Acoustofluidics and Whole-Blood Manipulation in Surface Acoustic Wave Counterflow Devices

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Supporting Information

ABSTRACT: On-chip functional blocks for sample preprocessing are necessary elements for the implementation of fully portable micrototal analysis systems (μTAS). We demonstrate and characterize the microparticle and whole-blood manipulation capabilities of surface acoustic wave (SAW) driven counterflow micropumps. The motion of suspended cells in this system is governed by the two dominant acoustic forces associated with the scattered SAW (of wavelength λ): acoustic-radiation force and acoustic-streaming Stokesian drag force. We show that by reducing the microchannel height (h) beyond a threshold value the balance of these forces is shifted toward the acoustic-radiation force and that this yields control of two different regimes of microparticle dynamics. In the regime dominated by the acoustic radiation force (h ≲ λ), microparticles are collected in the seminodes of the partial standing sound-wave arising from reflections off microchannel walls. This enables the complete separation of plasma and corpuscular components of whole blood in periodical predetermined positions without any prior sample dilution. Conversely, in the regime dominated by acoustic streaming (h ≳ λ), the microbeads follow vortical streamlines in a pattern characterized by three different phases during microchannel filling. This makes it possible to generate a cell-concentration gradient within whole-blood samples, a behavior not previously reported in any acoustic-streaming device. By careful device design, a new class of SAW pumping devices is presented that allows the manipulation and pretreatment of whole-blood samples for portable and integrable biological chips and is compatible with hand-held battery-operated devices.

Integration of multiple functional elements onto a single device is one of the keys to the development of truly portable micrototal analysis systems (μTAS). Toward this goal, surface acoustic wave (SAW) devices are one of the most promising microfluidic technologies for both miniaturization and integration of ultrafast fluid handling components. There is a large and growing amount of literature demonstrating that SAW-fluid interactions can drive a great variety of μTAS operations such as fluid actuation, mixing, centrifugation, cell sorting and lysis, thermal treating, and biosensing to name but a small selection. Importantly, all these functions are compatible with hand-held devices, since SAWs can be generated by on-chip microfabricated transducers operated with miniaturized battery-powered electronics. Furthermore, these operations were demonstrated both in digital microfluidics and within closed microchannel platforms, where the vast majority of operations have been demonstrated in the former and the later typically specializes in cell sorting and focusing applications. In particular, in the realm of microchannel devices a few truly integrated active micropumping mechanisms were proposed, with the most promising solutions including electro-hydrodynamic, magneto-hydrodynamic, and SAW-driven acoustic counterflow. Among these, SAW-driven counterflow is particularly attractive owing to its low voltage requirement and ability to pump fluids with no specific requirement on fluid properties. In counterflow devices (Figure 1), SAWs propagate from the microchannel outlet toward the inlet. The filling dynamics result from a cascading process; SAWs induce fluid atomization, then the resulting droplets collect in front of the meniscus and subsequently coalesce resulting in a net advance of the bulk fluid.

For complete sample-to-answer point-of-care systems, devices must include the integration of sample pretreatment and analysis stages. The starting sample for many biomedical

Received: July 4, 2014
Accepted: September 26, 2014

Figure 1. Schematic of the SAW counterflow device (not to scale).
applications is whole blood, and pretreatments such as plasma-blood separation and/or mixing with reagents (e.g., antibodies, lysis buffers) are generally required. In order to design these components it is necessary to first understand the internal fluid flows and the dynamics of suspended microparticles that are generated by acoustic counterflow. This is the focus of the present article. When SAWs come into contact with a fluid in its wave path, a sound wave is refracted at the Rayleigh angle into the liquid owing to the mismatch of sound velocities between the fluid and the substrate. SAWs are then attenuated within approximately ten wavelengths in water. On a longer spatial scale, sound waves are also damped due to the fluid viscosity and this dissipation generates a secondary stationary flow, known as acoustic streaming. Therefore, in SAW devices two forces act on the suspended microparticles: the acoustic streaming (Stokesian) drag force and the acoustic radiation force, due to direct interaction between the scattered sound wave and the particles. Detailed reviews of these two forces were published by Bruus, and Gedge and Hill. Here we show that by varying the microchannel height in SAW counterflow devices, the dominating force on particle suspensions can be switched between acoustic-streaming-driven drag force and acoustic-radiation force. We characterized each regime with synthetic monodisperse bead suspensions prior to exploiting these regimes for whole blood manipulation. Finally, we show that pumping whole blood in the acoustic streaming regime opens the possibility to simultaneously generate a gradient of the cellular concentration within the fluid front. Conversely, the alternate regime allows accumulation of blood cells in periodic bands with complete depletion in between. This separation method does not require any prior sample dilution that could hinder detection of low-concentration analytes.

**EXPERIMENTAL SECTION**

**Device Fabrication.** Devices (Figure 1) consist of two components: a lower 128° rotated Y-cut, X-propagating lithium niobate (LN) layer with a patterned interdigital transducer (IDT) for SAW excitation and an upper polydimethylsiloxane (PDMS) microchannel layer. The IDT has a single-electrode geometry with 24 straight finger pairs, $\lambda_{\text{SAW}} = 40$ μm periodicity, and an aperture of 17.5 $\lambda_{\text{SAW}} = 700$ μm which generate SAWs at a resonant frequency of $f_{\text{SAW}} \sim 96$ MHz along the major crystal axis. This corresponds to a wavelength of the sound wave in water of $\lambda_{w} = 15$ μm. The top layer is composed of a straight open-ended microchannel (300 μm width, 5 mm length) connected to an open reservoir (2 mm diameter, manually punched) via a prefilling chamber (500 μm width, ~1 mm length). The top and bottom layer were aligned so that the microchannel was centered with the IDT and the channel outlet was at 670 μm from the IDT front and joined via conformal bonding. Further fabrication specific details can be found in ref 50. Devices with microchannel heights ranging from 14 to 70 μm were tested, with the microchannel widths held constant at $w = 300$ μm since this was found to enable efficient pumping at the operating frequency used here ($w = 7.5 \lambda_{\text{SAW}}$).

**Device Testing and Operation.** Devices were connected to an rf generator (MXG Analog Signal Generator N5181A, Agilent Technologies) with an intermediate external amplifier (ZHL-5W-1, MiniCircuits). The SAW amplitude was calibrated as a function of the operating power by laser Doppler vibrometry (UHF-120 Ultra High Frequency Vibrometer, Polytec, Germany). A volume of 10 μl of fluid (water plus microparticle suspensions) was pipetted into the reservoir and pumped via SAW counterflow at fixed SAW amplitudes, ranging from 800 pm to 1.4 nm. Channel filling was recorded with a brightfield inverted microscope (Eclipse Ti, Nikon, Japan) equipped with a fast camera (A602-f, Basler, Germany). The microbead suspensions, 500 nm (L3280), 2 μm (L3030), 5 μm (79633) and 10 μm (72986) diameter particles (Sigma-Aldrich), were diluted with deionized water prior to use. μPIV data were recorded at 400 fps using 500 nm microbeads suspended in water with a concentration of 7.6 × 10^8 particles mL^-1. Ensemble-correlation μPIV code (Prana PIV, Virginia Polytechnic Institute and State University, 2012) was used to quantify velocity fields.

**Biological Sample Preparation and Analysis.** Mouse blood was used to mimic the corpuscular composition of human blood. Adult wild type mice (CS7BL/6J strain, Jackson Laboratory) were treated in accordance with the ethical framework of FP7 and with the guidance of the Animal Protection Law of the Italia Republic (D.L. 16/1992). Whole blood samples were drawn from the tail, and sodium citrate (3.8% in PBS solution) was immediately added in blood/citrate = 1:10 ratio to prevent coagulation. Whole blood was used without any further dilution or treatment and experiments were all carried within 6 h from collection. We used the Beer–Lambert law, stating that $c = -\log(T)/\varepsilon h$ where $c$ is the concentration, $T$ is the optical transmission, $\varepsilon$ is the absorptivity, and $h$ is the channel height, in order to quantify the relative spatial concentration of blood cells in the microchannel. Briefly, we first normalized the image intensity with respect to the empty channel (image pixel size: 2.7 μm). The logarithm of this value is proportional to the cell concentration. In order to get the relative concentration, we normalized this logarithm with respect to its mean value in the prefilling chamber before excitation of SAWs (when cell concentration is homogeneous and unaltered). Finally, we averaged spatially and temporally in the area of interest over a time span during which the meniscus position was stable.

**Simulations.** The sound-wave field distributions in the microchannels were simulated by finite element methods using commercial software (Multiphysics, COMSOL AB, Sweden). The three-dimensional (3D) geometry consisted of a 1 mm-long water-filled channel embedded in PDMS. In order to reduce the computational burden, we introduced the SAW effect in the acoustic pressure equations as a normal acceleration boundary condition, while lateral PDMS walls were modeled as a soft boundary condition. Underwater SAWs were considered as travelling waves with a planar wavefront and an exponentially decaying amplitude, as described by Frommelt et al. Water and PDMS sound speed and density were considered as found in Shi et al. Perfectly matched layers were introduced at the end of the water and PDMS domains to remove artificial reflections.

**RESULTS AND DISCUSSION**

The internal flows within SAW counterflow microchannels of varying heights, and the associated dynamics of the suspended monodisperse microparticles in the fluid were investigated (see the video in the Supporting Information). Figure 2 shows a representative measurement of the internal dynamics induced by SAW counterflow in 70 μm-high microchannels obtained by seeding the fluids with 500 nm beads. For this bead size ($d < \lambda_{w}$) and microchannel height ($h \gg \lambda_{p}$) the ultrasound...
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Figure 2. Representative flow streamlines reconstructed by μPIV in a 70 μm-high microchannel at different stages of fluid filling. Panel a shows the initial stage when the fluid is in the prefilling chamber and vortices are connected with the reservoir. Panel b shows a typical configuration in which the reservoir vortices (in white) are stretched into the microchannel and new front vortices (in yellow) start to nucleate close to the meniscus. In panel c, 1 mm of the channel is filled and the vortices are confined to the microchannel and begin to detach from the reservoir vortices. Panel d shows the last stage of the dynamics which is characterized by acoustic streaming close to the meniscus (left, yellow vortices extended for ~1 mm) and laminar flow at the channel inlet (right). Panel e schematically shows the microchannel geometry and marker positions (scale bars are 100 μm).

wavelength in water is $\lambda_f = 15 \upmu m$). Particle streamlines are not affected by the acoustic radiation force and accurately reproduce the acoustic streaming dynamics. The internal flow in these devices evolves through three different stages. First, as shown in Figure 2a, when the meniscus is located at the microchannel inlet, the streaming pattern is composed of a central jet separating two symmetrical vortices that continue into the reservoir, similar to what was observed at this operating frequency in digital microfluidic devices. As the fluid starts filling the microchannel, the vortical pattern elongates into the microchannel and a pair of symmetric vortices nucleates in front of the previous ones, as highlighted in yellow in Figure 2b. While the streamlines of the rear vortices extend into the reservoir (white streamlines), the new vortices are confined within the microchannel. The nucleation phase of the leading vortices ends when the meniscus is located approximately 1 mm into the channel, as shown in Figure 2c. During the remainder of the acoustic pumping, the front vortices are pulled with the meniscus without any qualitative variation in their geometry. Conversely, as the filling proceeds, the velocity of the rear vortices decreases until unidirectional laminar flow is seen at the inlet (Figure 2d). The boundary between the region of vortical streaming and the following laminar flow is at 1 ± 0.2 mm from the meniscus. The uncertainty of the vortical dimensions arises from the inertia of particles ejected from the jet (whose velocity is of the order of 1 mm s$^{-1}$) and the instability at the vortical breakup. This characteristic length is shorter than that of SAW-driven streaming in bulk liquids and microdroplets, probably owing to the low acoustic reflection coefficient at the top water-PDMS interface of the microchannel ($R = 0.23^{45}$) which introduces another source of loss of the ultrasound beam in water.$^{51}$

This flow pattern is not significantly affected by a change in SAW amplitude between 800 pm to 1.4 nm where SAW counterflow occurs, and the same flow pattern was observed for microchannel heights down to 21 μm ($h \gtrsim \lambda_f$). Within this range of microchannel heights, the acoustic-streaming drag force dominates the overall dynamics of 500 nm beads. It is worth noting that a secondary dynamics appears if the particle concentration is significantly increased to $3.8 \times 10^{10}$ particles mL$^{-1}$, reminiscent of what was observed by Manor et al. within a similar device configuration with a prefilled reservoir. This is characterized by particle accumulation in lines parallel to the meniscus near the substrate surface within the first ~300 μm from the meniscus. Instabilities in this local collection pattern were observed owing to the pumping process, leading to growth and breaking of these lines within the main vortical dynamics.

If the microchannel height is further decreased to 14 μm ($h \lesssim \lambda_f$), the dynamics of the 500 nm beads changes significantly. Microbeads proximal to the meniscus condense in a periodical two-dimensional (2D) pattern, shown in Figure 3a. The pattern displays well-defined bands parallel to the meniscus and separated by 27 ± 2 μm, while in the transversal direction the periodicity is 16 ± 2 μm. Particle-collection efficiency lowers as the distance from the meniscus is increased. After approximately 800 μm microparticles are homogeneously distributed in the channel and laminar flow is again observed. The measured longitudinal periodicity is not consistent with standard standing-wave devices that would show $\lambda_{SAW}/2 = 20 \upmu m$ or $\lambda_f/2 = 7.5 \upmu m$. In order to address the origin of this spatially periodic bead accumulation, we performed finite-element simulations of the pressure wave scattered by the SAW into the microchannel. Owing to the reflections off microchannel walls, simulations yield in a partial standing wave (Figure 3b) with a seminodal distribution with a periodicity of 27 μm along the $x$-direction and 18 μm along the $y$-direction. This is in agreement with the measured particle accumulation bands. We conclude that the acoustic radiation force dominates the dynamics in this regime. Note that the observed periodicity of the accumulation pattern must take into account both reflections off both top surface and lateral walls: changing these lengths and the operating frequency makes it possible to fine-tune the resulting pattern dimensions.

Since acoustic radiation force scales with micro bead volume while acoustic-streaming (Stokesian) drag force scales with micro bead radius, the threshold value for microchannel height between the two dynamics is particle-size dependent. Seeding with 5 and 10 μm beads, sizes similar to those of platelets and red blood cells, for both the case of 70 μm-high and 14 μm-high

dx.doi.org/10.1021/ac502465s | Anal. Chem. XXXX, XXX, XXX--XXX
channels, we observed the same dynamics reported above for the 500 nm beads. We remark that when the particle size is increased, however, difficulties can arise in seeding the frontal vortices in the case of 70 μm-high channels. During the first stage of streaming, particles equally seed the front and rear regions of the vortices, but the front region close to the meniscus starts depleting as the nucleation phase starts. This region is not completely forbidden to the particles. Once a particle enters this area, it follows the same vortical trajectories observed with 500 nm beads remaining trapped throughout the filling process. This suggests that depletion may be related to the acoustic streaming pattern evolution during the initial vortex nucleation phase.

Finally, we tested the application of these two schemes with whole blood in the two cases of 70 μm-high and 14 μm-high channels. Figure 4 illustrates that 14 μm-high channels allow blood micropumping by SAW counterflow and, at the same time, yield complete depletion of plasma from blood cells in periodic predetermined areas near the fluid front. This plasma-blood separation does not require any prior sample dilution, which is typically required by other microscale plasma-extraction systems\(^3\,^8,\,^39\) and can hinder the detection of low-concentration analytes. Since line width and periodicity of the depleted areas is controlled by the partial standing-wave pattern, this can be tailored by careful choice of the pumping frequency and microchannel characteristics. While the spacing of the depleted area along the channel length is the same for whole blood and the microbeads, blood-cell accumulation shows no transversal periodicity and collection efficiency decreases faster with distance from the meniscus.

Homogeneous cell distribution is observed after approximately ~200 μm. These differences are most likely related to the high concentration of blood cells, which introduce an increased attenuation of the sound wave\(^48\) and short-range interparticle interaction that can favor their packing\(^49\).

Figure 5 demonstrates the capability of the acoustic-streaming-dominated geometry (i.e., when \(h \geq \lambda\)) to generate a cell concentration gradient with significant cell dilution during counterflow pumping. This gradient, reported here for 70 μm-high channels, is V-shaped and localized near the meniscus within the vortical region. Transverse to the SAW-pumping direction, the concentration is approximately homogeneous within the central 100 μm, where the acoustic streaming jet is observed. Starting from the meniscus, where cell concentration is similar to that of the bulk sample, cell concentration diminishes as the distance increases, down to a minimum of 40 ± 10% occurring at approximately ~80 μm from the fluid front. This minimum position corresponds to the center of the vortices. Behind this region, the concentration increases rapidly up to a distance of ~250 μm from the meniscus, which corresponds to the SAW damping length, and then slowly to the end of the vortices. At 1 mm from the meniscus, where the vortices end, the concentration value is 83 ± 3%. These concentration values do not vary changing the SAW amplitude within the counterflow range (800 pm to 1.4 nm). The final cell concentration, therefore, is primarily determined by the geometry of the vortices and not by the fluid velocity in this region.

This blood dilution capability can be exploited to sample different hematocrit levels on-chip from small blood samples without buffer dilution. Owing to the connection between the gradient and the geometry of the vortices, but not their velocity, the pumping frequency can be used to tailor the area over which the gradient occurs.\(^14,\,^45\) This deterministic cell gradient generation was not observed in previous SAW acoustic streaming devices, where the streaming was typically applied to enhance the mixing of biomolecules.\(^3\,^7\) Previous cell manipulation methods based on acoustic streaming relied on asymmetrical SAW-microreactor configurations in which SAWs were used to actuate the fluid into a Batchelor flow instead of two symmetric vortices. Depending on the cell size and density, this type of flow allowed the concentration of specific cells in the center of a reactor while forcing others to the periphery.\(^17,\,^18\)

**CONCLUSIONS**

In conclusion, we have demonstrated acoustophoresis and associated whole-blood applications driven via surface acoustic
wave counterflow. We have shown that there are two alternate regimes of particle dynamics, each associated with the dominance of one of the two primary acoustic forces, the acoustic radiation force and the acoustic streaming drag force, which can be controlled by selection of the microchannel height. When acoustic radiation force dominates (i.e., when $h \lesssim \lambda_0$), microparticles down to 500 nm diameter are collected in the periodic seminode pattern of the partial standing sound wave resulting from the acoustic wave refraction into the liquid and multiple reflections at the channel walls. By exploiting this configuration, while pumping whole blood it is possible to simultaneously obtain a complete on-chip plasma separation in predetermined areas with no prior dilution of the sample. When acoustic streaming dominates (where $h \gg \lambda_0$), microbeads follow the vortical streamlines with a pattern evolving through three different phases. In this regime we can generate a cell concentration gradient in whole blood samples within the vortical area. Switching between these two regimes is possible by suitable design of microchannel dimensions thus opening the way to the integration of whole-blood pretreatments in μTAS devices.
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