BEAD-ASSISTED ACOUSTIC DIFFERENTIAL EXTRACTION OF SPERM CELLS IN DILUTE SAMPLES FOR POTENTIAL FORENSIC ANALYSES

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ABSTRACT

Here we describe an improved method of separating sperm cells from lysed epithelial cells via acoustic cell trapping. In particular, we show that in the processing of samples with low numbers of sperm cells, addition of polymeric beads to the sample[1] allowed for better performance in terms of higher trapping efficiency versus our previous acoustic differential extraction (ADE) results[2]. We report an increased throughput of 30 µL/min (1.8 mL/hour) and reduced sample concentration requirement (1 sperm cell/µL), Separation of low sperm count samples in epithelial cell lysate allowed unambiguous STR typing of the sperm donor.

KEYWORDS:

Acoustofluidics, bulk acoustic wave, cell separation, forensic analyses.

INTRODUCTION

Acoustic cell trapping is a sub-field of the burgeoning acoustofluidics discipline wherein localized ultrasonic standing waves (USW) are established in microfluidic devices to achieve liquid-phase, contact-free particle separations with high efficiencies[3].

In collaboration with the Laurell group, we have previously applied acoustic trapping to mock sexual assault samples, wherein sperm cells were separated from epithelial cell lysate via acoustic differential extraction (ADE)[2]. This proof-of-principle device required relatively high sample concentrations (~500 sperm cells/µL) and has a low processing throughput (1 µL/min), limiting its applicability to evidentiary samples (for which a processing backlog exists[4]) that may contain less than 1 sperm cell/µL in ≥1 mL total volume. In this work, we built an acoustic trapping system that includes a glass-PDMS-glass (GPG) resonator chamber with an external PZT transducer, which provided better performance in terms of increased flow rate up to 30 µL/min. In the separation of dilute semen sample, assisting polymeric beads were added into the sample solution to aid the formation of trapping aggregate and thus increase the trapping efficiency.

EXPERIMENTAL

Piezoelectric transducer assemblies were built from lead zirconate titanate (PZT) piezoelectric ceramic (SMD10T04F5000S111, Steiner and Martins Inc.) that was diced, scored, and mounted on printed circuit boards (PCBs) using conductive epoxy. During acoustic trapping the transducer was actuated near 5.4 MHz and ~30 V_{pp}. using a 20 MHz function generator (Agilent 33220A) and custom amplifier[5]. The temperature of the bottom glass plate near the PZT was monitored using a miniature type T thermocouple (Physitemp) and a thermocouple to analog converter (Omega Engineering). Channels were cut through a ~290 µm PDMS film (Roger HT-6240 solid silicone) using CO_2 laser ablation (VersaLaser 350, Universal Laser Systems) and then plasma bonded between two layers of ~180 µm cover glass (Corning). Access holes were cut through glass cover using laser ablation. A localized USW was established in glass-PDMS-glass (GPG) microfluidic resonators between the top and bottom glass layers (Fig. 1). The resonance frequency $\boldsymbol{f}$ of the USW was dependent on the height of the cavity $\boldsymbol{h}$ (i.e. the thickness of the PDMS layer)(Fig. 2), as predicted by the 1D resonance approximation, $\boldsymbol{h=n\lambda/2= nv/2f}$, ($\boldsymbol{n}$=1,2,3...) where $\boldsymbol{v}$ is the speed of sound in water at 38 ºC.

Neat semen samples were diluted with 0.1% TWEEN 20 (Sigma) and labeled with 10 mM Syto 11 (Invitrogen). Female lysate was prepared by first eluting epithelial cells from buccal swab samples and then lysed as previously described [6]. The polymeric bead solution was prepared by diluting 6 µm violet polystyrene beads (Polysciences) in 0.1% TWEEN 20. Mixed samples were prepared by spiking calculated amount of diluted semen sample and bead solution into female epithelial cell lysate. All samples were trapped at flow rate of 30 µL/min, driven by syringe pump (Cetoni). Fluorescence microscopy was used to distinguish between violet beads and Syto 11 labeled sperm cells (Fig. 3). Cells and beads were quantitated via hemacytometer (Glasstic, KOVA slide II, Hycor Biomedical). DNA from cell samples was isolated via solid phase extraction (Qiagen), STR PCR amplifications were performed (Applied Biosystems, Identifiler), and samples were analyzed by multi-color capillary electrophoresis (ABI 310, Applied Biosystems).

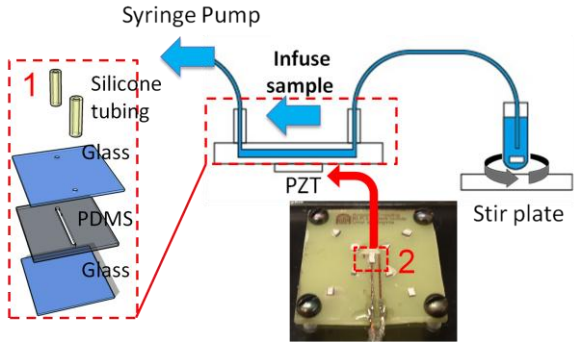

Figure 1. *System of acoustic trapping. Glass-PDMS-Glass (GPG) resonator chip (1) is fabricated by laser ablation and subsequent plasma bonding. Fluidic connections were made via PTFE tubing inserted into silicone tubing affixed with silicone adhesive. Completed GPG chip was clamped against a PZT transducer mounted on a printed circuit board (2).*

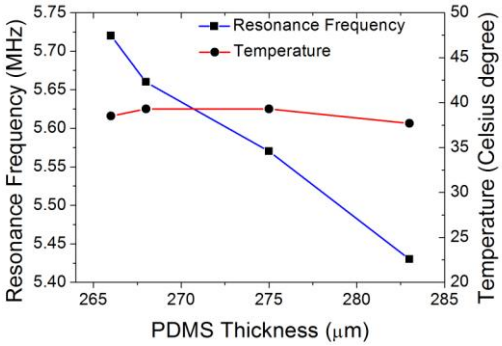

Figure 2. *Resonance frequency & working temperature vs. thickness of the PDMS layer. Temperature remained 38±0.5 degrees among all four assays. Resonance frequency at each PDMS thickness was determined by detecting the maximum temperature change*[8].

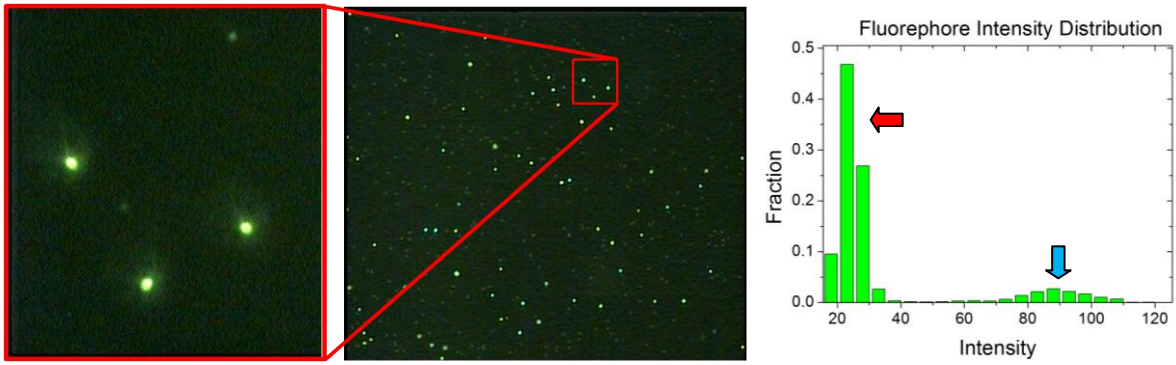

Figure 3. *Comparison of fluorescence intensity of beads versus syto-11 stained sperm cells. (A) Fluorescence images and (B) fluorophore intensity distribution for mixture sperm cells (red arrows) and polymeric beads (blue arrows).*

RESULTS AND DISCUSSION

In semen samples of 500 sperm cells/μL, a trapping efficiency (# retained cells / # total infused cells × 100 %) of 82 ± 9 % was achieved at a flow rate of 30 μL/min, while a sample containing 1 sperm cell/μL resulted in a trapping efficiency of only 18 ± 3 % (Fig. 4). The decrease in trapping efficiency for dilute samples can be attributed to difficulties in aggregate formation[1], and the concomitant reduction in the attractive inter-particle secondary radiation forces (SRF) that aid cell retention[7].

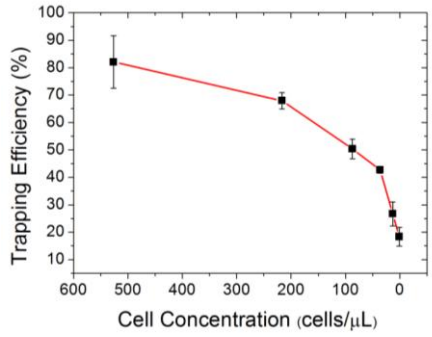

Figure 4. *Dependence of trapping efficiency on cell concentration (n=3). Samples were infused at a flow rate of 30 μL/min and sperm cells were quantitated via hemocytometer.*

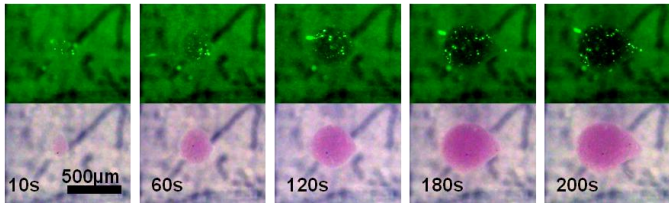

Figure 5. *Visual monitoring of bead-assisted sperm trapping. Still images from video of a trapping experiment are shown at 10, 60, 120, 180, and 200 second time intervals. The process was simultaneously monitored in fluorescence (upper row) and bright field (lower row) microscopy. The bright spots in the fluorescence images are fluorescently-stained sperm cells. Samples were infused at 30 μL/min for total volume of 100 μL.*

To increase the trapping efficiency of diluted samples, we added inert polymeric beads (6 µm diameter) to increase the probability of inter-particle collisions that lead to a bead-sperm cell co-aggregation (Fig. 5), after the "seeding" strategy by Hammarström et al.[1]. The trapping efficiency of dilute (1 sperm cell/µL) samples increased to 85 ± 4 % when a concentration of ~400 beads/µL was added to the sample. A study of bead-assisted ADE was performed on a dilute, mixed sample of 5 sperm cells/µL in female epithelial cell lysate. After recovery of the trapped cells, DNA was isolated via solid phase extraction and amplified for short tandem repeat (STR) analysis for human identification. Full profiles of male DNA (sperm cells) with no detectable female peaks were generated, indicating successful separation of the male and female components and negligible inhibition from the beads (Fig.6).

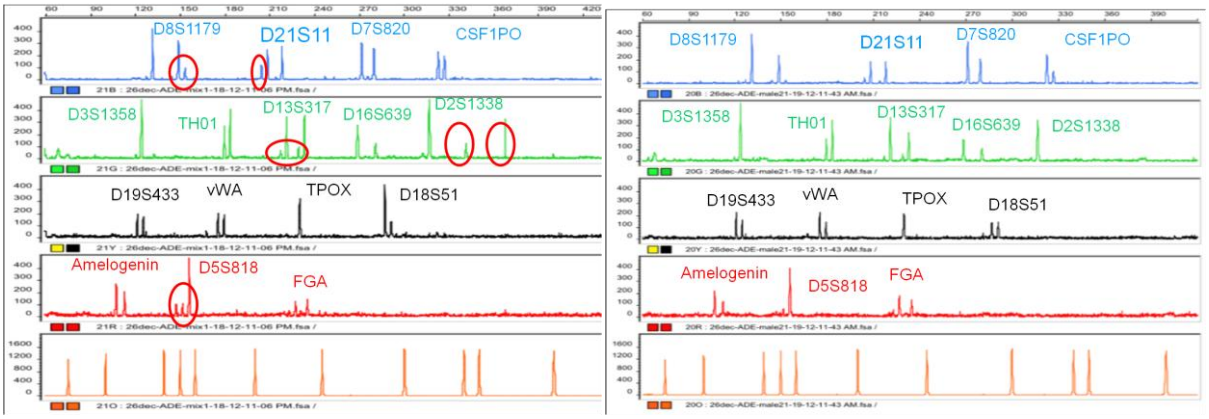

Figure 6. *STR amplification of DNA in untreated mixed sample (left) and isolated sperm cells (right). Female peaks (red circles) were present in the admixed sample but absent in the STR profile from isolated sperm cells*

CONCLUSION

In summary, we developed an acoustic trapping system consisting of a PCB-mounted PZT transducers and GPG resonators. Processing throughput was increased to 30 µL/min over our previous system[2] and the trapping efficiency of dilute sample was increased by nearly 5 fold using bead-assisted acoustic trapping. The addition of polymeric beads was proven compatible with STR PCR analyses. Acoustic trapping was utilized to remove female epithelial cell DNA from an aggregate of trapped sperm and achieve a distinct male STR profile. The acoustic trapping platform described here is a promising avenue toward expedited and lower cost processing of sexual assault forensic evidence.

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