EFFICIENT LEUKOCYTE ISOLATION BY DENSITY-GRADIENT CENTIRFUGATION VIA DUAL-CHAMBER PNEUMATIC SIPHONING

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ABSTRACT

In this paper we present a centrifugo-pneumatic siphon valving (CPSV) with a novel dual pneumatic chamber which is tailored for use with a blood processing method, Density Gradient Centrifugation (DGC). This new valve geometry stabilizes the position of the phase interface at high spin rates; thus permitting efficacious layering of blood onto a density gradient medium (DGM).

KEYWORDS: Lab-on-a-Disc; Centrifugo-pneumatic siphon valves; blood processing; density gradient medium.

INTRODUCTION

The centrifugal microfluidic Lab-on-a-Disc (LoaD) concept has shown particular benefits to blood processing [1-5]. DGC, a common bench-top method for blood processing, has been adapted for these LoaD systems [3-5]. In this technique whole blood is initially layered on top of a DGM (e.g. Ficoll). In a centrifugal field (typically above 400g), the denser red blood cells (RBCs) sediment through the DGM while the white blood cells (PBMC layer) and plasma remain stratified above.

As all liquids are exposed to the same centrifugal field, the availability of efficient valving techniques is key for LoaD platforms. A wide variety of techniques have been developed including externally actuated valves [1]. However, rotationally actuated valves – where varying the centrifugal force experienced by the fluid actuates the valve – are most common in centrifugal microfluidics [6]. CPSVs, which are a low-pass valve actuated through a reduction in rotational speed, offer a number of advantages compared to other schemes. For example, as the siphon is primed using energy stored in the compressed gas chamber, no hydrophilization of the siphoning channel is required; thus reducing cost of manufacture and enhancing shelf life [7]. As these valves are low-pass, they will remain closed at the high spin-rates associated with DGC and will be opened when the rate of rotation is dropped below a certain threshold. Recently, we used CPSV valving for peripheral blood mononuclear cell (PBMC) extraction [4]; we actuated two siphon valves using a single pneumatic chamber, thus allowing us to split whole blood into its constituent components (plasma, PBMCs and RBCs).

OPERATION AND THEORETICAL MODELLING

In this former implementation we used a CPSV with a conventional, contiguous pneumatic chamber. During blood loading, the increased pressure head in the sedimentation chamber displaced DGM into the pneumatic chamber; this moved the PBMC radially outward, resulting in sample loss as blood was unevenly layered into the DGM. To maintain the stability and location of the DGM-blood interface during blood loading, we therefore developed a dual-chamber design (Fig. 1). The first, smaller chamber fills with displaced liquid even at low spin-rates. The much larger second chamber provides sufficient compression volume, but stays free of liquid as it is connected through a narrow and long, radially inbound channel. Hence, above a critical rotational frequency, the fluid interface remains stable and will not be displaced; this in turn permits more efficient blood loading.

The compression of gas and displacement of liquid into the pneumatic chamber can be modeled based upon Boyle's law [6] considering the geometry of the structure. At the equilibrium between the rotationally induced centrifugal pressure head and the pneumatic counter pressure, we establish a relationship for the frequency, ω,

$$\rho \bar{r} \Delta r \omega^2 = \left(\frac{V_T}{V_T - V_D} \right) P_T \quad \Rightarrow \quad \omega = \sqrt{\frac{\left(\frac{V_T}{V_T - V_D} \right) P_T}{\rho \bar{r} \Delta r}} \qquad (1)$$

at which the disc must rotate to displace liquid volume, V_D, into the pneumatic chamber. The pneumatic chamber has a defined total volume, V_T, and has initial pressure, P_T, (assumed to be atmospheric). As the volume of displaced fluid, V_D, entering the pneumatic chamber is equal to the fluid displaced out of the sedimentation chamber, this volume can be related, using the geometries of the sedimentation and pneumatic chambers, to the position of the fluid interface in the sedimentation chamber (h_U) and the position of the fluid interface in the pneumatic chamber (h_L). Thus, the mean radial position $\bar{r} = 0.5 \cdot (h_U + h_L)$ and liquid level difference $\Delta r = h_L - h_U$ are a function of the displaced volume, V_D. Therefore, variations in interface position, h_U, can be derived from the volume of fluid displaced V_D, and in turn, using Eq. 1, related to the rotational frequency, ω, required to reach an equilibrium condition (Fig 2(a)).

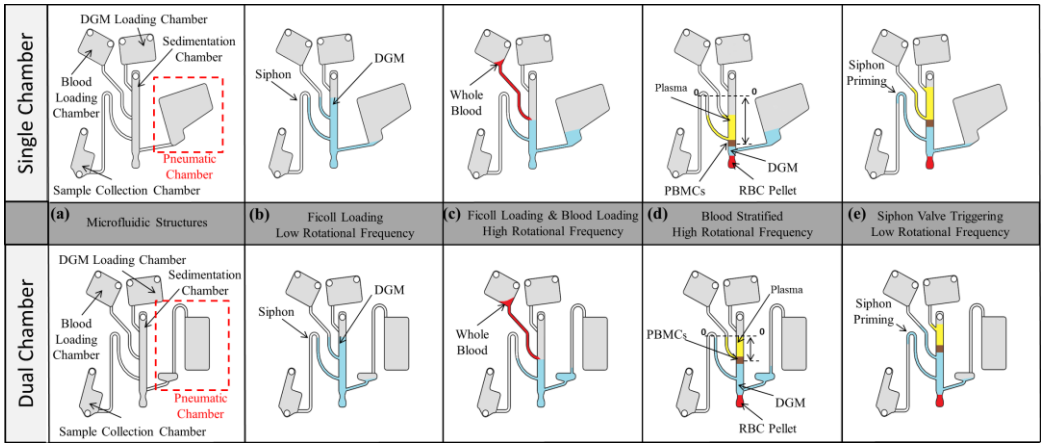

Figure 1: Blood stratification process in chambers with single (top row) and dual (bottom row) pneumatic chamber configurations. Note the different locations of the (brown) PBMC layer relative to the datum location at high spin speeds (d). In the conventional CPSV configuration the PBMC layer is offset below the outlet valve. In the split-chamber design the change in PBMC location is limited as only a minimal liquid level must be displaced to allow the system to reach equilibrium.

MATERIALS AND METHODS

The microfluidic disc was fabricated from six layers, three of 1.5mm thick plastic (PMMA) and three layers of pressure sensitive adhesive (PSA). Chambers are created by removing material from the central layers of PSA and PMMA. Microchannels to connect chambers are defined in the top PSA layer. Microchannels were backed by a second PSA layer to increase hydrophilicity of the microchannels and also to improve optical contrast of images acquired.

To test with blood, 25 µl of DGM (Ficoll) is preloaded in sedimentation chamber. Next, 18 µl of whole blood (diluted 1:1 with buffer) is layered on the DGM at high rotational frequencies (accelerating rapidly to 60 Hz). As the blood loads, the air in the pneumatic chamber is compressed and DGM enters the pneumatic chamber to lower the DGM interface. In the case of the split-pneumatic chamber, as the disc passes 40 Hz, the fluid interface height stabilizes and cannot be displaced significantly downwards. For the single pneumatic chamber, the DGM-blood interface is further moved as the blood is loaded (Figure 1). For each configuration, reducing the rate of rotation triggers the CPSV, resulting in the siphoning of PBMCs and plasma into the sample collection chamber.

The new valve configuration was also validated using dyed water (Figure 2). To simulate DGC, the chamber was initially loaded with 40 µl of liquid. This volume fills the sedimentation chamber without priming the siphon valve. The disc was then accelerated to 60 Hz and then decelerated in steps of 5 Hz. At each frequency an image of the disc was acquired. These images were later analyzed (ImageJ) to determine the position of the fluid interface height, $h_{U,}$ in the sedimentation chamber.

RESULTS AND DISCUSSION

As shown in Figure 2(a), the performance of the dual-chamber CPSV valve agrees well with theoretical modeling. The reduction in the aspect ratio of the pneumatic chamber – in this paper effected by using a microchannel to link two distinct chambers – results in a rapid change in fluid interface height, $h_{L,}$ with minimal liquid displacement into the pneumatic chamber. Once the rate of rotation is sufficient to displace fluid into this constriction, there is minimal additional fluid displacement required at higher rate of rotation for the system to reach an equilibrium state.

In the DGC blood processing method, this aspect of performance has a number of advantages. Initially, the displacement of the defined volume of DGM into the pneumatic chamber results in the creation of a very stable DGM-blood interface (Fig. 3). As the interface is stable despite the introduction of additional blood, this allows a very controlled layering of blood; a critical aspect for efficient DGC. Additionally, as the pneumatic chamber is pre-filled with DGM before RBCs begin to sediment, minimal additional DGM liquid is displaced into the pneumatic chamber during blood layering; thus RBCs will not be displaced into the pneumatic chamber. In the configuration presented here, this, in turn, ensures

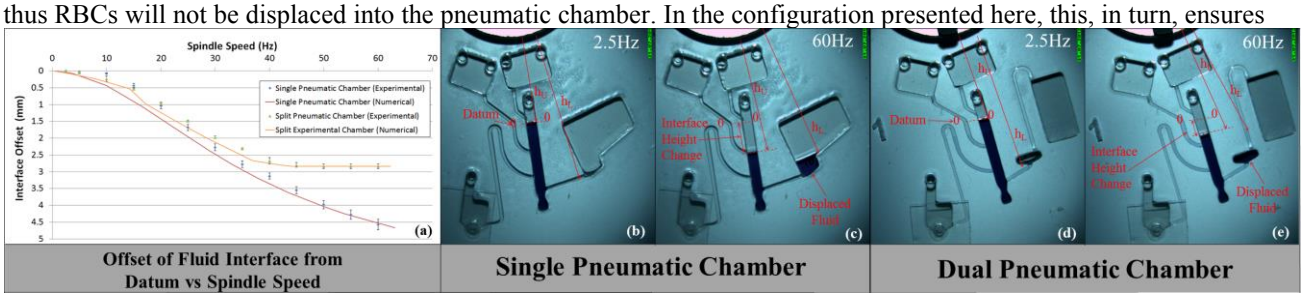

Figure 2: Experimental measurements (n = 4) and numerical simulation using water (dyed for visualisation). (a) The interface height change in the sedimentation chamber from the datum height is calculated numerically and measured experimentally. Above 40 Hz the split pneumatic chamber reaches an equilibrium condition. (b-e) Relative liquid levels at low and high centrifugal forces in both chamber configurations. Distances h_U and h_L are measured from the centre of rotation.

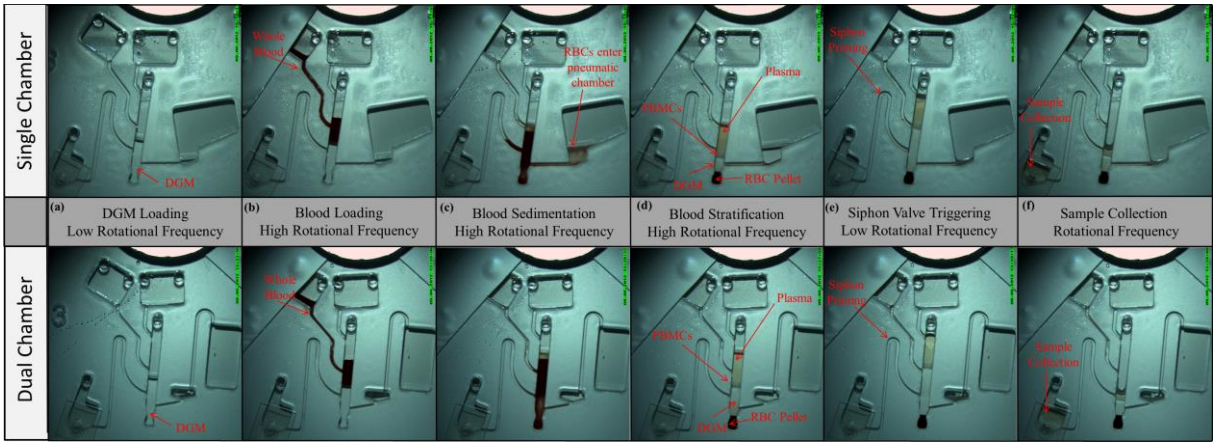

Figure 3: Comparison of blood centrifugation in configurations with single and split (dual) pneumatic chambers. Note that in the single chamber design (top) RBCs enter the pneumatic chamber due to increased liquid displacement (c). This does not occur in the dual pneumatic chamber design (bottom). In Fig 3(d) note the different PBMC locations in the two designs above and below the siphon outlet.

that RBCs will not be ejected from the pneumatic chamber into the sedimentation chamber during valve priming. Finally, during blood processing, the PBMC layer is held in a defined location rather than being displaced downwards (to the extent it can often be displaced below the outlet micro-channel).

SUMMARY AND OUTLOOK

In addition to improved loading efficiency, the dual-chamber design offers further advantages. From an aspect of microfluidic integration, increasing the volume of the secondary chamber without changing the first, smaller chamber allows the total volume of the pneumatic chamber to be increased (thereby enabling liquid loading and valve actuation at lower centrifugal force) without changing the geometry of the sedimentation chamber or the displacement of liquid in the system. Moreover, the secondary chamber can be located arbitrarily far away from the sedimentation chamber, thus making best use of valuable disc space.

Our new, dual-chamber CPSV-based blood processing structure offers increased stability of blood stratification (leading to enhanced extraction performance), increased design flexibility (by permitting use of remote sections of precious disc real estate) and improved operational flexibility (by permitting easy tailoring of valve actuation frequencies). Additionally, by avoiding the loss of RBCs into the pneumatic chamber, it could be possible to add secondary measurements such as hematocrit into this structure. In the future we hope to incorporate this optimized blood processing structure into integrated LoaD cartridges for applications such as whole blood differential counting and HIV diagnostics [8].

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