Elastomeric Negative Acoustic Contrast Particles for Capture, Acoustophoretic Transport, and Confinement of Cells in Microfluidic Systems

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Supporting Information

ABSTRACT: We present a particle-based method for the immunospecific capture and confinement of cells using acoustic radiation forces. Ultrasonic standing waves in microfluidic systems have previously been used for the continuous focusing of cells in rapid screening and sorting applications. In aqueous fluids, cells typically exhibit positive acoustic contrast and are thus forced toward the pressure nodes of a standing wave. Conversely, elastomeric particles exhibit negative acoustic contrast and travel toward the pressure antinodes. We have developed a class of elastomeric particles that are synthesized in bulk using a simple nucleation and growth process, providing precise control over their size and functional properties. We demonstrate that the biofunctionalization of these particles can allow the capture and transport of cells to the pressure antinodes solely via acoustic radiation forces, which may enable new acoustics-based cell handling techniques such as the washing, labeling, and sorting of cells with minimal preparatory steps.

INTRODUCTION

The number of miniaturized medical devices for biosensing, cell sequestration, and sorting applications has soared in recent years as a result of their functional versatility, capacity to analyze small sample volumes, and promise for clinical translation.1,2 Acoustofluidics, which relies on the actuation of a piezoelectric transducer to excite a microfluidic device to resonance, has merited considerable attention as a convenient means for cellular handling without imposing deleterious effects on cellular viability.3−5 Through the action of acoustic radiation forces in laminar flows, acoustofluidics can continuously direct cells into tightly focused streams, which provides an attractive alternative to centrifugation (e.g., for removing lipids from blood)6 and hydrodynamic focusing for flow cytometric analysis.7,8

Previous acoustics-based microchip systems have employed resonance pressure waves to selectively focus (i.e., concentrate) and manipulate cell populations on the basis of their morphological characteristics (e.g., size, compressibility, and density) using standing bulk acoustic waves and surface acoustic waves.9,10 In these systems, acoustic radiation forces drive cells with differences in such characteristics to the pressure nodes of standing waves at dissimilar rates, enabling continuous sorting at multijunction outlets, for example; however, no system has confined cells to the pressure antinodes of an acoustic standing wave. This study describes the use of elastomeric particles for the recognition (binding), transport, and confinement of cells to the pressure antinodes in a simple acoustofluidic system as an example of a new class of cell-handling techniques.

Particles migrate to the nodes or antinodes of an acoustic standing wave, depending on their acoustic contrast factor

\[ q_p = \frac{5\beta_p - 2\beta_f}{2\beta_p + \beta_f} \]

in which variables \( \rho \) and \( \beta \) represent density and compressibility and subscripts \( p \) and \( f \) represent the suspended object (e.g., particle or cell) and the fluid, respectively.11,12 Particles with a positive \( q_p \) migrate to the pressure node of a standing wave, whereas particles with a negative \( q_p \) migrate to the pressure antinodes.2 Mammalian cells generally exhibit positive \( q_p \) values in aqueous fluids,8 whereas elastomeric particles generally exhibit negative \( q_p \) as a result of their high compressibility and relatively low densities.13,14

For the half-wavelength resonance mode of a fluidic cavity, the pressure node and antinodes correspond to the centerline and the side walls of the channel, respectively. As depicted in Figure 1, elastomeric particles bound to cells can cause the cells to be transported to the pressure antinodes. To achieve this phenomenon, the acoustic radiation forces acting on the elastomeric particle(s) must be greater those acting on the cell(s) to which they are bound.2 The magnitude and direction
of these forces are determined by the size, density, and compressibility of the components of the assembled complexes as well as the fluid properties. This dependence is represented in Figure 1c, which indicates the maximum φ_p of an elastomeric particle capable of transporting a typical mammalian cell (13.0 μm in diameter) to a pressure antinode of an acoustic standing wave (details in Supporting Information). In practice, the elastomeric material bound to a cell may comprise multiple particles with the same effective volume. Thus, to transport cells controllably to pressure antinodes in acoustic standing waves, it is desirable to synthesize particles with precise size, sufficiently low density, high compressibility, and a robust capacity for readily forming complexes with cells.

We previously reported the preparation of elastomeric particles using homogenization and microfluidic techniques and demonstrated that they can be used to transport target proteins and positive φ_p microbeads to the antinodes of an acoustic standing wave. Although convenient, homogenization techniques provide limited control over the size distribution and properties of the resulting particles. In contrast, microfluidic syntheses provide precise control over the dispersity in particle size, but suffer from limited yields. Herein, we present the preparation of a new class of elastomeric silicone particles for the precise spatial control of cells in acoustofluidic devices. We describe how their synthesis by nucleation and growth is easily scalable to generate large quantities of particles that enable immunolabeling for specific binding to cells. As a demonstration of their use, we show that elastomeric particles can confine mammalian cells to the antinodes of an acoustic standing wave, which may provide the basis for several new acoustics-based cell-handling techniques.

**EXPERIMENTAL SECTION**

**Fabrication of Acoustofluidic Device.** Acoustofluidic chips were made from silicon and glass components using conventional microfabrication techniques: (i) photolithography and (ii) deep reactive-ion etching to generate flow channels (~250 μm deep), (iii) anodic bonding to adhere the patterned silicon chip to a Pyrex glass lid (Borofloat 33, glass B, Schott AG), and (iv) plasma treatment to bond poly(dimethylsiloxane) (PDMS; Sylgard 184, Dow Corning) ports for the inlet and outlet tubing connections. Inlets and outlets were designed on the back side of the chip to facilitate visual access to the channel by an upright microscope (Zeiss Axio Imager 2, Carl Zeiss, AG) with a fluorescent lamp (X-cite series 120Q, Lumen Dynamics). Tubing connections were installed into the bored holes of the PDMS ports and were interfaced with high-purity silicone tubing (9628TS4, McMaster-Carr Supply Co.). A lead zirconate titanate (PZT) transducer (841, APC International; 2.93 MHz resonance frequency) was bonded underneath the flow channel using cyanoacrylate glue. The PZT element (0.5, 5, and 30 mm thickness, width, and length, respectively) was actuated at ~2.96 MHz, corresponding to the half-wavelength resonance mode of a channel 250 μm in width via a sinusoid waveform from a function generator (DG1022, Rigol Technologies, Inc.) connected through a power amplifier (25A250AM6, Amplifier Research, Corp.). An oscilloscope (TDS2000C, Tektronix, Inc.) measured the peak-to-peak voltage applied to the PZT, which was 31 V in all experiments.

**Synthesis of Elastomeric Particles.** Monomers (totaling 1 mL, i.e., a 1:100 molar ratio of tetramethyl orthosilicate (TMOS; 98% purity, Sigma) to dimethoxydimethylsilane (DMODMS; ≥99.5% purity, Sigma)) were added to 10 mL of 2.2% HCl and were stirred at 500 rpm for 2–18 h. A molar excess of NH₂OH (Sigma) was then added with continuous stirring. Particles could be rendered fluorescent by adding 30 μL of 3.1 mM Nile red in acetone (Sigma) directly prior to the addition of NH₃OH. Approximately 1 × 10⁹ particles were functionalized by resuspending them in 1 mL of 1xPBS, adding 25 μL of 33.3 μM fluorescent (Alexa-Fluor 488 or 546-conjugated) streptavidin (SA), stirring at 100 rpm for 0.5 h, and storing overnight at 4 °C. Prior to use, particles were washed and resuspended in fresh 1xPBS (further details in Supporting Information).

**Cell Preparation.** Acute myelogenous leukemia (KG-1a) cells were cultured in Iscove’s modified Dulbecco’s medium (Invitrogen) with 20% fetal bovine serum supplemented with 50 μg/mL streptomycin, 10 μg/mL penicillin, and 50 μg/mL fungizone (Gibco). Prior to analysis, cells were stained with calcein AM from a live/dead cell viability kit (Invitrogen). Cells (1 × 10⁶) were incubated in a cold wash buffer with 25 μL of stock biotinylated CD34 antibodies (mouse anti-human mAb (clone 581), Invitrogen) for 0.5 h. After being washed, a concentrated mixture of SA-adsorbed elastomeric particles and biotinylated polyethylene microbeads or biotinylated anti-CD34-labeled KG-1a cells were gently agitated in an end-over-end rotator for 1 h prior to injection into the acoustofluidic device.
RESULTS AND DISCUSSION

Biofunctional Elastomeric Particles. We prepared silicone gel particles using a simple nucleation and growth process whereby monomers were hydrolyzed in a low-pH solution, thus forming nucleation sites for the rapid and uniform growth of particles upon catalysis. Monomers were initially immiscible in water, but became miscible after hydrolysis. Excessive hydrolysis resulted in the formation of nonuniformly sized oligomers and thus polydisperse particles. We thus controlled the duration of hydrolysis to achieve a narrow size distribution compared to that of particles prepared by homogenization techniques (18 vs 51% CVs, respectively; n = 50 particles, Figure 2a).  

![Figure 2](image_url)

Figure 2. Elastomeric particles obtained through nucleation and growth synthesis. (a) Optical micrographs of elastomeric particles (diameter ≈ 13 μm) used for the confinement of cells. (b) Elastomeric particles (red, with adsorbed SA labeled with Alexa-Fluor 546) transported to the antinodes of an acoustic standing wave within a microfluidic channel (no flow). The locations of the channel walls (dashed lines) and the features of the acoustic standing wave (solid lines) are denoted.

The reaction mixture contained two types of monomers: one that can form two siloxane linkages (i.e., DMODMS) and one that can form four siloxane linkages (i.e., TMOS). The large ratio of DMODMS to TMOS resulted in the formation of particles exhibiting elastic properties and displaying negative φ_p in water, as evidenced by their transport to the pressure antinodes of an acoustic standing wave (Figure 2b). A major advantage of this nucleation and growth synthesis is the ability to tune the size of particles by altering the concentration of monomers used during the growth process or the duration of the polycondensation reaction. Moreover, the nucleation and growth synthesis enables the facile incorporation of monomers with a variety of functional groups. These features are attractive compared to homogenization and microfluidic-based synthesis approaches because particles with programable properties (i.e., size, modulus, and surface chemistry) can be easily manufactured in large quantities for a variety of acoustofluidic applications.

Acoustic Confinement of Polystyrene Microbeads to the Pressure Antinodes. For an initial evaluation of the elastomeric particles, we studied their interaction with biotinylated polystyrene microbeads (Spherotech) as surrogates for cells owing to their inherent positive φ_p behavior in physiological buffers and ample surface binding sites. We functionalized elastomeric particles by physically adsorbing SA to their surfaces to permit binding to biotin groups on the polystyrene microbeads. As controls, we performed acoustophoresis separately on the elastomeric particles and the polystyrene microbeads. The elastomeric particles exhibited negative φ_p behavior in the acoustofluidic chip under flow by migrating to the pressure antinodes (Figure 3a). We note that high flow rates and multinodal systems with protective hydrodynamic sheath flow can help reduce the aggregation and buildup of negative φ_p particles as observed in Figure 2b. Polystyrene microbeads exhibited positive φ_p behavior by migrating to the pressure node (Figure 3b). After binding the SA-adsorbed elastomeric particles to the biotinylated polystyrene microbeads, the bound complexes were displaced to the pressure antinodes of the acoustic standing wave (Figure 3c, see also Figure 2 and Videos 1 and 2 in the Supporting Information), indicating that elastomeric particles prepared by nucleation and growth and adsorbed with SA are capable of capturing positive φ_p objects and transporting them to the pressure antinodes.

Acoustic Confinement of Cells to the Pressure Antinodes. We evaluated the ability of elastomeric particles with adsorbed SA to capture KG-1a cells presenting biotinylated antibodies against the CD34 cell surface antigen (Supporting Information Figure 1) and to transport those cells to the antinodes of an acoustic standing wave. After coincubation with gentle agitation, elastomeric particles were bound to KG-1a cells (Figure 4a,b). Once bound, the complexes were injected into an acoustofluidic chip, where they migrated to the pressure antinodes upon excitation of the PZT (Figure 4c,d; Videos 4 and 5 in the Supporting Information). As a control, KG-1a cells without bound anti-CD34-biotin separated from elastomeric particles and focused along the pressure node (Figure 4d (left), Figure 4e; Video 3 in

![Figure 3](image_url)

Figure 3. Fluorescence intensity profiles of particles across the microchannel cavity under flow. The distributions of (a) SA-adsorbed elastomeric particles (diameter ≈ 13 μm) at 15 μL/min, (b) biotinylated polystyrene microbeads (diameter ≈ 5.2 μm) at 100 μL/min, and (c) complexes of particles in a bound to particles in b at 100 μL/min are shown across the acoustofluidic channel, where W denotes the width of the microchannel (250 μm). See the Supporting Information for the corresponding micrographs of particles in the microchannel.
OUTLOOK

We describe the use of elastomeric silicone particles for the capture and transport of cells to the pressure antinodes of an acoustic standing wave. This approach provides (1) gentle forces on cells and particles, (2) implementation in continuous laminar flow streams, (3) the ability to focus cells tightly under a range of flow rates, even during flow reversal, and (4) discriminating forces that act on both labeled and unlabeled cell populations. Looking forward, the combination of acoustic standing waves and biofunctional and elastomeric particles holds promise for sorting cells via acoustophoresis. However, to achieve more robust binding between elastomeric particles and cells, we believe other approaches for biofunctionalizing elastomeric particles may be necessary. Isolating cells from complex biological fluids is critical to many diagnostic and therapeutic systems. We envision a miniaturized and continuous flow-sorting system in which cells biospecifically immunolabeled with elastomeric particles would displace to, and flow along, pressure antinodes, separating them from unlabeled cells that would be focused to, and flow along, the pressure node. In this approach, the separated populations of cells could be collected in a continuous fashion at high flow rates at a downstream channel trifurcation. Such a method could provide advantages over systems such as fluorescence-activated cell sorting in that it would not require copious volumes of purified sheath fluid or serial cell-by-cell inspection (e.g., by fluorescence spectrophotometry).

ASSOCIATED CONTENT

Supporting Information

Method for binding between SA-adsorbed particles and biotinylated anti-CD34 labeled KG-1a cells. Model for the properties of elastomeric particles needed to capture and confine cells to the antinodes of an acoustic standing wave. Corresponding micrographs of elastomeric particles and polystyrene beads from Figure 3. Videos of polystyrene microbeads bound to elastomeric particles being displaced to the pressure antinodes (Videos 1 and 2). Video of unbound KG-1a cells focusing on the pressure nodes (Video 3). Videos of KG-1a cells bound to elastomeric particles being displaced to the pressure antinodes (Videos 4 and 5). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES


Supporting Information). Green-stained KG-1a cells presenting biotinylated antibodies bound to SA-adsorbed, nonfluorescent elastomeric particles and separated from red-stained (PKH26, Sigma) KG-1a cells without anti-CD34-biotin (Figure 4f). At low flow rates (e.g., less than 50 μL/min), isolated complexes at the pressure antinodes generally remained immobilized against the walls of the microchannel, thus enabling the possibility of on-chip washing, staining, and secondary labeling of cells. At high flow rates (e.g., 200 μL/min in Figure 3c), shear forces cause the complexes to flow down the microchannel along the sidewalls, thus allowing their collection through a downstream microchannel trifurcation.


