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Effect of Therapeutic Ultrasound on Acoustically Sensitive Microcapsules

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Abstract— **In the area of therapeutic ultrasound activated drug delivery, difficulties exist in designing a carrier that responds to ultrasound for triggering and imaging but also provides adequate treatment potential. In this paper, we report on a novel acoustically sensitive microcapsule reservoir that can be activated with therapeutic ultrasound for payload release and can be potentially tracked using imaging. It is being designed for increased longevity and is not planned for the circulation. Here, we describe its unique formulation and demonstrate effects of therapeutic ultrasound on it at 1MHz using a combined optical-acoustic setup on a microscope. We see membrane bulging and damage for small and large capsules with both continuous and pulsed ultrasound. We also show some preliminary work on understanding the mechanism behind these effects. The reservoirs show potential for future ultrasound activated release and imaging while being patent in form and function over several weeks.**

I. INTRODUCTION

For 2010, NCI SEER Cancer Statistics Review reports 207,090 women will be diagnosed with breast cancer and 39,840 women will die of the disease [www.cancer.gov/statistics]. These mortality numbers have seen only a 2% decline in 20 years even though diagnosis is up by 29% [1]. To improve these outcomes, a new paradigm of minimally invasive and/or locoregional cancer therapies has been rapidly evolving. Ultrasound drug delivery researchers are contributing to this and are making great strides in localized therapies. Here, the energy has been used to trigger drugs from carriers like micelles, liposomes and microspheres due to energy dependent thermal and/or cavitational effects [2]. Therapeutic ultrasound has also been used to trigger drug release under image guidance (both therapy and imaging). This is a new area of localized treatments that integrate therapeutic interventions with diagnostic imaging [3]. In this context, the use of microbubbles ultrasound contrast agents (UCAs) is very popular [3]. UCAs are clinically available, tiny lipid/polymer-shelled gas bubbles [4]. They undergo volume changes under sonication and also generate an acoustic signal for imaging. Their micron size (1-5μm) makes them ideal for ultrasound (MHz) imaging [3]. These microbubbles are also used in drug delivery by either co-administering drugs and microbubbles or drug-loading of the microbubbles in their shell [3], [5]. Many examples of lipid/polymer-shelled microbubbles have been reported for drug delivery [4], [3]. Non-thermal cavitational effects of ultrasound result in the disruption of the microbubbles and formation of micropores in cell membranes, facilitating passive diffusion and intracellular accumulation of the drug [2].

The main difficulty in these ultrasound therapies is in designing a drug delivery scheme that will not only be responsive to ultrasound for triggering and imaging but also provide adequate treatment potential such as - high payload and good extravasation. Microbubble carriers provide excellent image contrast and enhance transport of drugs [4], but have low-payload [4] and are cleared quickly from the body (20 min) [3]. Thus, treatment needs to take place shortly after its intravenous injection [3]. Additionally, their micron size does not allow effective extravasation [5]. To improve on these issues with microbubble carriers, Rapoport *et. al.* [6], developed a multifunctional echogenic drug delivery system to comprise of doxorubicin loaded polymeric micelles for drug delivery and nanobubbles, perfluorocarbon microbubbles for imaging. Dayton *et. al.*, [7], on the other hand, used perfluorocarbon nanoparticles with a liquid core to improve extravasation. These had decreased echogenicity in solution but improved when deposited in a layer. Most of these approaches are intravenous techniques and are activated in the vasculature; hence are particularly good for well perfused tumors. However these could have difficulties in treating larger tumors with ischemic areas. Further their carrier formulations are quite complicated and difficult for immediate clinical implementation.

In this paper, we develop novel drug microcapsule reservoirs and demonstrate their activation under therapeutic ultrasound. These reservoirs are functionalized by ultrasound contrast agents (UCAs) and drug-like substance to potentially offer both image contrast and drug delivery with ultrasound. They are also designed for high payload and increased longevity. These are termed as *Acoustically Sensitized Microcapsules (ASMs)* and are not planned for the circulation. We demonstrate in this paper the preparation process of these ASMs, their response to therapeutic ultrasound and their mechanical strength.

Microcapsules have been used most commonly in cell encapsulation for treatment of metabolic disorders [8]. For instance, when microcapsules encapsulating islet cells were implanted into diabetic rats, they corrected its state for 15 weeks [9]. In other studies, they remained patent in form and function for 6-20 weeks [10]. Their immunosuppression is heavily influenced by materials used, purification and

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production processes along with implantation devices used [10]. Microcapsules can be simply injected as they are difficult to mechanically disrupt [8], [10]. Their size can be tailored from a few μm-mm [8]. Other advantages are high drug payloads, surface modification, and reproducibility [11]. These types of microcapsules will form the basis for the preparation of ASMs.

II. METHODS

A. Preparation of Acoustically Sensitive Microcapsules (ASMs):

We prepare ASMs using atomization (FIG 1a, [12], [8]) and ink-jet techniques ([13]). Atomization creates microcapsules in the range of 100-1000 μm and ink-jet creates ASMs in the range of 30-100 μm. To create ASMs, drug-like substance (ex. blue dextran) along with M-UCAs was suspended in sodium-alginate. One important selection was the size of the ASMs. It is designed such that the size is large enough for encapsulating UCAs and also small enough for future use in intra-tumoral administration. For a given viscosity, the coaxial air flow (Fig 1) shears the sodium alginate/UCA mixture into droplets. The size of the droplets can be controlled by the air and suspension flow-rate along with the dimensions of the atomizer. The atomizer needle assembly is a concentric 24G needle surrounded by a 16G needle. Typically, a 1.5% sodium-alginate solution (μ=500cP,γ=45dyn.cm) containing a 10% (v/v) solution of UCAs (2$\pm$0.5μm) are atomized into a 1.5% $CaCl_2$ bath. The calcified sodium-alginate beads are then washed with 0.9 % NaCl twice. These beads are impermeable. In order to confer selective permeability, beads are then suspended in a 0.1% poly-lysine (MW=25,000) for 3-30 minutes. After 2 saline washes, they are suspended in 0.1% (v/w) low viscosity alginate solution for 4 minutes. The resulting suspension is then suspended in a 55mM sodium citrate solution (3 mins) in order to liquefy the alginate core. Once prepared, the larger capsules were quantified for mechanical strength using a custom indentation system. The system estimates the stiffness (Young's modulus) of single ASMs by applying small forces in a sinusoidal manner and records resulting displacements.

Microcapsules in the range of 30-100μm were fabricated using our ink-jet system (not shown, Microfab's Jet-lab System). It consists of a CCD camera (30 fps), a piezoelectric print-head, a triggering unit, a fluid delivery unit, and a PC for modification of waveform parameters like Voltage (V), dwell time (t_d) and frequency (f). The print-head has an aperture of 60 μm and is rated for μ <40 cP and γ <72 dyn/cm. The ink-jet fires a low viscosity alginate/UCA into 1.5% $CaCl_2$ solution for bead formation. The steps for conferring selective permeability are identical to the atomization procedure.

In this paper, the main parameters that were varied were ASM size and presence/absence of M-UCAs for the ultrasound experiments (see below). Only atomized impermeable beads were used.

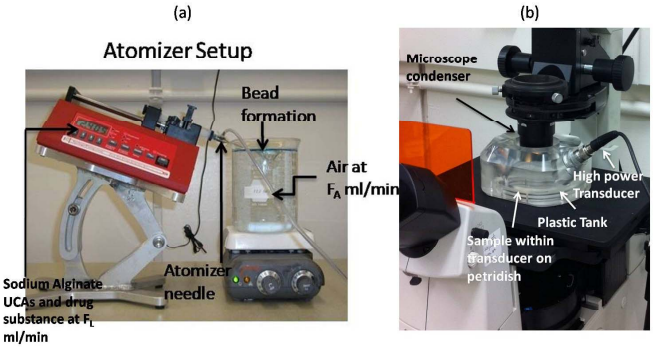

Fig. 1. (a) Shows the atomization chamber and needle assembly to create large ASMs (100-1000 μm). (b) Sonication setup showing a biological microscope, plastic tank with bottom cutout for a petridish on which ASMs within gelatin are placed. Also shown is the high power transducer used to apply continuous and pulsed wave ultrasound with its acoustic focus in-line with the optical focus of the microscope.

B. Effect of Therapeutic Ultrasound:

ASMs were mixed into a solution of tissue mimicking gelatin material to make small cylindrical transparent constructs (diameter: 20mm, height: 3mm). These constructs were mounted on a biological microscope (Nikon Ti-S) (Fig 1b). A single element ultrasound transducer (1MHz, Valpey Fisher Inc. at 45$\deg$) was powered using a RF power amplifier (ENI240L) and an arbitrary waveform generator (1281A, Tabor Electronics). It was then programmed with continuous wave or pulsed schemes and was used to sonicate the construct positioned on the microscope. Care was taken to ensure that the optical and acoustic foci (confirmed using a needle hydrophone (Ondacorp Inc.) were at the same location. The microscope camera was used to capture events observed. Choice of the ultrasound pulses is important to engage permeability changes. [3], [4] have used long low pressure pulses to translate UCAs in the vasculature and high pressure pulses to engage cavitation (just UCA oscillation or collapse). In this study, we use 10-40s of continuous wave (CW) ultrasound (center frequency = 1MHz or 2.25 MHz, power = 0.5W) to help translate UCAs and further nucleate energy for membrane damage. We also demonstrate the effect of pulsed ultrasound (center frequency = 1MHz, power = 35mW, $I_{avg} = 7mW/cm^2$) on these ASMs.

Overall power of the pulse was quantified using an ultrasound power meter (Ohmic instruments Inc.) that works on the radiation force balance technique. The lateral and axial beam profiles of the high power transducers were estimated using our custom built 3D hydrophone measurement system. This was done to reveal focus locations, lengths and extents of the transducers being used for sonication.

III. RESULTS AND DISCUSSION

Figure 2 shows an image of UCAs embedded within gelatin. They display a central white core with a dark ring. The figure also shows a sample ASM with UCAs and model-drug embedded. You can clearly see the UCAs within the ASM. We also prepared ASMs of several sizes using the atomization (see Fig 1) and ink-jet. We show ASMs

from the 30μm through 1000μm range. Once these were prepared, they were mounted on the Nikon microscope for simultaneous sonication and visualization using the setup in Fig 1b. Sonication was done with 2 different high power transducers: 1MHz and 2.25 MHz. Their focal widths, depths and extents were measured using our custom LABVIEW based hydrophone system. From the axial beam profile of the 2.25MHz transducer (not shown), the focal extent at 90% intensity was measured as 3 mm, the -6dB beam-width was measured at 0.8 mm and the focus location was at 31.8mm from the surface of the transducer. Similar measurements were made for the 1 MHz transducer (focal extent: 5mm; -6dB beamwidth: 4.2mm and focus: 25mm). These measurements assisted in the alignment of the transducers in the sonication setup on the microscope.

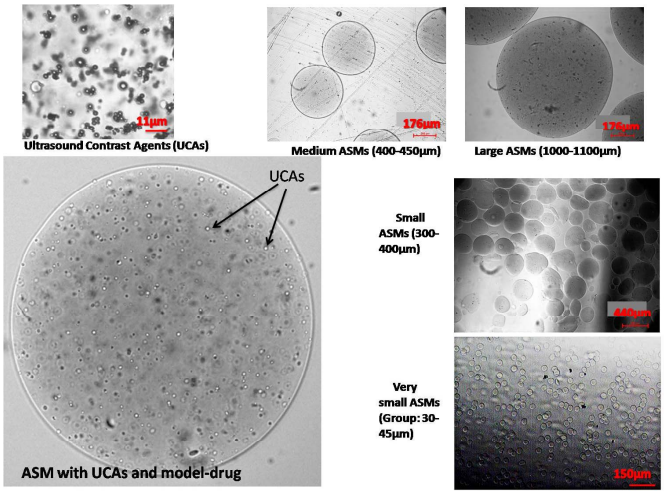

Fig. 2. Acoustically Sensitive Microcapsules with UCAs and model-drug substance prepared with Ink-Jetting (30-100μm) and Atomization ($\geq$100μm).

In Fig 3, we see that upon sonication with continuous wave ultrasound between 10-40s (frequency: 1MHz, power: 0.5W), small ASMs (order of 300 μm) undergo definite visual changes in the membrane; especially the ones that have UCAs encapsulated within them. With 10s of sonication membrane bulging was noted and 40s of sonication caused rupture of the membrane. Control ASMs prepared without UCAs did not experience rupture or bulging under similar conditions indicating that the UCAs were critical in nucleating ultrasound energy to cause membrane changes.

Fig 4 shows the effect of pulsed ultrasound (frequency: 1MHz, power: 35mW, $I_{avg} = 7mW/cm^2$) on these ASMs demonstrated using fluorescence imaging. We tagged blue dextran with a fluorescent particle for clearer visualization. We observed that bulging of the membrane was also evident when duty cycles as low as 1% were used. 100% duty cycles, i.e., continuous wave ultrasound again caused rupture. Currently we could not see the fluorescent dextran leakage into the surrounding gelatin after rupture, as the ASMs were embedded in gelatin. Gelatin has extremely small pore size (on the order of nm). Future experiments will involve

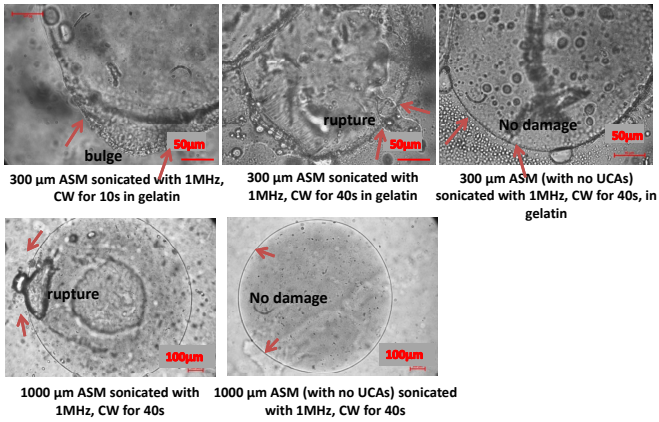

Fig. 3. Top Row: Effect of continuous wave therapeutic ultrasound on small ASMs embedded in gelatin. Ultrasound parameters: 1MHz high power transducer, 10-40s continuous wave, power=0.5W. Bottom Row: Effect of continuous wave therapeutic ultrasound on large ASMs embedded in gelatin. Similar ultrasound parameters were used. Also shown is the effect without the use of UCAs.

matrigels or chitosan matrices (to offer large pore size) and tracking of the particles over distance for mass-transport studies.

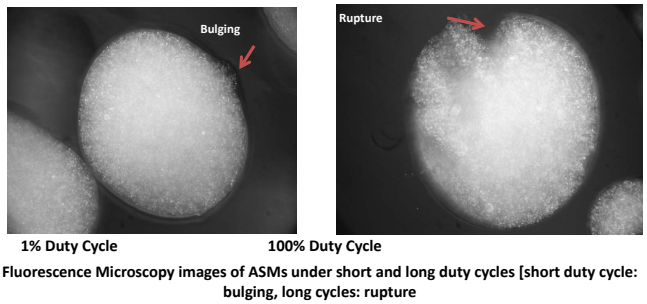

Fig. 4. Effect of pulsed wave therapeutic ultrasound on large ASMs embedded in gelatin. Ultrasound parameters: 1MHz high power transducer, 40s of pulsed ultrasound at 1% and 100% duty cycles, power=35mW, intensity (I_{avg}=$7mW/cm^2$).

We hypothesize that 10-40s of continuous wave (CW) ultrasound assisted in first translating UCAs to the ASM boundary and then helped in nucleating the energy to cause membrane damage. We have tried to capture this effect and we show a preliminary result in Fig 5. The figure shows accumulation of UCAs at the boundary of the ASMs over time (10s) upon continuous wave ultrasound sonication. We also show the movement of the UCAs deeper into the membrane structure with time. Both these effects are possibly caused by radiation force. We also show UCA expansion and contraction over time due to the continued ultrasound exposure. Both these effects combined could be responsible for membrane bulging and damage.

Lastly, mechanical measurements shown in Fig 6 revealed that stiffness of the ASMs varied significantly with size, % alginate used in the preparation and permeability. Permeable capsules measured $<$10KPa in Modulus estimates whereas impermeable beads were on the order of 100 KPa for the same size. We used impermeable beads in this study but at

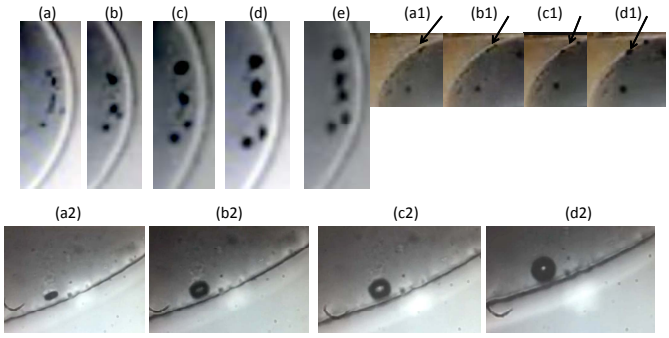

Fig. 5. (a-d): Effect of low power continuous wave ultrasound causing accumulation of the UCA at the boundary of the ASM over time (10s). Only one section of the ASM boundary is shown. (a1-d1) Movement of the UCAs into deep into the membrane structure again caused by radiation force. (a2-d2): Effect of low power continuous wave ultrasound on a UCA at the boundary of the ASM. The UCA is seen to be under contraction and expansion.

an alginate concentration of 1%. It is anticipated that the pulse sequences used above will be size and % alginate concentration dependent and will have to be adapted to these changing scenarios.

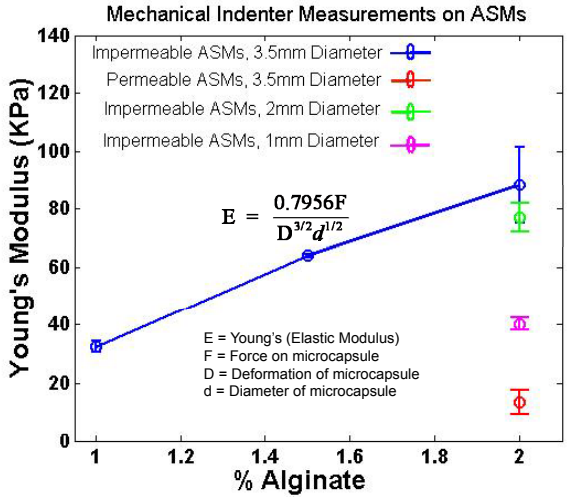

Fig. 6. Youngs modulus estimates of large ASMs based on indentation force and displacement data using a custom built indentation system (not shown).

IV. CONCLUSIONS AND FUTURE WORKS

In this paper, we demonstrate the preparation of a new drug delivery agent (Acoustically Sensitive Microcapsule-ASM) and the effect of therapeutic ultrasound on it. The ASM is unique in its construction and formulation and is being developed to target ischemic areas of large tumors with image guidance. The ASMs were designed to have high payload (because of its large size: ten's of microns to a mm) with high mechanical strength (see Fig 6) to withstand future intratumoral administrations into the tumor for increased longevity. We demonstrate some of the extreme effects of therapeutic ultrasound on these ASMs by using 10-40s of continuous wave ultrasound at 1MHz using a combined optical-acoustic setup on a microscope. This causes membrane breakage and damage only to those capsules that had UCAs present within them. Control capsules with no UCAs saw no damage at all indicating that the UCAs helped in nucleating ultrasound energy. We saw similar effects over small and large capsules. Preliminary measurements of the effect of pulsed ultrasound indicate that membrane bulging could be engaged at low duty cycles rather than rupture. This bulging could potentially cause stretching of the membrane and release of drug from within. However this aspect was not tested in this study. Some preliminary results were shown to understand the basic mechanism behind this rupture showing that UCAs could be manipulated using continuous wave ultrasound to move to the ASM boundary and oscillate at the boundary. Future work will involve design of specific pulse sequences to control the movement of UCAs more specifically and cause membrane bulging to release encapsulated drug substance for the purpose of using these ASMs for drug delivery over several weeks.

V. ACKNOWLEDGMENTS

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