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Acoustic Droplet-Assisted Particle Ejection through and from Agarose-Gel-Filled Petri Dish

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***Abstract*— This paper presents a novel way of ejecting particles (microspheres) carried by liquid droplets through and from Agarose-gel-filled Petri dish with high-intensity focused-ultrasound generated from self-focusing acoustic transducers working on the 3rd, 5th and 9th harmonic resonant frequencies of 1-mm-thick lead zirconate titanate (PZT) sheets. With different designs, the ejection spot area (related to focal size) is varied, so that the number of particles per ejection may be precisely controlled.**

Keywords—focused ultrasound, acoustic lens, self-focusing acoustic transducer, tunable focal size, droplet ejection

I. Introduction

There is unmet need to extract cells from mono-layer cells cultured on a solid surface in gel-based media without using a scoop, knife or pipette that leaves unwanted damage on the extraction edges, resulting in scars on the tissue grown out of the cells. With the mechanical tools, it is also difficult to control the number of cells extracted, due to large tool size and poor precision and repeatability of the manual operation.

A focused ultrasound (FUS) offers a solution to this need, as it can produce ejection of cells contained in liquid droplets from a solid surface with minimum impact on the edges surrounding the ejection spot. Ultrasound propagates through liquid and solid, and the FUS transducer does not have to be in physical contact with the substrate where cells are grown. The number of cells that are ejected by a FUS transducer depends on the focal size of the FUS, which can be very small and is very precise and repeatable.

In this paper, we present our proof-of-concept demonstration of FUS-based ejection of particles from a solid surface with the FUS transducer not in direct contact with the particle-containing solid substrate. Specifically, we use our self-focusing acoustic transducers (SFATs) based on Fresnel air-cavity lens [1], and show that different amounts of microspheres can be ejected out of the surface of a Petri dish filled with Agarose gel through varying the focal size of SFAT. For this paper, we design SFATs to operate at different frequencies, and use multiple SFATs for different focal sizes. However, with special design, the focal size of a single SFAT can also be electrically tuned [2].

II. Device Design

A. Focusing with Fresnel Air-Cavity Lens

The SFATs are built on PZT substrates (Fig. 1), which effectively produce ultrasound when a sinusoidal voltage signal with thickness-mode resonant frequency is applied onto the top and bottom circular electrodes sandwiching the PZT substrate. On the top electrode, we add a Fresnel acoustic lens consisting of Parylene-sealed annular-ring air cavities alternating with non-air-cavity ring areas on the electrode (with conformal deposition of Parylene). The rings are designed into Fresnel half-wavelength bands (FHWB) for 5 mm focal length, whose boundary radius R_n is given by [3]:

$$R_n = \sqrt{n\lambda \times (F_n + \frac{n\lambda}{4})}, \quad (1)$$

where λ and F are the wavelength in water and the designed focal length, respectively. This way, the path-length difference from two boundaries of a Fresnel ring band to the focal point (5 mm above transducer center) equals half wavelength. Utilizing acoustic impedance mismatch between air (only 0.4 kRayl) and solid/liquid (over 1 MRayl), all acoustic waves leading to destructive interference (in rings where $R_n<R<R_{n+1}$, n = 1,3,5,…) will be blocked by air cavities, where constructively interfering acoustic waves (in rings where $R_n<R<R_{n+1}$, n = 0,2,4,…) can propagate through Parylene layer of the lens (which is used for electrical insulation and acoustic matching), producing focused ultrasound of high intensity to eject droplets from air/water interface.

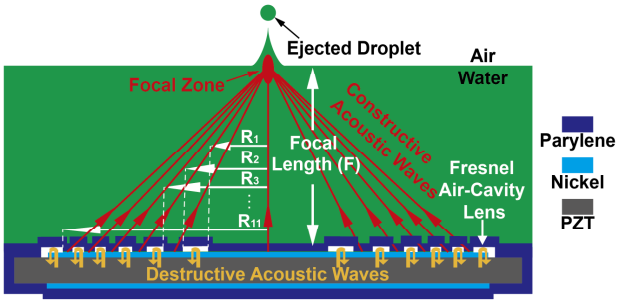

Fig. 1. Cross-sectional schematic of a SFAT-based droplet ejector, showing how the Fresnel annular-ring air-cavity reflector lens works.

B. Varied Focal Sizes through Harmonic Operation

The focal size of SFAT can be approximated by the width of its outermost ring band (if its boundary radii are much larger than its width) [4], and becomes smaller if the designed operating frequency is higher, as explained below. For focal length F much longer than wavelength λ (which is true in our cases), equation (1) can be approximated as

$$R_n \cong \sqrt{n\lambda F}. \quad (2)$$

For total N Fresnel ring boundaries, we get from (2)

$$R_N^2 - R_{N-1}^2 \cong N\lambda F - (N-1)\lambda F = \lambda F. \quad (3)$$

From algebra relationship, we also have:

$$R_N^2 - R_{N-1}^2 = R_N^2 - (R_N - \Delta R)^2 = 2R_N\Delta R - \Delta R^2, \quad (4)$$

where ΔR is the outermost ring width. If $R_N \gg \Delta R$, which is usually true for N > 3, ΔR^2 term in (4) could be ignored, and by comparing (3) and (4) we get

$$\Delta R \cong \frac{\lambda F}{2R_N} \cong \frac{\lambda F}{2\sqrt{N\lambda F}} = \sqrt{\frac{\lambda F}{4N}} = \sqrt{\frac{cF}{4Nf}}, \quad (5)$$

where f and c are frequency and sound velocity in medium, respectively. Equation (5) shows that with the same designed focal length and same number of rings, the focal size (which can be estimated by the outermost ring width) will be smaller when the SFAT is working at higher frequency, due to shorter wavelength, which is verified in finite element method (FEM) simulations (Fig. 2). Thus, we designed SFATs with 6 rings working at the 3rd (6.60 MHz), 5th (11.00 MHz), and 9th (20.96 MHz) harmonic thickness-mode resonant frequencies on 1-mm-thick PZT substrates. The actual measured resonant frequencies are 6.90, 11.65 and 20.99 MHz, respectively, which result in focal lengths that are slightly different from the designed values, but have negligible impact on the focusing efficiency. The device dimensions and simulation results are shown in Table I and Table II, respectively.

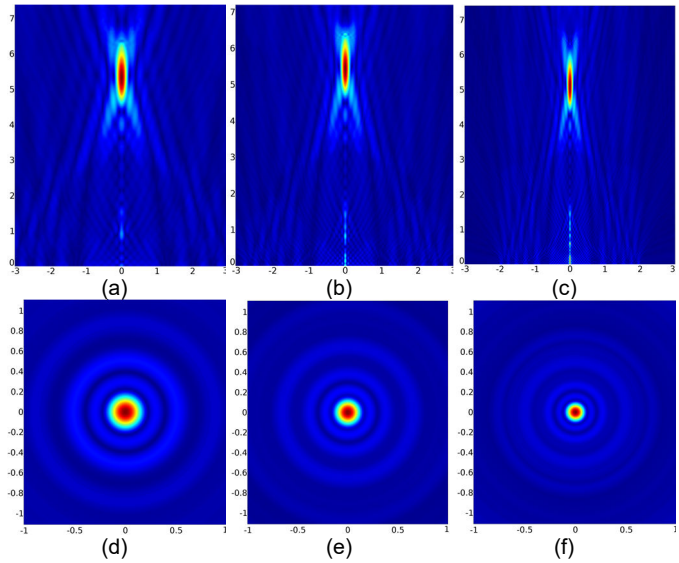

Fig. 2. FEM-simulated relative acoustic pressure distribution: on the central vertical planes for SFATs (designed for three different harmonics at 6.60 MHz, 11.00 MHz and 20.96 MHz) working at (a) 6.90 MHz, (b) 11.65 MHz, and (c) 20.99 MHz, respectively; and in the focal planes for the same SFATs working at (c) 6.90 MHz, (d) 11.65 MHz, and (e) 20.99 MHz.

TABLE I. DEVICE DIMENSIONS OF THE DESIGNED SFATS

Transducer size	Parylene thickness	Air cavity height
16 x 16 x 1 mm^3	16 μm	3.5 μm
Active area diameter		
3rd harmonic	5th harmonic	9th harmonic
7.44 mm	5.64 mm	4.02 mm

C. Focusing through Agarose-Gel-Filled Petri Dish

When a Petri dish (made of Polystyrene with its bottom plate being 0.75 mm thick) containing Agarose gel is immersed in water between SFAT and the water's top surface (Fig. 7b), the acoustic waves produced by the SFAT propagate through the water, Petri dish's bottom substrate, and Agarose gel, interfering with each other. The waves constructively interfere at the focal point with slightly larger focal size and slightly attenuated peak pressure at a slightly closer focal point (Fig. 4 and Table II), compared to the case of having no Petri dish. This is due to the fact that acoustic impedances of Petri dish (2.52 MRayl, attenuation coefficient 0.285 dB/cm-MHz [5]) and Agarose gel (1.58 MRayl, attenuation coefficient 0.07 dB/cm-MHz [6]) are close to the water's acoustic impedance (1.48 MRayl), so that there is little reflection at the interfaces of different media.

The effects of 1% (w/v) Agarose gel thickness on the peak acoustic pressure have been simulated (Fig. 3a), from which we see that the pressure changes periodically as the gel thickness increases and reaches local maxima at each incremental increase of the gel thickness every half wavelength. A thickness of 0.98 mm was thus chosen so that almost optimal peak pressure could be achieved for all the three frequencies. The gel thickness is realized by pouring 7.2 mL melted Agarose gel solution into a 90-mm-diameter Petri dish, based on our experiments shown in Fig. 3b.

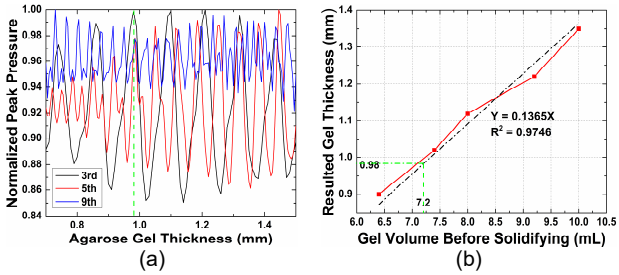

Fig. 3. (a) FEM-simulated normalized peak pressure versus 1% (w/v) Agarose gel thickness. The green dashed vertical line shows the selected thickness of 0.98 mm. In this simulation, the top of the gel is fixed at 4.5 mm above SFAT surface. (b) Experimental results on the relationship between gel volume before solidifying and the resulted gel thickness after solidifying at the center of a Petri dish with 90 mm diameter.

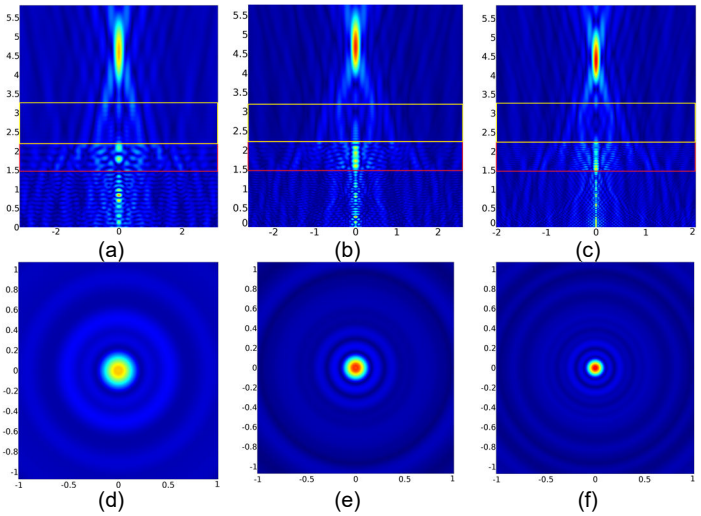

Fig. 4. FEM-simulated relative acoustic pressure distribution when the bottom of a Petri dish (with 0.75-mm-thick bottom plate, red line) filled with 0.98-mm-thick 1% Agarose gel (yellow lines) is 1.5 mm above SFAT surface in water: on the central vertical plane for SFATs working at (a) 6.90 MHz, (b) 11.65 MHz, and (c) 20.99 MHz, respectively; and in the focal planes for the same devices working at (c) 6.90 MHz, (d) 11.65 MHz, and (e) 20.99 MHz. The color ranges are adjusted to be the same as those in Fig. 2.

TABLE II. SIMULATION RESULTS

Focusing Parameters		Working Frequency (MHz)		
		6.90	11.65	20.99
Focal Length (mm)	In Water	5.34	5.39	5.08
	Through Dish & Gel	4.59	4.69	4.38
Focal Size (μm)	In Water	190.9	144.2	102.4
	Through Dish & Gel	198.6	149.0	103.4
Normalized Peak Pressure	In water	100%	100%	100%
	Through Dish & Gel	73.8%	88.9%	88.8%

III. EXPERIMENTAL RESULTS

A. Hydrophone Measurement of Peak Pressure

The SFATs are microfabricated according to the steps described in [7], in which the air cavities are fabricated through surface micromachining involving a sacrificial layer made of photoresist. The sacrificial photoresist is dissolved by acetone through release holes on Parylene which are sealed by another Parylene deposition (Fig. 5d to 5f). To measure the peak acoustic pressure at the focal point, a commercial hydrophone (Onda HGL-0085) fixed onto a manual 3-axis stage is scanned around along the central vertical axis to find the focal point, while the transducer is driven with pulsed sinusoidal signal with 50 V_{pp} at the resonant frequencies of each device. Then the same experiments are repeated with a Petri dish filled with 0.98-mm-thick Agarose gel with the bottom of Petri dish about 1.5 mm above the surfaces of SFATs. From the measurements (Fig. 6), we see that the Agarose-gel-filled Petri dish attenuates the acoustic pressure by 31.9%, 18.4% and 3.6% for the 3rd, 5th, and 9th harmonic SFATs, respectively, and the measured focal lengths are close to the simulated values (Fig.4 and Table II).

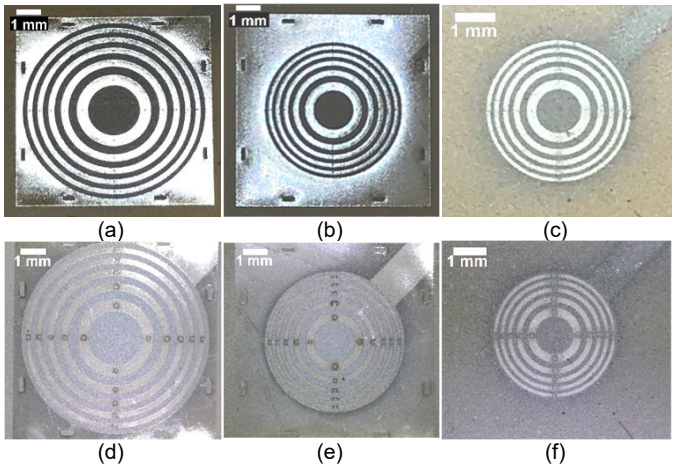

Fig. 5. Photos of fabricated devices on PZT substrates working at (a) 6.90 MHz, (b) 11.65 MHz, (c) 20.99 MHz, showing the air-cavities (shiny areas), and the same devices working at (d) 6.90 MHz, (e) 11.65 MHz, (f) 20.99 MHz under a digital microscope, showing air cavities (light grey areas), Parylene covered electrode (dark grey areas), and sealed release holes.

B. Droplet Ejection Experiments

The SFATs are then tested to eject water droplets in water with and without Petri dish filled with Agarose gel. During the tests, each transducer placed in a beaker filled with water is driven by pulsed sinusoidal signals of 200 V_{pp} (for the 6.90 MHz and 11.65 MHz transducers) or 400 V_{pp} (for the 20.99 MHz transducer) at the resonant frequency, at a pulse repetition frequency (PRF) of 20 Hz. The pulse width is selected so that only one droplet (without satellite droplets) may be ejected per ejection. To capture the ejected droplets, a red light-emitting diode (LED) driven by pulsed signals with the same PRF serves as a light source for stroboscopic observation of the ejection process with a certain delay after device actuation (Fig. 7a), while a camera whose frame rate is set to be the same as the PRF is attached at the end of a long-range microscope focused on the water surface where ejection happens for image recording. In experiments without Petri dish, the water surface is adjusted to the focal plane (Fig. 1). In experiments with Petri dish, the dish is held by a 5-axis precision manual stage, with a thin layer of water above the gel. Then the position of the Petri dish is adjusted until ejection can happen (Fig. 7b).

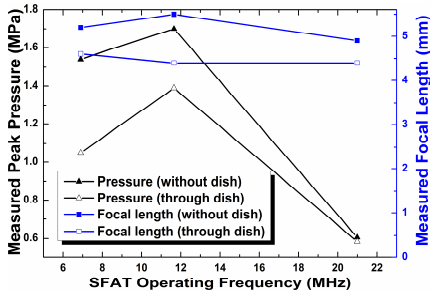

Fig. 6. Measured peak acoustic pressure at the focal point and focal length from different SFATs with and without the Agarose-gel-filled Petri dish.

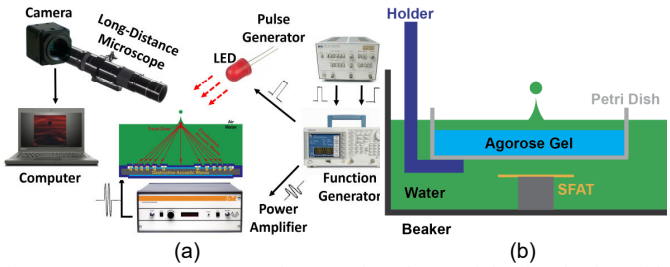

Fig. 7: (a) Measurement set-up for capturing photos of droplet ejection with optical strobing; (b) cross-sectional diagram showing the ejection set-up with a Petri dish filled with Agarose gel.

We have successfully observed ejection of a single water droplet per pulse without (Fig. 8a to 8c) and with the Petri dish (Fig. 8d to 8f). The diameter of ejected droplets and minimum pulse width needed for ejection to happen are summarized in Table III, from which we see that the droplet diameter (related to focal size) and the needed minimum pulse width are larger and longer with the Petri dish (than without it) due to some acoustic loss in the Petri dish and Agarose gel.

C. Droplet-Assisted Particle Ejection

We choose 10-μm-diameter Polystyrene microspheres to simulate grown cells. To embed the microspheres onto Agarose gel through self-assembly, we pour a thin layer of water on top of the gel, and fully suspend microspheres in methanol with sonication. Then the methanol with the microspheres is poured into water layer, and the microspheres form a uniform layer at water/methanol boundary, most of where a monolayer of microspheres is formed (Fig. 10a). When the solution is almost dried, we gently press the microspheres against the gel with a

spatula to increase the microspheres' adhesion on the gel. Then we use the set-up shown in Fig. 9a, and collect the ejected droplets (Fig. 9b) with a cover slip held above the water surface (Fig. 10b to 10d). All the SFAT-ejected droplets contain microspheres, and the diameter of collected microsphere agglomerates on cover slips (determines the number of microspheres per droplet) is related to droplet size (Fig. 10e). During 10 minutes of operation (2 droplets per second), no temperature rise or visible gel damage is observed.

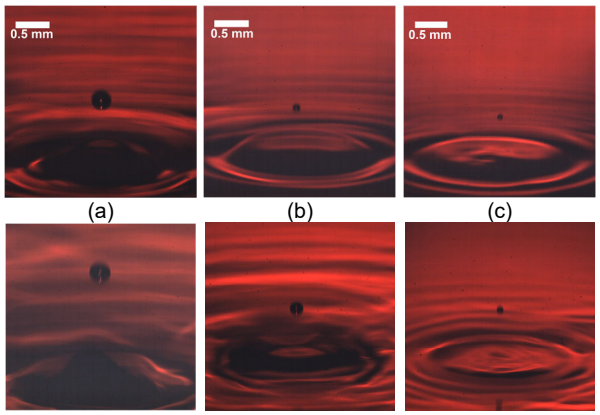

Fig. 8. Photos showing water droplets ejected without the Petri dish by our SFATs working at (a) 6.90 MHz, (b) 11.65 MHz, and (b) 20.99 MHz and that with the Petri dish for SFATs working at (d) 6.90 MHz, (e) 11.65 MHz, and (f) 20.99 MHz, having same scale as first three photos. The background circles are ripples on the water surface.

TABLE III. DRIVING PULSE WIDTH AND DROPLET DIMENSIONS

Ejection Parameters		Working Frequency (MHz)		
		6.90	11.65	20.99
Droplet Diameter (mm)	Without Dish	300	120	95
	Through Dish & Gel	330	190	105
Threshold Pulse Width (μs)	Without Dish	37.7	16.3	15.3
	Through Dish & Gel	94.2	60.1	33.4

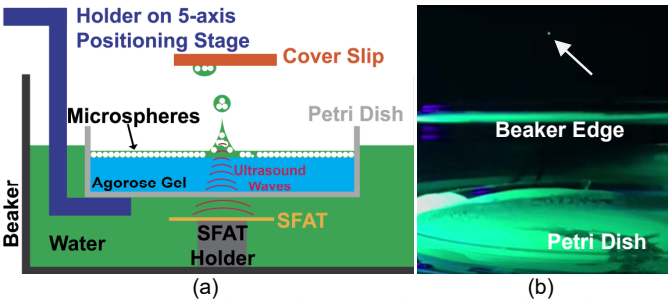

Fig. 9. (a) Cross-sectional diagram showing the droplet-assisted particle ejection set-up. (b) Photo of an ejected droplet carrying fluorescent microspheres under black light, ejected from the Petri dish by the 6.90 MHz SFAT and flies above the beaker edge.

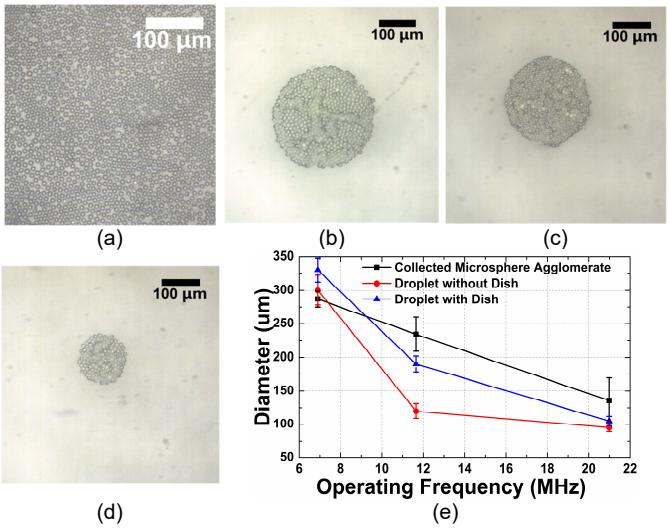

Fig. 10. Microscope photos of (a) microsphere monolayer on the gel surface; collected microsphere agglomerates on plastic cover slips, ejected by SFATs working at (b) 6.90 MHz, (c) 11.65 MHz, (d) 20.99 MHz, respectively. (e) Diameters of collected microsphere agglomerate on cover slips, ejected droplets without Petri dish and ejected droplets with Petri dish, versus SFAT operating frequency.

IV. SUMMARY

We have designed and fabricated SFATs (working at three different harmonic resonant frequencies having different focal sizes) that can produce high intensity focused ultrasound through and from Petri dish filled with Agarose gel, and can eject droplets of different sizes to carry different number of particles embedded on the gel surface. The transducers could be useful for precise extraction of cells without causing damage to the surrounding cells or the culture medium.

ACKNOWLEDGMENT

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