ACOUSTIC WHOLE BLOOD PLASMAPHERESIS CHIP FOR PSA MICROARRAY DIAGNOSTICS

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ABSTRACT

An acoustic device for generating high quality blood plasma for PSA microarray diagnostics is presented.

KEYWORDS: Plasmapheresis, PSA, Blood, Acoustophoresis

INTRODUCTION

Generating high quality plasma from whole blood is of major interest for many biomedical analysis and clinical diagnostic methods. The handling and processing of fluids with high cell content, like whole blood, in microfluidic separation devices has proven to be a major challenge often resulting in diluted samples or poor througput. Diverse micro-technology techniques have been developed to enable separation and concentration of the different components in whole blood [1]. Extensive efforts have also been spent on the development of chips that integrate plasma generation with subsequent miniaturized diagnostic tests. In this abstract, an acoustophoresis based separation chip that prepares diagnostic plasma from whole blood and its clinical application are presented.

THEORY

Previously presented microfabricated silicon acoustic separator chips has not been able to process high particle concentrations, partly due to the very high acoustic forces required to concentrate particles into a band narrow enough to enable separation in a laminar flow via flow splitting [2, 3]. A new acoustophoresis chip has been developed to deal with this limitation. Since the particles exhibits tighter focusing the longer time they remain in the acoustic field, the separation channel was elongated in a meander type of fashion, figure 1. An acoustic force of higher magnitude is thus not necessary, as the radiation force instead acts for a longer duration forcing the cells gently into a focused band. The impact of the longer separation channel was investigated with four different chips with four different channel lengths.

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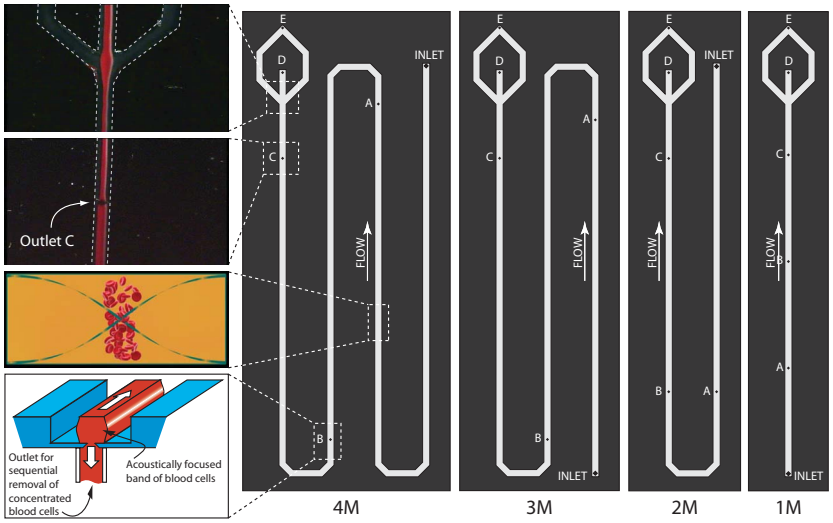

Figure 1. The principle of plasmapheresis. Acoustic standing waves gather blood cells in the pressure node located in the middle of the separation channel. Enriched blood cell fractions are removed through outlets A-C, thus decreasing the hematocrit gradually in the channel. The remaining focused blood cells exit through outlet D while the clean plasma fraction is withdrawn from exit E.

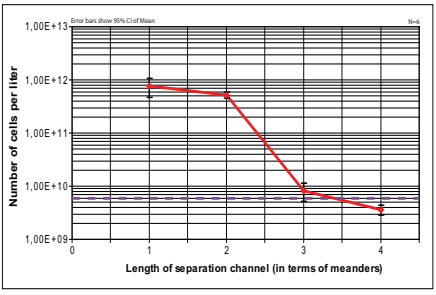

Figure 2. Graph showing the amount of cells per liter clean plasma separated from whole blood (Hct 40%). The purple dotted line show the upper limit of numbers of erythrocytes per liter plasma for transfusion set by the Council of Europe. That limit, which is set to $6x10^9$ erythrocytes per liter, was surpassed by the 4M plasma separator which generated plasma of cell counts of $3.65x10^9$.

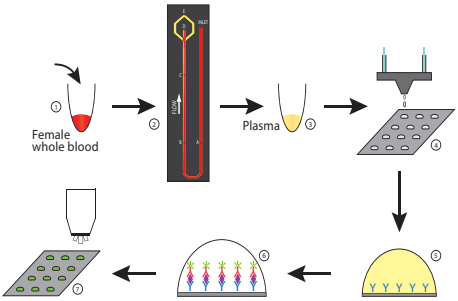

Figure 3. Schematic of the all chip based whole blood plasmapheresis and PSA diagnostics. 1) spiking of PSA in female whole blood, 2) ultrasonic standing wave driven microchip plasmapheresis, 3) plasma is collected via injector sample loops, 4) microarraying of PSA antibody, 5) microchip incubation in obtained plasma, 6) sandwich assay, 7) fluorescence readout.

Additionally, several extra outlets were added along the separation channel. These outlets, placed in the middle of the separation channel, allow blood cells al-

ready focused to be removed without removing a large part of the blood plasma. The cellular content in the suspension is thus lowered gradually in sequential steps, until only cell free plasma remains.

RESULTS AND DISCUSSION

Results show that the only design that proved to produce plasma with sufficiently low cell content to fit the suggestions of the Council of Europe, was the four folded separator, 4M in figure 2. The total number of cells in the sample was 3.65×10^9, which is well below the standards which has an upper limit of a red cell count of $<6 \times 10^9$ cells/L.

The plasmapheresis microchip was then successfully linked to our porous silicon sandwich antibody microarray chip for Prostate Specific Antigen (PSA) detection, figure 3. PSA was detectable from the generated plasma via fluorescence readout at clinically significant levels of 0.19-21.8 ng/ml with good linearity ($R^2 > 0.99$) without any signal amplification, figure 4. By combining USW microfluidics and protein microarray technology, an all microchip based PSA detection from whole blood is obtained in a novel lab-on-a-chip technique.

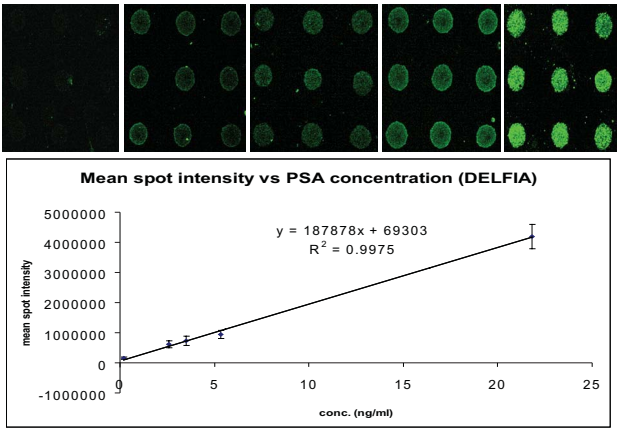

Figure 4. Microarray result of the titration series of blood plasma derived from PSA spiked female whole blood. The images shown were obtained from the porous silicon sandwich antibody microarray. Mean spot intensities and standard deviations (error bars) were calculated from the spots in the inset images detected via a 20x lens. The PSA concentrations on the x-axis were obtained by the reference DELFIA assay of the same plasmapheresis treated samples.

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