BLOOD SEPARATION BASED ON PRESSURE DRIVEN MEMBRANE DEFLECTION

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ABSTRACT

We present for the first time a compact blood separation structure for centrifugal microfluidic lab-on-a-disk platforms. Flow-induced deflection of a liquid membrane is used to gate the centrifugally driven flow through a microfluidic structure. While the flow is stopped, blood plasma and cells are separated by centrifugally induced sedimentation. After separation is completed, the flow resumes, providing up to 80 % of the plasma content to an overflow chamber. The entire process completes in less than two minutes.

KEYWORDS: Blood Plasma Separation, Centrifugal Microfluidics,

INTRODUCTION

Separating cell-free plasma from whole blood is an essential step in many diagnostic assays. For microfluidic point-of-care devices it is important to develop separation structures that work reliably, fast, occupy little space and are compatible with large scale production techniques. Centrifugal platforms are particularly suited to perform the separation of plasma and cellular components due to the higher density of blood cells compared to plasma. Several blood separation structures for centrifugal microfluidics have been presented in the past, e.g. using either siphons [1] or long throttle channels [2]. In contrast to these approaches the here presented device exploits self-induced membrane deflection to control liquid flow under rotation. Additionally, the fluidic structure possesses a compact footprint in the radial direction, does not require surface modifications, and it has proven to be compatible with standard compact disk injection molding techniques.

THEORY

The here presented structure is constituted by two azimuthally arranged chambers which are sequentially positioned in the radial direction of a disk shaped substrate These chambers are in fluidic communication via two independent channels, which for the sake of clarity will be referred as liquid and air channels.(Fig. 1).

Liquid samples are introduced to the inner chamber via an inlet port distant from the channels and the structure is subsequently sealed from atmospheric pressure. Rotation of the disk substrate forces liquid to flow through the liquid channel to the outer chamber. If the latter chamber is dimensioned sufficiently shallow (typically 50 to 500 μm), a liquid membrane forms, dividing the structure in two fluidically separate compartments a and b (Fig. 2 a).

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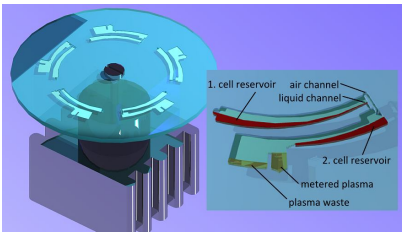

Figure 1: Setup of the test point, consisting of motor and disk with separation structures. The insert shows the design of the separation structure.

This gas pressure difference ΔP_{ab} deflects the liquid membrane towards the air channel. Once the membrane reaches the air channel, liquid enters the channel and the membrane breaks, leaving a liquid plug in this channel (Fig. 2b). In a given window of rotational frequency, the liquid plug is stabilized by the interplay of centrifugal pressure and gas pressure $\Delta P_{ab,}$ which also stabilizes the liquid residing in the liquid channel. During this phase, and despite the substrate being rotated, no liquid is transferred between the two chambers (stopped-flow phase), but heavier components present in the liquid will sediment against the outer walls of the chambers. Increasing the spinning frequency increases the centrifugal force on both, the liquid plug contained in the air channel and the liquid residing in the liquid channel. Once a given frequency threshold is reached the balance between the two independent liquid columns is disturbed and liquid will flow radially outwards from one of the channels, while simultaneously air escapes quickly in the opposite direction via the other channel to compensate for the gas pressure increase. Depending on the ratio of liquid to chamber volumes this process may occur once or several times until all liquid has been displaced from the inner to the outer chamber. (Fig. 2c).

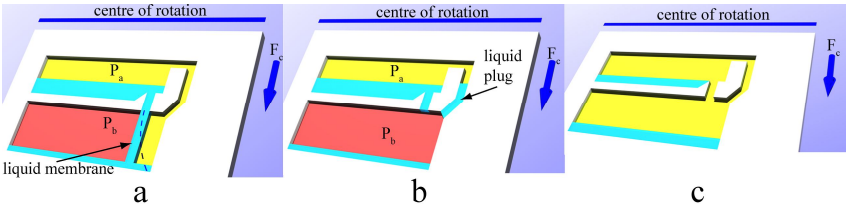

Figure 2: Principle of membrane deflection for flow control. Under rotation liquid flows from the inner to the outer chamber, forming a membrane that divides the structure in two compartments with pressures P_a and P_b, respectively. The pressure difference deflects the membrane along the dashed path (a). When the liquid membrane reaches the air channel it disrupts, leaving a liquid plug in the channel and the flow stops (b). Increasing the spinning frequency destabilises the previous balance and liquid is displaced to the outer chamber (c).

The chambers are designed such that during the stopped-flow phase all cellular components sediment in designated compartments (1. cell reservoir and 2. cell

reservoir, Fig. 1), allowing only the plasma to overflow to a metering chamber once the flow resumes.

EXPERIMENTAL

All structures used in this work have been produced engraved and cut by CO_2 laser ablation of PMMA cast sheets (Repsol, Spain). Engraved disk substrates were subsequently sealed to standard DVD halves using a 30 micron thick heat activated bonding film (Elga Europe, Italy). Figure 3 shows the steps of the blood separation process.

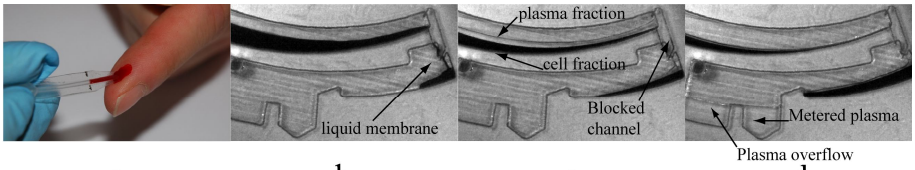

Figure 3: Blood is drawn directly from a fingertip (a) and filled in the separation structure. Then all inlets are sealed. The disk is spun at 50 Hz and a liquid membrane forms that bends towards the air channel (b). Once the membrane reaches the air channel it disrupts, leaving a liquid plug blocking the channel and stopping the liquid flow. The system is kept in this state for 60 s to perform the full separation of plasma and cells (c). After the separation has been performed, the disk is accelerated to 85 Hz in order to remove the liquid plug and resume flow. Plasma overflows to the plasma chamber and is metered (d).

RESULTS AND DISCUSSION

With this structure up to 80 % of the total plasma content was extracted in less than 2 minutes. The amount of residual erythrocytes was below 17 cells per µl of extracted plasma, demonstrating the suitability of the structure. Our structure meters the plasma and provides 1 µl of plasma for subsequent analysis steps.

CONCLUSIONS

We have developed a novel, compact blood separation structure for centrifugal lab-on-a-chip systems based on flow-induced membrane deflection. Starting with initial whole blood volumes of typically 5 µl, our structure meters 1 µl of highly pure plasma for subsequent assay steps. Furthermore the here presented device uses a novel membrane deflection mechanism that can be used to control liquid flow in centrifugal microfluidic networks.

REFERENCES

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