Microfluidic Cell Sorters for Stem Cell Separation and Size-Profiling Using Pressurized Laminar Flows at High Spatial-Temporal Resolution

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Abstract:

The sorting and isolation of stem cells from a medium is of great importance to the biomedical community. The focus of this project is to develop an acoustically driven, polymeric microfluidic cell sorter that will separate particles according to size-based differential migration with high spatial-temporal resolution. We modeled the acoustic field within a 150 μm high SU-8 chamber with rectangular cross sections (12.5 mm x 25 mm) sealed with 200 μm, 500 μm, and 1000 μm PDMS matching and reflective layers. We measured the energy coupled into a fluid-filled chamber of the same dimensions with 200 μm, 500 μm, and 1000 μm PDMS matching and reflective layers by the PZT to verify the model. The results show that a 200 μm thick PDMS matching layer increases the energy coupled into the chamber, while a PDMS reflective layer decreases the energy coupled into the fluid.

Introduction:

Stem cells are the future of regenerative medicine because they can develop into many types of cells. However, obtaining a stem cell "line" is often controversial because it involves the destruction of an embryo. Stem cells derived from liposuction aspirates circumvent this controversy. However, utilization of this source requires that stem cells be separated from endothelial cells and adipocyte precursors.

Microfluidic channels are promising for sorting applications: flows in microfluidic channels have a low Reynolds number, allowing laminar flow. Laminar flow prevents the shifting of suspended particles between fluid layers, thus preventing mixing of cells during sorting.

Acoustic standing waves provide an accurate, low-power method of separation that causes minimal damage to cells. These standing waves exert a primary acoustic radiation force on suspended particles (Figure 1a). In the radiation force equation (Figure 1b), if the sign of F_r is negative, the particles will migrate toward the pressure nodes, and, if positive, toward the pressure antinodes. Because of the volume dependence of the force, larger particles experience a greater force and reach pressure nodes and antinodes faster than smaller particles.

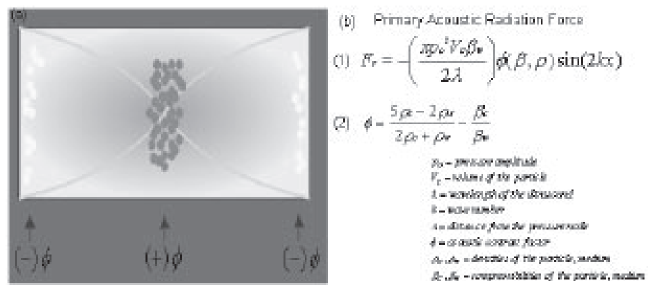

Figure 1: (a) Acoustic standing waves exert a primary acoustic radiation force on suspended particles. (b) Equations for (1) the primary acoustic radiation force and (2) the π factor.

Simulation:

The cell sorting efficiency depends on the acoustic energy coupled into the microfluidic channel.The acoustic performance within a fluid layer of 150 μm thickness with rectangular cross-sections (12.5 mm x 25 mm) was modeled for the instances in which the energy generated by the PZT transducer was coupled through 200 μm, 500 μm, and 1000 μm thick matching

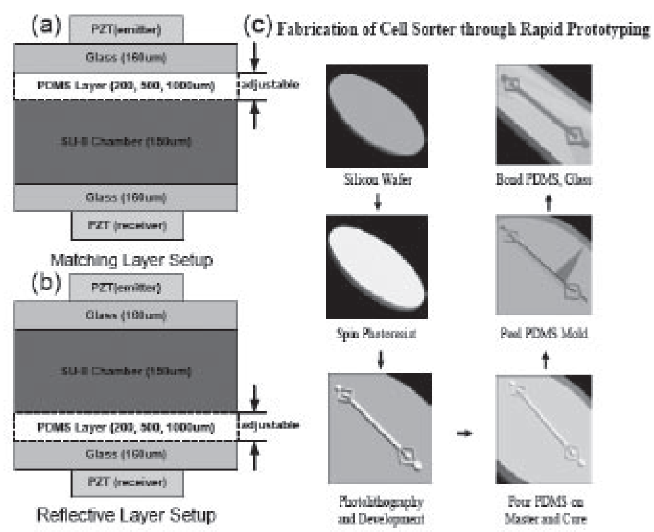

Figure 2: (a - b) Layer configurations for acoustic energy coupling studies. (c) Fabrication of cell sorters through rapid prototyping.

layers of PDMS (Figure 2a). The energy density model showed a 1000 μm increase in the thickness of PDMS resulted in an energy loss of 22% and a maximum pressure and velocity decrease of 5%.

The acoustic performance within the same fluid layer was modeled for instances in which 200 μm, 500 μm, and 1000 μm thick layers of PDMS were used as reflective layers to generate acoustic standing waves (Figure 2b). The energy density model predicted that a 1000 μm increase in PDMS thickness would result in a 17% energy gain and a pressure amplitude increase of 25%.

Experimental Procedure:

A 150 μm thick SU-8 structure with rectangular cross-sections (12.5 mm x 25 mm) was fabricated on a 170 μm thick glass slide (Figure 2c). The SU-8 chamber was then filled to capacity with water and closed with a glass slide to which 200 μm, 500 μm, and 1000 μm layers of PDMS were attached. One PZT was then attached to the back of each of the glass slides.

In order to determine the effect of PDMS as a matching layer, the PZT attached to the glass slide covered with PDMS served as the emitter, and the PZT attached to the other glass slide served as the receiver. For each thickness of the PDMS matching layer, the energy coupled through the device by the emitting PZT was measured by the voltage generated by the receiving PZT. In order to determine the effect of PDMS as a reflector layer, the roles of the two PZTs were reversed, and the energy coupled through the stack for each thickness of PDMS was measured.

Experimental Results:

A PDMS matching layer of up to 200 μm thickness increased the energy coupled into the chamber. However, the energy coupled into the chamber decreased with increasing PDMS layer thickness beyond 200 μm (Figure 3). These results are largely consistent with the results of the simulation, thus demonstrating that a PDMS matching layer generally decreases energy coupled into the chamber, unless the layer provides optimal impedance matching.

The energy coupled into the chamber decreased with increasing thickness of the PDMS reflector, and maximum energy was coupled into the chamber with only the glass backing acting as a reflector (Figure 4). These results show that the acoustic reflection properties of PDMS are inferior to those of glass.

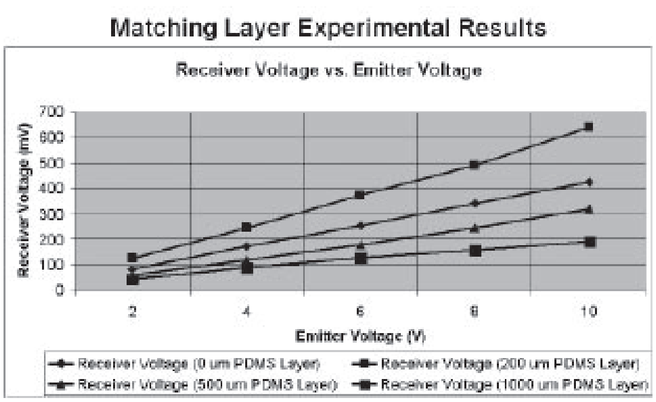

Figure 3: Matching layer experimental results.

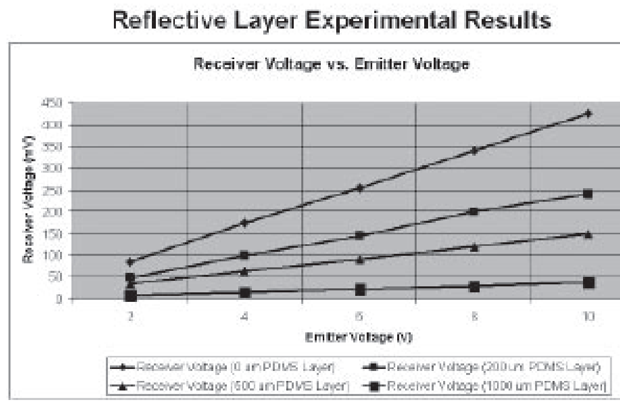

Figure 4: Reflective layer experimental results.

Conclusions and Future Work:

A PDMS matching layer of up to 200 μm thickness increases the energy coupled into a chamber by providing optimal impedance matching among the layers between the PZT and the fluid. However, matching layers of thickness greater than 200 μm decrease the energy in the fluid layer because the absorption of energy is greater than the energy gain acquired by optimal impedance matching. A PDMS reflective layer decreases energy coupled into the fluid layer by absorbing instead of reflecting the energy contained in the standing wave.

Based on these results, the design of the cell sorter can be optimized, involving the addition of a 200 mm PDMS layer above the bottom glass slide and a glass reflector plate above the channel, to achieve separation of stem cells from background particles of different sizes at high spatial-temporal resolution.

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