Acoustofluidic Medium Exchange for Preparation of Electrocompetent Bacteria Using Channel Wall Trapping

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With an improved etching process, we were able to produce a thin wall between two microfluidic channels, which, upon excitation, can generate streaming fields that complement the acoustic radiation force and therefore can be utilized for trapping of bacteria. Our novel design robustly traps Escherichia coli at a flow rate of 10 µL minute\(^{-1}\) and has a cell recovery performance of 47 ± 3 % after washing the trapped cells. To verify that the performance of the medium exchange device is sufficient, we tested the electrocompetence of the recovered cells in a standard transformation procedure and found a transformation efficiency of \(8 \times 10^5\) CFU per µg of plasmid DNA. Our device is a viable low-volume alternative to centrifugation-based methods and opens the door for miniaturization of a plethora of microbiological and molecular engineering protocols.

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Acoustofluidic Medium Exchange for Preparation of Electrocompetent Bacteria using channel wall trapping †

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Transformation, i.e. reprogramming of bacteria by delivering exogenous genetic material (such as DNA) into the cytoplasm, is a key process in molecular engineering and modern biotechnology in general. Transformation is often performed by electroporation, i.e. creating pores in the membrane using electric shocks in a low conductivity environment. However, cell preparation for electroporation can be cumbersome as it requires the exchange of growth medium (high-conductivity) for low-conductivity medium, typically performed via multiple time-intensive centrifugation steps. To simplify and miniaturize this step, we developed an acoustofluidic device capable of trapping the bacterium Escherichia coli non-invasively for subsequent exchange of medium, which is challenging in acoustofluidic devices due to detrimental acoustic streaming effects.

With an improved etching process, we were able to produce a thin wall between two microfluidic channels, which, upon excitation, can generate streaming fields that complement the acoustic radiation force and therefore can be utilized for trapping of bacteria. Our novel design robustly traps Escherichia coli at a flow rate of 10 μL min⁻¹ and has a cell recovery performance of 47 ± 3% after washing the trapped cells. To verify that the performance of the medium exchange device is sufficient, we tested the electrocompetence of the recovered cells in a standard transformation procedure and found a transformation efficiency of 8 × 10⁵ CFU per μg of plasmid DNA. Our device is a viable low-volume alternative to centrifugation-based methods and opens the door for miniaturization of a plethora of microbiological and molecular engineering protocols.

1 Introduction

Recombinant DNA technology allows the modification of organisms on the genetic level and transferring a manifold of functions that have evolved in nature. An essential step in many genetic engineering workflows is the delivery of exogenous genetic material into a bacterial cell, a process in the course of which the receiving bacterium is transformed. For genetic material to enter the cell, it must first pass the cell envelope, which in the case of the model bacterium Escherichia coli (E.coli) consists of two layers, the outer membrane and the cytoplasmic membrane. To cross this envelope, cells need to be conditioned to form holes in the envelope, or "made competent", which is typically achieved by transferring cells out of growth medium and into specific aqueous solutions, which either contain calcium ions or are of particularly low conductivity. In the case of a chemical transformation, pores in the cells’ membrane are formed through the interplay of chemicals and a heat shock, whereas during electroporation pores form in the cell membrane during an electric shock. Once the pores have formed, the genetic material can enter the cell, where it can either propagate independently from the chromosome in the form of a plasmid or is introduced into the chromosome and in the process causes specific mutations (e.g. recombineering). To be successfully electroporated, cells are briefly (5 – 10 ms) exposed to a strong (∼ 12.5 – 15 kV cm⁻¹) electric field and thus need to be suspended in a low conductivity medium, to prevent strong currents and subsequent Joule heating that would impact cell viability. This requirement is in strong contrast to growth media, where dissolved salts and other charged molecules required for cell growth lead to high electric conductivity. Therefore, medium exchange is an indispensable preparatory step for each form of transformation, and protocols for preparing competent cells usually include three cumbersome and time-consuming centrifugation-based washing steps. After each centrifugation, the cell-free supernatant is removed, and cells are resuspended.
in the respective specific solutions. For the highly efficient electroporation, this is typically ultrapure water. Given its crucial role in transformation, a procedure to reliably, and in an automated and ideally miniaturized fashion, condition cells for DNA uptake would represent a breakthrough for a plethora of microbiological and molecular engineering protocols, including such transformative methods as multiplexed automated genome engineering (MAGE). Recently, several research groups have developed methods for continuous medium exchange in microfluidic systems. These methods are based on particle trapping, dragging particles through a liquid interface, continuous buffer exchange, droplet merging, and fluid relocation. The forces utilized to achieve medium exchange are manifold. The most commonly used forces are dielectric, magnetic, hydrodynamic, and acoustic. However, each of these methods exhibits fundamental limitations, such as the requirement of low flow rates or particle labelling. In this work, medium exchange is achieved by first trapping bacterial cells by acoustic forces and subsequently flushing the channel with a new medium such as ultrapure water. The contactless manipulation of particles using acoustic forces, acoustophoresis, is one of the most used techniques for particle manipulation because it is non-invasive, label-free, flexible in design, and biocompatible. Acoustophoresis is based on the acoustic radiation force and the drag force from acoustic streaming. The acoustic radiation force results from scattering of ultrasonic waves on the surface of a particle. Its magnitude mainly depends on the particle size, the density and compressibility difference between the particle and its surrounding medium, and the amplitude of the acoustic pressure. In bulk acoustic wave (BAW) devices, an acoustic standing wave is generated in the microfluidic channel by matching the acoustic wavelength in the fluid to the channel’s dimensions. Particles with a positive acoustic contrast, which is typical for biological samples, accumulate in the pressure nodes of a 1D standing wave. There are several ways to trap particles with acoustic forces. One way is to adjust the shape of a microfluidic chamber such that radiation forces are generated that point against the flow direction. Further, two 1D standing fields can be generated perpendicular to each other in a square-shaped cavity. Finally, acoustic modes inside glass capillaries can be utilized to retain particles against a flow at a specific position.

In this work, we developed a new approach to trap particles in a BAW device. By etching two microfluidic channels close to each other, we get a thin silicon wall between the two channels. This wall can freely vibrate upon excitation and attracts particles to its displacement antinode. In contrast to all methods discussed before, we press the particles against an interface which has the potential to increase the trapping efficiency.

One major drawback of acoustophoresis is its limitation concerning particle size. The manipulation of particles below a critical particle radius is impeded by the drag force resulting from acoustic streaming. Acoustic streaming is a time-averaged nonlinear viscous effect that originates from the harmonic forcing of the fluid. Boundary-driven streaming affects the particles through the Stokes drag force, competing with the acoustic radiation force. The latter prevails if the particle has a radius larger than a critical radius, which is around $1\mu m$ in prominent BAW systems. This complicates the acoustic manipulation of bacteria, e.g., the biotechnological model organism and workhorse $E. coli$, as the critical radius overlaps with the typical cell dimensions. In general, to allow for efficient focusing of small particles in BAW devices, the acoustic streaming field needs to be suppressed or exhibit a particular shape; the vortices of the acoustic streaming field have to point towards the positions at which particles should be focused. Such a desired acoustic streaming field can be achieved either by square-shaped channel or by tailoring the acoustic impedance gradient. In a former publication, we utilized square-shaped channels to focus $E. coli$ revealing a major difficulty to operate at high flow rates. With our new design, we generate a complementary streaming field, which assists the acoustic radiation force with particle trapping, thus allows us to manipulate particles that have sizes near the critical particle radius and outperforms our previous work by a factor of 25 in flow rate.

While acoustic-based separation has been shown before, these processes were based on the effect of acoustic forces on larger cells and the absence of an effect on the smaller bacterial cells. By contrast, in this work, we use acoustic forces to trap small bacterial cells themselves and thus enable, for the first time, semi-continuous medium exchange. Furthermore, we analyze our approach’s underlying mechanisms using finite element simulations to enable the direct optimization of our design. We find that particles can only be trapped efficiently with a wall thickness below $20\mu m$. The fabrication of such small structures within a channel of $190\mu m$ depth has only been achieved by wet etching, which is limited in design by the crystal orientation. For manufacturing using a much less restrictive method, we optimize our deep reactive ion etching (DRIE) process, which enables us to produce devices with a wall as thin as $13\mu m$ in $190\mu m$ deep channels. At a flow rate of $10\mu Lmin^{-1}$ we are able to trap ~70% of the bacteria. In a subsequent electroporation step in a benchtop electroporator, 1% of the initial bacteria are successfully transformed with an antibiotic resistance harboring plasmid. Our new design shows significant potential for future applications of BAW devices in a variety of research fields.

## 2 Operating Principle

The acoustofluidic device consists of a microfluidic liquid channel and an air backing chamber that are in close proximity and separated by a thin silicon wall (fig. 1). The thin wall can be excited by a piezoelectric transducer (piezo), which leads to bending of the wall in its eigenmodes. This design approach is advantageous as it allows us to shift the pressure node inside the liquid chamber towards the thin silicon wall and by that enables medium exchange through successful trapping of particles at the wall’s displacement maximum.

The vibrations of the thin wall depend on its clamping and material properties, the fluid properties on both sides of the wall, and the device geometry. Due to the complexity of the boundary conditions it is difficult to analytically compute the exact vibrational modes, which nevertheless can be approximated with a
two-dimensional plate model\cite{54,55}

The force responsible for attracting small (relative to the acoustic wavelength), spherical, and solid particles towards the wall is called acoustic radiation force (ARF). For an inviscid fluid, it is given by the negative gradient of the Gor’kov potential\cite{59}

\[ \mathbf{F}_{\text{rad}} = -\nabla U, \tag{1} \]

which can be expressed as:

\[ U = \frac{4}{3} \pi r^3 \left( \frac{1}{2} \left( \langle r^2 \rangle \frac{f_0}{c \rho} - \frac{3}{4} \rho f_1 \langle r^2 \rangle \right) \right), \tag{2} \]

with the particle radius \( r \), the incident acoustic pressure field \( p \), the acoustic velocity field \( v \), the fluid speed of sound \( c \), the density of the fluid \( \rho \), the monopole and the dipole scattering coefficients \( f_0 \) and \( f_1 \), respectively. \( \langle \cdot \rangle \) denotes time averaging

\[ \langle x \rangle = \frac{1}{T} \int_{t_1}^{t_1+T} x dt, \tag{3} \]

where \( T = \frac{2\pi}{\omega} \) is the period of oscillation, with angular frequency \( \omega \). The Gor’kov potential is derived under the assumption that boundaries are far away from the region of interest, which is not the case here; however, a recent study of Baasch et al.\cite{52} showed that the validity can extend to close proximity to a boundary. In case of some cells, the Gor’kov potential is even valid, when the particle is touching the wall.

In Acoustofluidics, the acoustic energy density is often used as a benchmark for the device’s performance and, therefore, used in our numerical analysis. The average acoustic energy density \( (\mathcal{E}_{\text{ac}}) \) is given as\cite{55}

\[ \mathcal{E}_{\text{ac}} = \frac{1}{V} \int_V \left( \frac{1}{2} \rho \langle v^2 \rangle + \frac{1}{2} \kappa \langle p^2 \rangle \right) dV, \tag{4} \]

with compressibility \( \kappa \) and volume \( V \).

Another force that needs to be considered in our acoustofluidic device is acoustic streaming, which affects a particle through a drag force

\[ \mathbf{F}_d = 6\pi \eta r (\mathbf{v}_{\text{str}} - \mathbf{v}_{\text{prt}}), \tag{5} \]

with the fluid dynamic viscosity \( \eta \), particle radius \( r \), streaming velocity \( \mathbf{v}_{\text{str}} \) and particle velocity \( \mathbf{v}_{\text{prt}} \). The magnitude of the streaming velocity can be estimated as\cite{55}

\[ |\mathbf{v}_{\text{str}}| = \psi \frac{v^2}{c}, \tag{6} \]

with the first order acoustic velocity \( v_a \), the fluid speed of sound \( c \), and the geometry dependent factor \( \psi = \frac{3}{8} \) for a standing wave parallel to a planar wall.

As can be seen in equations\cite{2} and\cite{5} the acoustic radiation force and the drag force scale with \( r^3 \) and \( r \) respectively. The critical radius at which drag and acoustic radiation force balance each other can be estimated by

\[ r_c = \sqrt{\frac{3\psi}{2\Phi}} \delta, \tag{7} \]

with \( \delta \) as the viscous boundary layer thickness

\[ \delta = \sqrt{\frac{2\gamma}{\omega \rho}} \tag{8} \]

and \( \Phi \) as the acoustic contrast factor

\[ \Phi = \frac{1}{3} f_0 + \frac{1}{2} f_1 = \frac{1}{3} \left[ \frac{5\hat{\rho} - 2}{2\hat{P} + 2} - \hat{k} \right], \tag{9} \]

in which relative compressibility \( \hat{k} = \frac{\kappa}{\kappa_0} \) and density \( \hat{\rho} = \frac{\rho}{\rho_0} \) reflect the ratios between particle \( (\cdot)_p \) and fluid \( (\cdot)_f \) properties. The resonance frequency of our device that we used for excitation was around 2 MHz, leading to a viscous boundary layer thickness of \(~0.4 \mu m\) in water. To approximate the acoustic contrast factor of \( E. \ coli \), we took the buoyant density of \( E. \ coli \) analysed by Baldwin et al.\cite{59} and the acoustic impedance of cells derived by Olofsson et al.\cite{60} and ended up with a value of \( \Phi \approx 0.07 \). Thus, the critical radius for cells in our system is \(~0.95 \mu m\). Since \( E. \ coli \) cells are very close to that critical radius (approx. 2 \mu m in length, 1 \mu m in diameter) it is essential to account for acoustic streaming effects to obtain trapping capabilities.

### 3 Materials and Methods

#### 3.1 Device fabrication

We produced the acoustofluidic chips utilizing cleanroom processes. Microfluidic channels (192.6 ± 10 \mu m depth, 198.0 ± 5 \mu m width of the liquid channel and 100.3 ± 2 \mu m width of the air channel) were etched into a silicon wafer (500 ± 10 \mu m thickness). First, we used photolithography (Resist: S1828, Shipley, 4000 rpm; Developer: AZ 351B, Microchemicals, development time 30s) to transfer the designs from a chrome mask onto the silicon wafer. Next, we put the wafer into an inductively coupled plasma deep reactive ion etching (ICP-DRIE) machine (Esstrellas, Oxford instruments). With a modified high rate process, we achieved a selectivity (ratio of etch rate between photoresist and silicon) of around 220 and straight channel walls with an angle of \(~87.7^\circ\). We increased the passivation time, decreased the ICP and HF power allowing us to achieve significantly improved aspect ratios compared to current literature. Then, we anodically bonded a glass wafer (700 \mu m thickness) to the silicon wafer. Finally, we diced the wafer stack into small rectangles (16 mm x 15 mm) employing a wafer saw (DAD3221, Disco corporation) and glued to the backside of the devices using conductive epoxy (H20E, EPO-TEK). Copper cables (0.15 mm diameter) were attached to the piezo with conductive silver paste and glued to the chip with instant glue to increase mechanical stability.
3.2 Experimental Setup

Acoustic waves in the BAW device are coupled into the system by exciting the piezo with a high-frequency amplified (High Wave 3.2, Digitum Elektronik) AC signal from a wave generator (AFG-2225, GW INSTEK). The impedance of the piezo varies with its excitation frequency. Since the voltage at the piezo is dependent on its impedance, it is verified using an oscilloscope (UTD2025CL, Uni-Trend Technology). Flows inside the microfluidic channels are controlled by pressure pumps and flow sensors (Flow EZ, Fluigent). We studied the movement of green fluorescent polystyrene (PS) particles (Flow EZ, Fluigent). We used a high-speed camera (HiSpec 1, Fastec) to visualize the fast particle motions. Depending on the input power, we used frame rates between 20 – 200 fps.

3.3 Bacterial Growth and Maintenance

To visualize the bacteria, we used an E. coli MG1655 strain that constitutively expresses the gene for green fluorescent protein mNeonGreen. The gene was inserted into the ydgH gene locus of the chromosome resulting in strain bBPL227. We examined fluorescent E. coli cells under a fluorescence microscope with 470/40nm excitation and 525/50nm emission bandpass filters. To prepare bacteria for experiments, 3mL of Lysogeny Broth (LB) (Becton Dickinson) was inoculated with bBPL227 from a cryostock and grown overnight in a shaking incubator (Multitron Pro, INFORS HT) at 37°C and 200 rpm, until the culture reached the stationary phase. We added glycerol to a final concentration of 15% (vol/vol), split the culture into 100µL aliquots and stored them at −80°C. For each experiment, an aliquot of stock solution was thawed and transferred to 25mL LB in a 250mL Erlenmeyer flask and incubated in a shaking incubator at 37°C and 200 rpm. The cell culture was grown for 2 – 3 hours until mid-exponential phase (typically at an optical density at 600nm of 0.5). The cells are then harvested and put onto ice for the experiments.

3.4 Numerical Model

We built a 2D numerical model of the yz-section-plane (Fig. 2 (c)) of the chip and evaluated it in COMSOL Multiphysics (version 5.4) to analyze the device’s frequency response. At resonance frequencies, we investigated the wall displacement as well as the Gor'kov potential and streaming velocity. First, a mesh study was conducted to determine the converged mesh parameters (ESI Fig. S1). After the initial frequency-domain study of the Thermoviscous Acoustics and Solid Mechanics interface, a stationary study of the Creeping Flow interface at the resonance frequency was carried out using the solutions of the initial study as inputs. With this study, we were able to derive the streaming velocity. A detailed description of the numerical model is provided in the supplementary material.

3.5 Medium Exchange Protocol

Our medium exchange protocol expands the functionality of our acoustofluidic device from bacteria trapping to medium exchange. The protocol consists of three steps, namely: capture, wash, and release. In the first step, we filled the channel with cell solution at a flow rate of 10µLmin⁻¹ and activated the acoustic trapping of the cells through wall vibration. After two minutes, we stopped the flow of cell solution and flushed the channel with ultrapure water at a flow rate of 10µLmin⁻¹ for 30 seconds to wash the cells and remove remaining cell suspension liquid. Finally, we turned off the acoustic excitation and increased the flow rate of ultrapure water to 20µLmin⁻¹ for 60 seconds, which, given lack of acoustic forces during this step, allowed to remove the cells from the chamber while being suspended in the new medium.

The medium exchange protocol’s execution is computer controlled by a custom-written Python script to synchronize the acoustics and regulation of flows.

3.6 Determination of Bacteria Capturing and Release Efficiency

The device should capture and release as many bacteria as possible while replacing the original medium with ultrapure water for electroporation. We collected the liquid leaving the chip during each step in tubes. The collected bacteria solution was then weighed and diluted with LB to a suitable concentration (2000x – 50000x), and 40µL of each diluted sample was plated on OmniTray™ plates (Nunc, Thermo Fisher Scientific Inc.) with eight virtual wells by a liquid handling robot (EVO200, Tecan). Af-
For performing electroporation experiments, we measured the conductivity and leads to a low time-constant in the micropulser. For example, remaining growth medium drastically increases the time constant from the electroporator is a function of the sample conductivity and was used as a measure of success of the medium exchange. Immediately after the pulse, 980µL of room temperature SOC medium was added to the cuvette for cell recovery. The complete volume was transferred into a tube and placed in a shaking incubator (37°C, 200 rpm) for one hour to allow expression of the antibiotic resistance gene on the plasmid without allowing for growth to resume. Afterwards, we plated 40µL of the cell suspension on LB agar plates containing 50µg mL⁻¹ of the antibiotic carbenicillin. The plates were incubated overnight at 37°C and CFUs were determined the next day.

4 Results & Discussion

4.1 Numerical analysis of the particle attraction

We carried out numerical studies to get insight into the physical phenomena of the initial design that lead to particle attraction at the thin silicon wall between the water and the air channel. To obtain the accurate channel dimensions required for the numerical simulation, the device was diced perpendicularly to the channel, and the cross-section was evaluated using optical microscopy. The measured dimensions were then implemented in our numerical model (Fig. 2(c)). As can be seen in figure 2(b), we were able to etch straight channel walls enabling us to produce nominally walls as thin as 13µm with an etch depth of 192µm. At the bottom of the channel, the silicon wall still had a width of 6µm, which is thick enough to prevent the whole structure from collapsing during the acoustic excitation.

Given that the effect of acoustic streaming cannot be neglected in BAW systems for manipulating bacteria, we considered this by refining our mesh at the water-silicon interfaces and, by that, account for the viscous boundary layer (Fig. 2(d)). Subsequently, a study in the frequency domain was performed with excitation of acoustic streaming simulations, respectively, on a PC with 32 GB RAM and Intel Xeon E 2186G processor. (e) Frequency spectrum of acoustic energy density and wall displacement. A strong resonance is clearly visible at 1.8MHz. (f) Displacement of the wall (disp), (g) Gor’kov potential (U) with acoustic radiation force (white arrows) and (h) streaming velocity (vstr) at fres = 1.8 MHz.

![Numerical modelling of the acoustofluidic device](image-url)
frequencies ranging from 1.6 to 2MHz, as for this spectrum, the strongest particle attractions had been suggested in preliminary experimental investigations. As a measure for the device efficiency, we chose the average acoustic energy density, i.e. acoustic energy density (Eq. 4) integrated over the cross-section of the liquid channel and divided by its area. Similarly, we computed the average wall displacement. Simulation results show a global maximum in the average acoustic energy density and the average wall displacement at 1801 kHz with additional minor resonances being visible at slightly higher and lower frequencies (Fig. 2 (e)). The subsequent detailed experimental analysis confirmed the initial observations as well as the results of the numerical simulation by demonstrating strong particle attraction in the investigated frequency range. However, we were unable to experimentally reproduce the exact relative strength of the resonances, which might be caused by the idealized material parameters used as inputs for our 2D simulations.

We performed the second stationary study at the resonance frequency that revealed the highest average acoustic energy density (1801 kHz). Taking both studies into account, we generated plots for the wall displacement (Fig. 2 (f)), Gor’kov potential with acoustic radiation force as white arrows (Fig. 2 (g)) and streaming velocity (Fig. 2 (h)). The displacement plot shows that the maximal displacement of the wall is at half its height. At this point, a minimum of the Gor’kov potential (Eq. 2) is located. Thus, the acoustic radiation force (Eq. 1) is directed in positive y-direction throughout the entirety of the channel. Close to the wall, the direction of the force changes slightly towards the absolute minimum of the Gor’kov potential, which leads to an acoustic radiation force that pushes particles towards the thin wall.

Particles at the Gor’kov potential minimum that are larger in radius than \( r_c \) (Eq. 7) remain there due to the Acoustic Radiation Force. However, if the particle radius is close to or below \( r_c \) as it is the case for E. coli, streaming effects need to be considered. The streaming velocity plot reveals that particles are also dragged towards the thin wall by acoustic streaming with two counter-rotating vortices forming close to the silicon wall. Both vortices meet at the position of minimal Gor’kov potential. Therefore, even when the particles reach the point of minimal Gor’kov potential and thus are no longer subjected to the acoustic radiation force, they will still be trapped in the acoustic streaming vortices nearby the thin wall.

### 4.2 Capturing and Release of Bacteria

We performed experiments without flow to observe the forces acting on the bacteria and determine suitable excitation frequencies. We changed the frequency in steps of 1kHz in a range from 1 to 4MHz to experimentally determine resonance frequencies that lead to bacteria attraction. As the strongest observable particle attraction occurred at a frequency of 1.89MHz, this parameter was applied for all subsequent experimental procedures. To perform medium exchange with the device, we operated it while applying a flow of cells. Specifically, we implemented a three-step protocol including a capture step during which the bacterial cells are trapped at the wall by the acoustic forces, resulting from the ARF, and the drag forces, resulting from the acoustic streaming, followed by a wash step in which the surrounding medium is exchanged while the acoustic and drag forces still trap the cells, and finally, a release step in which the forces are no longer applied and cells can re-suspend into the surrounding liquid (Fig. 3 (a)). We ran the protocol and determined the efficiency of each step (ESI† video 2). We observed accumulation of cells at the acoustic pressure nodes at the vibrating wall (Fig. 3 (b)). Over three experiments, on average, we were able to capture 70% of the cells that passed through the corresponding channel section during the time of application of acoustics. An additional 20% of the captured cells were lost in the washing step, indicating that wash duration and flow rate require further optimization. Finally, up to 52% and on average 47 ± 3% of the cells that entered the chip initially were successfully washed and released into the new medium (Fig. 4).

Next, we performed the assay without acoustics to verify that the released bacteria stem from the trapping and not from side effects such as leakage between the cell suspension inlets or unspecified retention of bacteria in the channel. In contrast to the experiment with acoustics, we observed no cell accumulation at the wall and found the cell concentration at the outlet and inlet were nearly identical at 1 · 10^8 cells per mL during the first step ("capturing", but due to the absence of acoustic forces effectively just flushing of the tubing and chip with cell suspension). This suggests that no cells were actively trapped in the channel. Further
thermore, during washing with ultrapure water, we still measured a bacteria concentration of $1 \times 10^7$ cells per mL in a total volume of 5µL, which is in agreement with the assumption that at the beginning of the wash step the inner chamber of the device (approx. 1µL of volume) was still filled with cell suspension. At the same time, this suggests no substantial leakage of cells from the cell suspension channel into the main channel during the wash step. Finally, during the third step of the protocol without acoustics, the cell concentration is 60-fold lower than during the capturing step. The strongly reduced cell number during the third step confirmed that the washing time and flow rate was sufficient to exchange close to all the residual volume in the chip and thus avoid cross-contamination between the cell suspension and the released cells in the new medium.

In summary, the protocol allows the medium exchange for ~$4 \times 10^6$ cells in a volume of 20µL per round. The protocol takes only 210 seconds and is thus much quicker than repetitive centrifugation with durations of up to 20 minutes per step.

4.3 Functional Analysis of Medium Exchange

After capturing, washing and releasing bacterial cells, we determined whether the medium exchange from growth medium to ultrapure water was sufficient for biological applications. Therefore, we mixed the released cells in the liquid in which we had recovered them with the DNA of a 2.7 kB reference plasmid, pUC18 and electroporated them using a micropulser. Cells released from the acoustofluidic device exhibited a time constant of between 5.6 and 5.8 ms. In general, a time constant above 5 ms is a strong indicator for high transformation efficiency and indicates that the media was, in fact, exchanged very effectively. We performed electroporation in the micropulser on a dilution series of LB in ultrapure water to estimate the achieved grade of medium exchange. The time constant of around 5.7 ms reached by our sample corresponds to a dilution of 1:256 of LB in ultrapure water. We next examined successful transformation by plating the electropulsed cells on selective media. We measured around 40'000 successfully transformed colonies per electroporation of our acoustically washed cells, resulting in a transformation efficiency of $10^6$ transformations per microgram of plasmid, which is perfectly acceptable for transformation events. This high ratio of transformants further indicates that our medium exchange method does not negatively impact the cell viability. The reduced number of transformants relative to a standard lab protocol originates from a surplus concentration of added DNA as well as a decreased cell concentration compared to typical lab protocols. The cells released without acoustics as well as those without medium exchange yielded fewer than 250 CFUs per run on selective plates. The significantly higher transformation efficiency of cells washed with our device highlights that the presented acoustofluidic device is highly beneficial for medium exchange and in particular for the preparation of cells for electroporation, as often required in biotechnology. With our new device and our simplified protocol, we present a quick and viable alternative to centrifugation which allows us to also handle small sample volumes.

5 Conclusion & Outlook

In this work, we presented a new acoustofluidic device design with a novel trapping principle that outperforms similar approaches in terms of throughput and particle size. The device’s capabilities were then successfully demonstrated by semi-continuous medium exchange for the model bacterial workhorse E. coli from growth medium to ultrapure water. As an example of successful medium exchange, we electroporated the cells prepared with our acoustic device and showed successful transformation with plasmid DNA. In contrast to centrifugation, our device works well for microliter volumes and can easily be automated and integrated into microfluidic systems. It presents a crucial step towards automating transformation. Furthermore, we carried out numerical studies to get an insight into the physical phenomena that lead to particle trapping at the thin vibrating silicon wall. The simulations revealed that at a frequency close to the experimental resonance frequency, the thin silicon wall located between the water and the air channel has its maximal displacement, while the liquid channel exhibits a maximum in average acoustic energy density. A Gor’kov Potential minimum forms at half of the wall’s height, leading to strong particle attraction towards this point. Additionally, counter-rotating streaming vortices are generated, which limit the movement of particles close to the wall. Both effects lead to trapping of particles even in the range of the critical particle radius. In contrast to other designs, we can utilize straight channel walls for the particle trapping, thus preventing contamination with bubbles.

The device’s performance has been determined through experimental medium exchange. At a washing flow rate of 10µL min$^{-1}$ we successfully trapped 70 ± 5% of the bacteria. After a washing step, 47 ± 3% of the total bacteria were released into the new medium - ultrapure water. From these released bacteria, 40'000
were successfully transformed after electroporation with a pUC19 plasmid in a micropulser, leading to a high transformation efficiency of approximately 10^6 µgDNA⁻¹.

In the future, we will try to improve the device performance by using thinner walls, for which the etch process needs to be further optimized. Furthermore, we will test new innovative designs to increase the throughput as well as sample volume simultaneously.

Our device demonstrated the capability to transfer complex biological protocols into miniaturized dimensions. We believe that our novel approach is useful for a wide range of applications, including medium exchange, particle coating, and cell concentration.

Conflicts of interest
The authors declare no competing financial interest.

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Supplemental Material for "Acoustofluidic Medium Exchange for Preparation of Electrocompetent Bacteria using channel wall trapping"

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Figure ESI 1: Mesh study of the COMSOL Model. We introduced five mesh parameters reflecting different aspects of the mesh. (I) Maximal longitudinal element size on the mesh junctions. (II) Length of the mesh refinement layer, generated for the viscous boundary layer. (III) The number of rows within the mesh refinement layer. (IV) Width of the elements in the mesh refinement layer. (V) Maximal element size throughout the whole mesh. As long as the mesh refinement layer is set higher than the viscous boundary layer, it does not influence the overall quality of the mesh. With $E_{\text{ave}}$ as the average energy density in the water channel and DoF the number of degrees of freedom. We chose the following mesh parameters for all simulations in this work, corresponding to an error below 1% in respect to the finest mesh: (I) 200, (II) 1, (III) 15, (IV) 1.2, (V) 50.

Supplemental Material

Numerical Model

We used the Solid Mechanics interface to model the solid components of the model (silicon, glass, piezoelectric element). To account for damping, we modelled the glass as a linear elastic material with complex Lamé parameters. The silicon was modeled as an anisotropic linear elastic material with its elasticity matrix. The piezoelectric element was modeled with a Piezoelectric Material model. Finally, a thin elastic layer was introduced at the interface between the piezoelectric element and silicon to model the glue layer (spring constant per unit area). Please refer to table ESI 1 for all material parameters used in the numerical model.

To account for the piezoelectric effect, the Electrostatics interface was applied to the piezo-
Table ESI 1: Table of the material properties and damping factors.¹

| Parameter | Symbol and value | Unit       |
|-----------|------------------|------------|
| **Glass** |                  |            |
| Density   | $\rho_{\text{glass}} = 2240$ | [kg/m³]    |
| Lamé parameters | $\lambda = 23.1 \cdot 10^9 \cdot (1 + i/2420)$ | [N/m²]    |
|           | $\mu = 24.1 \cdot 10^9 \cdot (1 + i/2420)$ | [N/m²]    |
| **Silicon** |                  |            |
| Density   | $\rho_{\text{silicon}} = 2240$ | [kg/m³]    |
| Stiffness matrix | $C_{ij} = (1 + i0)C_{ij}$, with |            |
|           | $C_{11} = C_{22} = C_{33} = 166$ | [GPa]      |
|           | $C_{12} = C_{13} = C_{23} = 64$ | [GPa]      |
|           | $C_{44} = C_{55} = C_{66} = 80$ | [GPa]      |
| **Piezo** |                  |            |
| Quality factor | $Q_{\text{piezo}} = 100$ |            |
| Damping   | $\tan_{\text{damp}} = 0.003$ |            |
| Density   | $\rho_{\text{piezo}} = 7700$ | [kg/m³]    |
| Stiffness matrix | $c_{ij}^{\text{piezo}} = 0.965 \cdot (1 + i/Q_{t})C_{ij}$, with |            |
|           | $C_{11} = C_{22} = 155, C_{12} = 94.1$ | [GPa]      |
|           | $C_{13} = C_{23} = 79.9, C_{33} = 110$ | [GPa]      |
|           | $C_{44} = C_{55} = C_{66} = 27.3$ | [GPa]      |
| Coupling matrix | $e_{ij}^{\text{piezo}} = (1 + i(1/(2Q_{t}) - \tan_{\text{damp}}/2))d_{ij}$, with |            |
|           | $d_{31} = C_{32} = -5.48, C_{33} = 13.6$ | [C/m²]     |
|           | $d_{24} = C_{15} = 9.55$ | [C/m²]     |
| permittivity tensor | $\epsilon_{ij}^{\text{piezo}} = (1 - i \cdot \tan_{\text{damp}})\epsilon_{ij}$, with |            |
|           | $\epsilon_{11} = \epsilon_{22} = 929, \epsilon_{33} = 518$ | [C/m²]     |
| **Glue layer** |                  |            |
| Spring constants | $k_{11} = 2.06 \cdot (1 + 0.1 \cdot i)/5 \cdot 10^{-6}$ | [N/m²]     |
|           | $k_{22} = 8.94 \cdot (1 + 0.1 \cdot i)/5 \cdot 10^{-6}$ | [N/m²]     |

electric element. Charge conservation was implemented for the whole domain and an electric potential of 40 V was applied to one side of the piezoelectric element. Subsequently, the Solid Mechanics interface and the Electrostatics interface were coupled by the Piezoelectric Effect interface.

The Thermoviscous Acoustics interface was applied to both channels. The materials inside both channels were modeled with the standard material parameters provided by the software.

The Thermoviscous Acoustic-Structure Boundary interface was implemented to couple the Solid Mechanics interface and the Thermoviscous Acoustics interface at the water-silicon
and the air-silicon interfaces.

To incorporate the acoustic streaming in our simulations, we extended the procedure described in,\textsuperscript{2} where they used the limiting velocity approach. We followed,\textsuperscript{3} resolving the streaming in the boundary layer together with accounting for the Stokes drift. The Creeping Flow interface was assigned to the water domain. Source terms were enforced through Volume Forces across the domain. Finally, the second-order pressure field was constrained by setting a Pressure Point Constraint to zero at an arbitrary point of the domain.

**Python script for control of experimental setup**

We wrote a Python script that communicates with the pressure pump and the wave generator. We used the programming interface provided by the supplier (LineUP Series SDK, Fluigent) for communication with the pressure pumps and flow rate sensors. We used a self-written Python library to communicate with the wave generator (the library for the AFG-2225 wave generator can be found on GitLab: \url{https://git.bsse.ethz.ch/pruppen/afg2225-library}).

**Electroporator - time constant and conductivity**

During the electric pulse, a $10\,\mu F$ capacitor is discharged, creating an exponentially declining voltage with time constant $\tau$. Inside the electroporator, a $600\,\Omega$ resistor is placed in parallel with the sample cuvette along with a $30\,\Omega$ resistor in series with the sample cuvette.\textsuperscript{4} The time constant, that is displayed on the electroporator screen is thus a function of the sample conductivity, as indicated in the following equation:

\begin{equation}
R = \frac{600\,\Omega \cdot (30\,\Omega + R_s)}{630\,\Omega + R_s},
\end{equation}

\begin{equation}
\tau = C \cdot R,
\end{equation}

with the equivalent resistance of the resistor circuit $R$, the sample resistance $R_s$ and the
micropulser capacitance $C$.

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