Acoustically Driven Microfluidic Applications for on-Chip Laboratories

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***Abstract*—Employing surface acoustic waves (SAW), we induce acoustic streaming within minute amounts of fluid on a planar piezoelectric chip. Apart from the SAW generating interdigital transducers, this chip can also be equipped with various other functional elements like heaters, sensors and alike. Because the SAW driven micro fluidic system can be electrically addressed, we end up with a fully programmable micro laboratory for chemical and biological applications. Depending on the amplitude of the SAWs, the acoustic streaming can be very efficiently used to mix and stir the fluids, or – at higher amplitudes- to actively actuate whole droplets or closed fluid volumes across the chip surface. Various applications are given, which demonstrate the uniqueness and power of our approach.**

I. Introduction

Reducing the dimensions of macroscopic biological or chemical laboratories is advantageous for the following reasons: The small scale allows for the integration of various processes on one chip analogous to integrated microelectronic circuitry. Thus, manual handling, e.g. transferring reagents from one process step to the next, can be reduced. Such an integration is the prerequisite for a fully automated data management system covering all steps of a given process. Furthermore, the required reagent volumes are reduced thus saving both material costs and process time as many of the time consuming amplification steps for biological substances can be omitted. Finally, the miniaturization results in enhanced precision by providing more homogenous reaction conditions and in shorter reaction times, as less sample volume is present at higher concentrations.

So far, in most of the micro fluidic systems liquids are confined and moved in tubes or capillaries. Usually, the application of such systems is restricted to continuous flow processes [1]. Small amounts of liquid cannot be handled separately in tubes, as these need to be completely filled in order for the pumping mechanism to work properly. Most of the work in macroscopic laboratories, however, is carried out as a batch process. A typical example is the mixing of two reagents or dissolving a substance in a liquid. One would measure the required amounts in separate beakers, and then pour the substances in a third beaker while agitating with a stirrer.

Micro fluidic devices for sophisticated lab applications usually comprise different components: First, the small amounts of fluids need to be confined to some kind of containers or reactors, holding specific amounts of the liquid. These containers and reactors are then connected via miniaturized tubes or channels, which are operated by small pumps and valves. The whole system additionally needs to be interfaced to the outside world. The smallness of a micro fluidic chip (tube diameters are typically of the order of 100 µm or less), ensures that only tiny amounts of reagents are needed for a chemical or biological reaction, on the other hand, however, it also causes complications that are not of relevance in macroscopic fluid handling systems.

As usual for fluidic problems, one has to first regard the Navier-Stokes-Equation, describing the flow in a hydrodynamic system. It is a nonlinear equation in the velocity components, reading

$$\rho\frac{\partial\vec{v}}{\partial t}+\rho(\vec{v}\bullet grad)\vec{v}=-grad(p)+\eta\Delta\vec{v}+\vec{f} \qquad (1)$$

Here, $\vec{v}$ is the velocity field of the flow, η the viscosity and ρ the mass density of the fluid. p denotes the pressure that a fluid element experiences from its surroundings and $\vec{f}$ is an externally applied body force driving the flow. The term $\rho(\vec{v}\bullet grad)\vec{v}$ describes the inertia of the fluid element and $\eta\Delta\vec{v}$ marks the viscous term. The interaction between the fluid confined to a 'lab-on-a-chip' and the tube walls leads to hydrodynamic features that usually are comprised into a single number characterizing the flow behavior in a fluid, the Reynold's number *Re*, describing the ratio between the inertial and the viscous term:

$$\mathrm{Re}=\frac{\rho v^2}{l}\frac{l^2}{\eta v}=\frac{\rho v l}{\eta} \qquad (2)$$

l denotes a typical length scale in the system under consideration, for example the channel diameter. For a micro fluidic system, *Re* is usually a small number, indicating the little importance of inertia in the problem. The most prominent consequence thereof and hence the most important difference

to a macroscopic fluid volume is probably the lack of turbulent flows in a micro fluidic system. The transition between turbulent and laminar flow is usually occurring at a threshold Reynold's number $Re \approx 2000$. Given $l \approx 10$ µm, v ≈ 100 µm/sec, and the material parameters of water ($\rho=10^3$ kg / m^3, $\eta=10^{-3}$ kg / (msec)), we end up at $Re \approx 0.001$. For a lab-on-a-chip application, this smallness usually causes severe problems. For instance, mixing of two fluids or stirring a liquid to enhance homogeneity or to speed up a chemical reaction, is a very difficult task for a purely laminar flow system. Also, pumping of a low Reynold's fluid is difficult, as the interaction of the fluid with the vessel walls mimics a high viscosity. We will show, that many of these obstacles can be overcome by employing our acoustic methods.

II. Surface Acoustic Wave induced streaming

Flow in a micro fluidic system can be achieved by the interaction of the fluid and surface acoustic waves (SAW), propagating at the surface of a solid chip. The driving force behind this interaction and the resulting acoustically driven flow is an effect called 'acoustic streaming'. It is a consequence of the pressure dependence of the mass density ρ of a fluid, leading to a non-vanishing time average of the acoustically induced pressure. Although acoustic streaming is a well-known effect for a long time [2] in macroscopic, classical systems, little attention has been paid on it, so far, in terms of miniaturization.

Here, we report on SAW streaming in a fluid residing directly on top of a planar chip. SAW have been first described in combination with earthquakes [3]. Meanwhile, reduced to the significantly smaller nano scale, they found their way into much friendlier fields: SAW devices are widely used for RF signal processing and filter applications and became a huge industry in mobile communication. Also, SAW are a vital part in basic research on the nanoscale. Here, the optical and electronic properties of semiconductor nano systems have been intensively investigated over the last two decades [4]. SAW are especially convenient to excite on piezo electric substrates. A well defined wavelength and frequency can be excited if a specially formed pair of electrodes is deposited on top of the substrate. Such electrodes are usually referred to as interdigitated transducers (IDT). A high frequency signal applied to such an IDT is then converted into a periodic crystal deformation. If fed with the right frequency

$$f = \frac{v_{SAW}}{\lambda}, \qquad (3)$$

a SAW is launched. Here, v_{SAW} denotes the sound velocity of the respective substrate, and the wavelength λ is given by the lithographically defined periodicity of the IDT. Typical wavelengths of technically exploited SAW range from about λ=30µm at $f \approx 100$ MHz. If a second IDT was placed downstream the substrate surface, a so-called delay-line would be formed. Both transducers, their design, and the substrate properties thus act as a high frequency filter with a predetermined frequency response. They are lightweight, relatively simple and low cost, and can be produced very reproducible, which explains their massive use in high frequency signal processing like mobile telephony.

Most of the energy propagating in a SAW (usually more than 95%) is of mechanical nature. Viscous materials like liquids absorb a lot of this mechanical energy at the surface of the chip. The interaction between a SAW and a liquid on top of the substrate surface induces internal streaming, and, as we will point out below, at large SAW amplitudes this can even lead to a movement of the liquid as a whole [5]. In Fig. 1, we depict the basic interaction between a SAW and a fluid on top of the SAW carrying substrate:

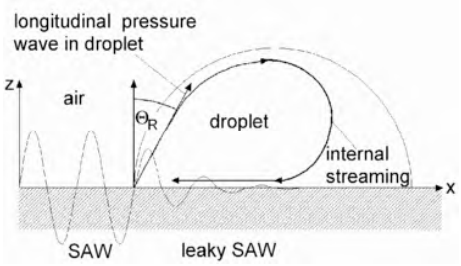

Figure 1. Sketch of the acoustic streaming acting on a small droplet on the surface of a piezoelectric substrate. The acoustic energy is radiated into the fluid under an angle Θ_R, leading to internal streaming in the small fluid volume.

The SAW is approaching from the left and entering the fluid covered region (represented by a droplet in this case) of the chip. There, it becomes attenuated by a viscous damping mechanism, which leads to the excitation of a sound wave in the fluid itself. Phase conservation requires that this sound wave is entering the fluid under an angle Θ_R, very much like for a diffracted beam in optics. SAW streaming then leads to an internal streaming pattern within the droplet, the exact shape of which is determined by Θ_R and the geometry of the fluid volume.

III. Surface Acoustic Wave Mixing

The internal streaming as shown in Fig. 1 can be very efficiently used for mixing smallest amounts of fluid. In Fig. 2, we show a series of two snapshots, about half a second apart, taken for a 50 nl droplet. Here, some fluorescent dye had been deposited at the chip surface just before the water droplet was placed on top.

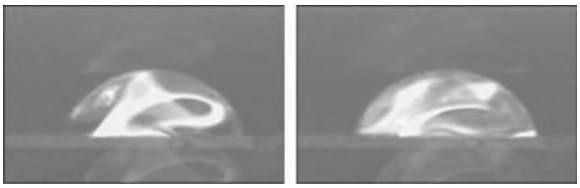

Figure 2. SAW induced internal streaming in a small water droplet (side view, approx. 50 nl). A powdered fluorescent dye on the surface of the chip is dissolved by SAW agitation, and rapidly fills the whole droplet volume.

Not only is the dye dissolved by the internal streaming, it is also distributed across the whole volume of the droplet. It should be noted at this point, that the SAW induced streaming

still is laminar, as the Reynold's number is so small. The complex flow pattern, however, strongly supports complex material folding lines which in turn facilitate a quick mixing [5]. Moreover, by switching the SAW frequencies and or directions during the mixing, different material folding lines are generated which then in turn further improve mixing [6]. This can be seen in Fig. 3, where we plot the SAW induced mixing behavior in a small volume of fluid, where fluorescent latex beads had been added as tracer particles. Two different SAW are entering the fluid volume from the top and the right side, and both SAWs are modulated in terms of their amplitude at two different frequencies ν and ν_0. The amplitudes and acoustic frequencies in that case are equal. For a certain ratio between the modulation frequencies (depending on the volume and geometry of the mixed sample), perfect mixing is achieved in very short times. This is due to the fact that for this parameter set (for details see [6]) the condition for quasi chaotic advection is met. The lower panel exhibits the results of a numerical model description of the problem for the same set of parameters.

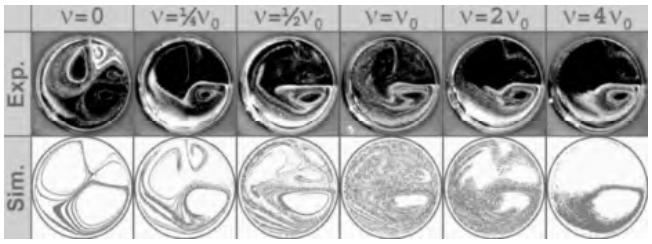

Figure 3. Experimental (upper panel) and theoretical investigation of SAW driven mixing in a small fluid volume. Two SAWs enter the fluid volume from top and right, both at the same acoustic frequency and intensity. Their amplitudes, however, are modulated at two different frequencies ν and ν_0 which can be analytically calculated for a given geometry of the system [6].

A typical and very important application for such SAW driven mixing is the use of SAW in so called microarrays. Microarray hybridization experiments are mostly based on quite small sample volumes being confined between the microarray itself and a cover slip or lifter slip on top of the narrow fluid layer. Under such conditions, the system is governed by the rules of micro fluidics, i.e. by the regime of small Reynold's numbers. Here, diffusion is the only source for moving sample molecules towards their target spots. However, for a typical macro molecule as it is used in microarray hybridization experiments, the diffusion constant is very small. Hence, driven by diffusion only, travelling over typical distances on a microarray may take them a very long time. Additionally, the slow time constants associated with the diffusion limit lead to pronounced depletion effects which strongly influence the dynamics of an hybridization assay. In this report, we describe a novel technique to overcome the diffusion limit in microarray hybridization experiments. Surface acoustic waves on a piezoelectric substrate are coupled into the sample fluid on a microarray where they act as a very efficient agitation source. We demonstrate that this way the diffusion limit can be overcome, leading to a remarkable increase in signal intensity and homogeneity in fluorescence labelled microarray assays.

Here, SAW driven mixing proved a very powerful tool to significantly enhance the hybridization process and to also be extremely beneficial to the homogeneity and overall sensitivity of the microarray readout.

For microarray production, large sets of genes are arrayed on small surface areas using high throughput robotic platforms. In most cases, the substrates for DNA microarrays are conventional microscope slides with dimensions of about 75 mm by 25 mm. Up to several thousand spots of oligonucleotides or cDNA probes with known identity cover the slide in a checkerboard pattern. In gene expression profiling assays, the ratio of binding of complementary nucleic acids from test and control samples is determined. This allows a parallel, semi quantitative analysis of transcription levels in a single experiment. In a standard microarray experiment the sample solution is sandwiched between the DNA microarray and a cover slip, forming a capillary gap of about 20...100 µm in thickness and several centimeters in width. Especially in case of low concentrated cDNA molecules representing the low expressed genes, the immediate vicinity of the corresponding probe spot will be quickly depleted. Without active agitation, diffusion is the only mechanism for the DNA strands to be transported to their complementary spots. However, on the scale of several centimeters, diffusion is a notoriously slow process for molecules that are the size of the DNA strands discussed here. It has been estimated that it would require weeks for the hybridization reaction to reach equilibrium [7].

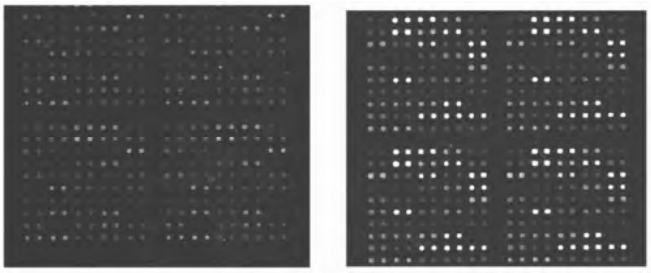

Figure 4. Comparison of Cyanine-5 (rat kidney cDNA) signal intensities of cover glass (left) and micro agitated experiments (right) shows an increase of the mean signal intensity by a factor of 6. Background values are identical [7].

IV. Droplet Actuation

For somewhat higher SAW amplitudes, the acoustic streaming effect leads to a strong deformation of the liquid surface and a momentary asymmetry in the wetting angles left and right with respect to the SAW impingement.

A. Droplets as virtual test tubes

Especially for small droplets, this leads to a movement and an actuation of the whole droplet into the direction away from the SAW, as shown in Fig. 4. In this sense, one can regard an IDT on a piezoelectric substrate as an integrated nano pump. Moreover, employing a chemical surface modification, one can define hydrophobic and hydrophilic regions on the chip surface, acting as anchors or fluidic tracks for small droplets. By a chemical modification of parts of the chip surface (e.g. silanization employing an OTS based surface chemistry) we

are able to create patterns of preferred wetability (hydrophilic regions), being separated by regions of surface chemistry, where fluids are repelled (hydrophobic areas). Employing lithographic techniques borrowed from semiconductor microelectronics, we thus can create completely flat, two-dimensional fluidic networks, where liquids are confined to virtual tracks, reservoirs and reaction chambers by surface tension alone.

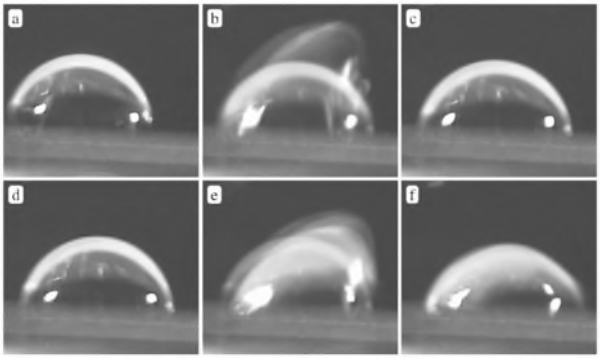

Figure 5. A small (V=50 nl) droplet under the influence of a pulsed, intense SAW impinging from the left for six different times. In(b) and (e), one clearly sees a strong deformation of the droplet under influence of the SAW. This leads to an actuation of the droplet as a whole.

B. Programmable Lab on a Chip

As an example for the versatility of this approach in terms of lab-on-a-chip applications, we show in Fig. 5 a series of snapshots of a programmable micro fluidic chip with integrated planar pumps.

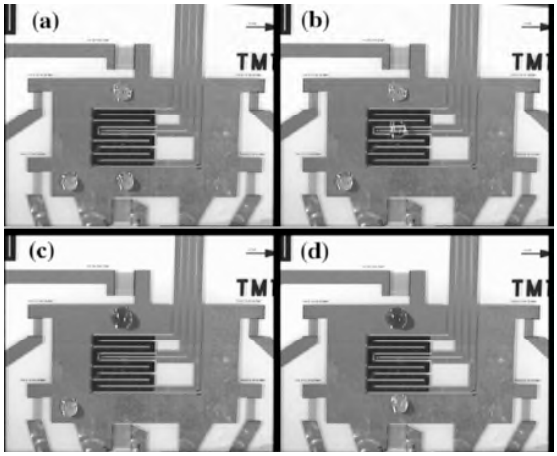

Figure 6. Series of snapshots of a fully programmable SAW driven lab on a chip. In this case, six independent IDT are used to launch SAWs along the different directions on the chip, and to thus actuate a few droplets on itss surface. The droplets (approx. 100 nl each) can be moved independent from each other, being merged, mixed, and transported to, e.g., a heater being evenly integrated on the chip. Color changes indicate the ocurence of a chemical reaction in the example.

Here, we have used three different droplets of different chemical solutions which exhibit a color change when merged and mixed. It should be noted that while the droplets are actuated and eventually merged, in their bulk, the SAW streaming leads to extremely fast chemical reactions as compared to a diffusion-only driven process.

C. Polymerase Chain Reaction on a Chip

Having a completely programmable micro fluidic chip at hand, one can aim towards more complex assays like, e.g., polymerase chain reaction (PCR) on a chip [8]. Here, however, comparably high temperatures are involved during the protocol. Such high temperatures are not compatible with the 'open geometry' of our droplet based fluidics. To avoid evaporation – especially during the 95° Celsius cycle of a PCR process - we cover the aqueous sample solution with a mineral oil layer.

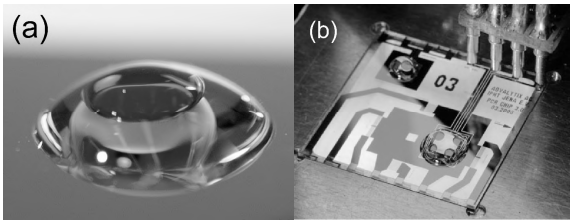

Figure 7. (a) virtual liquid test tube' for high temperature application of the planar SAW driven fluidics. To avoid evaporation of the aquaeous sample solution (red), the droplet has been covered by a thin mineral oil layer. Both represent a fluid test tube for further processing of the sample. (b) Programmable microfluidic biochip for multi-spot PCR in top view. Apart from SAW driven nanopumps for the fluid actuation, the chip also hosts a heater and a thermometer. The chip is connected to the outside via pogo contacts. Underneath the transparent piezoelectric substrate, a microscope objective is mounted for fluorescence monitoring of the PCR progress employing an intercalating dye [8].

The sample contains all the 'PCR-mix' including template, primers, and polymerase for a successful amplification of a small amount of genetic material. The sample volume in this case is well below 10 micro liters, a 'virtual liquid test tube' as depicted in Fig. 7a. Here, the sample droplet has a red color for better visualization. As the programmable biochip is fabricated employing standard planar lithographical technology, we can also include additional functional elements like thermometers and heaters. The optical transparency and the lack of self fluorescence of the substrate materials used further adds valuable for this lab-on-a-chip application [8]. In Fig. 7b, we show a typical PCR chip. In the lower right corner, an oil droplet is seen, with in this case four different sample droplets, each holding a different sample. From below, an optical system is attached to monitor the progress of amplification using an intercalating dye. For details of the PCR protocol, the sensitivity of the technology, and typical experimental results we refer the reader to [8].

V. BLOOD FLOW ON A CHIP

Studying cell or platelet adhesion (hemodynamic) in small capillaries and arteries is a particularly challenging topic, since it is difficult to be mimicked in an in-vitro system and hard to access by optical or mechanical means. As the diameter of capillaries and arteries become as small as one micrometer, the Reynolds number becomes very low making the pumping

mechanism extraordinary difficult. This calls for a new pumping principle when designing a in vitro model to study the basic principles taking place during blood flow. Fig. 8 describes the principle of a micro flow chamber, which is directly built on a SAW chip (µ-FCC). This system then resembles a type of "artificial blood vessel". The fluid on the chip is confined to a trajectory or a virtual container by a chemical modulation of the surface wetability using hydrophobic/hydrophilic surface functionalization employing soft lithography. The heart of the chip, the nanopump is driven by a surface acoustic wave (SAW) causing the liquid flow by inducing surface acoustic streaming (see Fig. 1). The amplitude of the SAW in the liquid decays exponentially within a characteristic length scale of a few micrometers.

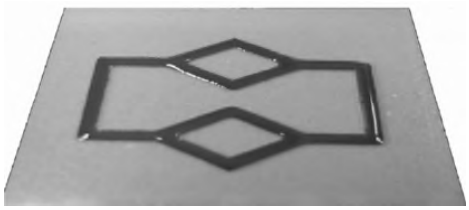

Figure 8. An 'artificial blood vessel' on a chip. Employing chemical surface functionalization, the fluid can are confined to lithographically defined hydrophillic areas on the chip surface. To simulate a blood vessel, typical branching and different length scales of the fluidic reservoirs are easily achievable. One or more IDT (not shown in the figure) are used to induce internal streaming, and in certain regions also defined shear force fields.

Therefore, considering the total length of a typical channel (4 mm) the pump basically acts as a point-like source, driving the liquid to flow according to conservation of mass. The flow chamber has no dead volume, allowing for the investigation of even expensive or rare substrates with a volume 100 to 1000 times less than usually required. The particularly small sample volume of only 8µl is probably the most prominent advantage of our nanopump driven planar flow chamber chip. Moreover, the chip components are inert and entirely compatible with biological systems and the system is free of all movable parts making the handling extremely simple. The complete optical transparency of the $LiNbO_3$ substrate allows for individual cells to be tracked over long periods of time, when the µ-FCC is mounted directly onto a fluorescence microscope.

Lanes ranging from a few µm up to 10 mm can be realized and the open structure on a free surface allows for direct access to the channel at all times (e.g. for the addition of antibodies or drugs). The technique also provides maximum freedom for mimicking all possible vessel architectures and conditions as they may exist in nature with curved, branched or restricted vessels. Finally, the SAW based pumping system of our chip guarantees for a homogenous, easily controllable laminar flow whose profile covers the complete range of physiological flow and shear conditions from 0 to several $10000s^{-1}$ [8]. Finally, no restrictions are put on the surface functionalization. Artificial lipid membranes, protein coats and even confluent cell layers have been successfully be prepared on our chips [9].

VI. Proteins under Shear

Using this set up, we studied the effect of shear flow on a protein called von Willebrand factor (vWF). It is a glyco-protein being synthesized and stored in endothelial cells, and has found to play an important role for blood coagulation, particularly in regions where the shear rates are high. Under normal conditions, it assembles into multimers (biopolymers) which when stretched can reach sizes as long as 100 µm. The monomeric length is unusually large (~100nm) and contains 2050 amino acids residues. Intuitively, one would expect that blood platelet adhesion is always decreasing with increasing shear force applied to the platelet. Surprisingly, vWF mediated adhesion, on the other hand, is strongly enhanced under high shear-flow conditions [10,11]. vWF was spread over the hydrophilic track of the flow chamber chip an exposed to various shear flows. Images of vWF at concentrations typical for human blood ($c \approx 2\ \mu g/ml$) are presented in Fig. 6. At shear rates between $\dot{\gamma} \approx 10$ and 1000 s^{-1}, the biopolymer exhibits a compact conformation (Fig. 9 left). The size of the VWF globules was estimated within our fluorescence setup to be $d \approx 2\ \mu m$, clearly showing that it consists of more than one monomer. This compact conformation remains unchanged as long as the shear rate is maintained below a certain (critical) value $\dot{\gamma}_{crit} \sim 5000\ s^{-1}$. Increasing the shear rate above $\dot{\gamma}_{crit}$ induces a shape transformation of the VWF fibers from a collapsed to a stretched conformation of length $l \approx 15\mu m$ (Fig. 9 right). This transition is reversible, as we observe an immediate relaxation of the protein to its compact conformation when the flow is turned off.

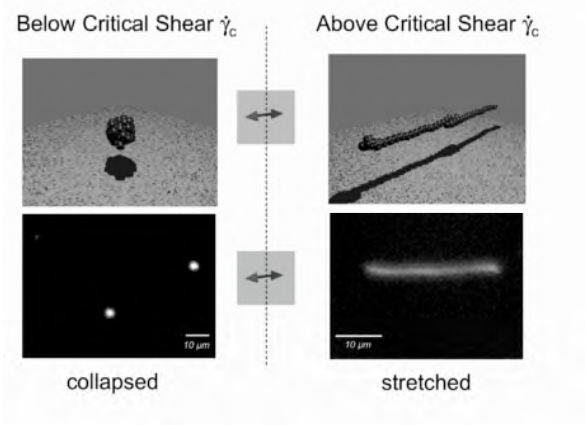

Figure 9. Proteins under Flow. The blood clotting protein vWF is exposed to various shear flow conditions. Only when a critical value is exceeded the protein elongates in a reversible fashion. The unusually high shear rates could not be explained by conventional hydrodynamic modells, instead a new theory was developed [11] . The upper panels represent a sketch of this situation, whereas the lower panels are fluorescence microscopy images taken of a single polymer under flow.

The fact that the transition occurs at such a high shear rate cannot be understood from previous studies on linear chains under good solvent conditions. For example, it has been shown that DNA of roughly the same length as the vWF fibers studied here will exhibit drastic changes of elongation for shear rates as low as 30 s^{-1} at a viscosity of 1cP (i.e. water or the phosphate buffer employed here). Our studies, however,

indicate that significant changes in conformation occur only at shear rates $\dot{\gamma} \geq 5000 s^{-1}$. This value is more than two orders of magnitude higher than that reported for DNA. We assume that strong attractive interactions between monomers hold the VWF fiber tightly together, even under strong shear conditions. Therefore, we proposed that a single VWF biopolymer forms a compact or collapsed structure similar to a folded protein (Fig. 6 left) [10] . This model explains the counterintuitive observation mentioned at the beginning of this paragraph. Under high shear flow, vWF mediates blood platelet adhesion more effectively. In its collapsed state, all binding sites are buried inside the coil, leading to a strongly suppressed binding affinity of the protein.

Once the critical shear is exceeded, vWF stretches into a long thin fiber exposing all its binding sites. If vWF touches the surface in this conformation, which is highly unlikely in small capillaries, it will immediately be immobilized and serve as a "sticky" grid for free floating blood platelets. Since forces, conformation and function are so closely related, we call this "self organized blood clotting".

VI. Conclusions and Summary

In summary, we described a novel technique to manipulate smallest amounts of liquid on a chip. Employing surface acoustic waves on a piezoelectric substrate, we are able to actuate individual droplets along predetermined trajectories, or induce acoustically driven internal streaming in the fluid. This internal acoustic streaming can efficiently be used to agitate, mix and stir very small liquid volumes, where the low Reynold's number usually only allows for diffusive mixing. As for typical applications, we described a programmable microfluidic chip for droplet based assays, to perform high resolution micro liter polymerase chain reaction (PCR). The technique is equally well suited to actuate or agitate small amounts of liquids either in closed volumes or in an open, droplet based geometry. An example for such a closed though 'open' volume is given in the context of a model for the investigation of blood flow on a chip. Here, we showed the feasibility to even grow a cell culture on predetermined areas of the chip, representing a flat version of a blood vessel. The shear force induced mechanical activation of an important biopolymer for wound healing and cell adhesion is one of our latest unpreceeded applications of our quite universal SAW fluidic chip. The combination of the SAW actuated droplet based fluid handling and SAW driven fluidics in closed volumes opens a wide field of many different applications. Many more applications, and many more visualizations of the technology described above can be looked up on either http://www.Advalytix.de or http://www.physik.uni-augsburg.de/exp1.

Acknowledgment

I would like to thank a few colleagues personally, without whom a lot of the experiments that we presented would have been impossible. First, we like to thank Matthias Schneider and his brother Stefan for the fabulous work on the von Willebrand factor and blood vessels on a chip. Stefan Thalhammer supplied much of the biological background and technological solutions, especially in the field of cytogenetics. Thomas Frommelt became obsessed with the art of SAW-driven mixing during his PhD thesis in Augsburg. Jürgen Scriba and Christoph Gauer made the commercialization possible by co-founding Advalytix AG, Munich. Peter Hänggi, Peter Talkner and Michael Schindler and Daniel Köster, Roland Hoppe and Kunibert Siebert were a great team with whom to work and to discuss our experimental findings.

This work has been financially supported in part by the Deutsche Forschungsgemeinschaft (SFB 486, SFB 492, SPP1164, and SPP1253), in part also by the Cluster of Excellence "Nano Systems Initiative Munich", NIM), and partly through the Elite Network Bavaria (CompInt) and the "Innovative Medical Research Program at the University of Münster. We also thank the Bayerische Forschungsstiftung for financial support under the programs FORNANO and FOROXID, as well as a grant entitled "Programmable cytogenetic sub-microliter lab-on-a-chip". Finally, we thank the Center for Nanoscience at the LMU in Munich, the University of Augsburg and Olympus Advalytix, Munich for constant support.

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