PLASMA GENERATION AND LABEL-FREE MONONUCLEAR CELL SEPARATION FROM WHOLE BLOOD BY ONE-STEP ACOUSTIC FOCUSING

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ABSTRACT

We present the first one-step acoustic separation of high quality plasma and mononuclear cells (MNCs) from undiluted human whole blood. This enables centrifugation-free integration in analytical instrumentation.

KEYWORDS: Whole blood separation, Mononuclear cells, plasma separation, label-free

INTRODUCTION

Fractionation of whole blood is essential to analyse biomarkers in plasma and for separation of white blood cells (WBCs) for analysis or cell therapy. Today, blood samples are commonly sent to central laboratories where fractionation is performed using centrifugation, e.g. using a density medium, to separate specific WBC subpopulations. This process is slow, prone to contamination, bulky and ill-suited for in-line integration. Previous work on acoustic plasma generation required several consecutive separation steps [1], required diluted whole blood [2], or >1% of the blood cells remained in the plasma [3]. Similarly, MNCs have been acoustically separated from diluted whole blood [4], but never from undiluted samples. In this abstract we show that high quality plasma as well as MNCs can be separated from undiluted human whole blood by one-step acoustic focusing.

EXPERIMENTAL

The separations were performed in the AcouWash system (AcouSort AB), an automated acoustic focusing system. The AcouWash utilizes a piezoelectric transducer, glued to a glass chip, to generate an acoustic standing half wave between the walls of a microchannel (figure 1). The trifurcation at the end of the channel separates the acoustically focused blood cells in the centre of the channel from the plasma that remains along the sides (figure 2A-C). MNCs were separated using Histopaque 1119 as an acoustic impedance barrier in the centre of the channel. Histopaque has a higher acoustic impedance (density times speed of sound) than the plasma which reduces the focusing of MNCs to the centre of the channel, leaving a large fraction of the MNCs on the side (figure 2D). Whole blood of 38-45% haematocrit entered the chip at 20-50 µl/min and plasma was extracted through the side outlet at 5-15 µl/min.

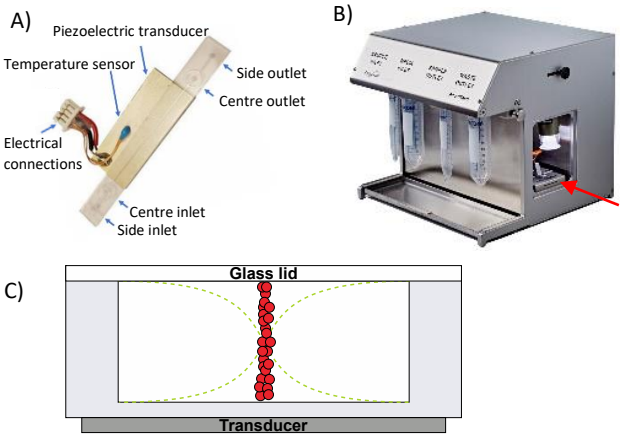

Figure 1: Design of the acoustic glass chip (A) and the AcouWash system (B). The red arrow indicates where the chip is connected. (C) shows an illustration of the channel cross section where the standing acoustic wave focuses red blood cells (RBCs) to the node in the centre.

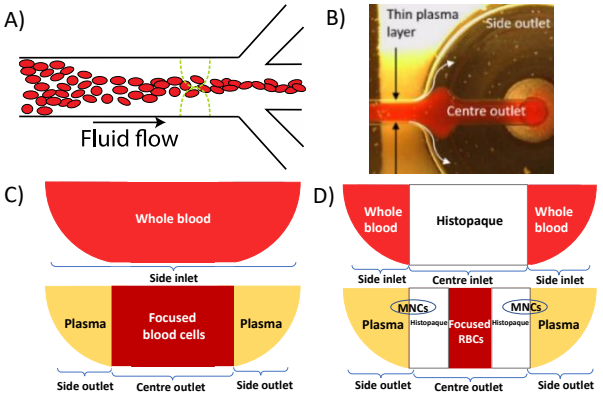

Figure 2: Sketch (A) and microscope image (B) of the acoustically focused blood cells which exit through the centre outlet while plasma is recovered from the side outlet. The two separation setups before and after the liquid passes the acoustic field are shown in (C) and (D). In the last setup, Histopaque, which has higher acoustic impedance than the plasma, is used as a barrier to reduce the MNCs focusing to the centre of the channel.

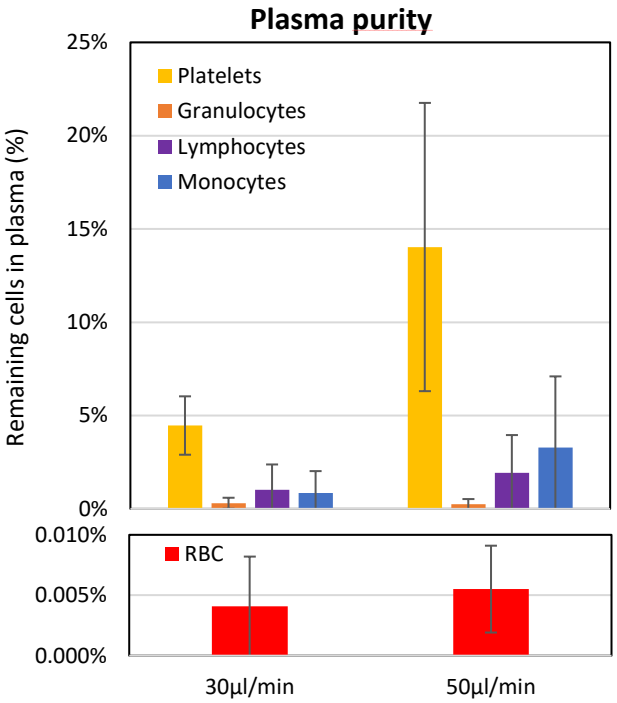

Figure 3: Purity of the generated plasma measured as the cell concentrations in the plasma compared to the input sample for sample throughputs of 30 and 50µl/min.

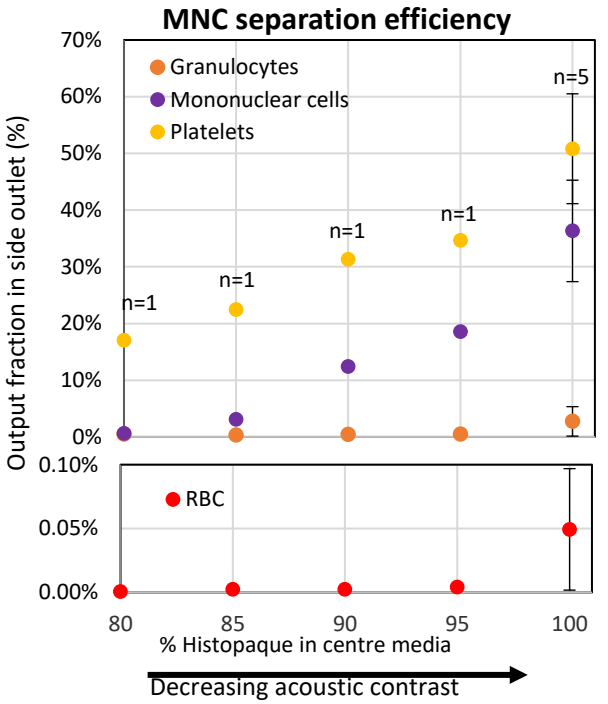

Figure 4: Number of cells in the side (plasma) outlet compared to the number of cells in both outlets combined.

RESULTS AND DISCUSSION

The cell content of the generated plasma and cell fractions was evaluated by staining both the side and centre outputs for platelets (CD61), WBCs (CD45), RBCs (CD235a) and counting the number of cells by flow cytometry. Less than 0.006% of the RBCs remained in the plasma after the separation, together with 4.5-15% of the platelets (figure 3). The generated plasma was slightly diluted by the system's priming liquid. Increasing the acoustic impedance in the central inlet by Histopaque led to a separation efficiency of up to 36% for the MNCs while depleting 99.95% of all RBCs (figure 4).

CONCLUSIONS

The results presented here show that high quality plasma can be generated by one-step acoustic focusing and that MNCs can be separated label-free directly from undiluted human whole blood. The methods are automated and gentle to delicate samples and facilitate in-line integration in analytical instrumentation.

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