ACOUSTOPHORESIS
- A SOUND APPROACH TO CHIP BASED CELL HANDLING –

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ABSTRACT

The basic principles of acoustic cell and particle manipulation in chip integrated acoustic standing wave resonators is highlighted. Our recent developments of applications in the bioanalytical and medical area are overviewed and current developments of microchip acoustophoresis in clinical applications as well as in flow cytometry is reported.

KEYWORDS: Acoustophoresis, acoustic standing wave, cell separation, cell handling

INTRODUCTION

The use of acoustic standing wave forces has in recent years become a viable strategy for advanced cell manipulation and separation (acoustophoresis). With the transition to a microchip format where the acoustic resonator became an integral part of a microfluidic system [1-3] a wide variety of acoustophoretic unit operations have emerged that allow cell and particle separation, buffer medium exchange, acoustic valving, affinity bead based extraction, acoustic trapping, cell interaction studies, etc [4].

The bioanalytical field and clinical diagnostics have become key applications areas for microchip acoustophoresis and are now being researched at an increasing rate. Early work on clinical applications in our group demonstrated principles for lipid microemboli depletion from blood recovered during surgery prior to autologous re-transfusion [5]. The potential for blood cell washing in the post surgery intensive care unit, eliminating high levels of inflammatory components and coagulation factors, was also proposed [6]. This progress triggered further work to explore acoustophoresis as a basic modality in the development of chip integrated strategies for cells and particle handling..

EXPERIMENTAL

Our group has in the past years developed several applications on chip integrated cell and particle manipulation based on two fundamental operational modes of acoustophoresis, i.e. binary acoustophoresis and Free Flow Acoustophoresis (FFA) [7]. A key to these developments is that the direction of cell movement by means of acoustic standing wave forces can be performed in plane with the chip, enabling integration with more advanced microfluidic networks.

Binary acoustophoresis utilizes the fact that the acoustic properties of the species to be separated display either a positive or negative primary acoustic radiation force (analogous to positive and negative dielectrophoresis) relative its counter part, which either drives the cells into the acoustic pressure node (blue) or into the antinodes (yellow), Fig 2., whereas FFA rely on the fact that different cells migrate at different speeds to the pressure node in the channel centre, enabling a separation by multiple flow splitters at the chip outlet, Fig 3. The cell specific primary acoustic radiation force is dependent of the cell size, compressibility and density.

Our developments of continuous flow based buffer exchange of a cell/particle population has emerged to be a simple but powerful modality and has direct implications in clinical applications, eliminating time-consuming manual centrifugation steps, hence opening the route to automated sample processing in the routine lab.

Fig. 2 Cross-section of a flow channel. Particles in suspension without (left) and with an active acoustic standing wave (right). Yellow particles experience a primary acoustic radiation force to the pressure anti-node and blue particles a force towards the standing wave pressure node. By tuning the density of the carrier buffer a binary separation condition can be obtained, enabling separation of normally non-separable polymer particles (right) where 3 um PS (red) go in the side outlets and PMMA (white) are collected in the centre outlet. Similarly cells that normally do not separate can be differentiated by tuning the buffer.

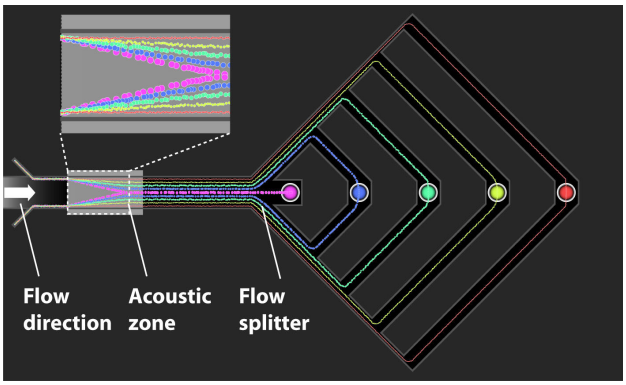

Fig 3a. Free Flow Acoustophoresis, (FFA). Larger particles migrate faster into the channel centre.

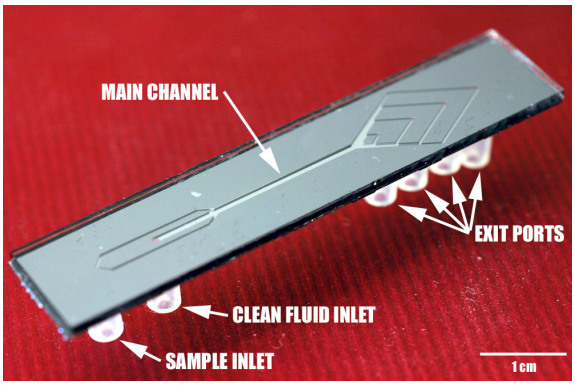

Fig 3b. 1st chip generation for Free Flow Acoustophoresis (FFA) with outlets for four fractions.

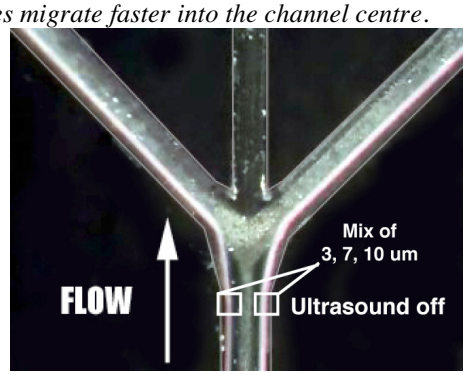

Fig 3c. Without ultrasound all particles are laminated along the channel sidewalls [7]. Copyright 2007 American Chemical Society

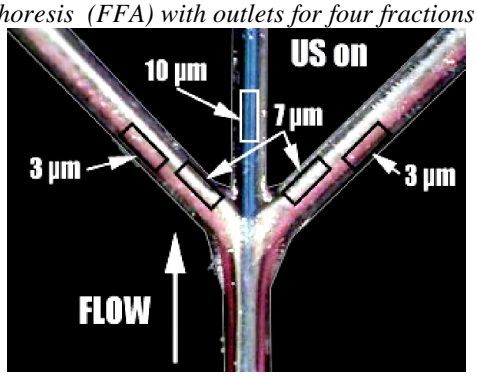

Fig 3d. Ultrasound active (FFA) with 3 um red, 7 um white & 10 um blue PS particles now separated in clear bands [7]. Copyright 2007 American Chemical Society

Cells undergoing acoustophoresis experience a low mechanical stress. Our studies on human stem cells and blood components as well as studies by other groups confirm that acoustophoresis does not induce any detectable stress [8]. In he clinical applications of blood washing we have shown that no hemolysis can be attributed to acoustophoretic processing of blood [5], whereas conventional centrifugation induces hemolysis. These findings have opened the route to a wide range of clinical applications. One such application in our recent work describes whole blood acoustophoresis where we perform plasma extraction, with clinically approved quality [9]. The plasmapheresis platform is currently being integrated with cancer biomarker microarrays for on-line multiplex immunoassaying, including secondary detector antibody incubation.

Acoustophoresis has also been developed to purify/extract components from complex matrices using affinity probe activated microbeads, i.e. Affinity Acoustophoresis. By laminating a complex sample mixed with activated beads to the side inlets of an acoustophoresis channel and with a centre inlet carrying a clean buffer, beads with the bound targeted species can be moved into the clean buffer flow for further down stream identification. We have demonstrated affinity specific extraction of phospopeptides on metal affinity microbeads in complex biological samples using affinity acoustophoretic bead washing and clean-up prior to MALDI-MS readout [10]. The acoustophoresis based buffer exchange platform can also be configured to perform multiple buffer exchanges [11] in a single sequence on a chip, which later has been implemented in a system for sequential elution of peptide mixtures from strong cation exchange beads at step wise increased pH, also pH dependent elution of surface bound peptides from spermatozoa.

Affinity acoustophoretic bead extraction of specific bacteriophages from a phage library has also been performed, demonstrating an optional and improved purification modality as compared to conventional magnetic bead based phage display selection. The acoustophoretic selection process offers a simplified and accelerated process for phage selection as demonstrated by targeting a grass pollen allergen (Phl p5) specific antibody in a phage library [12].

The fact that cells display individual migration velocities in the acoustic standing wave field allows for bead and cell specific differentiation. Based on this we have developed a Free-Flow Acoustophoresis (FFA) platform that enables on-line separation of multiple beads sizes. Fig. 3d shows the first data on FFA presented by our group, separating a mixture of 3, 7 and 10 um beads. Based on this set-up we have demonstrated the separation of erythrocytes, leukocytes and thrombocytes using buffy coat as the put sample.

Pre-processing of cell samples in clinical diagnostics and therapeutics is a key research target area performed in The CellCARE research environment at Lund University (www.cellcare.lth.se), which addresses 4 clinical application areas in immediate collaboration with clinical research. 1) Neuronal stem cell separation for treatment of Parkinsons disease, 2) Extraction of peripheral blood stem cells for transfusion in blood disorders, e.g. leukemia and lymphoma, 3) Extraction of circu-

lating tumors cells in prostate cancer for diagnostics & treatment follow-up [13] and 4) Blood component preparation in blood banking. Our developments of acoustophoresis in these clinical areas also targets the removal of trombocytes from apheresis products and production of WBC-free trombocyte fractions.

A recent key development was the demonstration that high performance acoustophoresis can be performed in standard etched glass channels [14], thereby opening the route to simple large scale fabrication and cost effective chip solutions that can be integrated with industrial analytical systems. This has fuelled the development of sample preprocessing steps for analytical instrumentation. Acoustophoresis, and its ability to be tethered to downstream analytics has thus recently been addressed by our team together with BD Biosciences, and we have for the first time reported coupling of acoustophoretic buffer switching of cells as a modality for down stream sample processing in FACS analysis of labelled cells, still in their labelling buffer [15]. The buffer switching eliminates the centrifugation step prior to FACS analysis. Acoustophoresis is an evident development route for a set of modalities in processing cell samples prior to FACS analysis and hence eliminating the influence of the human factor in the centrifugation step as well as to speed up the analytical process.

Acoustic standing wave resonators can also be designed in microfluidic systems to retain/trap and enrich cellular, bacterial or other bioparticle species prior to down stream diagnostic readout. This has been demonstrated in the enrichment and purification of sperm cells in forensic samples [16]. We have also realised simple disposable glass capillary systems for acoustic cell trapping [17] that allows for detailed cell/cell or cell drug interaction studies in sub microlitre volumes that can be linked directly to microchip based sample preconditioning and MALDI MS/MS analysis [18].

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