

Editorial

## An indispensable tool for ultrasound based diagnostics and therapies - Microbubbles

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### Abstract

Microbubbles (MBs) are micrometre sized gas spheres comprising a biocompatible shell that provide vascular contrast for diagnostic ultrasound (US) imaging. MBs volumetrically oscillate in an ultrasonic field and scatter acoustic energy over a range of frequencies that can be separated from the tissue response. MBs can also provide organ perfusion rates by imaging their “wash-in” to a region of interest which can be correlated to vascular flow. When driven at higher acoustic pressures, localized biological effects can be induced, including increased tissue permeabilization, thermal effects and localised release of drugs that can be encapsulated in the MBs themselves. Both hydrophobic and hydrophilic drugs can be loaded on to MBs e.g. through the use of liposomal carriers or direct attachment of drug molecules to the bubble shell. Since the early 2000s, MB-based technologies have been well researched, though there was significant regulatory push back starting in 2006 based on a controversial clinical trial. From that point, both physicians and researchers have consistently demonstrated the robust safety of MBs as ultrasound contrast agents and their significant clinical utility. Within the last 5 years, more indications have been approved. A recent first-in-man clinical trial of therapeutic US with MBs reversibly opening the blood brain barrier has also been shown to be safe in amyotrophic lateral sclerosis patients. The following article outlines the coupling of US and MBs as a diagnostic and therapeutic platform with a particular focus on their application to the therapy of surgical diseases.

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### Clinical use of ultrasound

For almost 20 years, microbubbles (MBs) have been widely used as ultrasound contrast agents (UCA) after receiving the United States Food and Drug Administration (FDA) approval for left ventricle opacification for echocardiography. Since then, an immense amount of research has been dedicated to further develop MBs as not only UCAs but therapeutic agents as well. FDA approval for wider indications has been impeded by safety concerns, but these have now been assuaged through extensive pre-clinical and clinical testing.<sup>1,2</sup> For example, a retrospective study of over 23,000 patients showed that the use of MBs was safe with an adverse event rate lower than 1 in 10,000.<sup>2</sup> The documented safety of MBs has led to new FDA approval in 2016 for characterization of focal liver lesions in adult and pediatric patients, though the same approval in Europe occurred over a decade earlier.

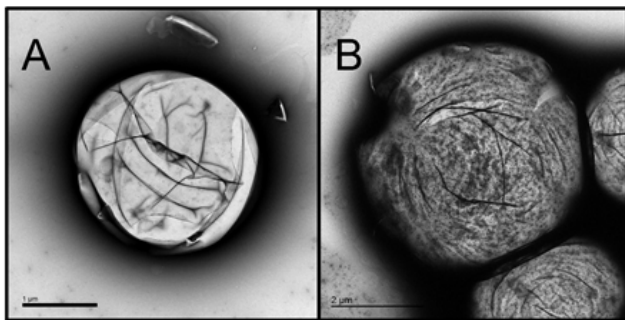
Contrast enhanced ultrasound (CEUS) is a versatile imaging platform that requires no ionizing radiation, is low-cost, has increasing regulatory acceptance and provides real-time imaging of both superficial and deep anatomical structures. The clinical uses of diagnostic ultrasound (US) without contrast agents for interventional

procedures range from US-guided biopsies to hemodynamic evaluation of pelvic and thoracic organs, as well as guided therapeutic injections. More recently, the application of US for therapeutic purposes has ranged from high intensity US for localized heating (with or without MBs) to lower intensity US combined with MBs or other cavitation nuclei to induce bioeffects.<sup>3-5</sup> These bioeffects are primarily caused by the dynamic behavior of bubbles that, promotes the permeabilization of nearby tissue through a variety of mechanisms.<sup>6</sup> The combined US and MB platform provides a range of approaches for diagnostic and therapeutic applications.

### Further potential for microbubbles in diagnostic applications

For clinical applications, MBs are typically ~2 µm in diameter and encapsulate inert, low solubility gases (Figure 1). Commercial formulations use biocompatible phospholipids or albumin to coat the MBs and stabilize them against dissolution. MBs are strong acoustic scatterers and also can produce highly non-linear echoes that enable CEUS imaging. The linear echoes of tissue can be separated from the non-linear echoes of the contrast agent which can

help, for example, elucidate vascular density or perfusion volume in the liver.<sup>7</sup> CEUS perfusion imaging is able to detect small changes in blood flow, enabling clinicians to e.g. quantify the effect of anti-angiogenic therapies.



**Figure 1.** Transmission electron micrographs of (A) a typical echo contrast microbubble encapsulated with a phospholipid shell and (B) a microbubble with super paramagnetic nanoparticles attached to the shell.

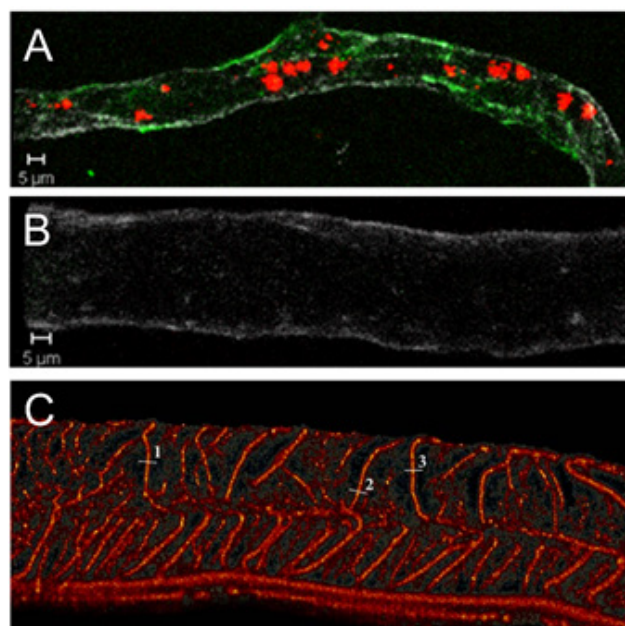
Following the success of MBs as UCAs, the development of more sophisticated MBs for molecular imaging has shown clinical promise. The surface of a MB can be chemically modified to attach targeting ligands that can adhere to specific receptors on the endothelium. For example, MBs functionalized with antibodies that target inflammatory markers such vascular epithelial growth factor 2 or vascular cell adhesion molecule 1 showed a marked increase in signal compared to non-targeted MBs (Figure 2A and B).<sup>8,9</sup> MB based molecular imaging not only reveals the presence of a specific biomarker but shows the extent of the diseased tissue, thus, making it an ideal diagnostic tool for surgeons. One can further imagine a MB that, in addition to having a targeting ligand, can carry a therapeutic payload. In turn, the release of the drug may be controlled by the US device providing controlled and localized release. The development of MBs as a molecular imaging probe has opened the door for new indications for CEUS.

A negative aspect of US imaging is the poor spatial resolution which is limited by the wavelength of sound. This wavelength is dependent on and inversely scales to the driving frequency (e.g. a 7MHz transducer has a ~200 μm wavelength and the resolving power of wavelength/2). Clearly, the resolution is insufficient to visualize the microvasculature which gives critical information on a range of disease states. Addressing this limitation of US imaging was done by leveraging the echo difference between tissue and MBs, similar to CEUS but with the addition of sophisticated signal post-processing to track a single bubble signal. Ultimately, an image can be constructed reducing the diffraction limit by nearly 10-fold and elucidating vessels >20 μm (Figure 2C).<sup>10</sup> With the advent of super-resolution US imaging, the observation of the micro-vessels in the organs such as the brain, kidney and cancerous tumors is being realized. The clinical needs this technology addresses are still being established and clinician input is critically needed.

### Clinical utility of therapeutic microbubbles

The response of US activated MB can be characterized in two regimes, inertial and non-inertial cavitation. At low to mild acoustic intensities, MBs oscillate stably in a pressure field and generate fluid microstreaming that can e.g. increase the local convection of drugs into tissue. Shear forces are also generated on nearby cells that can induce

reversible cell poration. At higher acoustic intensities, inertial cavitation occurs, characterized by large amplitude volumetric oscillations that typically lead to rapid bubble destruction and much more significant physical effects on the surrounding tissue. During rarefaction, bubbles can expand to many times their initial radius so that, upon compression, they collapse violently creating shock waves, and potentially high speed liquid jets that can penetrate tissue. The key US parameters that dictate the MB response is pulse duration, frequency and pressure. On commercial diagnostic systems, these parameters are not controlled by the user to ensure patient safety, rather the user has control over the type of imaging probe, the set image focus and the mechanical index. Often, in MB therapeutic applications, a single-element focused transducer is used to control the spatial location of high acoustic intensities to prevent adverse off target bioeffects. Both cavitation regimes have important utility for enhanced therapeutic delivery in surgical diseases.



**Figure 2.** (A) Example of molecular imaging using adherent microbubbles (red) to E-selectin (green). (B) The control for (A) where the endothelium (grey) is shown of an E-selectin knock-out mouse. No microbubbles adhere when the target receptor is not present. (C) Super resolution ultrasound imaging of a rat spinal cord. The colour-map indicates the microbubble density. The brighter the region the greater the number of bubbles and the vessel diameters for profiles 1, 2 and 3 are 21 μm, 19 μm and 20 μm, respectively. (A) and (B) adapted from ref.<sup>14</sup> with permission. (C) adapted from ref.<sup>10</sup> with permission.

Inertial cavitation for drug delivery has been widely shown in pre-clinical studies to promote the intratumoral distribution of drugs. Researchers have shown that stimulated MBs can move even macromolecular therapeutics and nanoparticles more than 200 μm.<sup>11</sup> Careful consideration of the target application must be taken before deciding the desired MB cavitation regime. For example, opening the blood brain barrier to enable deep penetration of a therapeutic is accomplished using non-inertial cavitation that can lead to reversible opening of the tight junctions. Alternatively, inertial cavitation may be more appropriate for targeting liver or pancreatic lesions. One such example using inertial cavitation is for the delivery of the oncolytic adenoviruses (Ads).<sup>4</sup> This macromolecule is a

potent anti-cancer therapeutic but suffers from poor tumor penetration when administered intravenously. Prior work showed that when Ads are co-administered with clinically approved MBs and stimulated with focused US the infection rate of the tumor cells improved over 30-fold. This kind of immunotherapy plus US and MBs offers a robust solution to poor tumor extravasation.

Another emerging area for therapeutic MBs is for vascular diseases. Acute limb ischemia due to an occluding thrombus requires urgent treatment. Current clinical interventions are time-consuming and require repeated surveillance typically with computed tomography angiography. US and MBs have proven to be a viable treatment alternative that supports the dissolution of calcified blood clots and improves penetration of thrombolytic plasminogens.<sup>12</sup> An ongoing phase II clinical trial is investigating the safety and clinical feasibility of US-stimulated MBs for thrombolysis.<sup>15</sup> Additionally, another application arises from the increased frequency of femoral artery puncture to access the vasculature in interventional cardiology and interventional radiology. Perforation to the vessel combined with the influence of arterial pressure can form a perfused blood sac that communicates with the vessel lumen. To avoid open surgery to repair the vessel, MBs loaded with magnetic nanoparticles and thrombin are being developed. Magnetically susceptible MBs are able to be trapped due to an externally applied magnetic field preventing the outflow of thrombin from the blood sac. The MBs are imaged to visualize the delivery of the coagulant. Collaborations with physicians and engineers will continue to unlock the potential uses of the US and MB drug delivery platform.

## Clinical developments and challenges

Commercial US and MB technologies are on the horizon. In 2017, the first-in-man trial of US-triggered targeted drug delivery in tumors was demonstrated that showed enhanced delivery of doxorubicin to unresectable liver tumors.<sup>5</sup> This study did not use cavitation nuclei, but it was an important step showing utility of therapeutic US. More recently in 2019, the first-in-man trial of blood brain barrier opening with MR-guided focused US showed that the use of MBs was feasible and safe for all amyotrophic lateral sclerosis patients in the trial.<sup>15</sup> These MB technologies and others not mentioned have already and continue to be a realized clinical tool that should be integrated into the first-line of patient care.

The greatest challenge facing MB-based technologies is from the international regulatory bodies. It will be critical for researchers, biologists, engineers, physicists and clinicians to have clear and open communication with regulators to prevent poorly devised clinical trials. Importantly, there is still much room for optimizing many of the previously mentioned diagnostic and drug delivery systems and it will be a significant challenge to develop these systems in a timely and cost-effective manner that will require substantial contributions from both the end users (clinicians) and designers (engineers).

## Conflicts of interest

None.

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