On-Demand Cavitation from Bursting Droplets

Breaking down biological barriers with microexplosions.

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Introduction

For many parents, the first image of their child in the womb is provided by diagnostic ultrasound. In addition to providing a moment of joy for the expecting parents, ultrasound images allow doctors to safely monitor fetal development. For most people, this is their first and only experience with biomedical ultrasound; however, the capabilities and impact of biomedical ultrasound is far more extensive. For example, ultrasound can be used to detect changes in blood flow associated with cardiovascular disease as well as facilitate the transport of drugs locally into the brain to treat neurological disorders. Many of these novel applications of biomedical ultrasound rely on ultrasound-driven bubble activity (acoustic cavitation), whether subtle and stable (stable cavitation) or intense and short-lived (inertial cavitation). Thus, clinical translation depends on the ability to control microbubble formation and activity in the body.

It has been estimated that the acoustic pressure required for bubble formation in the body exceeds 10 MPa (>100 atm). At these pressures, control of bubble activity is impractical and, consequently, the associated bioeffects are unpredictable. A greater level of control can be achieved by injecting bubble nuclei, such as microbubbles coated with stabilizing agents (i.e., ultrasound contrast agents). However, microbubbles injected intravenously are rapidly cleared from circulation by dissolution and the immune system, severely limiting their availability for biomedical applications.

In recent years, phase-change perfluorocarbon (PFC) droplets have emerged as a viable alternative to microbubbles for creating bubble activity in vivo. Compared with microbubbles, PFC droplets are more stable in vivo, can circulate longer, and can be made to escape the bloodstream, enabling extravascular bubble production. The droplet is composed of a liquid PFC with a boiling point that is lower than the body temperature and therefore exists in a superheated state when in the body. Subjecting the superheated droplets to ultrasound of sufficient pressure amplitude triggers a liquid-to-vapor phase conversion (**Figure 1**). The resultant gas cavity grows explosively within microseconds, increasing in volume by more than two orders of magnitude! In a sense, ultrasound serves as a remote detonator to trigger the vaporization process in a highly localized and predictable manner.

Envisioned by the late Dr. Robert Apfel (ASA past president and Gold Medal winner; 1998), the vaporization of PFC droplets using medically relevant ultrasound frequencies was first demonstrated experimentally by Kripfgans et al. (2000). These first observations of acoustic droplet vaporization (ADV) captured the imagination and scientific curiosity of physicists, chemists, and engineers, leading to exciting new research pathways in fundamental and translational science (Sheeran and Dayton, 2012). This article provides an overview of past and current work in this area, including studies on the physical mechanisms of ADV, approaches to con-

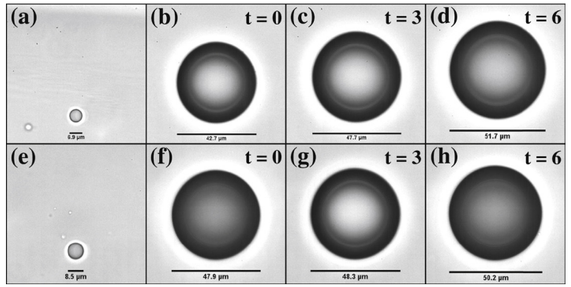

Figure 1. *Acoustic droplet vaporization (ADV) of individual phase-change microdroplets with ultrasound.* a *and* e*: Before images of microdroplets.* b-d *and* f-h*: Observed bubbles seconds after phase change. Reprinted with permission from Sheeran et al. (2011).*

trol acoustic cavitation (i.e., bubble formation and resultant oscillations) created by ADV, and potential biomedical applications of these novel droplets.

Physics of ADV

ADV Inception

The physical mechanisms behind the ADV process have long been a mystery. Depending on the boiling point of the perfluorocarbon used to prepare the droplets, the ADV pressure threshold can vary significantly. The threshold is a function of the ultrasound frequency and pressure, with decreasing pressures needed at higher frequencies. This is in stark contrast to the typical relationship reported for acoustic cavitation inception where higher pressures are needed at higher frequencies. The droplet size also plays an important role, with lower pressures required for larger droplets. All of these confounding factors have baffled scientists, who have turned to high-speed imaging methods to capture the initial stages of the ADV process.

In a series of recent experiments by Shpak et al. (2013), the ADV process with micron-size phase-change droplets was imaged on a nanosecond time scale. **Figure 2** shows images of ADV imaged at 18.5 million frames per second. The authors characterized the ADV process into three regimes: (1) oscillatory translations of the droplet due to the acoustic impedance mismatch between the droplet and the surrounding liquid; (2) nucleation of the liquid core and expansion of the vapor bubble while the ultrasound is ON; and (3) continued growth while the ultrasound is OFF (Shpak et al., 2013). The nucleation of the liquid core occurred during the peak negative pressure phase of the ultrasound pulse and appeared to be governed by a homogeneous nucleation theory. Microscopic voids formed from the thermal motion of the liquid core served as the nuclei necessary to grow into a vapor bubble once sufficient tensile stress was applied.

This again brings into question the frequency dependence of the ADV threshold. A homogeneous nucleation theory would suggest that lower frequencies would subject the liquid to tensile stresses for longer periods of time, thus allowing more time for a microscopic void to form and increase the probability of nucleation. One could therefore assume that lower frequencies would be more ideal to initiate the ADV process.

Additional high-speed imaging experiments by Shpak et al. (2014) have addressed this discrepancy and provided insight into physical mechanism(s) of ADV. Their results suggest that ADV is initiated by superharmonic focusing of the ultrasound wave by the droplet. The high excitation pressures used for ADV lead to increased nonlinear distortion of the propagating ultrasound pulses. These higher order harmonic frequencies present in the ultrasound wave can couple more effectively into the droplet. This leads to a focusing effect inside the droplet where high peak negative pressures can be obtained. Essentially, the incident ultrasound wave is amplified inside the droplet and this amplification is strongly dependent on the ultrasound frequency. This nonlinear wave propagation and pressure amplification inside the droplet can begin to explain results that show an inverse relationship between the ADV pressure threshold and frequency of the ADV pulse. It is important to note that the above studies were performed with micron-size phase change droplets and it is still unknown whether the proposed vaporization mechanisms are valid for droplets on the nanoscale. Researchers have shown that the ADV threshold is much higher (>5 MPa) for nanodroplets and reported inconsistent results on the frequency dependence.

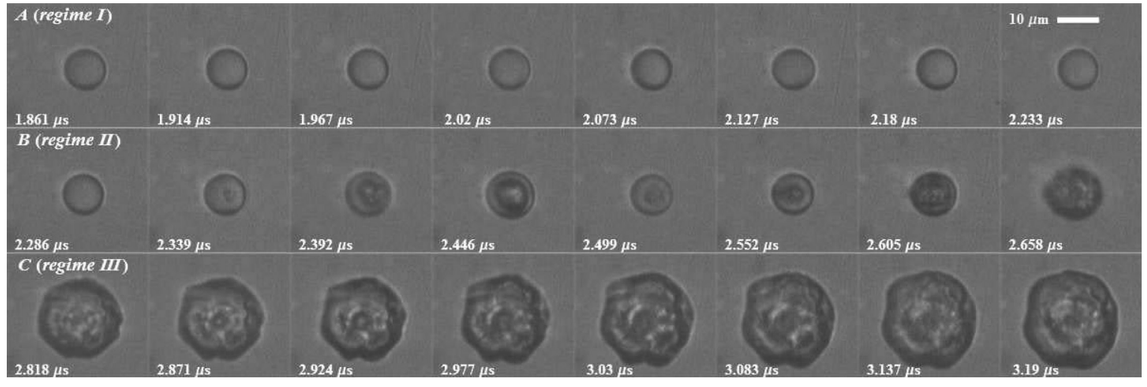

Figure 2. *High-speed imaging (18.5 million frames per second) of an individual phase-change microdroplet during ADV.* A*: Regime I;* B*: Regime II;* C*: Regime III. See text for additional description of the ADV process. Reprinted with permission from Shpak et al. (2013).*

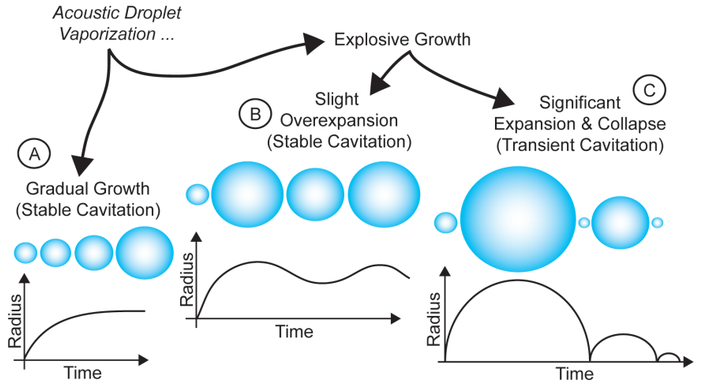

Figure 3. *The fates of bubbles after ADV.* A *and* B*: Bubbles undergoing stable cavitation after ADV. The gradual bubble growth after ADV can lead to stable bubble formation with no additional oscillations after passage of the ultrasound pulse. Explosive growth after ADV can lead to more extensive bubble motion and oscillations after passage of the ultrasound pulse. A slight overexpansion of the bubble can lead to bubble oscillations at its resonance frequency.* C*: Significant expansion and collapse (transient cavitation) can also occur after the explosive growth phase of the bubble. This leads to an energetic collapse that can lead to bubble fragmentation and destruction.*

Post-ADV Bubble Dynamics with Microdroplets

Once formed, bubbles will oscillate in response to transmitted ultrasound. The bubble oscillations can be classified as stable (i.e., sustained over many cycles with a radial expansion ratio < 2) or inertial (radial expansion ratio > 2 followed by energetic collapse). Stable cavitation is less damaging than inertial cavitation and more useful for imaging applications, whereas inertial cavitation can be used for a variety of therapeutic applications. The cavitation activity is dictated by several factors, including droplet size, boiling point of the PFC core, and the ultrasound frequency and pressure. Studies to understand the relationship between these factors and the dynamics of the resultant vapor bubble are currently underway.

Studies with micron-size droplets have generally uncovered a rapid growth of the bubble during the ultrasound pulse due to ultrasound-driven heat transfer followed by a slow growth regime. Sheeran et al. (2014) have shown that vapor bubbles formed from ADV overexpand and undergo free oscillation at the resonant frequency of bubble that can be detected acoustically. These scenarios are depicted in **Figure 3a** and **b**, where ADV can be followed by gradual or explosive growth of the bubbles leading to stable cavitation.

Similarly, Fabiilli et al. (2009) have shown that ADV can be achieved with or without inertial cavitation, supporting the idea that ADV of micron-size droplets can be achieved with or without explosive growth and collapse of vapor bubbles. This leads to the aforementioned reasoning that the bubbles formed from ADV of micron-size droplets do not respond rapidly to the ultrasound frequencies in the 1- to 10-MHz range used for ADV. Typically, a phase-change droplet transitions into a bubble with a 5-fold increase in diameter. Therefore, a 1- to 10-μm-diameter droplet would convert into a 5- to 50-μm-diameter bubble. The resulting free bubble resonant frequencies would be 1.35 – 0.12 MHz, respectively. Analogous to a forced mechanical oscillator, the bubble would be insonified well above resonance in a mass controlled regimen and therefore not respond significantly to the remainder of the ultrasound pulse. For high-speed imaging experiments, researchers have predominantly imaged larger droplets (>5 μm) for ease of viewing under the microscope. The resultant bubbles from these droplets would have large diameters (>25 μm) and low resonant frequencies (<0.3 MHz), which could explain the results depicting slow growth.

Post-ADV Bubble Dynamics with Nanodroplets

Phase-change nanodroplets have shown different dynamics post-ADV compared with their micron-size counterparts. Reznik et al. (2013) showed that bubbles formed after ADV undergo rapid growth and collapse. This is similar to transient or inertial cavitation and different from the gradual growth of bubbles seen with micron-size droplets. Other post-ADV fates were seen after the transient growth/collapse phase such as dissolution, recondensation of the liquid

core, coalescence, fragmentation, or free oscillations.

Preliminary results from our laboratory show that nanodroplets immediately undergo an explosive growth and collapse at the ADV threshold. Using a acoustic detection scheme similar to that of Ammi et al. (2006), we detected the acoustic emissions from individual nanodroplets at the ADV threshold. The results show evidence of postexcitation (i.e., after the ultrasound pulse had passed) broadband acoustic emissions. Because ADV occurs during the peak negative phase, the bubble is subjected to high tensile stresses that can drive an explosive growth phase where the bubble can reach high expansion ratios (i.e., $R_{max}/R_0 > 2$). Then, once the ultrasound wave has passed, the bubble collapses due to the in-rushing fluid surrounding the bubble. This is shown in **Figure 3c** where ADV is followed by explosive growth, collapse of the bubble (inertial cavitation), and radiation of broadband acoustic emissions similar to a hand clap or door slam.

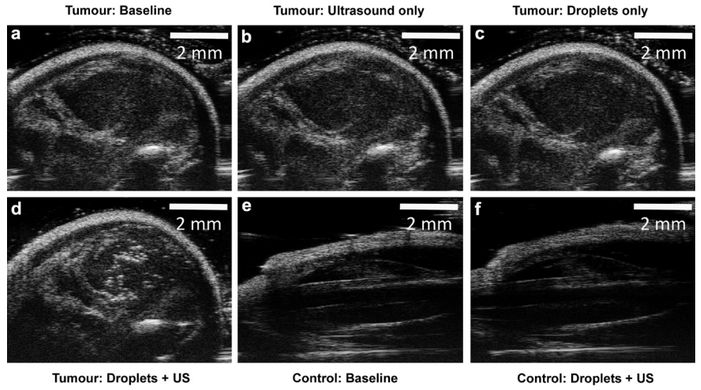

Figure 4. *In vivo demonstration of ADV with phase-change nanodroplets in a mouse tumor and imaging of the resultant microbubbles with a diagnostic ultrasound imaging system. The tumor was implanted in the hind limb of one leg and the other leg was used for baseline images without a tumor present.* a *and* b*: Control ultrasound images of the leg with the tumor before introduction of nanodroplets.* a*: Baseline image of the tumor.* b*: Resultant image after exposure to a 1-ms burst of ultrasound at 10 MHz with a peak negative pressure of 6 MPa before nanodroplet injection.* c*: Baseline image of the tumor after injection of phase-change nanodroplets. ADV has not been initiated at this point and therefore the lack of increased contrast emphasizes that the nanodroplets are stable on injection in the mouse.* d*: Detection of microbubbles 1 hour and 20 minutes postinjection of nanodroplets and exposure to a 1-ms burst of ultrasound at 10 MHz with a peak negative pressure of 6 MPa.* e*: Baseline image of the other leg without a tumor.* f*: Image after application of a 1-ms burst of ultrasound at 10 MHz with a peak negative pressure of 6 MPa. The lack of contrast enhancement in the nontumor leg indicates that nanodroplets were only able to extravasate into the tumor and not into the normal tissue. Reprinted with permission from Williams et al. (2013).*

Applications of ADV

Diagnostic Applications

Because bubbles volumetrically pulsate in response to ultrasound waves, they act as excellent scatterers during diagnostic ultrasound imaging. This provides an increased contrast enhancement in locations where bubbles are present. Kripfgans et al. (2002) proposed to use phase-change droplets as "sound beacons" for the improvement of diagnostic and therapeutic ultrasound fields by phase aberration correction. Individual bubbles formed after ADV can be used as point sources of ultrasound that can be used to predict changes in sound speed along the propagation path that leads to image distortion.

Ideally, phase-change nanodroplets are the most attractive option for diagnostic applications because they won't transition into large enough bubbles to block blood flow. Additionally, they are small enough to escape the vascular space and accumulate in tissue. **Figure 4** shows the results from a study investigating the use of phase-change nanodroplets for the extravascular imaging of cancer. Williams et al. (2013) showed that it was possible to inject nanodroplets into mice with tumors implanted in one of their hind limbs and initiate ADV inside the tumor. Their results suggest that the nanodroplets are indeed stable inside body and able to leave the blood stream and accumulate in tumors. Nanodroplets were injected into the tail vein over 20 minutes with an injection pump and allowed to circulate for 1 hour before the initiation of ADV. On application of a 1-ms burst of ultrasound at 10 MHz with a peak negative pressure of 6 MPa, increased image contrast can been seen within the tumor (**Figure 4d**), indicating the successful conversion of nanodroplets into microbubbles. The authors then performed an identical exposure with a 1-ms burst of ultrasound at 10 MHz with a peak negative pressure of 6 MPa in the other hind limb with no tumor present (**Figure 4f**). The lack of contrast enhancement in the muscle of the other leg confirms that (1) nanodroplets were not still in circulation and (2) the nanodroplets were able to only extravasate into the tumor and not into healthy tissue.

Researchers have taken extravascular imaging one step further by incorporating molecular targeting onto the surface of phase-change nanodroplets. The surface of the nanodroplets can be decorated with targeting ligands and antibodies that can bind with certain receptors expressed on cells. Therefore, if there are known targets for certain diseased states, the nanodroplets can hunt out those cells and bind to them. Sheeran et al. (2013) have shown proof-of-principle results using nanodroplets for molecular imaging in vitro. Nanodroplets were able to bind to specific receptors on the cell membrane and imaged with ultrasound after ADV. This platform could be expanded to numerous molecular targets inside the body. Examples could include cancer detection and imaging the therapeutic response of a tumor to treatment.

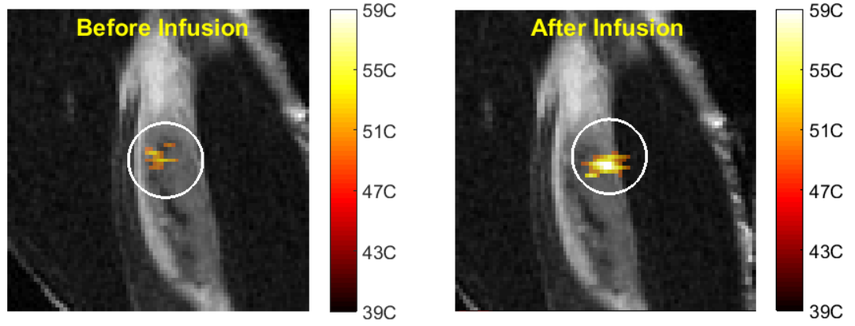

Figure 5. *Temperature maps in rabbit tumors after 30 seconds of sonication with high-intensity focused ultrasound (HIFU) using magnetic resonance (MR) thermometry. Sonications were performed before or after infusion of phase-change nanodroplets. The presence of nanodroplets resulted in an increased peak temperature and heating area. From Kopechek et al. (2014).*

Therapeutic Applications

Perhaps the earliest therapeutic application was to utilize ADV for blood vessel occlusion (Kripfgans et al., 2002). The dramatic volume change of microdroplets can be used to occlude the microvasculature by lodging bubbles in small blood vessels. A reduction in blood flow to specific tissue regions in the body by occlusion could be beneficial for thermal therapies. Local hyperthermia (thermal ablation) is very effective at killing cancer cells and essentially cooks the tumor. Ultrasound, radio waves, and microwaves are all forms of energy that can be used to heat up the tissue. One issue with these techniques is the loss of heat due to blood perfusion. The blood flow carries away thermal energy and acts as a heat sink to reduce energy deposition.

Drug delivery enhancement can also be achieved through decreased blood flow by allowing better diffusion of the drug into the tissue. Fabiilli et al. (2010b) demonstrated the incorporation of hydrophilic (water-soluble) drugs into micron-size droplets that can be released on ADV. This method was also extended to lipophilic (oil-soluble) drugs such as chemotherapeutics (Fabiilli et al., 2010a). The unique aspect of this work was the ability to utilize the perfluorocarbon liquid core as a drug reservoir that can be released upon ADV. The controlled release combined with the synergistic effects of reduced blood flow and tissue hypoxia could provide a promising drug delivery platform.

Phase-change nanodroplets have also been utilized for numerous drug delivery applications. Rapoport et al. (2011) demonstrated the use of phase-change nanodroplets for delivery of a chemotherapeutic agent to mouse tumors. The agent was loaded into the polymer shell of the nanodroplet, which was able passively accumulate in the tumor. On ultrasound exposure, the drug could be released to provide a therapeutic effect, which showed tumor regression and suppression of metastasis (i.e., spread of cancers in the body).

Enhancement of acoustic cavitation-based bioeffects is perhaps the greatest advantage of phase-change nanodroplets. Stable and inertial (transient) cavitation has long been known to cause numerous bioeffects due to the mechanical motion of the microbubble. High radial wall velocities, microstreaming, shockwave emission, fluid jetting near boundaries, and the build-up of high temperatures and pressures on collapse are all possible methods of acoustic cavitation-mediated bioeffects (Neppiras, 1980; Leighton, 1994).

The radiated acoustic waves during cavitation can also lead to an increased absorption of ultrasound energy by the tissue and accelerate heating. **Figure 5** shows the results from studies investigating the potential of phase-change nanodroplets for accelerating high-intensity focused ultrasound (HIFU) heating with reduced acoustic power (Kopechek et al., 2014). These experiments were performed in rabbits with tumors implanted in the hind limbs. HIFU exposures were performed before and after injection of phase-change nanodroplets and the temperature increase was monitored with magnetic resonance (MR) thermometry. Exposures with the nanodroplets present resulted in higher peak temperatures and a larger heating area, thus highlighting the potential use of nanodroplets for ultrasound-based thermal therapies.

In terms of breaking down biological barriers, Chen et al. (2013) demonstrated the use of phase change nanodroplets for a controlled disruption of the blood-brain barrier. The

blood-brain barrier limits the potency of drugs in the brain due to their inability to cross the barrier and enter brain tissue. Upon ADV of nanodroplets in the brains of mice, the bubbles were able to permeabilize the barrier and allow drug penetration in the brain through acoustic cavitation-based bioeffects. The results demonstrate that nanodroplets may be more efficacious in the disruption of the blood-brain barrier than preformed ultrasound contrast agent microbubbles. Nanodroplets led to a more homogeneous distribution of a model drug into the brain and showed enhanced cavitation properties.

Reversible cell membrane disruption with phase-change nanodroplets has also been shown to be possible. The ability to use acoustic cavitation to disrupt the cell membrane is called sonoporation. Burgess and Porter (2015) highlighted the possibility of using nanodroplets for the delivery of nucleic acids to cells using sonoporation. This in vitro study demonstrated that after ADV of nanodroplets, the bubbles were able to temporally open up cell membranes to allow passive diffusion of the nucleic acids into cells. The main conclusion was this process maintained high cell viability, highlighting the ability to trigger ADV in a way that won't destroy cells.

Future Outlook

In summary, phase-change micro- and nanodroplets provide an appealing platform for the creation of acoustic cavitation inside the body. Although described as an explosive process, careful planning in regard to the droplet size and ultrasound parameters can aid in determining the violence of the phase-change process. Gradual growth of the vapor bubbles may be more beneficial for diagnostic applications where explosive growth and collapse may be appropriate for therapeutic applications. Hopefully, the concept of microexplosions inside the body doesn't sound as crazy now.

Acknowledgments

The authors acknowledge funding support from the National Institutes of Health.

Biosketches

Mark T. Burgess graduated from the University of Cincinnati in 2010 with a BS degree in Biomedical Engineering. He then enrolled in the Department of Mechanical Engineering at Boston University, received his MS degree in 2013, and is currently pursuing his PhD. His thesis work is focused on understanding the acoustic cavitation properties of phase-change nanodroplets and their relationship with sonoporation. After graduation in Fall 2015, he plans on joining the Ultrasound Elasticity Laboratory at Columbia University as a postdoctoral research scientist under the supervision of Dr. Elisa Konofagou.

Tyrone Porter is an Associate Professor in the Departments of Mechanical Engineering and Biomedical Engineering at Boston University. In 2006, he founded the Nanomedicine and Medical Acoustics Laboratory (NanoMedAL) with the goal of engineering novel stimuli-responsive particles for medical applications. Dr. Porter earned a Bachelor's degree in Electrical Engineering from Prairie View A&M University in Texas and completed his graduate work in the Department of Bioengineering at the University of Washington. He was awarded the Frederick V. Hunt Postdoctoral Fellowship and the R. Bruce Lindsay Award from the Acoustical Society of America in 2003 and 2008, respectively.

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