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100% Single Cell Encapsulation via Acoustofluidic Printing Based on a Gigahertz Acoustic Resonator

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Abstract— **In this study, an acoustofluidic printing system for generation of single-cell droplets based on a gigahertz acoustic resonator was proposed and verified. The working area of the resonator has a typical dimension of 10×10 micrometer which is very suitable for single cell printing. Single cells were encapsulated in picoliter droplets and printed directly to a flat substrate without any significant influence on their viability. By combining an optic feed-back loop, a 100% single-cell encapsulation rate is achieved.**

Clinical Relevance— **This acoustic-based system has good biocompatibility and high encapsulation rate, which expands the mechanism of medical and biology studies.**

I. Introduction

Single cell analysis reveals the true diversity of biology at cellular level and emerges as a powerful tool for medical and biology studies [1,2]. However, previous cell studies are conducted with large populations of cell, and these results can only reflect average values summed over the responses of many cells. These approaches can be the source of misinterpretation which ignore the statistical nature of many cellular events [3]. Therefore, single cell analysis has gained a lot attention benefit from its accurate representation of cell-to-cell variations, minimal biological noise and convenience of studying rare cells [4]. To carry out single cell analysis, the first step is the encapsulation of individual cells.

The most common conventional approaches for isolating single cells are hydrodynamic trapping [5], laser capture microdissection (LCM) [6] and microscale cell manipulation [7]. Mostly these methods require plenty of time, and some need specialized electronics or optical equipment which are not common in a medical or biological laboratory and induce further obstacles for operation [4]. In addition, traditional LCM requires large sample volumes (milliliters), and use expensive instruments used by skilled operators [8]. In the recent years, these traditional technologies have been improved, and gradually developing towards microfluidics [9-12]. Dino Di Carlo et.al. developed a microfluidic-based dynamic single cell culture array that no surface modification is required and cell loading is done in less than 30 seconds [9]. It has a simple structure and good biocompatibility. However, there are challenges to control the number of cells per trapping chamber due to the random allocation process. Russell H. Cole et.al. proposed a novel technology that combines the flexibility and programmability of microliter dispensing with the scalability and single-cell sensitivity of flowing droplet microfluidics [10]. The core of this approach is a fluorescence-activated droplet sorter coupled to a specialized. This approach can generate arrays of high complexity quickly which will allow new kinds of analyses on single cells and combinations of different cell types. However, In the process of droplet sorting, a large amount of sample solution is wasted and the efficiency is extremely low. At present, many bio-printing methods have been developed to precise spatial positioning and isolation of mammalian cells including inkjet bioprinting [13], valve-based bioprinting [14] and laser-induced printing [15]. Bio-printing technologies enable powerful methods to address the challenges related to multiple potential applications in single cell research [16]. However, current cell printing systems still suffer from several issues including clogging, high costs, low encapsulation rate of cells and complexity of the instrumental setup.

In this paper, we proposed a novel acoustic bioprinting system which is able to produce single cell droplets. It utilizes a gigahertz acoustic resonator to induce a strong body force at the resonator-liquid interface, which then pushes the liquid upwards to overcome its surface tension and generates a stable and sharp liquid spike at the liquid-air interface [17]. The droplets that wrap the cells can be generated by contacting the liquid spike with substrate. To guarantee 100% single cell encapsulation rate, an optic feed-back loop was applied to the system. Thus, the sample solution waste is minimized. In addition, the liquid spike is formed in an open well and is always vertical to the device, thus, there is no concern about clogging or satellite droplets issues. Besides, since the fabrication process of resonators is CMOS-compatible, they can be easily integrated for high throughput applications.

II. Experimental

A. Instruments

Figure 1(A) illustrates the setup of our developed single-cell printing system. The ultrahigh frequency (2450MHz) solid-mounted thin-film piezoelectric resonator (SMR) is excited using a radio frequency signal (RFS) source. A reservoir made from PDMS is fixed on top of the resonator to store the liquid sample of cells. The target substrate is located above the sample reservoir which is precisely controlled by a 3-axis position system. A CCD camera is utilized to record the dispensing process in real time. To judge whether a droplet only wrap one cell, an optical system is adopted to monitor the droplets. A custom-developed LABVIEW program is used to

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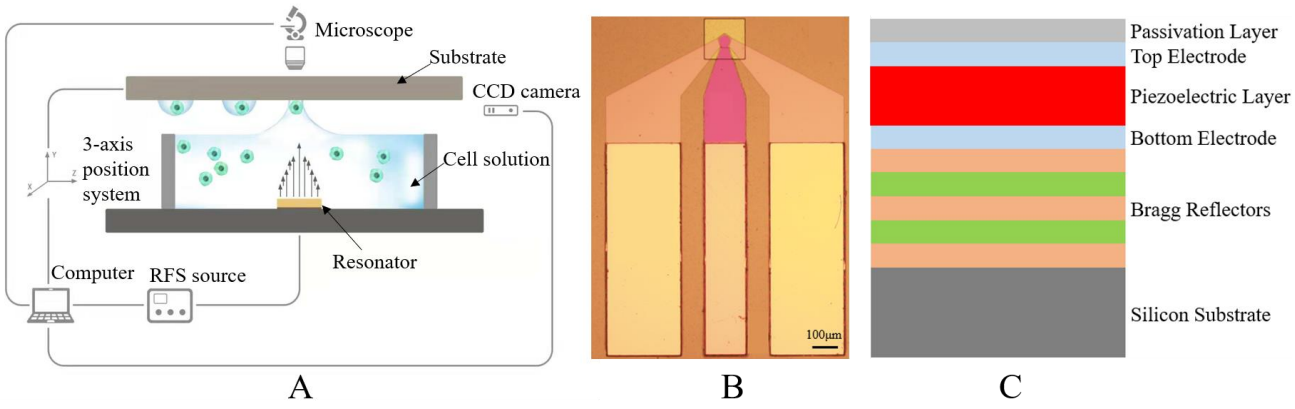

Figure 1 : (A) Schematic illustration of the cell-printing system. (B) Top view of the SMR. (C) The structure of SMR.

control the whole system. Figure 1(B) and 1(C) shows the structure of SMR, which consists of a sandwich piezoelectric structure with a Bragg reflector structure underneath fabricated by a standard micro electromechanical (MEMS) technology.

B. Method

The SMR is utilized to generate a stable liquid spike at the water-air interface. The basic working principle of SMR is inverse piezoelectric effect, which makes the resonator generate vertically propagated acoustic waves. The resonant region of the SMR is designed to be a regular pentagon which is a very asymmetrical shape so that the horizontal propagation can be greatly reduced. In addition, with the help of the Bragg reflection layers, part of propagating downward acoustic waves is reflected, thus enhancing the acoustic waves acting on the liquid. In order to investigate the amplitude distribution of the resonant region more intuitively, we carried out simulations using multi-physics finite element analysis software (COMSOL 5.0). The detailed process of the simulation has been reported in our previous literature [17] and briefly described here. As shown in Figure 2(A), the amplitude of the SMR is strongest at center and graduate attenuates around, which indicates the SMR can generate a peak shaped sound field. When the ultrahigh frequency acoustic wave travels into liquid, a body force (F_B) will be generated and drive a directed fluid motion along the propagation path of the acoustic wave [18]. It can be expressed as :

$$F_B(x,y,z) = \rho\beta\omega^2 u^2(x,y)e^{-2\beta z} \quad (1)$$

with

$$\beta = \frac{b\omega^2}{\rho c_l^3} \quad (2)$$

as the attenuation coefficient, where ρ is the liquid density, ω is the angular frequency of the acoustic waves, $u(x,y)$ is the vibration displacement magnitude at the resonator surface, c_l is the sound speed in the liquid, and $b = \frac{4}{3}\mu + \mu_B$, where μ and μ_B are dynamic viscosity and bulk viscosity of the liquid, respectively. Due to the exponential attenuation of body force along the z direction, the energy of body force is coupled into the liquid within a few micrometers above the device and propagate upward. At the water-air interface, a stable liquid spike will be produced through the acoustic streaming effect [19]. Figure 2(B) presents a liquid spike contacting with the substrate to generate the droplet. When the device is turned off, the liquid spike will fall back and leave a micro droplet on the substrate.

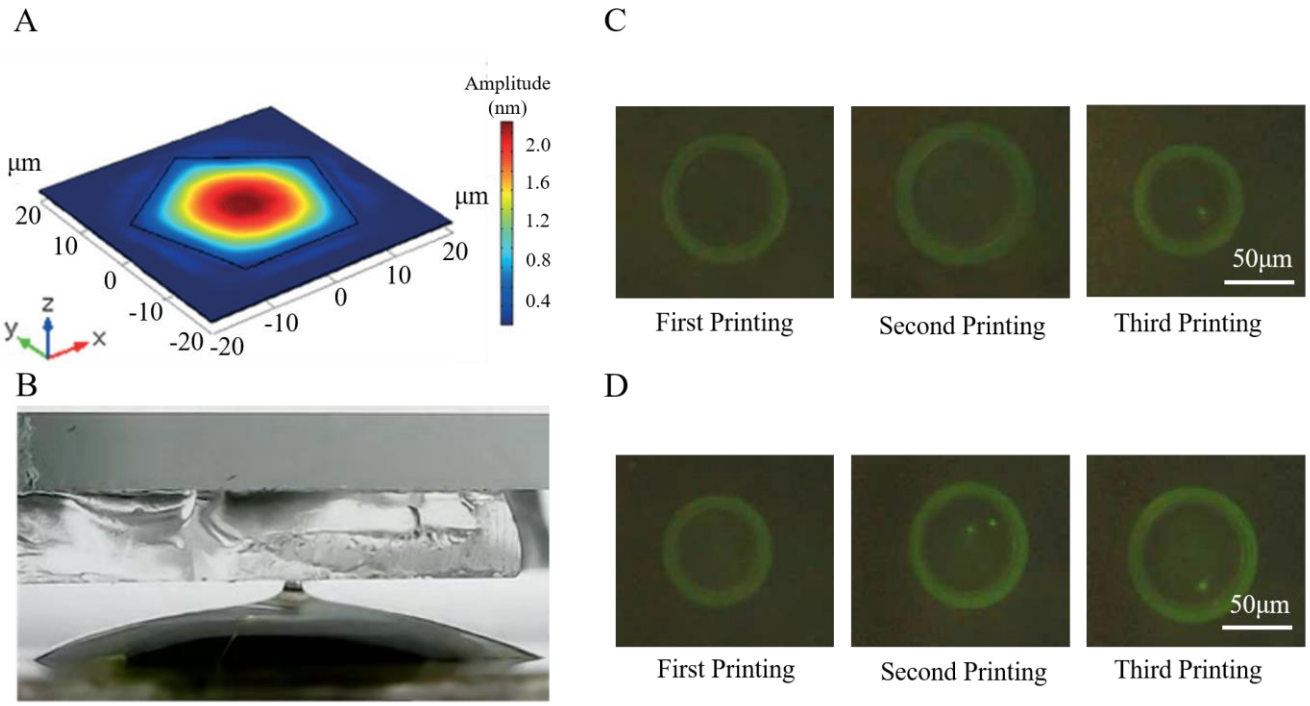

Figure 2. (A) Simulation result of acoustic field generated by an acoustic resonator. (B) The liquid spike at the air-liquid interface with its spike top contact with substrate. (C) and (D) Two cases of generating single cell droplets.

C. Quantification of cell density

The HeLa cells were obtained from GuangZhou Jennio Biootech Co., Ltd. And HeLa cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cell suspensions were used in this printing experiment. HeLa cells were randomly distributed in the extracellular medium, and the probability that a droplet contains k cells follows Poisson statistics, i.e.,

$$P_{k,\lambda} = \frac{\lambda^k e^{-\lambda}}{k!} \tag{3}$$

where λ is the average number of cells per droplet. In order to maximize the single cell encapsulation rate, the average number of hele cells per droplet needs to be equal to 1 (i.e., λ=1). Since our droplet printing system can stably generate droplets with the diameter of 100 μm, the cell density was selected as 2×10^6 cells per ml.

D. Two cases of generating single cell droplets

A feed-back loop is developed to enable the automatic cell printing. If the droplet is empty, extra printing will be performed at the same spot until a single cell droplet is generated. The substrate will then move to next spot and print another droplet through the 3-axis position system. Figure 2(C) shows a typical cell printing process flow. Three times printing is enabled a successful single cell patterning. Though we optimized the density of the cell solution, it happened that two cells could be printed together in one droplet (Figure 2D). In this case, controlled cell removal is required. Since our printing system is based on contacting the liquid spike with substrate, such contact could be used to remove cells from droplets as well. We can print again at the same spot to bring excess cells back to the sample reservoir, which guarantee the single cell patterning.

III. Results and Discussion

As shown in Figure 3(A), a 4×4 single cell droplet array was successfully printed through the automatic bio-printing system. As the step motor of the 3-axis position system has a precision of 5 μm in XYZ directions, the droplets can be spaced out and arrayed accurately, which facilitates subsequent single cell analysis. Meanwhile, for a given distance between the liquid sample and the target substrate, the acoustic power is optimized and set at a fixed value (200 mW to 400 mW). And the time for a single print is varied from 30 ms to 60 ms. Before the cell printing, calcein-AM and propidium iodide (PI) were added in the extracellular medium to characterize the cell viability. The cells in droplets did not emit red fluorescence (Figure 3B), which indicates that acoustic bio-printing does not have significant effect on cell viability. The presented results of our work are preliminary, and the larger single-cell droplet arrays could be completed by integrating multiple acoustic devices.

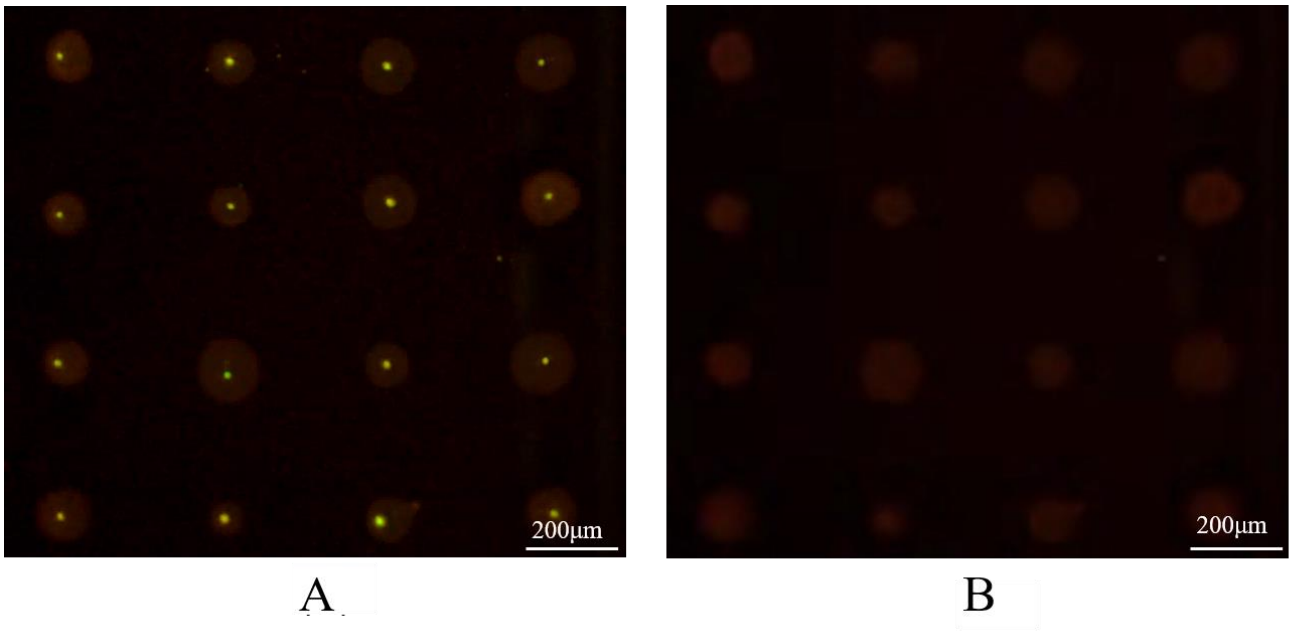

Figure 3. (A) Single cell droplet array in fluorescence field with 488nm excitation light. (B) Single cell droplet array in fluorescence field with 535nm excitation light.

IV. CONCLUSIONS

In this work, we successfully developed a single cell encapsulation and dispensing tool using gigahertz acoustofluidic printing approach. Droplets containing single cell were printed on the solid substrate by contacting the liquid spike generated by the acoustic jetting. The experimental result demonstrates 100% single-cell encapsulation rate by combining an optic feed-back loop with automatic image processing. The developed acoustic printing platform has good biocompatibility, high single cell encapsulation rate, and low power assumption, which can benefit many single cell researches for different biomedical applications.

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REFERENCES

[1] J. El-Ali, P. K. Sorger and K. F. Jensen, *Nature*, 2006,442, 403-411.
[2] P. S. Dittrich, K. Tachikawa and A. Manz, *Anal. Chem.*, 2006, 78, 3887-3908.
[3] R. M. Johann, *Anal. Bioanal. Chem*., 2006, 385, 408-412.
[4] Narayanamurthy, Vigneswaran, et al. "Microfluidic hydrodynamic trapping for single cell analysis: mechanisms, methods and applications." *Analytical Methods* 9.25 (2017): 3751-3772.
[5] J. R. Rettig and A. Folch, *Anal. Chem.*, 2005, 77, 5628-5634.
[6] Geigl JB, Speicher MR (2007) Single-cell isolation from cell suspensions and whole genome amplification from single cells to provide templates for CGH analysis. *Nat Protocols* 2: 3173-3184.
[7] Kvist T, Ahring BK, Lasken RS, Westermann P (2007) Specific single-cell isolation and genomic amplification of uncultured microorganisms. *Appl Microbiol Biotechnol* 74: 926-935.
[8] Moon, Sangjun, et al. "Drop-on-demand single cell isolation and total RNA analysis." *PLoS One* 6.3 (2011): e17455.
[9] Di Carlo D, Wu LY, Lee LP (2006) Dynamic single cell culture array. *Lab Chip* 6: 1445–1449.
[10] Cole, Russell H., et al. "Printed droplet microfluidics for on demand dispensing of picoliter droplets and cells." *Proceedings of the National Academy of Sciences* 114.33 (2017): 8728-8733.
[11] Zeng Y, Novak R, Shuga J, Smith MT, Mathies RA (2010) High-performance single cell genetic analysis using microfluidic emulsion generator arrays. *Anal Chem* 82: 3183-3190.
[12] Toriello NM, Douglas ES, Thaitrong N, Hsiao SC, Francis MB, et al. (2008) Integrated microfluidic bioprocessor for single-cell gene expression analysis. *Proceedings of the National Academy of Sciences* 105: 20173-20178.
[13] Gutzweiler, Ludwig, et al. "Large scale production and controlled deposition of single HUVEC spheroids for bioprinting applications." *Biofabrication* 9.2 (2017): 025027.
[14] Song, Y.S. et al. (2010) Vitrification and levitation of a liquid droplet on liquid nitrogen. *Proc. Natl. Acad. Sci. U.S.A.* 107, 4596-4600
[15] Raof, N.A. et al. (2011) The maintenance of pluripotency following laser direct-write of mouse embryonic stem cells. *Biomaterials* 32, 1802-1808.
[16] Tasoglu, Savas, and Utkan Demirci. "Bioprinting for stem cell research." *Trends in biotechnology* 31.1 (2013): 10-19.
[17] He, Meihang, et al. "An on-demand femtoliter droplet dispensing system based on a gigahertz acoustic resonator." *Lab on a Chip* 18.17 (2018): 2540-2546.
[18] D. J. Collins, Z. Ma and Y. Ai, *Anal. Chem*., 2016, 88, 5513-5522.
[19] Yu H, Zou Q, Kwon J W, et al. Liquid needle[J]. *Journal of microelectromechanical systems*, 2007, 16(2): 445-453.