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Carrier Medium Exchange through Ultrasonic Particle Switching in Microfluidic Channels

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This paper describes a method, utilizing acoustic force manipulation of suspended particles, in which particles in a laminar flow microchannel are continuously translated from one medium to another with virtually no mixing of the two media. During the study, 5-μm polyamide spheres suspended in distilled water, spiked (contaminated) with Evans blue, were switched over to clean distilled water. More than 95% of the polyamide spheres could be collected in the clean medium while removing up to 95% of the contaminant. Preliminary experiments to use this method to wash blood were performed. Red blood cells were switched from blood, spiked with Evans blue, to clean blood plasma. At least 95% of the red blood cells (bovine blood) could be collected in clean blood plasma while up to 98% of the contaminant was removed. The obtained results indicate that the presented method can be used as a generic method for particle washing and, more specifically, be applied for both intraoperative and postoperative blood washing.

Acoustic manipulation of suspended particles has previously been used to separate particles from a fraction of their medium, i.e., to increase the concentration of particles. In general, this has been done by moving the particles into one region of their medium and subsequently removing a fraction of the obtained particle-free medium.[1−6] The same principle can be utilized to separate two different particle types from each other, provided that their acoustic properties are such that the induced acoustic forces on the two particle types have opposite directions.[7−9] The primary acoustic radiation force, eq 1,[10] dominates the motion of suspended particles in the relevant frequency range of the investigation in this paper.[11] It should, however, be stressed that there are additional forces influencing the details in the behavior of the particles. These forces become more evident as the density of particles increase.[11,12]

$$F_r = -\left(\frac{\pi p_0^2 V_c \beta_w}{2\lambda}\right)\phi(\beta,\rho)\,\sin(2kx) \quad (1)$$

$$\phi = \frac{5\rho_c - 2\rho_w}{2\rho_c + \rho_w} - \frac{\beta_c}{\beta_w} \quad (2)$$

In eqs 1 and 2 the densities of the medium and the particles are denoted ρ_w and ρ_c, respectively, and the corresponding compressibilities β_w and β_c respectively, p_0 is the pressure amplitude, V_c is the volume of the particle, λ is the ultrasonic wavelength, ϕ (the acoustic contrast factor or simply the ϕ-factor) is defined by eq 2, k is defined by $2\pi/\lambda$, and x is the distance from a pressure node. The direction of the force is determined by the sign of the ϕ-factor, a positive ϕ-factor results in movement toward a pressure node and a negative in movement toward a pressure antinode correspondingly, as illustrated in Figures 1 and 2.

A medical application of acoustic particle separation, capitalizing on the different signs of the ϕ-factors for different particle types, has been proposed by Petersson et al.[8,9] Lipid particles (emboli) were separated from red blood cells (erythrocytes) in an effort to realize an intraoperative blood wash system. However, in some applications of blood washing, it is desirable to entirely remove the carrier medium, the blood plasma, from the red blood cells. This is, for example, the case when the plasma contains high levels of inflammatory, coagulation, and complement activation factors or drugs.[13,14] These contaminants must be removed before returning the erythrocytes to the patient. Currently, centrifuge-based methods are utilized to perform this procedure. However, centrifuges are a suboptimal solution to the removal of

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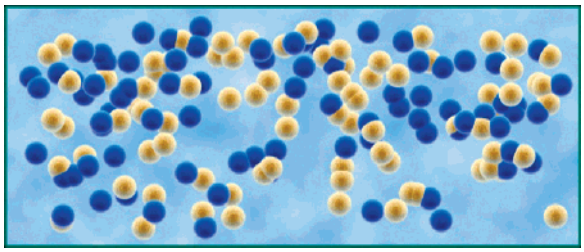

Figure 1. Schematic illustrations of a particle mixture in an enclosed chamber. The chamber holds the same proportions as the cross section of the main channel in the medium exchange chip. No accoustic force field is present in the cavity. The suspended particles have negative (yellow) and positive (blue) ϕ-factors.

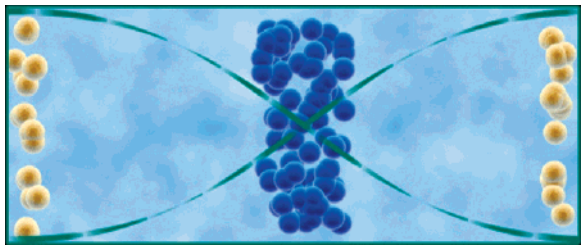

Figure 2. Acoustic standing wave (outlined with the pressure node in the center of the chamber) induced between the walls of the cavity. Under these conditions, the ϕ-factors of the particles will determine whether they move towards the pressure node or the pressure antinodes.

lipid microemboli in blood retransfusion during, for example, cardiac surgery. Lipid microemboli have been shown to cause capillary obstructions thereby affecting different organs such as the brain and the kidneys.[15,16] Furthermore, centrifuges expose the red blood cells to deforming forces,[17] demand large volumes of blood to be initiated, and are, in general, not continuous. It should be noted that acoustic standing wave manipulation of erythrocytes in microfluidic channels has previously been shown not to induce hemolysis, which indicates that the process conditions are mild.[9]

This paper proposes a method to switch particles from one medium to another using acoustic forces and laminar flow. Initial work on this topic was presented by Petersson et al.[18] Later, Hawkes et al. described a similar approach to translate yeast cells in a sheet flow domain by means of acoustic forces.[19] A brief description of the exchange process suggested by Petersson et al. follows. Particles in one medium enter a silicon microchannel with a rectangular cross section through side inlets. Another medium, without particles, enters through a center inlet. The two media form a laminar flow profile that remains stable throughout the length of the channel. When the channel is ultrasonically actuated, a half-wavelength standing wave is formed perpendicular to the direction of flow. If the density and compressibility of the

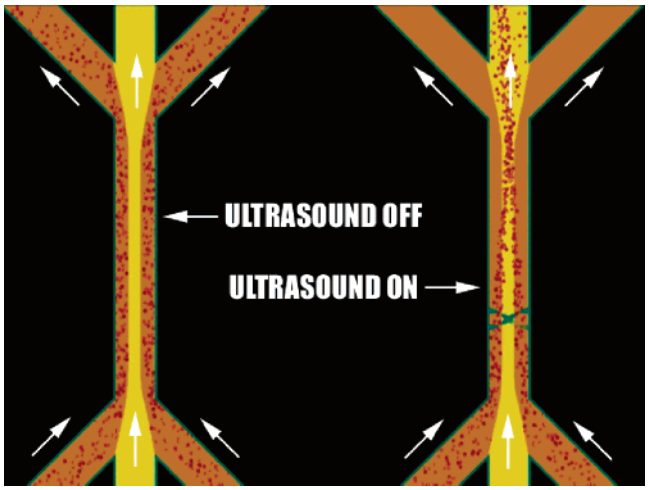

Figure 3. Schematic illustration of the medium exchange principle. The particles in the contaminated medium enter through the side inlets and exit through the side outlets when the ultrasound is turned off (left). If the ultrasound is turned on, as indicated by the schematic standing wave in the right microfluidic structure, the particles are switched over to the clean medium and exit through the center outlet together with the clean medium, whereas the contaminated medium still flows to the side outlets.

particles are appropriate, compared to the medium, they will move toward the pressure node in the center of the channel as they advance along the flow path. If the end of the channel is split into three outlet channels, the particles will exit through the center outlet together with the clean medium while the original medium exits through the side outlets as defined by the laminar flow lines. Some particle types move toward the pressure antinodes of an acoustic standing wave because of their acoustic contrast factors. In that case, the particle wash method can still be used if the suspended particles enter through the center inlet and the clean medium enters through the side inlets. As the acoustic standing wave is applied, the particles migrate into the clean medium along the walls of the channel.

With the ability to exchange the medium, the earlier presented intraoperative blood wash method can be extended to include both a wider range of intraoperative applications and postoperative applications. In the postoperative mode of operation, contaminated blood enters through the side inlets while clean blood plasma or saline solution enters through the center inlet (Figure 3). During the passage through the channel, the red blood cells move from the contaminated blood plasma into the clean medium in the middle of the channel and exit through the center outlet. The red blood cells can then be returned to the patient without risk of infusing potentially harmful substances. To address the issue of volumetric throughput, an arbitrary number of channels can be used in parallel.[20] Furthermore, the method provides a generic platform to exchange carrier medium for particles in suspension.

As soon as the contaminated medium is brought in contact with the clean medium, the undesired substances start to diffuse into the clean medium. Diffusion of particles and molecules across laminar flow borders in microchannels has previously been

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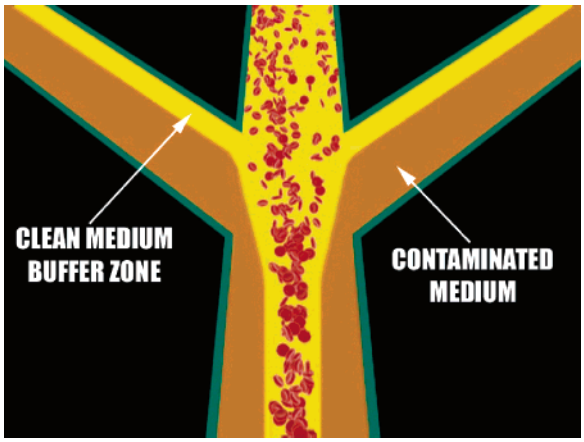

Figure 4. Schematic close-up of the outlet channel zone of the medium exchange chip. A buffer zone of clean medium in the side channels reduces the diffusion and convection based carry-over across the laminated fluid interface. The buffer zone is also visible in Figures 7 and 9.

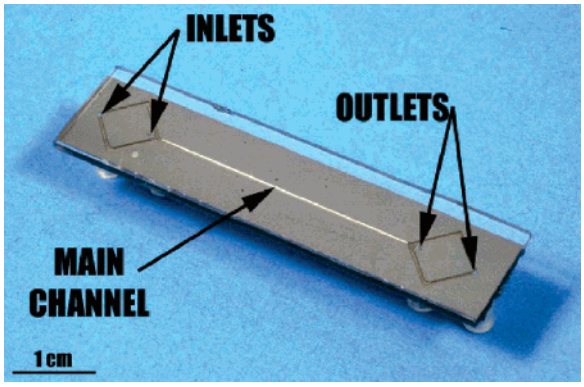

Figure 5. Top view of the silicon chip with bonded glass lid.

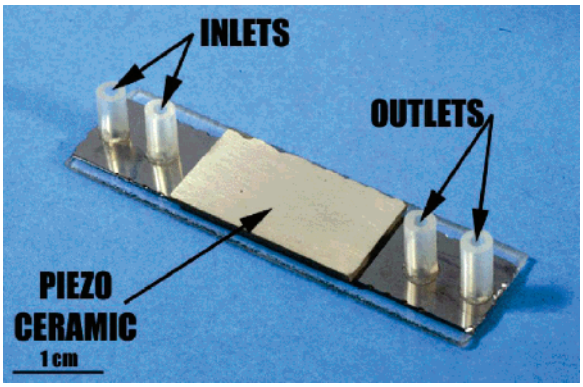

Figure 6. Bottom view of the silicon chip with attached tubing and piezo ceramic plate.

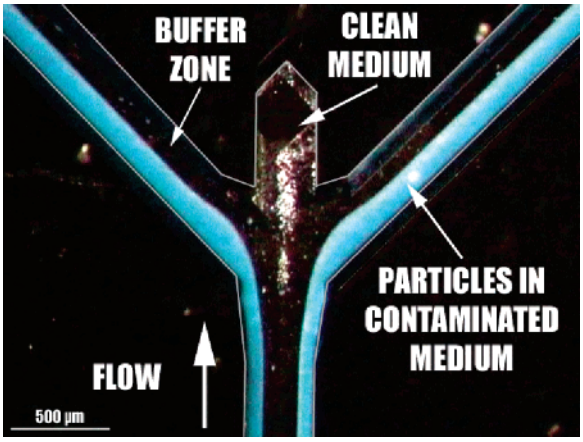

Figure 7. Microscope image of the main and outlet channels without ultrasonic actuation. Note that the white particles appear blue because of the Evans blue contaminated medium. White lines have been added to outline the channel boundaries.

extensively investigated by Weigl et al.[21,22] To suppress cross-contamination by diffusion, it is essential to minimize the time the two media are in contact; i.e., a relatively high linear flow rate is desirable. It is also possible to apply a higher flow rate through the center inlet and side outlets, relative to the side inlets and center outlet, thereby creating a diffusion buffer zone of clean fluid that spills over into the side outlet channels (Figure 4).

EXPERIMENTAL SECTION

The separation chip was microfabricated in silicon using conventional anisotropic wet etching. The rectangular cross-section main channel was 125 μm deep, 350 μm wide, and 30 mm long (Figures 5 and 6). At the beginning of the main channel, there was one center inlet channel and two side inlet channels. The side inlet channels originated from a common inlet. At the end of the main channel there was one center outlet channel and two side outlet channels with a common outlet. The channels were sealed by anodic bonding of a glass lid. Tubing and a piezoceramic plate were attached to the backside of the chip. It can be noted that although the acoustic signal is coupled into the microchip in a direction normal to the chip surface, the obtained standing wave is generated in a direction in-plane with the chip and orthogonal to the direction of flow in the microchannel. This is explained as follows; as the microchip is excited mechanically by the piezoceramic plate, a standing wave arises in the main channel as soon as the excitation frequency matches a $\lambda/2$ criterion for sound in the medium used, which equals the width of the main channel. Further information on the design, fabrication, activation, and experimental setup of the device has been reported by the Laurell group.[1,9] Silicon was used as the substrate for the microfluidic structure since it simultaneously offers excellent microfabrication features and displays mechanical properties well suited for low loss acoustic coupling.

The flow rates through the inlets and outlets were controlled using syringe pumps (WPI SP260P, World Precision Instruments Inc., Sarasota, FL). The total volumetric flow through the main channel was 0.27 mL/min or lower, resulting in a Reynolds number below 20, i.e., a truly laminar flow. The main channel was actuated via a piezoceramic plate (Pz26, Ferroperm Piezoceramics AS, Kvistgard, Denmark) operated at ~2 MHz, corresponding to its fundamental resonance mode. The activation voltage was chosen sufficiently high to focus at least 95% of the suspended particles into the center outlet (Figures 7 and 8). Evans blue (Merck, Darmstadt, Germany) was used to simulate a contaminant. Samples were collected from the outlets for analysis. The samples were first centrifuged, to remove the particles, where afterward the medium was collected and analyzed using a

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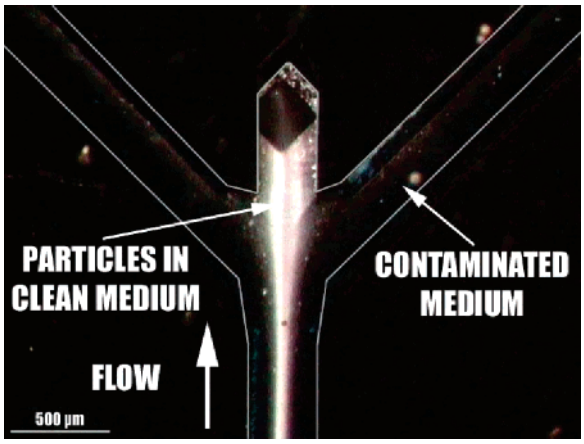

Figure 8. Microscope image of main channel and outlet channels with ultrasonic actuation. The white particles exit through the center outlet, now suspended in the clean medium. White lines have been added to outline the channel boundaries.

spectrophotometer (Genesys 10, Spectronic Unicam, Rochester, NY) to determine the concentration of Evans blue. The absorption measurements were made at 611 nm, corresponding to the absorption maximum of Evans blue. The medium exchange efficiency was defined as the fraction of the contaminant exiting the system through the side outlets. All sample collections and corresponding measurements were repeated six times.

Initially, the medium exchange efficiency was measured at varying driving voltages applied to the piezoceramic plate. This was made in order to determine to what extent the medium exchange efficiency was affected by the magnitude of the acoustic force field. Five-micrometer polyamide spheres (Orgasol, Autofina Chemicals, Philadelphia, PA), 1.5 vol %, suspended in distilled water, spiked with 90 μg/mL (94 μM) Evans blue, entered the main channel through the side inlets. The medium exchange efficiency was measured at driving voltages between 6 and 14 V_{pp} (peak-to-peak). No buffer zone was used (volumetric flow rate, 0.10 mL/min through all inlets and outlets).

Second, the separation efficiency was evaluated at different concentrations of suspended particles. The concentration of particles was varied between 1.5% and 6.0 vol % at constant driving voltage (10 V_{pp}) and contamination level (90 μg/mL, i.e., 94 μM). The effect of a buffer zone was evaluated in these and consecutive measurements (volumetric flow rate: 0.1 mL/min through common side inlet and center outlet, 0.17 mL/min through center inlet and common side outlet).

Third, the effect of different contaminant concentrations was studied. The amount of Evans blue added was varied between 90 and 360 μg/mL (94 and 375 μM) at constant driving voltage (10 V_{pp}) and particle concentration (1.5 vol %).

Furthermore, complementary measurements using bovine blood diluted by saline solution, 9 mg/mL, to 2 vol % erythrocytes and spiked with Evans blue, 90 μg/mL (94 μM), were made to confirm that the process was applicable for blood wash purposes (Figures 9 and 10). The clean fluid was bovine blood plasma, diluted to six times its original volume by saline solution. The driving voltage during the blood measurements was 10 V_{pp}.

RESULTS AND DISCUSSION

The medium exchange efficiency was clearly affected by the voltage applied to the piezoceramic plate (Figure 11). As the Evans blue molecules were too small to be significantly affected by the acoustic forces, the reason for the decline in medium exchange efficiency at increasing voltage must have been the stronger acoustic forces on the particles. Stronger forces caused more violent particle movements and presumably particle-induced mixing as the particles moved perpendicular to the direction of

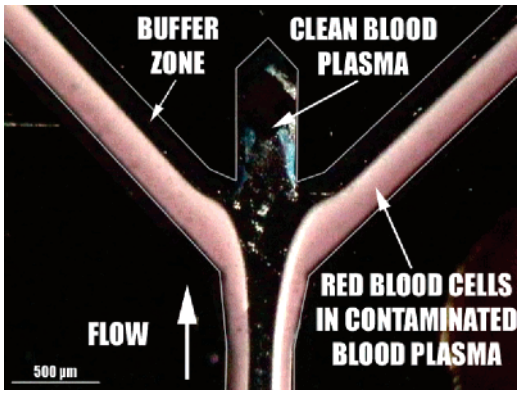

Figure 9. Microscope image of the main and outlet channels without ultrasonic actuation. Contaminated blood and clean blood plasma are observed in a laminated stream leaving the system in the same order as they entered. White lines have been added to outline the channel boundaries.

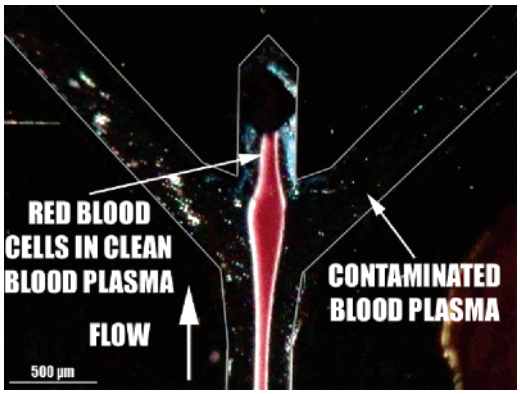

Figure 10. Microscope image of the main and outlet channels. When the ultrasound is applied, the red blood cells at the sides are switched over to the clean blood plasma in the center of the main channel. White lines have been added to outline the channel boundaries.

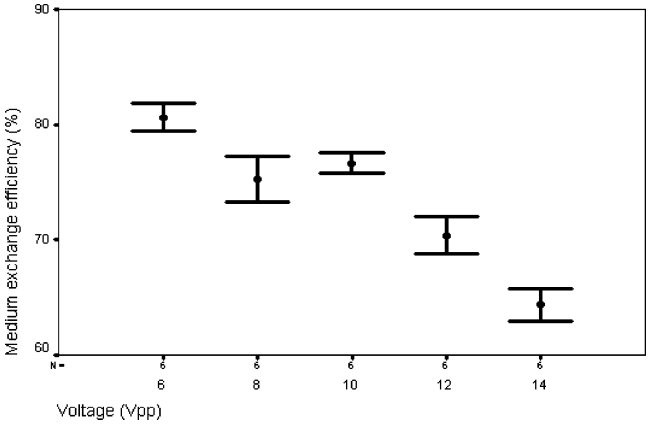

Figure 11. Medium exchange efficiency versus voltage applied to the piezoceramic plate. Particle concentration, 1.5 vol %; Evans blue concentration, 90 μg/mL (94 μM); flow rate, 0.10 mL/min through all inlets and outlets (95% confidence interval).

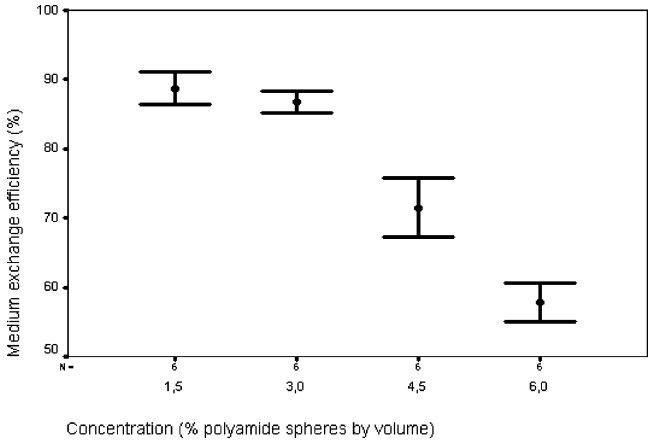

Figure 12. Medium exchange efficiency versus particle concentration. Activation voltage, 10 V_{pp}; Evans blue concentration, 90 μg/mL (94 μM); flow rate, 0.1 mL/min through common side inlet and center outlet, 0.17 mL/min through center inlet and common side outlet (95% confidence interval).

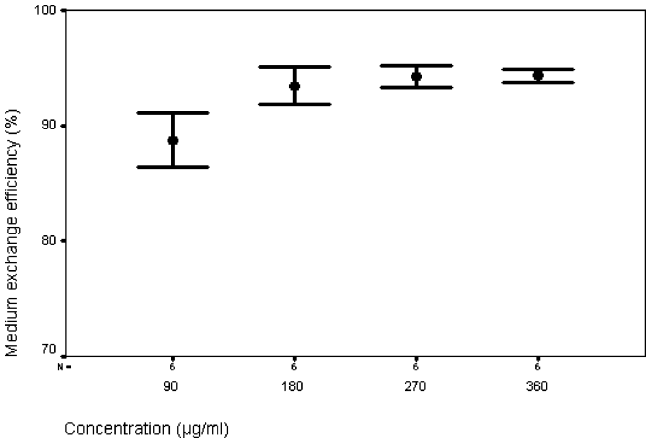

Figure 13. Medium exchange efficiency versus contaminant concentration. Activation voltage, 10 V_{pp}; particle concentration, 1.5 vol %; flow rate, 0.1 mL/min through common side inlet and center outlet, 0.17 mL/min through center inlet and common side outlet (95% confidence interval).

flow. The maximum medium exchange efficiency reached was ~80%, at 6 V_{pp}. However, the system stability was better at 10 V_{pp} since the particles were focused into a narrower band that did not spill over into the side outlets when flow disturbances occurred. Because of this, all further measurements were made at 10 V_{pp}.

While suspended in the contaminated medium, the particles absorbed contaminants that subsequently were carried with the particles into the clean medium. In combination with the particle-induced mixing, this factor also ought to have reduced the medium exchange efficiency of the device. The reduction was probably dependent on the tendency of the particle type in question to absorb the contaminant, as well as the shape and size of the particles. To suppress these effects, as well as diffusion across the laminar interface, the flow through the center inlet and the common side outlet was increased from 0.10 to 0.17 mL/min to create a buffer zone in the laminar flow profile as outlined in Figure 4. This action led to a considerable increase in medium exchange efficiency at 10 V_{pp}, from below 80 to ~90% (Figures 11 and 12). As expected, the medium exchange efficiency decreased as the particle concentration was increased since, in absolute amounts, more of the contaminant was carried over to the clean medium and the particle-introduced mixing was enhanced (Figure 12). To confirm that the particles were responsible for the cross-contamination, the system was operated without particles present in the contaminated medium; in this case, no contamination of the clean medium exiting the system was detected. The decrease in medium exchange efficiency at increased particle concentrations (Figure 12) can be expected to vary with the surface characteristics of the microparticles used and the molecular composition (hydrophobic, hydrophilic, isoelectric point/charge, etc.) of the contaminant and will have to be taken into account for specific applications. To counteract the drop in exchange efficiency at higher particle concentrations, the original suspension can be diluted before the medium exchange process. Inherent with the medium exchange principle is the fact that the particles can be enriched in the center outlet, to some extent accounting for an upstream dilution process. If a sufficient medium exchange efficiency is not accomplished, the clean medium buffer zone can be increased to reduce contaminant carry-over, and most importantly, the process can be repeated in a serial fashion.

Since the flow was laminar and the diffusion negligible, the contaminant should have remained along the walls of the channel independent of the concentration. To confirm this, the amount of Evans blue added was varied at constant activation voltage and particle concentration (Figure 13). The medium exchange efficiency was found to increase and become more stable at higher contaminant concentrations. This behavior supports the hypothesis that the main source of cross-contamination was the particle-induced mixing and particle surface absorbed contaminants. The fraction of the contaminant introduced into the clean medium in this way should decrease with increasing contaminant concentration. A stable medium exchange efficiency of close to 95% was reached at an Evans blue contamination level of 360 μg/mL (375 μM).

In the evaluation of the system's ability to switch red blood cells from contaminated blood to clean blood plasma, a clean buffer zone was utilized (volumetric flow rate: 0.1 mL/min through common side inlet and center outlet, 0.17 mL/min through center inlet and common side outlet). It was found that a stable medium exchange efficiency of 98% could be reached. The results indicated that erythrocytes were advantageous for carrier medium exchange by ultrasonic particle switching and that high medium exchange efficiency probably can be sustained even if the particle and contaminant concentrations are increased further. It has previously been shown that the acoustic separation method is harmless to the erythrocytes; i.e., it does not cause hemolysis.[9,20]

A most critical part of the device assembly was the coupling of the piezoceramic element to the backside of the chip. This was done by applying ultrasound gel to either of the components and subsequently pressing the two surfaces together.[9] Minor variations in the acoustic coupling in consecutive assemblies might have led to small deviations in the acoustic pressure amplitude in the main channel at the same activation voltage. Another factor affecting the data collected was small disturbances in the flow, introduced by the syringe pumps and the syringes. Furthermore, gases dissolved in the fluids sometimes formed bubbles in the

flow channel that disturbed the laminar flow profile slightly as they moved through the system. Altogether, these factors limited the accuracy of the measurements.

The proposed acoustic manipulation technique enables new microfluidic strategies to be employed in the field of bioanalysis. An important feature is the reasonable volumetric throughput of a few hundred microliters per minute whereby a wide span of applications in preclinical and potentially also clinical diagnostics may become an area of interest for the technology. As clinical applications are targeted, yet higher throughputs are requested. This can be addressed by scaling up the number of parallel medium exchange channels in a similar manner as reported by Jönsson et al.[20]

Not only does the microfluidic particle manipulation as demonstrated offer features of washing cells but equally important, cells or particles may be switched to a new medium wherein a biospecific reaction or activation may take place over a given period of time. Subsequently the particles are returned to their native medium, e.g., for biological readout. In more advanced sequential setups, it can be envisioned that particles transferred from the laminar flow lines at the sides of a manipulation channel to a new medium in the center of the channel are fed further on to the side inlets of an identical new manipulation channel in series, that is supplied with yet another bioactive fluid in its central region and the particles are again transferred to this fluid. This series of operations will in principle allow sandwich assays to be performed in a flow-through format starting with a crude biosample that is initially washed whereafter the assay is sequentially performed with, for example, a fluorescent readout in the last step. This procedure can be repeated and controlled by a binary mode of operation; i.e., the specific biochemical interaction is only allowed to occur if the acoustic focusing is switched on. In principle a programmable system of bioanalytical questions could be designed and simply by selecting the desired steps of serial biochemical interactions to be performed the requested readout would be provided.

CONCLUSION

The presented medium exchange method for suspended particles demonstrated an exchange efficiency of up to 95% for polyamide spheres in water spiked with Evans blue and 98% for red blood cells in whole blood spiked with Evans blue. It was concluded that the system performed best at relatively low activation voltages, low particle concentrations, and high contaminant concentrations. In combination with the previously presented particle separation method, two particle types can be separated from each other while one of the particle types is moved over to another medium.[9] In the blood wash application, this means that both lipid particles and small particle contaminants can be removed from the erythrocytes before returning them to the patient. This offers an opportunity to recycle blood in situations where shed blood is discarded today. Furthermore, the presented method offers a generic platform for carrier medium exchange and particle separation that could be used in, for instance, biomedical and chemical applications.

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