HIGH-THROUGHPUT ACOUSTIC SORTING OF CELLULAR-SIZED MICROPARTICLES IN 3D MICROFLUIDIC CHANNELS

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ABSTRACT

This manuscript presents high-throughput sorting of cellular-sized microparticles within a three-dimensional microfluidic channel by focused bulk acoustic wave (BAW) produced by a Self-Focusing Acoustic Transducer (SFAT). The focused ultrasound induces a substantially higher acoustic radiation force within the focal region, enabling sorting based on particle size and density. Unlike surface-acoustic-wave-based setups, the BAW-based technique uses a three-dimensional microfluidic channel through which a mixture of particles is transported, while SFAT(s) may be placed at multiple points along the channel for multi-stage sorting. The technique has been successfully used in sorting 50 µm microparticles, which are analogous to cancerous or differentiated Mesenchymal Stem Cells (MSC), from 30 µm microparticles, which are analogous to healthy MSC. The sorting results in 97.5% purity at the smaller microparticle outlet and a 97.2% recovery rate for the smaller particles. The technique allows sorting 650,000 smaller and 142,000 larger microparticles within a mere 10 minutes.

KEYWORDS

Bulk Acoustic Wave Sorting, 3D Microfluidics, Cellular-sized Sorting, Precision 3D Printing

INTRODUCTION

Cell analysis in stem cell research [1], tissue regeneration, tumor cell removal [2], and cancer diagnostics often require cell sorting based on the size and density, as accurate sorting of cells separates target cells from a heterogeneous mixture of cells. Cell sorting is typically done with fluorescence labels or magnetic beads, such as Fluorescence-activated cell sorting (FACS) [3] or Magnetically-activated cell sorting (MASC) [4]. These require labeling of cells with fluorescent or magnetic tags, which can affect the cells.

Label-free acoustic sorting has emerged as an alternative approach, but most acoustic sorting methods rely on the formation of standing waves to generate nodes, where cells are trapped. The trapping force is typically low across the channel, resulting in low sorting accuracy [5] and a relatively low throughput [6]. Another method of acoustic sorting is based on acoustic tweezers [7], which trap particular particles with a very high accuracy, but the sorting throughput is low [8].

Microfluidic sorting offers the advantage of small sample quantities, portability, and miniaturization [9]. However, most microfluidic systems are made of two-dimensional channels which limit the application of bulk acoustic waves to sorting. This paper presents a method for fabricating three-dimensional microfluidic channels using a 3-D Stereolithography (SLA) Printer with inlet and outlet ports designed for standard Olive connectors. The channels are designed to have a diameter of approximately 600 microns, facilitating the passage of particles of diverse sizes. Self-Focusing Acoustic Transducers (SFAT) generate focused bulk acoustic waves to create a region of intensified pressure at its focal zone. This region is precisely aligned to the channel, near the point immediately preceding the separation into two output channels. Consequently, all particles traversing the channel encounter an identical acoustic radiation force (ARF) from the SFAT.

COMPONENTS AND SETUP

The sorting system is composed of SFAT, a 3D-printed channel, inlet and outlet tubes, and push-pull syringe pumps (SyringeONE).

Self-Focusing Acoustic Transducer (SFAT)

Self-Focusing Acoustic Transducer (SFAT) is based on Fresnel Half Wavelength Bands (FHWB), which allows the passage of constructively interfering waves while blocking destructively interfering waves at the focal zone, through designing the annular rings with their radii being

$$R_n = \sqrt{n\lambda \times \left(F + \frac{n\lambda}{4}\right)}$$

where R_n, λ and F are the radius of the n^{th} ring, wavelength, and focal length, respectively. A high-pressure zone is generated at the focal zone, where the intensified acoustic field exerts large ARF on particles. As the ARF applied to a particle is related to the particle's size and density, size- and density-based sorting is possible. The acoustic patterns generated by the SFAT are simulated with Finite Element Modeling (FEM) through COMSOL, and Fig. 1a illustrates the impact of ARF on a particle positioned near the focal point, on a COMSOL simulation.

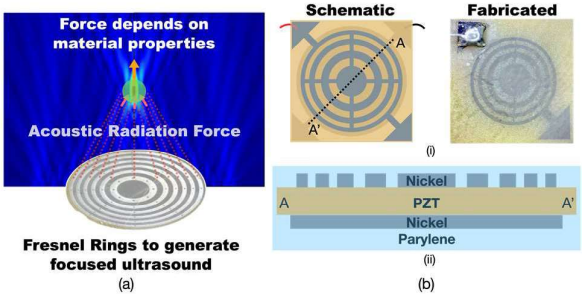

Figure 1. (a) Illustration of a particle influenced by acoustic radiation force from focused ultrasound, overlaid on a simulation depicting the high-pressure zone generated by Self-Focusing Acoustic Transducer (SFAT); (b) (i) Top-view photos of SFAT showing electrode patterned on PZT surface and (ii) Cross-sectional view of SFAT.

The SFAT is fabricated on a 1 mm thick Lead Zirconate Titanate (PZT) sheet by patterning the top and bottom nickel electrodes (Fig. 1b). The device is subsequently covered with 25-micron thick Parylene-D to safeguard the electrodes from exposure to water. Since the

fundamental resonant frequency of the thickness-mode vibration of the 1 mm thick PZT is 2.3 MHz, the FHWB annular rings are designed for 3rd and 9th harmonics, to make the wavelength comparable to the particle sizes used for sorting. We have fabricated two sets of SFATs; one designed for sorting particles larger than 100 µm in diameter using the third harmonic of a 1 mm thick PZT sheet (for 6.96 MHz) and the other using the ninth harmonic of the same PZT sheet (for 20.3 MHz).

3D Printed Microfluidic Channel

The particle sorting chamber is designed to have an inlet for particles and two additional inlets for introducing sheath flow, one positioned on either side of the particle inlet (Fig. 2). The flow rates at the inlets are chosen to ensure laminar flow within the channel, preventing the sheath liquid from mixing with the solution containing the microparticles. The chamber comprises two outlets: one inclined at a 45-degree angle (in the vertical direction) to expel the particles deflected by the SFAT and the other to collect the particles that have not been deflected by the SFAT.

The sorting chamber has channels with circular cross sections designed to be 600 µm in diameter and is printed on a FormLabs 3B+ SLA printer using the FormLabs Clear Resin V4 with a resolution of 25 µm in the thickness direction and 10 µm in the in-plane direction. The channel is printed at a 45-degree tilt in the z-direction, allowing the resin to drain and preventing channel clogging.

After the 3-D printing, the sample undergoes a washing process in an Isopropyl Alcohol (IPA) bath for 20 minutes using FormLabs Form Wash. Simultaneously, IPA is circulated through the channels via a syringe every 5 minutes to eliminate any residual resin. Subsequently, the sample is cured at 60°C for 15 minutes under ultraviolet light using a FormLabs Cure device.

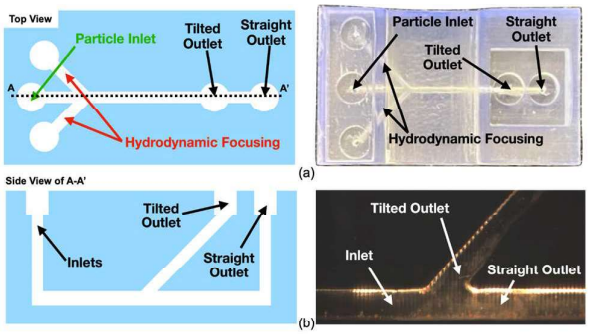

Figure 2. (a) Top-view schematic and photo of the stereolithography 3D-printed microchannels and (b) cross-sectional-view schematic and photo of the microchannels.

The cured sample exhibits surface roughness due to the printing resolution, hindering its complete transparency. Consequently, additional surface treatment is necessary to enhance transparency and facilitate visualization of the microchannels through a microscope. We sequentially treat surfaces with "Heavy Scratch Remover", "Fine Scratch Remover", and "Clean, Shine, and Protect Spray" from Novus Plastic Polish. Olive connectors are attached to the pre-designed grooves, which match the base of the olive connectors. Gorilla Glue is used to seal the connectors further, making them completely leakproof.

Sorting Setup using Push-Pull Syringes

A mixture of two distinct microparticles is kept in a glass beaker, with a uniform concentration maintained by a magnetic stirrer (Fig. 3). A push-pull syringe pump configured in the push mode is used to maintain the sheath flow rate. Two additional push-pull syringe pumps set in the pull mode are used to extract the particles from the two outlets at equal flow rates. The combined extraction rate at the two outlets is maintained above the sheath flow rate. The microparticles from the glass beaker are automatically transported to the sorting chamber due to the differential flow rates. Biocompatible fluorinated ethylene-propylene (FEP) tubing is used for all inlets and outlets. A long-range microscope equipped with a camera is used for visualization of the sorting process during the passage of the microparticles through the sorting zone.

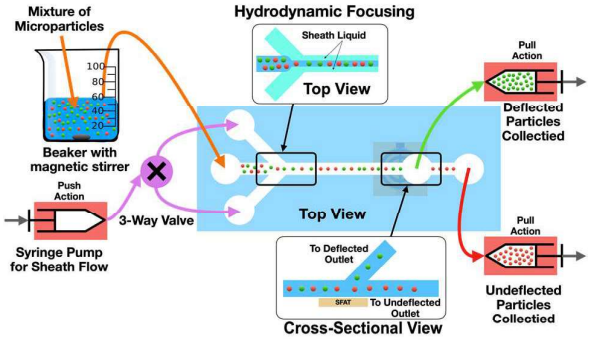

Figure 3. Experimental setup with push-pull syringes for testing hydrodynamic focusing and sorting of different particles by focused ultrasound produced by SFAT placed under the microchannel.

EXPERIMENTAL RESULTS OF SORTING MICROSPHERES

The setup shown in Fig. 3 is used to demonstrate particle sorting based on particle size and density. The setup is also used to sort healthy Mesenchymal Stem Cells (MSC) from cancerous/differentiated MSCs.

Size-Dependent Sorting

Multiple experiments have been carried out to demonstrate the particle sorting capabilities of the SFAT inside the microfluidic channel. Larger microparticles exhibit greater deflection due to the ARF and are subsequently collected in the Tilted Outlet. The 6.96MHz SFAT sorts polyethylene microspheres in the range of 50µm to 500µm (Fig. 4).

The SFAT is shown to be capable of separating microspheres with diameters of 50µm (green) from those with diameters of 100µm (red) when actuated with a sinusoidal signal from a Power Amplifier at 11.3V_{pp} (Fig. 4). Notably, the 100µm-diameter microparticles are deflected more than the 50µm-diameter microparticles, and are directed to the Tilted Outlet (Fig. 2b), while the smaller microspheres continue through the Straight Outlet. Figure 4 shows the trajectory of these two distinct particle types within the microchannel, with the particle concentration reduced to enable visualization of individual particles.

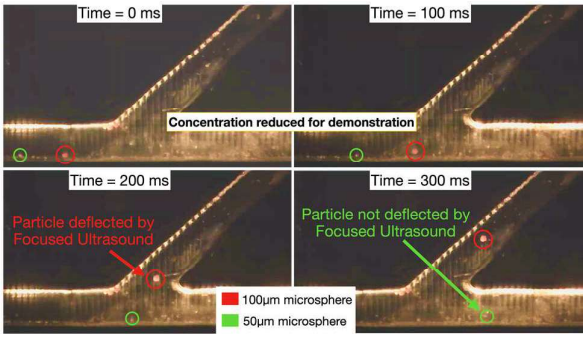

Figure 4. Cross-sectional-view photos showing sorting of 50µm and 100µm microparticles with a density of 1.06g/cc and larger particles being deflected further away from SFAT.

Density-Dependent Sorting

Experiments with microspheres of the same size but different densities have been carried out to visualize the impact of density on sorting. Across all experiments conducted to assess densities ranging from 1.025 g/cc to 1.13 g/cc, particles with lower densities exhibit a greater deflection.

To demonstrate sorting within the cellular size range, experiments have been conducted with 50-µm microspheres having densities of 1.025 g/cc (green) and 1.06 g/cc (red), using an SFAT operating at 20.3 MHz with 50 V_{pp} applied voltage. As can be seen in Fig. 5, a clear separation of the red and green microspheres is observed. The lower-density microspheres (green) are deflected more and subsequently collected at the Tilted outlet, while the red microspheres are deflected less and are collected in the Straight outlet.

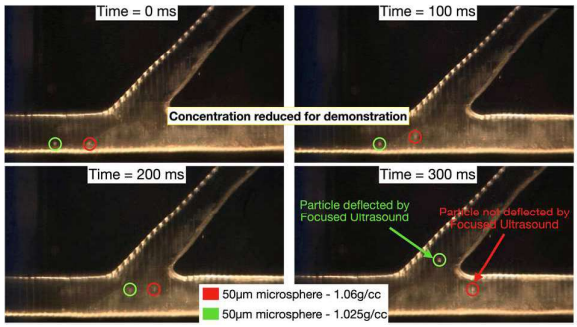

Figure 5. Cross-sectional-view photos showing sorting of 50µm microparticles with densities of 1.025g/cc and 1.06g/cc with less-density particles being deflected further away from SFAT.

Microparticle Sorting Emulating Mesenchymal Stem Cell (MSC)

We have conducted experiments with microspheres of 30 and 50 µm in diameter to emulate the sorting of healthy Mesenchymal Stem Cells (MSC) from unhealthy MSCs due to differentiation or becoming cancerous. Sorting such cells is needed to use healthy stem cells without having any differentiated/cancerous MSCs, which can disrupt the desired differentiation process. Healthy MSCs typically have a diameter of approximately 30 µm, while the diameter of the differentiated MSCs can reach about 50 µm.

Equal weights of the two types of microspheres are mixed and passed through the 3D microchannel for sorting. The 20.3MHz SFAT is actuated with a 60V_{pp} sinusoidal signal to separate the two types of particles at high throughput. Though we can confirm the sorting of 30µm microparticles (blue) that pass almost undeflected from the 50µm particles (green) that deflect to the Tilted outlet, through a long-range microscope (Fig. 6), precise measurement of sorting accuracy is not possible with a long-range microscope, due to the high flow rate and small particle size.

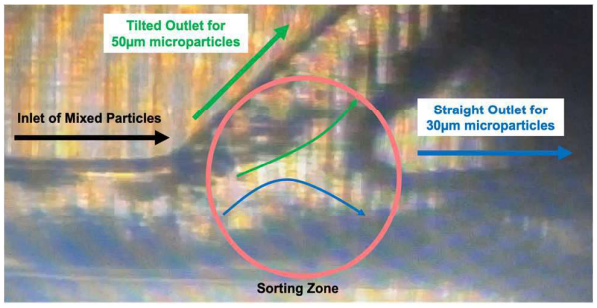

Figure 6. Photo through a long-range microscope demonstrating sorting of 30µm and 50µm microparticles emulating healthy and differentiated MSCs.

To assess the purity of the particles in the outlets and the recovery of each particle type, the sorting is conducted for ten minutes, followed by collection of the sorted particles in the outlet syringes. Then, the particles from each syringe are extracted onto a petri dish with grid on the bottom, where the number of cells can be counted using the manual grid counting technique, as illustrated in Fig. 7.

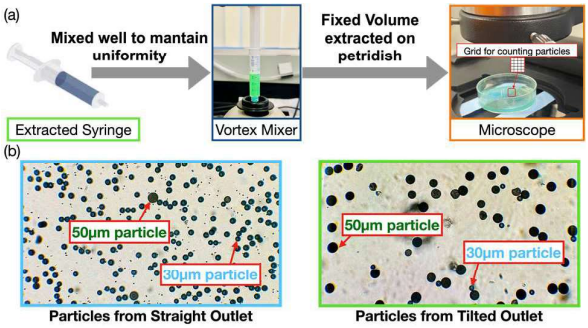

Figure 7. (a) Steps for distributing the cells (after sorting and collecting) over a Petri dish for grid-counting of microparticles. (b) Microscopic image showing high purity of 30µm blue microparticles in the straight outlet and 50µm green microparticles in the tilted outlet (Fig. 2)

The recovery is calculated with the following equation

$$Recovery\ of\ Particles = \frac{NPD}{NPD + NPU}$$

with NPD = Number of Particles in the Desired Outlet and NPU = Number of Particles in the Undesired Outlet, while the purity at an outlet is calculated with

$$Purity\ of\ Outlet = \frac{NDP}{NDP + NUP}$$

with NDP = Number of Desired Particles in an Outlet and NUP = Number of Undesired Particles in an Outlet. Table

1 summarizes the measured recoveries and purities at the outlets.

Table 1. Results of experiment mimicking extraction of healthy mesenchymal stem cells (MSCs) from infected MSCs

Accuracy Measure	Percentage
Recovery of Blue Particles	97.2%
Recovery of Green Particles	88.1%
Purity at Tilted Outlet	89.5%
Purity at Straight Outlet	97.5%

CONCLUSION

This paper presents a cost-effective, label-free, and high-throughput particle sorting based on focused Bulk Acoustic Waves (BAW) produced by Self-Focusing Acoustic Transducers (SFATs). The intensified Acoustic Radiation Force (ARF) provides high selectivity for sorting based on the size and density of the particles. The method has been extended to sort microparticles that mimic healthy and unhealthy Mesenchymal Stem Cells (MSC), demonstrating 97.2% recovery of particles mimicking healthy MSC cells and 97.5% purity at the Straight outlet. The SFAT is not placed inside the microfluidic channel, and can easily be replaced. The method can be readily extended to cells through 3D printing of biocompatible materials like FormLabs BioMed Clear Resin. The potential for cascading multiple stages of the SFATs over a microfluidic channel offers high purity and recovery in cell sorting. Consequently, this cost-effective, label-free, high-throughput sorting method can accurately sort cells of varying sizes based on their size and density.

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