CD4 CELL ISOLATION FROM BLOOD USING FINGER-ACTUATED ON-CHIP MAGNETOPHORESIS FOR RAPID HIV/AIDS DIAGNOSTICS

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ABSTRACT

With timely diagnosis and correct treatment, people living with HIV/AIDS can consider the disease as a chronic rather than a terminal illness. Still, in regions were HIV is endemic, rapid diagnosis is a challenge due to the complexity of the instrumentation required, the poor infrastructure in these countries, as well as the technical expertise required to carry out the diagnosis. This paper presents a microfluidic chip based approach allowing semi-quantitative CD4+ cell counting on a cheap, rapid, highly portable and instrumentation-free Point-of-Care HIV diagnostic device. Flow is driven by finger-pressing a flexible reservoir, and the target cells are immobilized through magnetophoresis. The fluidic test completes within ca. 30 seconds of sample application to the chip.

INTRODUCTION

HIV (human immunodeficiency virus) infection and the subsequent development of the associated symptoms know as AIDS (acquired immunodeficiency syndrome) remains an ongoing disease of pandemic proportions since its identification in the early 1980s. Yet, application of modern molecular and cellular instrumentation to the diagnostics of the infection, as well as availability of [highly active] anti-retroviral therapy ([HA]ART) have brought the infection to low penetrance in countries where these technologies and interventions are readily available. However, epidemiological statistics show that low- to mid-income countries consistently maintain the largest viral pool of HIV (Fig. 1), with Sub-Saharan Africa being the most heavily affected (69% of the 34 million global cases in 2011) [1]. Access to rapid and reliable diagnosis in these regions is low due to limited infrastructure, costs and technical know-how. Alternatives to the expensive "gold standard" are currently investigated to allow reliable diagnostics to be deployed in such regions. These "Point-of-Care" (PoC) devices aim to be capable of operating at remote villages with minimal power and personnel. ART can then be immediately delivered to a patient presenting as positive for HIV in these areas.

The target of the HIV virus is the T-helper cells of the patient. Progressive loss of these cells results in the suppression of the immune system and the pathology of AIDS. T-helper cells have a specific protein marker on the cell surface called CD4, and hence CD4 cell counts are a key clinical determinant for diagnosis of HIV patients and the initiation of treatment. While healthy individuals present a CD4 cell count of >1200 cells μl^{-1} in whole blood, common practice is that HIV positive patients begin ART treatment when this number falls below a specified threshold – currently set by the World Health Organization (WHO) at 500 cells μl^{-1} [2]. The ability to identify HIV patients with CD4 cell counts around this threshold is therefore critical. A number of devices are on the market that performs at the required level, but the instruments and/or per-test cost can be large, and the number of tests-per-day can also limit the use of the devices [3].

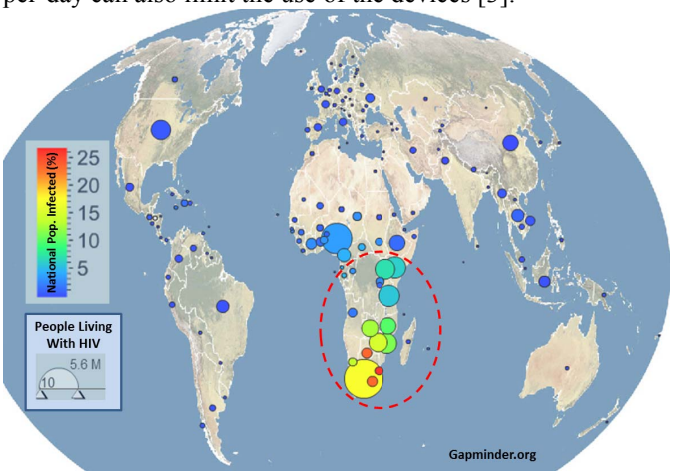

Figure 1: Global snapshot of the HIV pandemic in 2011. The diameters of the circles indicate the population numbers, while the color represents the percentage of a national population living with HIV. The area circled in red contains 50% of all people in the world living with HIV.

We present here a finger-press driven magnetophoretic CD4 cell isolation on an autonomous microfluidic chip for isolating CD4 cells from whole blood within 30 seconds of sample loading. The very simple, minimum operator-skill and instrument-free microfluidic procedure delivers accurate results and thus meets the criteria for deployment in resource-limited regions where HIV / AIDS is largely endemic, and monitoring remains a challenge. The strategy involves the incubation of whole blood with (super)para-magnetic beads that specifically bind to the CD4 epitope of T-helper cells. This sample is then introduced to a flui-dically primed microfluidic chip, and passaged a number of times past a magnetic capture chamber, specifically isolating the CD4 cells to a location removed from the bulk of the blood sample. Critically, the forces needed to drive both the loading of the sample, and the fluidic movements are generated by deflecting a fluidic reservoir connected to the main chamber *via* a flexible membrane. This can be achieved simply by depressing the membrane with the finger of the operator. Following isolation of the CD4 cells, they can be semi-quantitatively enumerated by visual inspection of the capture chamber.

SYSTEM DESIGN

The architecture of the chip displays the pneumatic fluidic reservoir (P1) at the base of the chip. P1 connects directly to the separation channel, a secondary reservoir (P2) is at the top of the separation channel and the sample input port is directly below P2. Midway down the separation channel is the CD4 capture locus. On the opposite side to the capture locus is a vent to allow any overflow to occur during the cell separation process (Fig. 2).

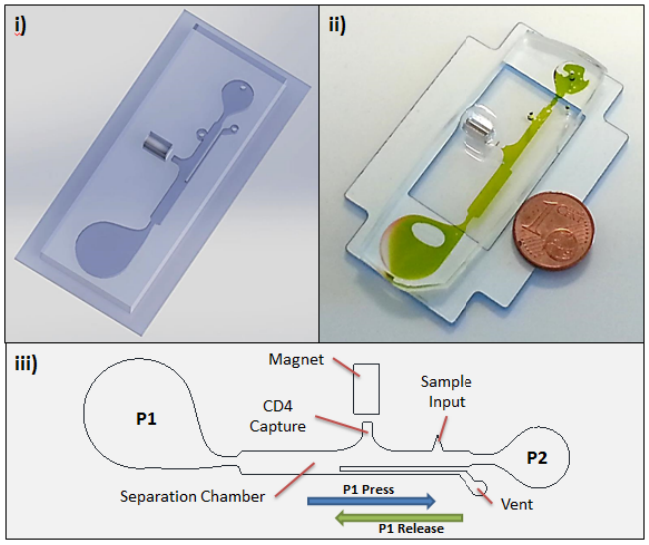

Figure 2: Finger-press actuated magnetophoretic CD4 isolation chip. i) 3D rendering of chip. ii) Image of a chip with the microfluidic features shown using green dye. iii) Schematic showing features of interest. Direction of flow when P1 is depressed or released is indicated with Blue and Green arrows respectively.

Microchannels (40 µm deep) are fabricated in PDMS [4] and adhered to a glass base. A permanent magnet is placed perpendicular to the pneumatically-driven flow of liquid (Fig. 2 iii), and the chamber is filled with priming buffer.

For biosafety reasons, we cannot handle HIV patient blood in our lab. Therefore finger-prick derived whole blood from healthy donors was first depleted of native CD4+ cells. It is then spiked with defined concentrations of fluorescently stained CD4-positive HL60 cells at medically relevant concentrations between 10 and 1000 cells μl^{-1}, and incubated for 3 min with paramagnetic beads specific to the CD4 epitope.

SYSTEM OPERATION

To load the chip, the pneumatic chamber (P1) is first depressed to reduce the internal volume of the chip. 4 µl of sample is applied to the loading inlet and P1 is released (Fig. 3 i-ii). The subsequent reconstitution of its initial volume generates a flow from the sample inlet, past the capture region, and into the P1 reservoir (Fig. 3 iii-iv). The actuation of the second reservoir (P2) compensates any shortfall in liquid volume, insuring that the chamber always remains fully primed. The vast background of non-magnetically tagged cells follows the laminar flow towards P1; only the magnetically tagged, CD4-positive cells are deflected to the base of the flow-free capture structure (Fig. 4). In order to sequester remaining CD4 cells to the capture chamber, P1 is again pressed to drive the sample back towards P2 (Fig. 3 v-vi). By releasing P1 the cycle re-commences. By repeating these steps a second time, a dual-pass actuation will complete in approximately 30 seconds. Quantification of recovered cells was carried out by fluorescence and bright-field microscopy (Figs. 4-5), but the chip is amenable to detection using a handheld LED-based UV-fluorescence detector.

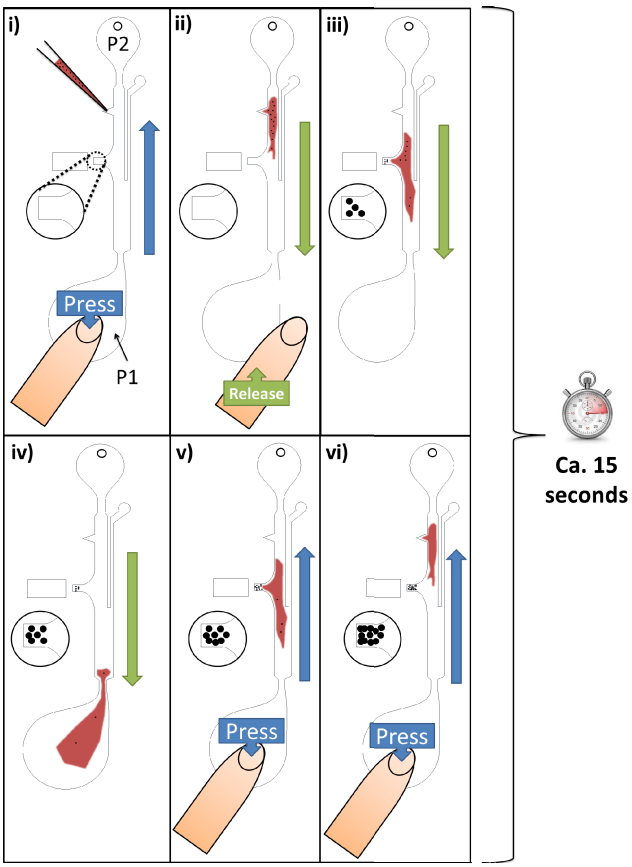

Figure 3: Operation of the magnetophoresis chip to isolate CD4 cells (shown as black circles) from whole blood. Direction of flow pressure is shown with Blue (towards P2) and Green (towards P1) arrows.

METHODOLOGY

Chip manufacture

The microfluidic chips used in this paper were formed from polydimethylsiloxane (PDMS; Dow Corning, MI) mixed at a ratio of 10:1 base and curing agent. The procedures for making a master and for securing the co-rotating magnet in the PDMS have been described in detail elsewhere [4-5]. Loading holes and vents were defined in the PDMS at appropriate locations using a dot punch. The

PDMS slab containing the microfluidic features was placed on a 25 × 60 mm glass coverslip and allowed to bond for 1 min. Finally, the glass / PDMS chip was mounted to a PMMA base. To prime the microchannels and structures, the chip was placed under vacuum for at least 1 hour, following which a large drop of priming buffer (phosphate-buffered saline [PBS] pH 7.4, 0.1% w/v bovine serum albumin [BSA], 1 mM EDTA) was immediately placed on the surface of the PDMS, covering both the sample port of the loading chamber, and the vent. Degas driven flow then primed the channels. Magnets used were NdFeB N45 cylindrical magnets, with a diameter of 3 mm and a height of 6 mm (Supermagnete, Germany). Magnets were placed in the molded cavities before the samples were loaded to the chip.

Blood Processing and Cell Culture

Blood was extracted directly from healthy donors *via* finger prick using 1.5 mm sterile lancets (BD Biosciences, NJ, USA). To prevent coagulation of the blood sample, 60 mM EDTA solution was immediately added to the sample to result in a final concentration of 6 mM EDTA in the whole blood. Blood was isolated and prepared fresh, directly before experimental use. Native CD4 cells were depleted using the T4 Quant Kit (Life Technologies, CA, USA) according to manufacturer's instructions.

HL60 cells (DSMZ, Braunschweig, Germany) were cultured in 75 cm^2 flasks in RPMI 1640 media, with 10% un-inactivated fetal bovine serum, 100 U mL^{-1} penicillin, and 100 µg mL^{-1} streptomycin. Cultures were maintained at 37 °C with 5% CO_2. Where indicated, cells were fluorescently stained with NucBlue Live Cell Stain (Life Technologies) according to the manufacturer's instructions.

Experimental samples composed of blood spiked with HL60 were treated with Dynabeads CD4 magnetic beads (Life Technologies). Incubations were carried out in a 2 mL Eppendorf tube, and final incubation volume was 200 µL per sample, composed of 140 µL incubation buffer (PBS pH 7.4, 0.1% w/v BSA, 1 mM EDTA), 50 µL experimental sample, and 10 µL Dynabead CD4. Incubation was performed at room temperature with rotation for 3 min. 4 µl of sample was introduced to the chip as described.

RESULTS

Following a dual-pass actuation of blood samples spiked with 4 concentrations of HL60 cells, CD4 concentrations as low as 10 cells $µl^{-1}$ in whole blood were detected. Furthermore, in the medically relevant diagnostic range of 10-1000 CD4 cells $µl^{-1}$ of whole blood, the system showed a linear fluorescent signal output corresponding to the concentration of cells. Over this range, the square of the Pearson correlation coefficient (RSQ) was calculated as 0.97 (Fig. 5-i). This suggests that the CD4 concentration in patient blood over diagnostic ranges lie within in the linear range of the chip performance – indicating that the strategy can lead to high predictability of CD4 count using a single data-point.

Furthermore, when the area of the space occupied with beads / cells is measured using the free image analysis software ImageJ (NIH, USA), a similar correlation to HL60 concentration (0.99) is observed (Fig. 5-ii). This suggests that imaging and quantification can be carried out using only bright-field optics.

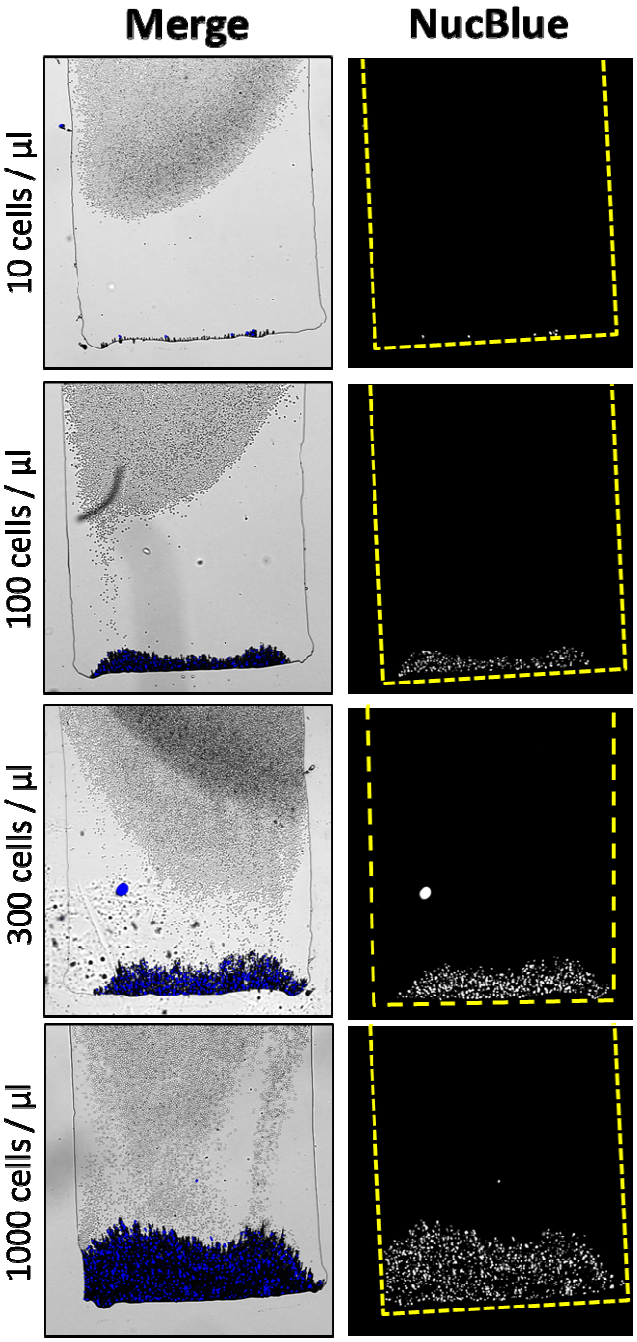

Figure 4: Results of finger-press pumped magneto-phoretic isolation of HL60 cells from CD4-depleted whole blood. Merged DIC / fluorescent images (left), raw fluorescent images (right). The edge of the capture chamber is shown as a yellow dashed line.

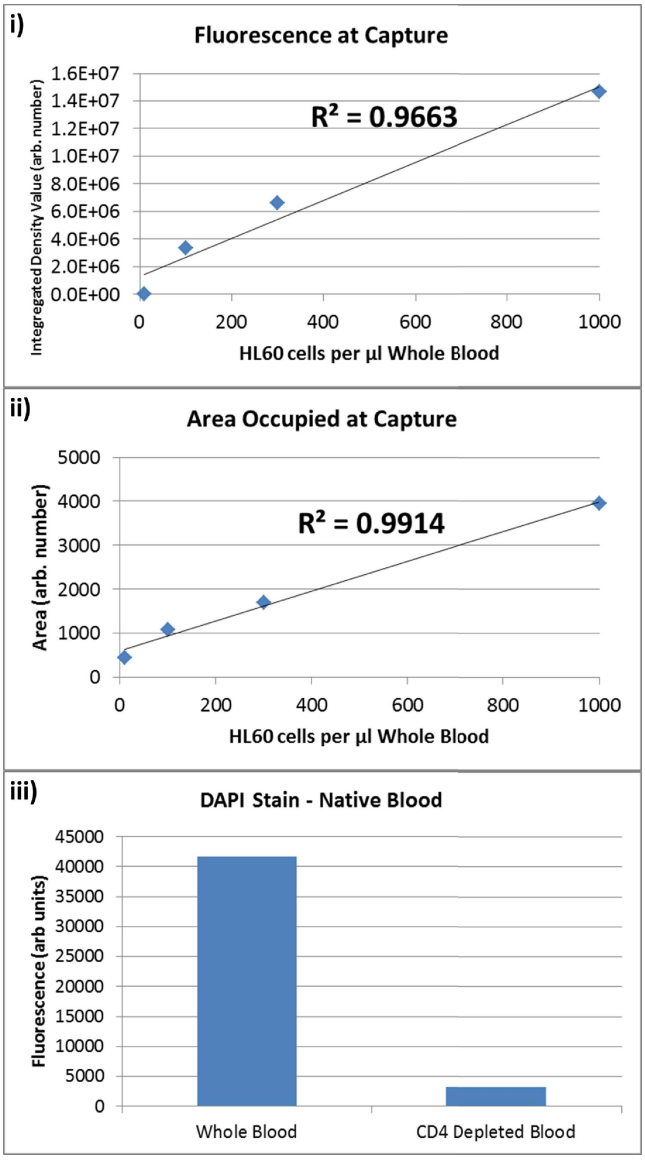

Figure 5: Fluorescent analysis of images from Fig. 4. i) The integrated density values (IDV) from identically sized region-of-interests (ROIs) from the images were calculated and shown relative to the number of HL60 cells spiked to the blood. ii) ROIs were generated on the brightfield images shown in Fig. 4. The area of these were compared to the number of HL60 cells spiked to the blood. RSQ was calculated on i) and ii) to show linearity of the data. iii) UV fluorescence was measured at the capture chambers of chips containing native- and CD4-depleted whole blood treated with DAPI.

In spite of the promising results using area calculation for the quantification of the data, a future analysis instrument may be more accurate using fluorescence imaging on a dark-field background. In this case, to keep the cost-per-test at a minimum, specific staining using expensive antibody based fluorescence markers should be avoided. However, as the chip will exclusively isolate CD4 cells using positive capture, when run under non-spiked native conditions a general UV based white blood stain will be sufficient to allow CD4 specific fluorescent readout without the need for expensive antibody binding reagents. To this end, we co-incubated native whole blood (both CD4-depleted and non-CD4 depleted) with DAPI (1 µg ml^{-1}) concurrently with the CD4 para-magnetic beads, and examined the resulting on-chip isolations under UV. A clear difference was observed in UV signal measured in the capture chamber between native and CD4-depleted blood (Fig. 5-iii).

DISCUSSION AND CONCLUSION

To enable rapid, cheap and simple HIV diagnostics based on CD4 cell count in a finger-prick volume of whole blood, we have developed an autonomous microfluidic chip capable of delivering a semi-quantitative diagnosis of HIV infection. Critically, this chip runs without instrumentation for flow control; and if deployed, would potentially require only a bright-field light source with an associated optical camera similar to that on mid-range mobile phones for readout. Compared to the complexity of devices currently on the market for PoC HIV diagnostics, the strategy presented here would be notably more affordable, albeit without a fully quantitative result. Nevertheless, in most cases, a medical practitioner on site needs only to know if a patient falls into a "treat" or "no-treat" category for the distribution of ART pharmaceuticals. Hence, given the simplicity of the presented device, the semi-quantitative nature of the data generated would be an acceptable caveat.

ACKNOWLEDGEMENTS

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