ACOUSTIC ENRICHMENT OF MICROVESICLES IN PLASMA
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ABSTRACT
We present for the first time an acoustofluidic method to enrich cell-derived extracellular vesicles (EV) from human plasma, enabling rapid access to a biological nano-compartment rich in biomarkers. The system was characterized using polystyrene nanoparticles. Human EVs from cell-free plasma were successfully enriched and analyzed using a FACS. The technique enables EV-recovery from small sample volumes, not possible with standard techniques. It also avoids the cumbersome standard method of hour-long ultracentrifugation steps and manual sample handling required to purify and enrich the 100 – 1000 nm large microvesicles.

KEYWORDS: Extracellular vesicles, microparticles, acoustic trapping, acoustofluidics, enrichment

INTRODUCTION
EVs are released from several different cell types. They contain protein, mRNA/miRNA, express antigens on their surfaces and are involved in a vast array of cell signaling processes, including RNA transfer between cells. Alterations in the EVs have also been connected to a long list of diseases, e.g. type 1 diabetes, cardiovascular diseases, cancer and Alzheimer’s disease [1]. Research efforts are currently focused on widening our understanding of the processes they’re involved in and utilize our knowledge to develop diagnostic tools.

Isolating EVs from blood is a laborious process and the standard method involves lengthy ultracentrifugation. The isolation process is slow, has a low EV-recovery (5-25%) and may damage the vesicles. There have been efforts in developing microfluidic approaches but they have mostly been based either on antibody capture of the EVs (making it impossible to extract intact EVs) [2] or filtration devices that clog after processing a small sample volume [3].

Acoustophoretic forces have been utilized for a vast array of applications, demonstrating the technique to be a gentle, non-contact cell handling method [4]. We have previously demonstrated acoustic trapping and enrichment of bacteria and nanometer-sized particles through the use of seed particles, wherein larger particles are trapped to initiate the capture and retention of nanoparticles/microvesicles using a combination of primary and secondary acoustic forces [5]. Since the technique is non-contact, the enriched cluster can be released for further processing downstream by deactivating the ultrasound.

EXPERIMENTAL
The acoustic trapping system can be seen in figure 1. A rectangular cross-section capillary was used as fluidic channel (2x0.2 mm² ID) and the piezoelectric transducer was actuated using at 10 Vpp, 4 MHz. The system was characterized using enrichment of 500 nm fluorescent polystyrene particles from buffer at 10 μl/min, washed microvesicles marked with fluorescent Annexin V in buffer at 25 μl/min and finally human, cell-free plasma at 10 μl/min. Nanoparticles and microvesicles were acoustically captured using secondary forces from 12 μm polystyrene seeding particles, preloaded in the acoustic trap prior to sample infusion. The enriched and trapped clusters were washed in the capillary and released in tubes for FACS-analysis.
RESULTS AND DISCUSSION

Figure 2 shows the FACS-results from the polystyrene nanoparticle enrichment for varying sample volumes. As expected, a linear increase in concentration can be seen as the processed sample volume is increased.

For the washed microvesicles, fluorescent images were acquired at different times during the enrichment, see figure 3, showing an increasing number of trapped microvesicles.

Figure 3: Fluorescent images of a continuous enrichment of microvesicles marked with Annexin V running at 25 µl/min. The images show the increase in fluorescence due to the increased number of microvesicles being acoustically trapped in a seeding cluster of 12 µm polystyrene beads (vaguely seen in the background image).
The result from the FACS-analysis of the enrichment of the microvesicles in human plasma can be seen in figure 4. The microparticle enrichment correlates to increasing sample volume, however, the behavior is not as expected. This is believed to be caused by aggregation mechanisms in the undiluted plasma.

**CONCLUSION**

The results show that it is possible to capture and enrich microvesicles using an acoustic trapping platform. Further optimization is, however, needed when working with undiluted human plasma, as the enrichment is not as stable as expected. This optimization is currently underway and future work includes adding an upstream acoustophoretic plasma generator that would enable fast microvesicle enrichment directly from whole blood.

**ACKNOWLEDGEMENTS**

This work was funded through Knut and Alice Wallenberg Foundation and the Swedish Foundation for Strategic Research.

**REFERENCES**

5. “Seed particle-enabled acoustic trapping of bacteria and nanoparticles in continuous flow systems. B. Hammarström et al. Lab Chip, 2012, 12, 4296-4304

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