of specialized contrast-specific US techniques, which allow a definite improvement in the contrast resolution and suppression of signal from stationary tissues.

CEUS with low transmit power allows real-time scanning with the possibility of prolonged organ insonation. Several reports have described the effectiveness of microbubble contrast agents in many clinical applications and particularly in the liver, spleen, and kidneys. CEUS allows the assessment of the macrovasculature and microvasculature in different parenchymas, the identification and characterization of hepatic and splenic lesions, the depiction of septal enhancement in cystic renal masses, and the quantification of organ perfusion by the quantitative analysis of the echo-signal intensity.

Other fields of application include the assessment of abdominal organs after traumas and the assessment of vesico-ureteral reflux in children. Finally, tumor-targeted microbubbles make possible the depiction of specific biologic processes. The bubbles greatly affect ultrasound backscatter and increase vascular contrast in a similar manner to intravenous contrast agents used in CT and MRI.<sup>1</sup>

## DISCUSSION

CEUS with the help of microbubbles is increasingly being used in the diagnostic radiology. It does not involve radiation.<sup>2</sup> There are a variety of microbubbles contrast agents. Microbubbles differ in their shell makeup, gas core makeup, and whether or not they are targeted.

### *Microbubble shell*

Selection of shell material determines how easily the microbubble is taken up by the immune system. A more hydrophilic material tends to be taken up more easily, which reduces the microbubble residence time in the circulation. This reduces the time available for contrast imaging. The shell material also affects microbubble mechanical elasticity. The more elastic the material, the more acoustic energy it can withstand before bursting.<sup>3</sup> Currently, microbubble shells are composed of albumin, galactose, lipid, or polymers.<sup>4</sup>

### *Microbubble gas core*

The gas core is the most important part of the ultrasound contrast microbubble because it determines the echogenicity. When gas bubbles are caught in an ultrasonic frequency field, they compress, oscillate, and reflect a characteristic echo- this generates the strong and unique sonogram in CEUS. Gas cores can be composed

of air, or heavy gases like perfluorocarbon, or nitrogen.<sup>4</sup> Heavy gases are less water-soluble so they are less likely to leak out from the microbubble leading to microbubble dissolution. As a result, microbubbles with heavy gas cores last longer in circulation.

Regardless of the shell or gas core composition, microbubble size is fairly uniform. They lie within a range of 1-4 micrometres in diameter. That makes them smaller than red blood cells, which allows them to flow easily through the circulation as well as the microcirculation.

### *Specific agents*

Optison, a Food and Drug Administration (FDA)-approved microbubble made by GE Healthcare, has an albumin shell and octafluoropropane gas core. The second FDA-approved microbubble, Levovist, made by Schering, has a lipid/galactose shell and an air core.<sup>4</sup>

SonoVue, made by Bracco (company), consists in sulphur hexafluoride microbubbles. It is mainly used to characterize liver lesions that cannot be properly identified using conventional (b-mode) ultrasound. The use of SonoVue to assess the early response to anti angiogenetic drugs in cancer chemotherapy has been studied, with promising results. Wider studies are ongoing.

Perflutren lipid microspheres (trade name Definity) are composed of octafluoropropane encapsulated in an outer lipid shell.<sup>5</sup>

Perflexane lipid microspheres (trade name Imagent or previously Imavist) is an injectable suspension developed by Alliance pharmaceutical approved by the FDA (in June 2002) for improving visualization of the left ventricular chamber of the heart, the delineation of the endocardial borders in patients with suboptimal echocardiograms. Beside its use to assess cardiac function and perfusion it is also used as an enhancer of the images of prostate, liver, kidney and other organs.<sup>6</sup>

### *Targeted microbubbles*

Targeted microbubbles are under preclinical development. They retain the same general features as untargeted microbubbles, but they are outfitted with ligands that bind specific receptors expressed by cell types of interest, such as inflamed cells or cancer cells. Current microbubbles in development are composed of a lipid monolayer shell with a perfluorocarbon gas core. The lipid shell is also covered with a polyethylene glycol (PEG) layer. PEG prevents microbubble aggregation and makes the microbubble more non-reactive. It temporarily “hides” the microbubble from the immune system uptake, increasing the amount of circulation time, and hence, imaging time.<sup>7</sup>

In addition to the PEG layer, the shell is modified with molecules that allow for the attachment of ligands that bind certain receptors. These ligands are attached to the microbubbles using carbodiimide, maleimide, or biotin-streptavidin coupling.<sup>7</sup> Biotin-streptavidin is the most popular coupling strategy because biotin's affinity for streptavidin is very strong and it is easy to label the ligands with biotin. Currently, these ligands are monoclonal antibodies produced from animal cell cultures that bind specifically to receptors and molecules expressed by the target cell type.

Since the antibodies are not humanized, they will elicit an immune response when used in human therapy. Humanizing antibodies is an expensive and time-intensive process, so it would be ideal to find an alternative source of ligands, such as synthetically manufactured targeting peptides that perform the same function, but without the immune issues. Destruction of microbubbles by ultrasound in the image plane allows absolute quantification of tissue perfusion.<sup>8,9</sup>

There are two forms of CEUS, untargeted (used in the clinic today) and targeted (under preclinical development). The two methods slightly differ from each other.

### ***Untargeted CEUS***

Untargeted microbubbles, such as the aforementioned SonoVue, Optison or Levovist, are injected intravenously into the systemic circulation in a small bolus. The microbubbles will remain in the systemic circulation for a certain period of time. During that time, ultrasound waves are directed on the area of interest.

When microbubbles in the blood flow past the imaging window, the microbubbles, compressible gas cores oscillate in response to the high frequency sonic energy field, as described in the ultrasound article. The microbubbles reflect a unique echo that stands in stark contrast to the surrounding tissue due to the orders of magnitude mismatch between microbubble and tissue echogenicity. The ultrasound system converts the strong echogenicity into a contrast-enhanced image of the area of interest. In this way, the bloodstream's echo is enhanced, thus allowing the clinician to distinguish blood from surrounding tissues.

### ***Targeted CEUS***

Targeted CEUS works in a similar fashion, with a few alterations. Microbubbles targeted with ligands that bind certain molecular markers that are expressed by the area of imaging interest are still injected systemically in a small bolus. Microbubbles theoretically travel through the circulatory system, eventually finding their respective targets and binding specifically. Ultrasound waves can then be directed on the area of interest. If a sufficient number of microbubbles have bound in the area, their

compressible gas cores oscillate in response to the high frequency sonic energy field, as described in the ultrasound article. The targeted microbubbles also reflect a unique echo that stands in stark contrast to the surrounding tissue due to the orders of magnitude mismatch between microbubble and tissue echogenicity.

The ultrasound system converts the strong echogenicity into a contrast-enhanced image of the area of interest, revealing the location of the bound microbubbles.<sup>10</sup> Detection of bound microbubbles may then show that the area of interest is expressing that particular molecular marker, which can be indicative of a certain disease state, or identify particular cells in the area of interest.

## **CONCLUSION**

CEUS is now a days becoming an imaging modality of choice for real-time evaluation of blood flow which does not involve radiation. Destruction of microbubbles by ultrasound in the image plane allows absolute quantification of tissue perfusion. Since microbubbles can generate such strong signals, a lower intravenous dosage is needed, micrograms of microbubbles are needed compared to milligrams for other molecular imaging modalities such as MRI contrast agents.

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