CONCENTRATION OF WHITE BLOOD CELLS FROM WHOLE BLOOD BY DUAL CENTRIFUGO-PNEUMATIC SIPHONING WITH DENSITY GRADIENT MEDIUM

David J. Kinahan, Macdara T. Glynn, Sinéad M. Kearney, and Jens Ducrée
Biomedical Diagnostics Institute, National Centre for Sensor Research,
School of Physical Sciences, Dublin City University, Ireland

ABSTRACT

Due to the pervasiveness of HIV infections in developing countries there exists a need for a low-cost, user-friendly point-of-care device which can be used to monitor the concentration of T-lymphocytes in the patient's blood expressing the CD4+ epitope. As a first step towards developing a microfluidic "lab-on-a-disc" platform with this aim we present the concentration of white blood cells from whole blood using a density medium in conjunction with centrifugo-pneumatic siphon valves [1]. Two such valves are actuated simultaneously, removing the bulk of plasma through the upper valve and the bulk of WBCs through the lower valve while leaving the vast majority of red blood cells in the centrifugal chamber.

KEYWORDS

HIV, Lab-on-a-Disc, White Blood Cells, CD4+ count, Centrifugo-Pneumatic Siphon, Density gradient centrifugation

INTRODUCTION

Although there is no vaccine for the prevention of HIV infection, the subsequent onset and severity of AIDS can be alleviated with Antiretroviral Therapy (ART). The ART induction guidelines issued by the World Health Organization (WHO) are based on monitoring the concentration of T-lymphocytes in the patient's blood expressing the CD4+ epitope [2]. In developed countries this is a standard procedure which uses commercial cell counters. However, these costly instruments require extensive supporting lab infrastructure and skilled operators. Hence there exists a need in the developing countries where HIV is endemic (particularly in rural areas) for a fast and accurate point-of-care device which can monitor the concentration of CD+ T-cells with minimal demands on the lab environment and operators [3]. Along with the clear medical and economic advantages to be derived from such a platform, recent studies have shown that the introduction of point-of-care testing significantly reduces the loss of patients to follow-up care [4-5].

Generally, the first step in evaluating CD4+ content is the purification of the white blood cells (WBCs) from patient's whole blood. This is most efficiently carried out by segregating the WBCs using density gradient centrifugation (DGC) protocols. Therefore the lab-on-a-disc paradigm is particularly suitable for adaptation of these techniques. Such centrifugal microfluidic platforms have been used for a broad number of biomedical applications including sample preparation, analyte detection, nucleic acid amplification (PCR) [6] and cell analysis [7].

The feasibility of DGC for concentration of WBCs on a disc-based platform has previously been demonstrated. Schaff *et al.* [8] used fluorescence to estimate the concentration of white blood cells. Park *et al.* [9] used thermally activated ferro-wax valves to separate the component parts of whole blood. However, opening these valves necessitated the disc to halt and the valves to be heated using a complex instrument equipped with an infra-red laser.

OPERATING PRINCIPLE, MATERIAL AND METHODS

In this work present the concentration of WBCs from whole blood using centrifugo-pneumatically primed siphon valves. These rotationally actuated valves have proven to be more reliable and less dependent on surface treatment and interfacial tension effects than conventional siphons [1,10]. In addition, they do not require external intervention to activate and they are very compatible with mass manufacturing techniques such as injection molding.

As demonstrated in Figure 1(a) fluid is loaded into the sedimentation chamber at a low rotational frequency (low Relative Centrifugal Force (RCF)). The rotational frequency is then increased (Fig 1(b)) such that the centrifugal force suppresses capillary flow and the siphon valve does not prime. At these RCF values air in pneumatic chamber is compressed and fluid partially fills this chamber. Thus the fluid height is lowered below the siphon crest height. Following completion of the centrifugation protocol the rotational frequency is reduced (Fig 1(c)) such that air in the pneumatic chamber expands and the fluid level rises above the siphon crest. The siphon valve primes and fluid exits the sedimentation chamber (Fig 1(d)).

The microfluidic disc was fabricated as shown in Figure 2(a) from six layers, 3 of PMMA and 3 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 by 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.

Figure 2(b) is a schematic of the microfluidic structure. Two separate loading chambers are used, one for Ficoll density gradient media (Sigma Aldrich) and one for whole blood (diluted 1:1 with buffer). Ficoll is pre-loaded such that it reaches the level of the blood inlet. This allows for efficient overlay of blood on the media in line with standard Ficoll protocols. The pneumatic chamber is connected to the sedimentation chamber by a microchannel. The dual siphon valve

system is configured such that following centrifugation and on actuation the lower valve removes WBCs, Ficoll and some plasma to one collection chamber while the upper valve removes the bulk of the plasma to the second collection chamber. The red blood cells (RBCs) remain in the sedimentation chamber.

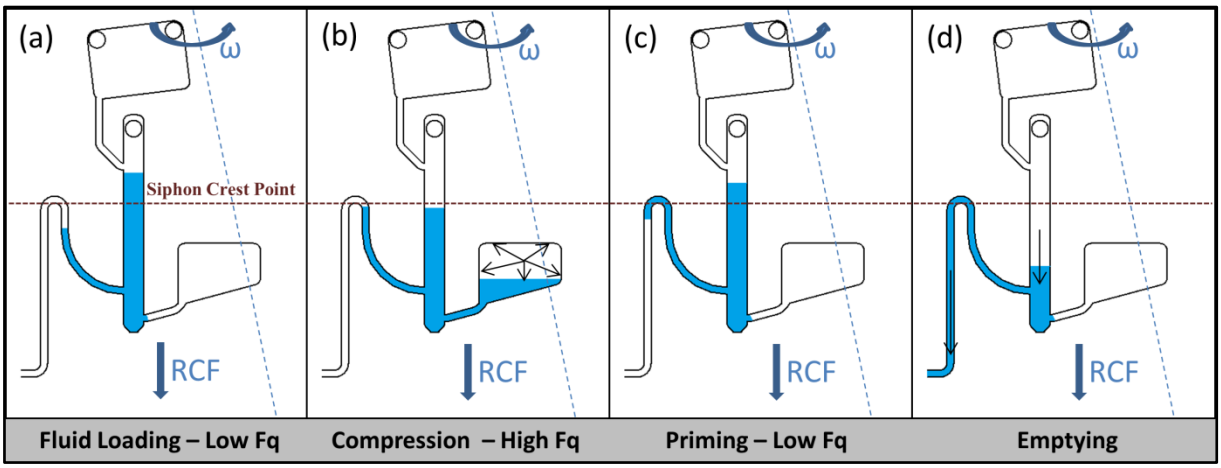

Figure 1. Operational principle of the centrifugo-pneumatic siphon.

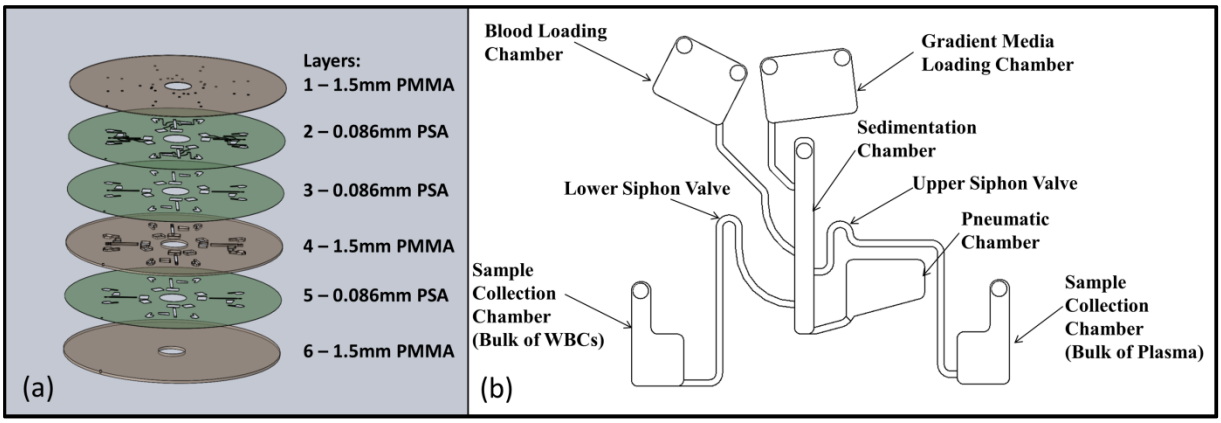

Figure 2. Schematic of WBC purification disc structure.

As shown in Figure 3(a), 25 µl of density gradient medium (Ficoll) is preloaded in sedimentation chamber. Next, 18 µl of whole blood (diluted 1:1 with buffer) is layered on the Ficoll at high rotational frequencies (Fig. 3(b)). As the blood loads, the air in the pneumatic chamber is compressed and some Ficoll media enters the pneumatic chamber. This drops the fluid height below the crest point of the siphon. The rotational frequency is then increased such that the bottom of the sedimentation chamber experiences an RCF of 400 g (Fig. 3(c)). During this step, the RBCs migrate to the bottom of the chamber. Plasma and WBCs, which are buoyant in Ficoll, create distinct stratified bands (Fig. 3 (d))

After centrifugation is completed, the rotational frequency, and thus the pressure head, is reduced so the air residing in the pneumatic chamber expands and pushes the fluid level above the siphon crests (Fig. 3(e)). The siphon valves are primed simultaneously and the fluid drains into the collection chambers (Fig. 3(f)). The upper siphon valve removes plasma to a designated collection chamber (Fig. 3(g)) while the WBCs, some plasma and some Ficoll is removed into the second collection chamber through the lower siphon valve (Fig 3(h)). The RBC component of the whole blood remains in the main sedimentation chamber.

RESULTS AND CONCLUSIONS

We have presented a new method where two centrifugo-pneumatically primed siphon valves are actuated simultaneously using the same pneumatic chamber. This valving strategy offers a number of key advantages compared to other available techniques As the siphons are pneumatically actuated it allows us to manufacture our disk from a hydrophobic material such as PMMA. Conventional siphon valves would require the use of a naturally hydrophilic material (which could promote cell adhesion), the addition of surfactants to biological samples or the application of a hydrophilic surface treatment to the material (which in some cases can degrade over time). In addition cetrifugo-pneumatically primed siphon valves do not require any external instrumentation for actuation, are amenable to mass production techniques such as injection molding and are compatible with the RCF forces required by the established DGC technique.

A significant disadvantage of centrifugo-pneumatically primed siphon valves is the surface area occupy on a disc. This can be particularly critical for highly integrated sample-to-answer systems where disc real estate can be a precious.

The use of a single pneumatic chamber to simultaneously actuate two valves allows increased concentration of the WBC layer (due to simultaneous plasma removal) while making a significant space saving over a cascaded system (where plasma and WBCs are could be isolated sequentially). In the case presented here the design precondition to have both siphon crest points at the same radius requires the upper siphon valve to take a circuitous route around the pneumatic chamber. This can be mitigated – and indeed the entire design be made smaller – in future designs by employing 3D type structures where microchannels and reservoirs are integrated into different vertical layers.

The density gradient centrifugation method presented here constitutes a key sample preparation step towards developing an integrated, easy-to-use, rugged and cost-efficient lab-on-a-disc diagnostic platform for accurate monitoring of CD4+ concentration for application in developing countries.

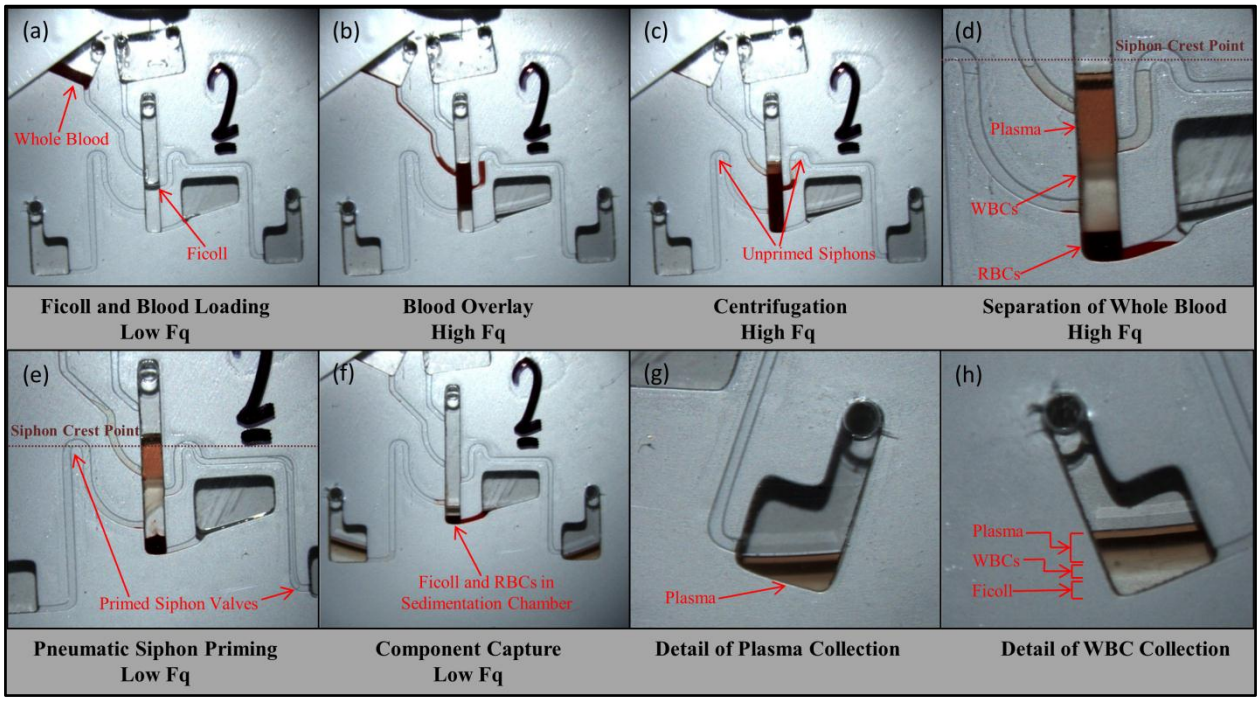

Figure 3. WBC concentration using the novel, dual siphon valve structure.

ACKNOWLEDGMENT

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CONTACT

David Kinahan, david.kinahan@dcu.ie, Jens Ducrée, jens.ducree@dcu.ie