AcouTrap 2
PLATELET MICROPARTICLE CAPTURE

The AcouTrap from AcouSort AB is a tabletop research platform for performing acoustic trapping. The central part of the system is an acoustic trapping unit where standing waves are used to trap and hold objects against a fluid flow. A motorized stage and two high resolution syringe pumps interface the trapping unit with 96-well microplates. The system is controlled by a computer through a graphical user interface where user-defined scripts can be used to automate the sample handling a fluid flow.

Introduction

Altered levels of platelet-derived microparticles (PMPs), a class of extracellular vesicles, are associated with several forms of cardiovascular disease. There is a lack of standardized isolation protocols as it is technically challenging to extract these submicron vesicles from plasma. The most common method is centrifugation that requires large sample volumes, is time-consuming, requires manual labour and results in low recovery.

Acoustic trapping is here used for isolating PMPs from small plasma volumes, demonstrating higher recovery and an automated sample processing compared to a common differential centrifugation protocol.

Figure 1: A schematic image of acoustic seed trapping of PMPs. 12 µm polystyrene particles are trapped in a 4 MHz standing wave and used to capture PMPs using secondary acoustic forces. T
Experimental

Human whole blood was centrifuged at 2x1600g for 15 min to produce cell-free plasma. The plasma was frozen at -80°C and thawed for processing either with acoustic trapping or a common centrifugation protocol (13000 xg for 2 min). The extracted PMPs were stained for CD42a and analyzed using flow cytometry.

Acoustic trapping was performed in the AcouTrap system using a 4 MHz standing wave in a 2 x 2 mm² glass capillary. 12 µm polystyrene particles were initially trapped to act as seed particles, see Fig.1. Cell-free plasma was diluted 1:4 in PBS and 50 µl was aspirated at 25 µl/min. The trapped cluster was washed with PBS and the ultrasound was turned off to dispense the captured vesicles for flow cytometry.

Results

PMPs stained with CD42-PE were injected into the system and trapped together with 12 µm polystyrene particles. Images were taken with a fluorescence microscope to show the accumulation of PMPs in the cluster, see Fig. 2.

Using pooled plasma samples, the consistency of the PMP recovery on different days was calculated based on the flow cytometry data. Fig. 3 shows a recovery of ~80% on the different days. Using 5-10 experiments in replicate, the PMP recovery was compared between centrifugation and acoustic trapping. The acoustic trapping protocol had roughly twice the recovery of the centrifugation, see Fig. 4.

Conclusion

Acoustic trapping enables rapid isolation of PMPs from small plasma volumes in an automated setup. Compared to differential centrifugation, the AcouTrap system achieved ~80% recovery and extracted higher numbers of PMPs.