CENTRIFUGAL MICROFLUIDIC SYSTEM FOR RAPID, LOW-COST HIV DIAGNOSIS: CD4+ T-CELL COUNTING USING AN INTEGRATED DVD PLATFORM

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ABSTRACT

HIV is a pandemic that currently threatens over 33 million lives worldwide and HIV/AIDS remains one of the major causes of death globally. The continued monitoring of the CD4+ T-lymphocytes count in HIV patients is necessary for proper treatment, although this testing is too expensive and complex for limited resource settings. We report on a novel integrated centrifugal (CD) microfluidic system for rapid and low-cost HIV diagnosis through automated counting of CD4+ T-cells for point-of-care applications. We demonstrate the integrated T-cell immunocapture and detection mechanism using a novel system comprised of a modified commercial DVD drive and polymer disc.

KEYWORDS

Centrifugal microfluidics, Immunocapture, HIV diagnostics

INTRODUCTION AND BACKGROUND

HIV/AIDS continues to be one of the leading causes of mortality, affecting many millions of people worldwide [1]. The presence of HIV infection can be diagnosed through the detection of antigens or antibodies in a patient's blood sample. Once a patient has been diagnosed with HIV, tests to determine the ratio of CD4+ T helper cells and CD8+ T-cytotoxic cells are used as a biomarker for identification of HIV progression in AIDS. These tests must be performed every 3 months in order to effectively monitor HIV-infected patients. However, the accurate diagnosis of HIV progression poses several technological challenges as the current clinical method utilizes flow cytometry, a complicated and costly procedure that is difficult to perform in the resource-limited environments of developing countries.

While significant progress has been made in applying miniaturized technologies to isolate CD4+ T lymphocytes based on immunoassays, the need for manual sample handling and external detection and sample processing has limited the method to be implemented in resource-scarce settings. The centrifugal (CD) microfluidic platform, with its intrinsic pumping and valving mechanisms, is an ideal choice for such an integrated point-of-care system [2]. In this paper, we introduce a CD-based platform that consists of a multi-layered disposable polymer disc and modified DVD drive with integrated fluid propulsion and detection capabilities.

DVD SYSTEM AND DEVICE DESIGN

We show here that a DVD drive can, with certain modifications, be turned into an improved DVD-based Laser Scanning Microscope (DVD-LSM) (Fig.1). The DVD-LSM operates in a similar fashion to a standard DVD drive, with the addition of a second photo detector module positioned above the DVD surface. In this way, cells or other absorbance-based reactions on the DVD surface can be detected via the decreased absorbance or scattered light reaching the two photodetectors from the DVD laser light source. Using this principle, we have manufactured a DVD-LSM prototype that incorporates a standard DVD reader with photo detector (detection), rotational control (sample handling), temperature control (optimized bioassay), and software (signal processing).

A simple U-channel design was used to test the surface treatment procedure and verify cell attachment and counting using the integrated software of the prototype instrument (Fig.2-3). The top polycarbonate layer containing the fluidic features is UV-bonded to the bottom multilayer disc that is pre-functionalized with epoxy-silane. To incorporate a capture-based biological assay onto the DVD platform, the surface of DVD discs were first functionalized to enable the deposition of neutravidin. Briefly, DVD disc were first incubated with a neutravidin solution for 30 minutes, followed by washing with washing buffers. Afterwards, streptavidin-coated beads were used to test the fuctionalization and to evaluate the imaging capabilities of the system. Furthermore, for cell-based experiments, neutravidin was covalently attached to the epoxy-silanized DVD discs by a standard bio-conjugation protocol followed by deposition of antibodies.

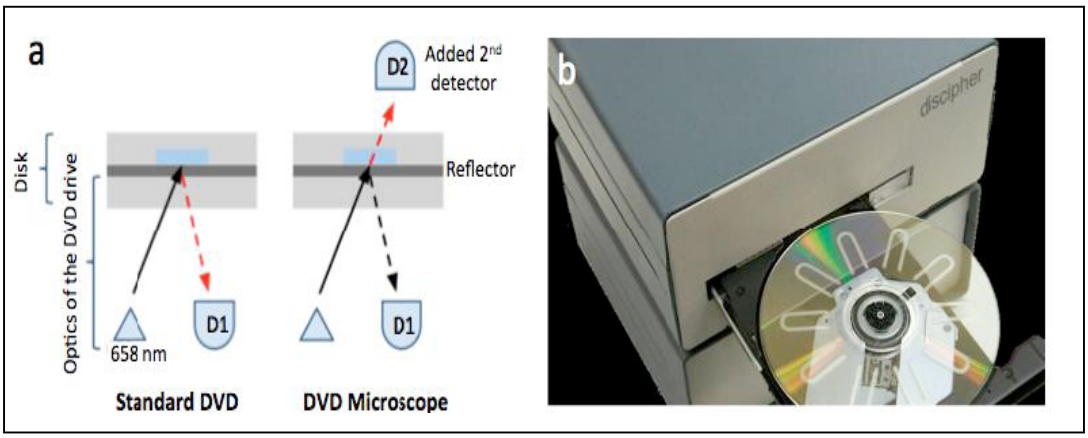

Figure 1. Optical Disc-based Laser Scanning Microscope (DVD-LSM). (a) The principle of DVD LSM: A second photo detector is added to a standard DVD reader to record scattered light. The disc consists of multilayer PC with reflectors. (b) The DVD platform: the prototype integrates rotational control, temperature control and DVD laser reader. The unit (18x20x28 cm) is driven by software residing on PC or Laptop and data is transfered via USB2.

RESULTS AND DISCUSSION

Microfluidics combined with DVD technology offers promising alternative for simple, fast and reliable diagnostics in a global health setting. In this work, we have developed an optical disc-based platform for analysis of biological samples by converting a low-cost DVD drive into a scanning laser microscope. The use of a DVD drive for imaging is similar to using a standard light microscope; both can generate the same kind of image. The primary benefit of the DVD drive being the ability to integrate various preparative steps on a rotating platform, and the ability to do the analysis in a low cost, fully integrated manner.

In initial testing, we were able to show that beads can be specifically and securely attached onto the surface of pre-functionalized DVD discs. Before the bonding of channels layer, neutravidin was attached onto the disc by physisorption. After bonding of the channels layer, the streptavidin beads were flowed into channels over spotted neutravidin followed by washing with washing buffer. Figure 2 shows the DVD system output microscopy image of 2.8 µm streptavidin beads, showing the specific and unspecific binding of beads with typical single bead resolution.

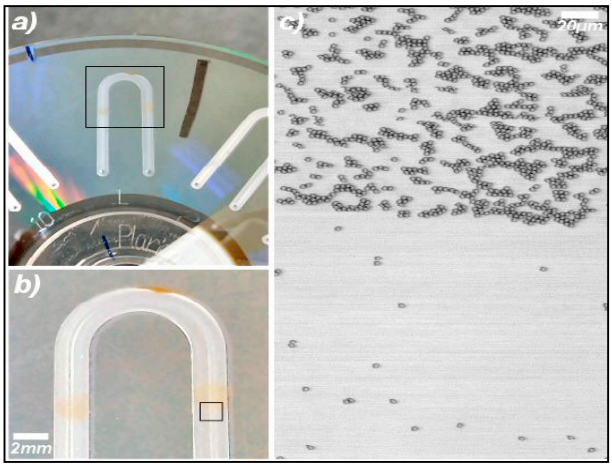

Figure 2. DVD microscope image. (a) Injection molded top PC layer with the microchannel features was UV-bonded to the bottom PC substrate disc with a semi-transparent reflector coating. (b) A simple U-channel design (150mmx2mm) used in this study. (c) DVD Image of 2.8um beads specifically attached (streptavidin-biotin interaction) inside a surface-modified microchannel.

To demonstrate practical applications in resource limiting settings, we tested immobilizing CD4+ T-lymphocytes for HIV diagnostics. For the cell experiments, the epoxy-silane was first incubated with neutravidin for 30 min and then with anti-CD4+ or CD8+ for additional 30 min. T2 cell lines that express only CD4+ and CD8- were used as a model cell line for the experiments. To increase the contrast, the cells were stained with Hematoxylin and eosin stain after being captured on the surface. We show specific binding of a CD4+ specific cell line, with negligible unspecific binding (Fig.3). Furthermore, more work is under way to integrate the steps of blood sample handling and processing in an integrated fashion.

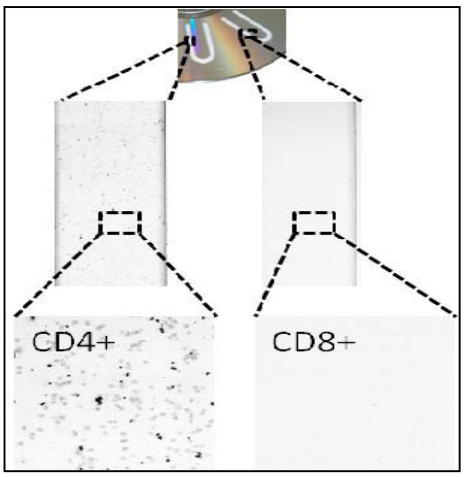

Figure 3. DVD platform readout of immuno-captured cells. The two u-channels were surface modified with anti-CD4 (left) and anti-CD8 (right) antibodies. The T2 cells (CD4+, CD8-) are specifically attached to the surface modified with anti-CD4 antibodies.

CONCLUSION

We have demonstrated a novel microfluidic system with integrated detection of CD4+ cell counts for HIV diagnosis. A stand-alone bench-top DVD-drive system was developed that integrates fluidic propulsion, microscopy-based detection and image processing. This integrated DVD-LSM system has the capability to perform absorbance-based measurements, in addition to the demonstrated light-scattering application of immunocapture counting.

Temperature control is also integrated into the prototype system, enabling the adaptation of various temperature-reliant biochemical assays. Environmental temperature control makes this system well-suited for installation in remote environments where temperature fluctuations would likely have an impact on diagnostic test result reproducibility. An integrated system is currently under development which will automate the surface modification, sample inlet and washing steps, along with the cell staining steps. This future integrated system will allow the user to input the blood sample into a pre-modified disc and view the cell count results without any additional steps, greatly simplifying the immunocapture detection process.

ACKNOWLEDGEMENTS

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