Lab on a Chip

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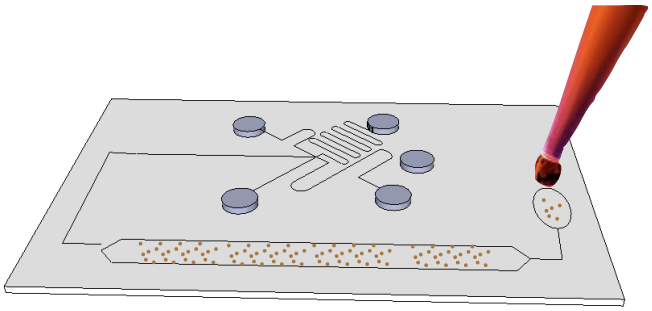
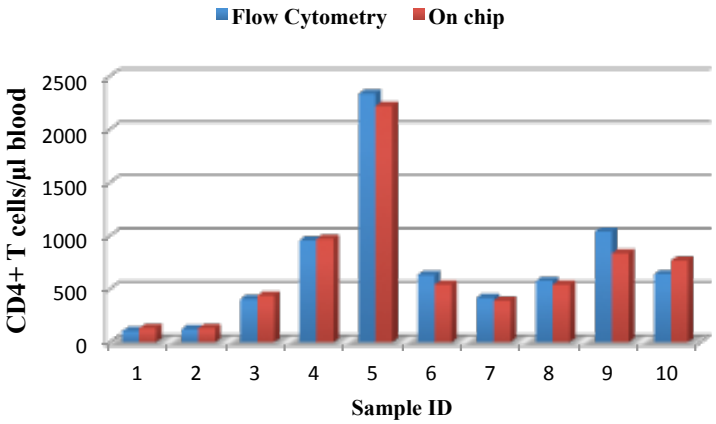
The ARTμS: a novel microfluidic, label-free CD4+ T-cell enumeration system for monitoring antiretroviral therapy in HIV patients

Qian Liu *et al*

Graphical Abstract

An integrated microfluidic device that enumerate CD4+ T-cell by its DNA content





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ARTICLE

The ARTμS: a novel microfluidic CD4+ T-cell enumeration system for monitoring antiretroviral therapy in HIV patients

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We report on a novel and cost-effective microfluidic platform that integrates immunomagnetic separation and cell enumeration via DNA-induced bead aggregation. Using a two-stage immunocapture microdevice, 10 µL of whole blood was processed to isolate CD4+ T-cells. The first stage involved the immuno-subtraction of monocytes by anti-CD14 magnetic beads, followed by CD4+ T-cell capture with anti-CD4 magnetic beads. The super hydrophilic surface generated during Polydimethylsiloxane (PDMS) plasma treatment allowed for accurate metering of the CD4+ T-cell lysate, which then interacted with silica-coated magnetic beads under chaotropic conditions to form aggregates. Images of the resulting aggregates were captured and processed to reveal the mass of DNA, which was used to back-calculate the CD4+ T-cell number. Studies with clinical samples revealed that the analysis of blood within 24 hours of phlebotomy yielded the best results. Under these conditions, an accurate cell count was achieved (R^2=0.98) when compared to cell enumeration via flow cytometry, and over a functional dynamic range from 106-2337 cells/µL.

Introduction

CD4+ T-cells in blood are the primary target of human immunodeficiency virus (HIV) [1]. In general, the CD4 count of a healthy adult/adolescent ranges from 600 to 1200 cells per microliter of blood, with the median of 828 cells/µL [2]. Infection with HIV results in fewer CD4+ T-cells, and the cell count needs to be monitored every three to six months after the patient is diagnosed as HIV positive in order to determine the eligibility for antiretroviral therapy (ART). CD4 level needs to be monitored during the ART to evaluate the effectiveness of treatment. According to the new HIV treatment guidelines provided by the World Health Organization (WHO) in June 2013, it is recommended that HIV patients start ART when their CD4 count falls below 500 cells/µL. In some special cases, for example, pregnant women, HIV-positive partners in serodiscordant couples, children younger than five, and people with HIV-associated Hepatitis B and tuberculosis, treatment should begin immediately [3]. According to the US Center for Disease Control (CDC), patients progress to stage 3 infection, i.e., acquired immune deficiency syndrome (AIDS) when they have a CD4 count below 200 cells/µL [4].

In the developed world, flow cytometry is the gold standard for CD4+ T-cells enumeration; however, it is not widely available in resource-limited areas where more than 85% of HIV patients live, primarily due to high cost, poor portability, and the sophisticated nature of the required training [5]. In 2012, 9.7 million people in low and middle-income countries received antiretroviral therapy, representing 61% of those who were eligible under the 2010 WHO HIV treatment guidelines. The Joint United Nations Program on HIV/ Acquired Immune Deficiency Syndrome (UNAIDS) aims to treat 15 million people currently living with HIV with antiretroviral treatment by 2015 [3]. While ART becomes more accessible, the diagnostic tests involved in monitoring the progression of HIV via CD4+ T-cells counts remain a hurdle due to the lack of lab capacity for CD4 - a major obstacle in accelerated HIV treatment scale-up [3]. Point-of-care (PoC) CD4 analysis can increase the number of people receiving treatment, as well as

the effectiveness of the treatment due to earlier diagnosis. A recent study has shown that after the introduction of a point-of-care CD4 device in Mozambique, the total loss to follow-up before initiation of ART dropped from 64% to 33%, and the enrolled patients for ART increased from 12% to 22% [6].

Effort has been exerted to define an affordable, less complicated alternative to standard flow cytometry. Some single-purpose flow cytometers have been specially designed for CD4 counting, such as the Becton Dickinson FACSCountTM and Guava Easy CD4™. While the instruments themselves are available at a reduced cost, the reagent cost remains the same, the fluorescent reagent involved is still difficult to store and transport, and the technical skill

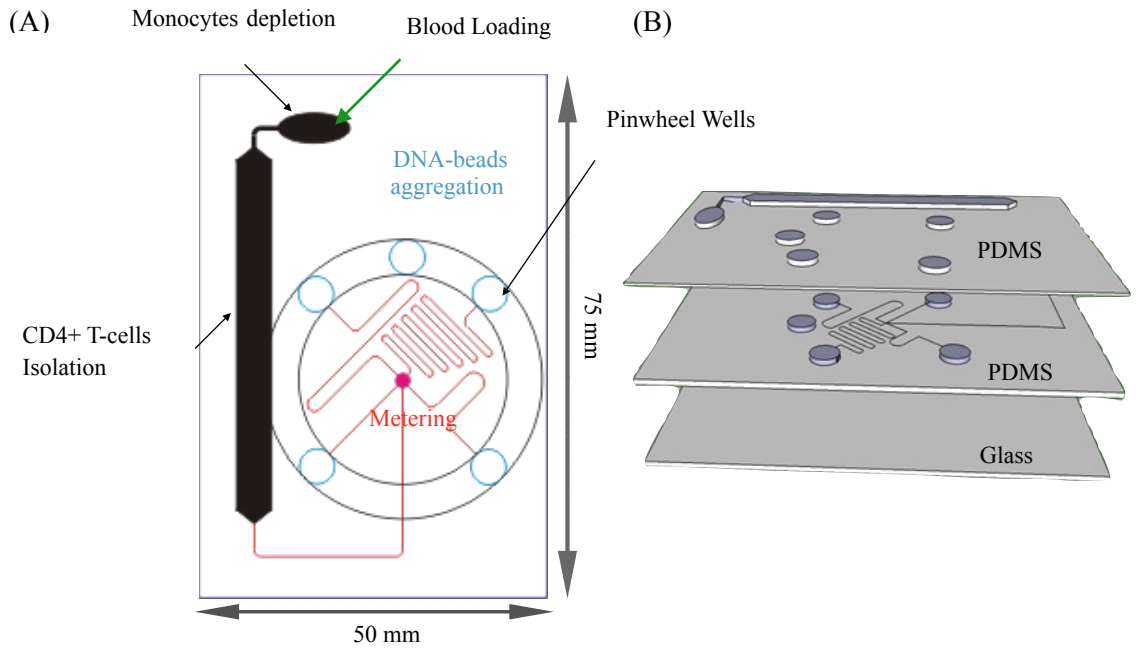

Figure 1. CD4+ T-cell counting chip design. (A) The microchip contains two domains: an immunocapture domain (black) and a DNA quantitation-cell counting domain (blue), connected by the metering region (red). The immunocapture domain consists of 2 chambers, for monocytes depletion and CD4+ T-cells isolation respectively. The isolated CD4+ T-cells were lysed in the same chamber, and the lysate were pumped into the metering region. The DNA quantitation-cell counting domain include 4 wells for metered cell lysate, and 1 for negative control (solely magnetic beads). (B) Side view. The device is fabricated using two layers of PDMS and one layer of glass.

requirement is high [7]. Alternatives outside flow cytometry have also been developed and recently reviewed [8]. In the microfluidic setting, fluorescence and quantum dots have widely been utilized as detection modes. Since quantum dots enhance fluorescence detection, the optical requirements for these systems are reduced [9-12]. Chemiluminescence is another useful tool to avoid complicated optics; with this approach, cells are first captured by anti-CD4, then sandwiched by a second antibody directed at CD3, which is attached to an enzyme that induces chemiluminescence. The luminescence generated is detected by a silicon photo detector and is converted to a photocurrent [13]. Watkins *et al.* developed an impedance-based microchip [14], where the CD4 capture chamber was functionalized with antibodies. Impedance pulses at the inlet count all particles, while pulses at the outlet count those not captured (i.e., non-CD4 cells), with the subsequent difference being the CD4 count. Mechanical sensors have been developed by functionalizing a quartz crystal sensor with anti-CD4 and, as CD4 cells bind, the mass of the quartz crystal is increased, thereby changing its resonance frequency, and the change can be correlated to a CD4 count [15]. Other proposed techniques include isolation of CD4 cells by anti-CD4 bound to magnetic beads and focused in a capillary tube; the height of the beads can be correlated to the cell count [16]. Macdara et al. have recently developed a portable finger driven device with a similar concept [17]. A 'lab on DVD' system has also been presented for CD4+ enumeration [18]. There are many excellent reviews on this topic [31-33].

Most of the existing point-of-care CD4 counting devices are based on counting whole cells, i.e., the CD4+ T-cells isolated by antibodies and then enumerated by various detection mode. To our knowledge, only one method has been described that doesn't count intact T-cells [19]. Cheng et al. isolated CD4+ T-cells isolated in a microfluidic device and, through lysis,

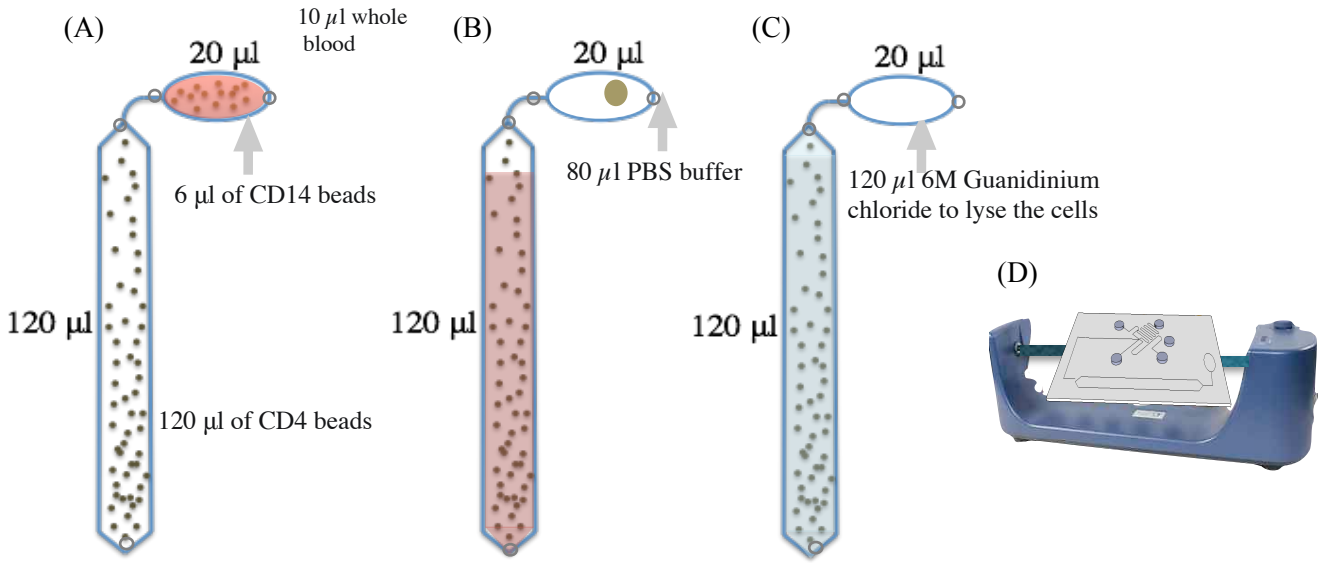

Figure 2. CD4+ T-cells isolation using antibody-coated magnetic beads. The Dynabeads® T4 Quant Kit was optimized for on chip cell isolation. (A) The two chambers were coated with CD14 beads and CD4 beads respectively, by loading the bead solution and then removing the liquid while holding the beads in place with a magnetic manifold. A total of 10 μl whole blood was pipetted in. The T-cell isolation was achieved by loading the chip on a lab tube rotator to enhance the interaction between the beads and cells. 6 μl of CD14 beads and 10 μl of whole blood were mixed for 10 minutes in the first chamber. (B) 80 μl PBS buffer was pipetted in the chip while holding the monocytes saturated beads by a magnet, the monocytes saturated cd14 beads were guided by a magnet to the outlet, then sucked up by paper. Then and the solution was mixed for another 10 minutes in the second chamber. After the CD4+ T-cells were isolated, 3 to 5 aliquots of 200 μl PBS buffer was loaded to wash away RBCs and unwanted WBCs. (C) 120 μl of 6M guanidinium-HCl solution was introduced into the CD4 chamber to lyse the captured CD4+ T-cells, the cell lysing and mixing was achieved by loading the chip on the same lab tube rotator for another 10 minutes. (D) Illustration of the chip mounted on a lab tube rotator for mixing. The released cell lysate was then metered and quantitated by the DNA-beads aggregation assay.

released and quantitated the intracellular ion concentration using impedance spectroscopy [19]. To go beyond the cell level, although adding one more step of lysing, could simplify the detection mode and reduce the assay cost. Over the last few years, we have been reporting on the 'pinwheel effect', a phenomenon that involves the quantitative chaotropic-driven adsorption of DNA onto paramagnetic silica in a rotating magnetic field (RMF), and its application to DNA quantitation and cell enumeration [20-23,34]. Simple image analysis of the induced bead−DNA aggregation allows for direct quantification of DNA in crude biological samples. The main advantage of this method is the cost, the DNA-bead aggregation requires only commercially-available silica-coated magnetic beads, which is much more cost-effective compared to fluorescence reagents or chemiluminescence method. Additionally, the core hardware for detection is an inexpensive camera, and we are in the process of swapping these out for Android cell phone cameras (as shown in our previous publication [21]) which enhances device portability for possible point-of-care use.

Here, we report on the **A**utomated **R**esult for **T**-cell counting **μ**fluidic **S**ystem (ARTμS), a microfluidic device that quantitates CD4+ T-cells based solely on DNA content, in concert with an integrated two-step immunomagnetic separation, on-board metering, and the pinwheel assay. The analysis of blood from HIV- or non-infected patients shows a strong correlation (R^2=0.98) in a wide dynamic range with the values generated by the gold standard, flow cytometer.

Experimental

There are 4 steps overall

1. Extract CD4+ T-cell from 10 ul whole blood
2. Lyse the captured cells with guanidinium-HCl solution
3. Meter the cell lysate into 4 chambers
4. Add silica coated magnetic beads into the 5 chambers (1 is negative control). Run the DNA-beads aggregation assay

Materials and Instruments

Sylgard® 184 silicone elastomer base. Sylgard® 184 silicone elastomer curing agent (Sigma-Aldrich). SU8-2150

(MicroChem), KOVA® GLASSTIC® SLIDE 10 with grids (Fisher Scientific). 6M guanidinium HCl 6.1. Dynabeads® CD14 2x concentrated. Dynal® Dynabeads® (Life Technologies™) CD4 5x diluted. Isotonic PBS buffer ph 7.4 with 1% BSA and .02% sodium azide. MagneSil® Paramagnetic particles (Promega). Corning glass slides 75x50 mm thickness of .96 to 1.06 mm (Fisher Scientific). Light mineral oil (Fisher Scientific). Harrick Plasma cleaner/ sterilizer PDC-32G. Cannon Rebel T1i high resolution camera. Versa CO_2 laser cutter.

Chip Fabrication

channels face the glass layer. The layers were bonded by treatment of air plasma oxidizer.

CD4+ T-cell Isolation and Lysing

The microdevice was prepared by introducing 120 µL of 5X diluted Dynabeads® CD4 to the CD4 chamber. The beads were maintained in the chamber via a manifold embedded with magnets, while the buffer solution was removed. 10 µL of unprocessed whole blood and 6 µL of Dynabeads® CD14 2x concentrated were introduced to the small 20 µL chamber. The chip was mixed via rotation at 6 RPM for 10 minutes before

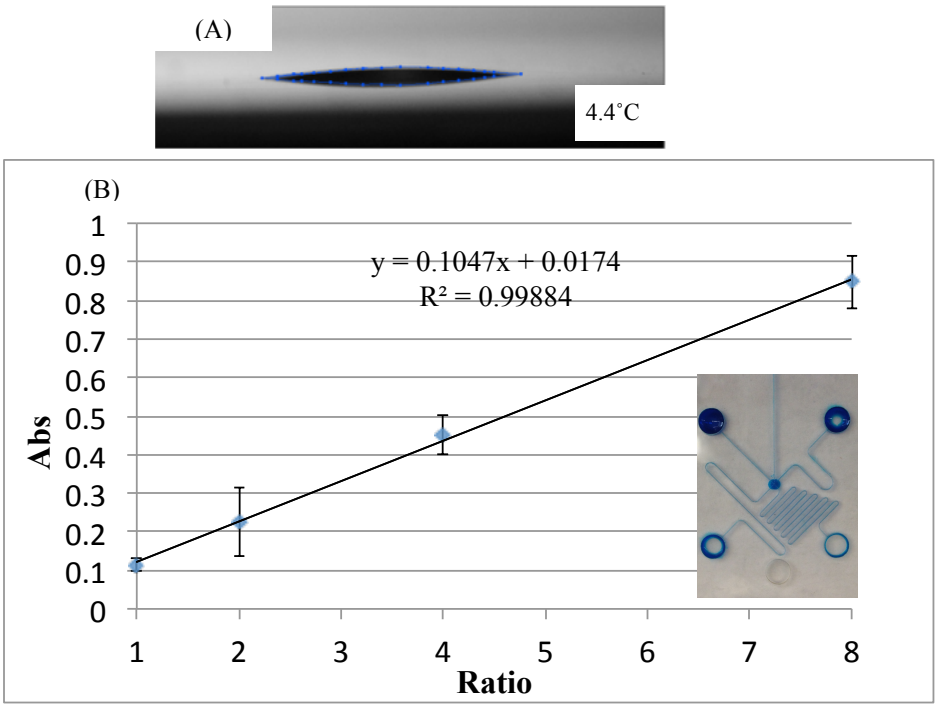

Figure 3. Resistance based metering. Syringe pump was used to pump cell lysate to the 4 chambers. (A) The chip is made of 3 layers, the first 2 PDMS layers were pre-fabricated for cell isolation and lysing, and the 3rd glass layer was bonded to the first 2 layers during the metering step. The PDMS and glass surface were treated by plasma oxidizer to create a super-hydrophilic surface (contact angle<5°), so that the fluid only stops when the channel meets the pinwheel well (capillary valving), to ensure the metering. Cell lysate were pumped into the metering region by a syringe pump (B) Erioglaucine dye was metered on chip, pipetted out, and examined by UV-Vis spectrometer, n=3 on 3 different chips, the inset shows one of the on chip metering result, the channel lengths are 120mm, 60mm, 30mm and 15mm respectively.

Both layers were created from a 10:1 ratio of PDMS monomer to curing agent and cured for 10 minutes at 115°C. The first layer was designed with a 20µL well for monocyte trapping, connected with a 120µL well designed for CD4 T-cell isolation and lysis. The second layer was laser ablated with a Versa CO_2 laser cutter from a sheet of cured PDMS. The second layer consisted of 5 separate wells and 4 channels of varying lengths, the metering channels were not cut through, and the

the fluid was pushed into the large 120µL chamber together with 80 µL of PBS buffer, while maintaining the captured monocytes in the small chamber via applied magnets. The fluid was then mixed with the CD4 coated beads for 10 minutes before all fluid was removed from the chamber while maintaining the beads with CD4+ T-cells in the chamber. 3-5 aliquots of 120 µL PBS buffer were used to rinse the chamber in order to remove any red blood cells or unwanted white blood cells. The CD14 beads with captured monocytes were

removed from the chamber. The cells were then lysed by adding 120 µL guanidinium HCl to the large chamber and mixed for 10 minutes. The lysate was then metered through the micro channels. The Dynabeads® used here can be shipped at room temperature and stored at 4 - 8°C for 2 years [35], and all the experiments were carried out at room temperature, making it desirable for usage in resource-limited regions.

Pinwheel assay and Data Analysis

Paramagnetic silica beads were added into each well for a total volume of 20 µL in each well, including a negative control containing only beads. The chip was placed in an oscillating magnetic field at 210 RPM for 3 minutes, 20 seconds in each direction (Figure 4). An algorithm was developed to correlate the percentage dark area in each well to the CD4 count, using a calibration curve. The percentage dark area (%DA) is defined as the Please refer to previous publications for the algorithm details [20-23,36].

Flow Cytometry Analysis

The blood samples were obtained from collaborators (Davis Lab) at the UVA Hospital, using BD FacsCaliber (two color laser) and MultiTEST CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC Reagent. These were samples that had been submitted for CD4 analysis, and were a mix of HIV+ and HIV- patient samples, with the patient information de-identified with respect to which samples came from HIV+ or HIV- patients. The only information we were given (after we completed our analysis) was the CD4 count. To evaluate the accuracy of the cell counts obtained from the microfluidic chip, a portion of the samples are also analyzed by standard flow cytometry to quantify the CD4+ T-cells. The CD4+ T-cells counts obtained from the developed algorithm are compared to the results obtained from the flow cytometry, and accuracy is evaluated based on a 1:1 ratio.

Results and discussion

The optimized device design is shown in Figure 1, and it consists of two domains - one for immunocapture and one for detection/cell counting. The immunocapture domain has two stages, one for immuno-subtraction of monocytes, and a second chamber for selective immunocapture of CD4+ T-cells. The Dynabeads® T4 quant kit was used to isolate CD4+ T-cells from 10 µL of unprocessed whole blood, and the isolation procedure was optimized based on the kit instructions and previous experiments [23]. The Dynabeads® are uniform, super-paramagnetic polystyrene beads with a diameter of 4.5 µm, coated with mouse monoclonal antibodies against CD14 or CD4 antigens, allowing for specific capture of monocytes and CD4+ T-cells, respectively. A small fraction of monocytes may express the CD4 antigen in addition to CD14, therefore a high efficiency monocyte depletion step is critical to ensuring accurate CD4+ T-cells counts, especially when counts are on the low end of the range. The CD4 immunocapture chamber volume was designed to optimize the dynamic range of the DNA-bead aggregation methodology. The DNA-beads aggregation assay displays the highest sensitivity when sample DNA concentrations are below 100 pg/µL [20]. Given an average mass of DNA per white blood cell of 6.25 pg [26], and that the CD4 count in HIV-positive patients could range from <10 cells/µL to >2000 cells/ µL, a small volume chamber could potentially result in DNA concentrations that falls outside of the functional range. The optimal CD4+ T-cell isolation results were obtained through active mixing of the blood with Dynabeads® via rotation (details in Figure 2 and in the *Experimental* section). In the first step, whole blood and CD14 beads are retained in the CD14 chamber during rotation; no leakage was observed due to the hydrophobic nature of the PDMS surface. During the second step, a back-flow problem was encountered from the CD4 chamber to the CD14 chamber as a result of inherent wetting of the channel. This was overcome by reducing the volume of PBS buffer in the 120 µL CD4 chamber to 80 µL, leaving an air plug in place, which served to minimize back flow. The solution was mixed via rotation at 6 RPM for 10 minutes for each chamber, although this mixing time may be reduced with a higher RPM lab sample rotator. After removing the unwanted cells from the chamber by loading PBS buffer, the captured CD4+ T-cells were lysed by 6M guanidinium HCl, which served as both the lysing solution and the chaotropic agent to induce DNA-beads aggregation.

The metering step utilized a capillary burst valve, specifically a geometric variation valve [25], which impedes the flow of cell lysate solution at the junction where the micro channel meets the open chamber (Figure 3B inset). The driving force (pressure provided by a syringe pump) ultimately overcomes the resisting capillary force, and the chip meters based on the resistance of the channels, which is dependent on channel length defined by equation (1) [28].

$$R=\frac{12\mu L}{d^3 w} f\left(\frac{d}{w}\right) \qquad (1)$$

The fluidic resistance, R, of a channel with a rectangular cross section, is defined by µ, the fluid viscosity, and L, w, d, which are length, width, and depth of the channel respectively. The f term is a dimensionless shape factor function that approaches unity when d<<w. Given that all other parameters remain unchanged in the 4 channels, the channel length is the only variable that defines resistance. Hence, varied metering channel lengths of 120 mm, 60 mm, 30 mm, and 15 mm provide a resistance ratio of 8:4:2:1 and, thus, a metered solution volume ratio of 1:2:4:8. The circle at the center of the

chip was rastered using laser ablation, and functions as a buffer zone to ensure equal liquid distribution into each channel.

Although the laser ablated channel is wedge shaped, we can reasonably apply equation (2), which describes the liquid interface in a rectangular channel. In this equation, P_A and Po represent the pressure inside and outside of the channel, σ is the surface tension, θ_s and θ_v are the contact angle with the sidewall and with the top and bottom ceilings, respectively, w and h are the width and the depth of the channel. Using light microscopy, the width was found to be 218±4.5 μm (n=3), with a channel depth of 791.25±15.95 μm (n=3). If a given solution is exposed to a device chemical surface with features dimensions that result in small contact angles for θ_s and θ_v, equation (2) indicates that the difference between P_A and Po will become large, thus, indicating that the capillary valve will retain the solution until enough pressure is provided to 'burst' the valve. Experiments showed that, when the surface was superhydrophilic (contact angle <5° as shown in Figure 3A), the metering worked as designed, i.e., the cell lysate flow was impeded at the junction where the channel met the well, until all the channels were filled, at which point they burst simultaneously.

$$P_A - P_O = -2\sigma\left(\frac{\cos\theta s}{w} + \frac{\cos\theta v}{h}\right) \quad (2)$$

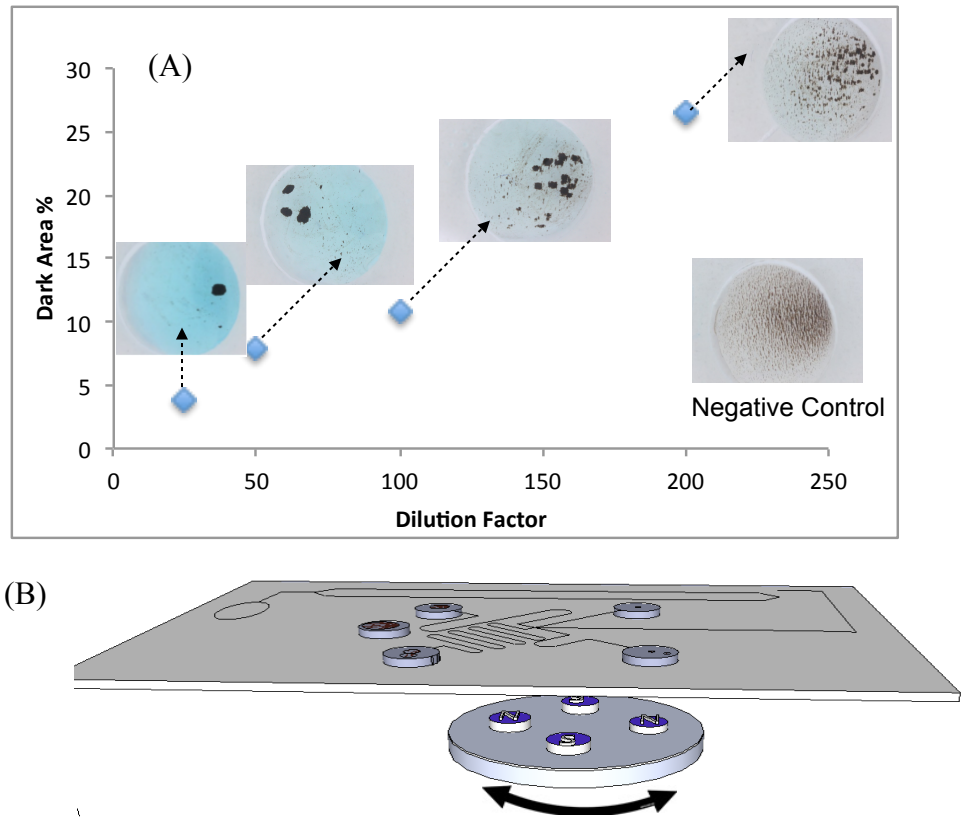

Figure 4. Illustration of the on chip DNA-beads aggregation assay. (A) An example of CD4+ T-cells quantified by DNA-beads aggregation on chip. To ensure an accurate count, each sample was serially diluted to generate a calibration curve. Dark area is defined as the pixels that make up the brown area. Dark area% is normalized by negative control (No DNA). The dilution factor indicates the cell concentration is 25, 50, 100 and 200 times diluted from the whole blood. (B) The rotating magnetic field set up for DNA-beads aggregation. The rotating magnetic field switch directions every 20 seconds, for a total of 3 min. The guanidinium-HCL solution was doped with Erioglaucine dye for illustration.

Studies have shown that there is a positive correlation between the hydrophilicity and bonding strength when treating PDMS and glass with oxygen plasma [27], when the surface is super hydrophilic, the bond is strongest. The plasma power and exposure time was optimized to obtain a superhydrophilic surface, with the metering performed before the PDMS surface reverted to its initial hydrophobic state. To assure accurate metering of 1 µL, 2 µL, 4 µL, and 8 µL into their respective wells, erioglaucine dye was added to the guanidinium HCl solution and metered through the chip, the metered solution was pipetted out from each well and diluted to the same final volume, followed by UV-Vis spectrophotometry analysis, the results were compared to a calibration curve to confirm the metered volume.

In order to prevent mixing, light mineral oil was pumped into the CD4 chamber to displace the cell lysate and pump the lysate to the pinwheel wells. The metering parameters were optimized, by pumping light mineral oil into the dyed guanidinium HCl-filled chip. When metering was complete, the dye-spiked guanidinium HCl solution was extracted from

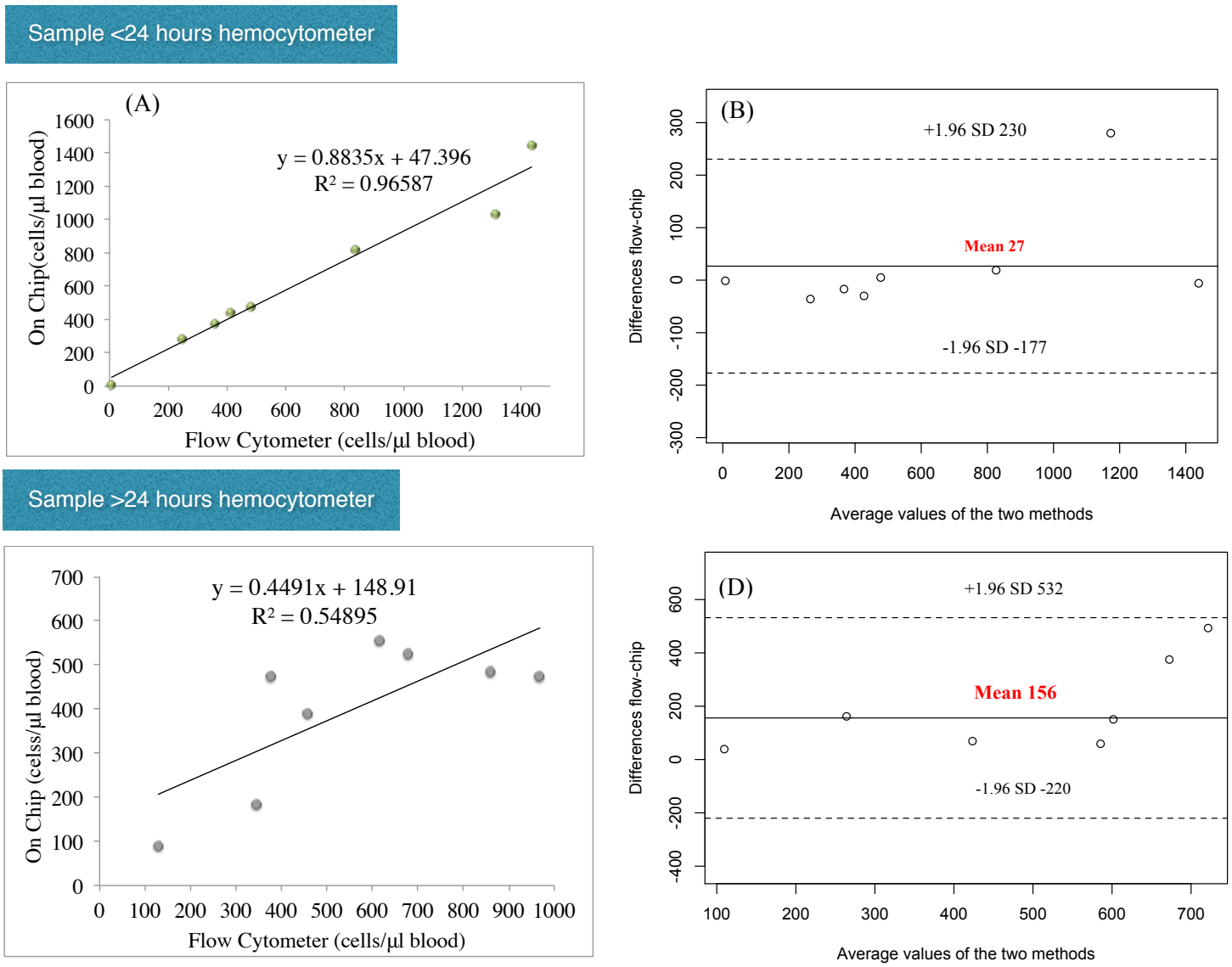

Figure 5. Clinical study results compared with flow cytometry. (A) On chip isolation and hemocytometer enumeration result correlate well with flow cytometry (R^2=0.966) when samples are processed within 24 hours after blood drawn (C) Aged samples (>24 hours) are poor target of immunomagnetic separation, therefore will affect the enumeration result. (B.D.) Bland–Altman methods comparing the cell count in the chip to absolute CD4 cell counts obtained by standard four-color flow cytometry.

the wells, diluted to 1 mL, and then analyzed using a UV-Vis spectrophotometer. Through comparison of the absorbance values for the recovered solution with a standard curve generated using the same dye spiked guanidinium HCl, the optimized volumetric flow rate was found to be 100 µL/min with a total dispensed mineral oil of 140 µL from the syringe ; Figure 3B inset picture shows the on chip metering result, the volume in each well is inversely correlated with the channel length.

DNA-Beads Aggregation

To this point, we have demonstrated the ability to remove monocytes and specifically isolate CD4+ T-cells from whole blood, lyse the isolated cells, and metered select volumes of the DNA-containing lysate. Following the metering step, different masses of silica-coated magnetic beads were added so that each well would have a total volume of 20 µL. Under chaotropic conditions exposed to a rotating magnetic field, the silica coated magnetic beads aggregate with the DNA from the lysed CD4+ T-cells. Aggregation is quantitative and correlates with the mass of DNA present in each well [20], and therefore, the CD4 count. After only 3 minutes exposing to the RMF, the wells were photographed with a high-resolution camera and the percentage dark area (%DA) was calculated; a higher %DA signifies a lower concentration of DNA present in the well. For each sample, an on-chip calibration curve was generated using 4 different dilutions. **Figure 4A** provides the DNA-bead aggregation results for an exemplary sample, while **Figure 4B** provides a schematic of the chip-based ARTµS set up.

Evaluation of HIV Patient Samples Using ARTµS

To test the effectiveness of the CD4+ microfluidic enumeration system, we determined CD4 counts in 26 clinical samples that had been evaluated by flow cytometry at the University of Virginia Health Systems Clinical Laboratory. To test the efficiency for chip -based isolation of CD4+ T-cells, cells captured within the CD4 chamber were stained with Sternheimer-Malbin stain, a supravital crystal-violet/safranin solution well-established for staining WBC's, epithelial cells, and urinary casts [29]. Early efforts to define the chip-based isolation efficiency showed poor correlation between the counts obtained by flow cytometry and those by hemocytometry. It became clear that the correlation between to two methods was strongly dependent on the age of sample (time-elapsed since blood draw). There was clear-cut delineation in the results from blood samples analyzed after 24 hr post-draw. This was supported by previous studies describing that blood samples stored at 2°C to 8°C or room temperature for >24 hours perform poorly when analyzed by immunoassay[23,24]. Given that, the Clinical Laboratory provided us with HIV patient blood samples that, when analyzed, would fall into two groups - within or outside of 24 hrs since blood was drawn. **Figure 5A** shows the result with samples analyzed within 24 hrs of blood draw, and the results show excellent correlation of the hemocytometry results with

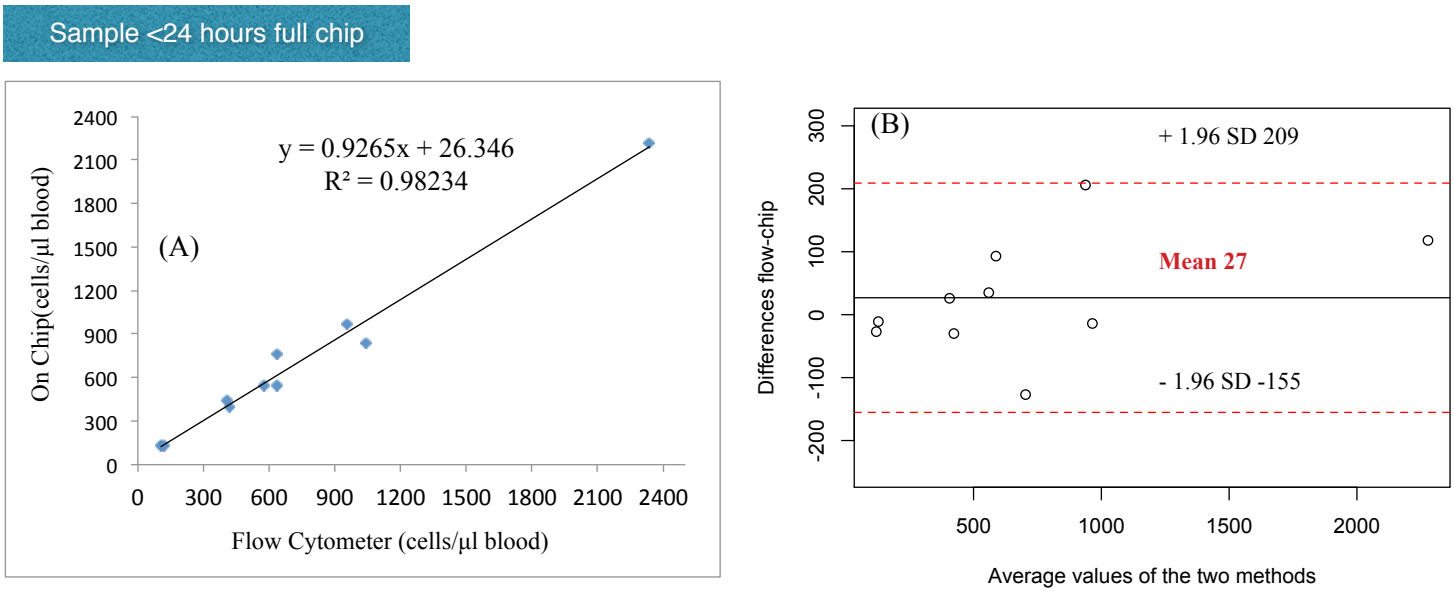

Figure 6. Integrated chip blood in, cell count out result. (A) Result shows strong correlation with flow cytometry (R^2=0.98). (B) Bland–Altman methods comparing the cell count in the chip to absolute CD4 cell counts obtained by standard four-color flow cytometry.

those from flow cytometry. The same number of blood samples (8) that had been stored for more than 24 hrs were analyzed by hemocytometry and flow cytometry, and the results given in Figure 5C confirmed our hypothesis regarding post-draw analysis time. The statistical gold standard for comparing two clinical methods is the Bland-Altman plot [30]. Results of the evaluation of the data by this method is shown in Figures 5B&D, which clearly show a positive bias of 27 cells/μL, and 95% limits of agreement of 230 cells/μL and -177 cells/μL with samples analyzed in <24 hours post-draw. This contrasts samples analyzed after >24 hours post-draw showed a bias of 156 cells/μL and 95% limits of agreement of 532 cells/μL and -220 cells/μL.

With these encouraging results, we tested the efficiency of the microfluidic system for complete processing of <24 hours post-draw blood samples (specific CD4+ isolation, lysis and metering, DNA-bead aggregation) on the integrated chip-based ARTμS, i.e., *blood in-cell count out*. The results in Figure 6A indicate that, for 10 blood samples, an excellent correlation was obtained with the flow cytometry results. The Bland–Altman plot results are given in Figure 6B and indicate that these samples exhibit an acceptable positive bias of 27 cells/μL, and 95% limits of agreement of 209 cells/μL and -155 cells/μL. Given the new ART initiation threshold of 500 cells/μL, the ARTμS assay correctly categorized all samples determined by flow cytometry to be eligible for the ART. Hence, while still is the breadboard stage, the ARTμS has the potential to provide a rapid and cost-effective alternative to flow cytometry.

Conclusions

In summary, we have developed a microfluidic system integrating the DNA-beads aggregation assay to accurately quantitate CD4+ T lymphocytes in a fast low cost manner. The correlation with the gold standard flow cytometry was excellent (R^2=0.98). This chip holds potential as an alternative for CD4+ T-cells counting in resource-limited regions. however, the need for a plasma oxidizer is limiting this device as a rapid deployment solution for HIV diagnosis, we are looking into permanent super-hydrophilic coating for PDMS, in order to make the process more automatic and less time sensitive, an integrated system is designed to encompass the chip and the RMF setup. In addition, we are looking to integrate the immunocapture and DNA-beads assay into a centrifugal device, with automated sample loading and metering process, the challenge lies in designing appropriate spin speed and duration to capture pure CD4+ T-cells efficiently.

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Notes and references

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