Point-of-care CD4 Cell Counting for Monitoring HIV/AIDS Patients in Resource-confined Regions

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ABSTRACT

We developed a prototype of optical imaging-based point-of-care (POC) devices that can detect CD4+ T-lymphocytes in human blood for HIV/AIDS monitoring. The proposed portable cell-counting system, *Helios CD4*, can acquire sample images and analyze particles or cells automatically, by using a simple imaging module and a sample cartridge with a three-dimensional (3D) helical mini-channel. This device has advantages over the existing devices because of its small size and simple scanning mechanism. A performance evaluation was conducted by comparing the cell count obtained using *Helios* with that obtained using PIMA, one of the most widely used POC CD4+ cell counter.

1. INTRODUCTION

The 2014 UNAIDS report states that approximately 37 million people worldwide are infected with human immunodeficiency virus (HIV), which leads to acquired immunodeficiency syndrome (AIDS). In particular, these HIV/AIDS patients are concentrated in developing regions such as Africa and Southeast Asia. AIDS-related mortality has been declining owing to the introduction of antiretroviral therapy (ART). However, the gold standard method for measuring CD4 lymphocytes, i.e., flow cytometry, is complicated and expensive to use, especially in resource-limited areas. Therefore, new methods for counting CD4 cells based on image cytometric analysis have emerged. Many types of microfluidic point-of-care (POC) devices have been developed for monitoring HIV/AIDS by counting CD4+ T-cells. However, researchers continue to develop a more efficient low-cost device that can enhance patient comfort by reducing the number of visits to clinics and by using gentler blood sampling due to reduced blood volume requirement for monitoring HIV/AIDS in resource-poor settings (Kirby *et al*., 2015). We aim to contribute to these efforts at improving the situation in developing countries and to ultimately promote human health by developing a portable CD4 cell analyzer for monitoring the effectiveness of ART therapy in HIV/AIDS patients.

2. MATERIALS & METHODS

2.1. Fabrication of Sample Cartridge

We fabricated a sample cartridge with a thread-like microgroove on its cylindrical surface. The microgroove, covered with a transparent adhesive tape, can form a helical mini-channel with variable channel width and depth. Blood was introduced to the channel through a hole at the end of the screw. The foot area of the sample cartridge with a three-dimensional (3D) helical mini-channel was smaller than the area of the conventional planar microchannel. The cartridge had a length of 66 mm and a diameter of 6 mm. The height and width of the helical mini-channel are 100 μm and 600 μm, respectively. Figure 1 shows the sample cartridge with the helical mini-channel. A DC motor was installed to rotate the cylindrical sample cartridge at various speeds. Multiple images of the sample particles in the channel were obtained using a camera synchronized with the motor. A nut and bolt mechanism was used, which facilitated scanning of a large volume of sample along the helical mini-channel by simply rotating the cartridge this greatly simplified the operation of the related electromechanical parts.

2.2. Prototype Development

We engineered a prototype of a portable cell counter, *Helios CD4*, based on the experimental setup that we built using commercially available optical components for measuring CD4+ T-lymphocytes in human blood cells. The optical part comprised a CCD camera (DMK21BU618, The Imaging

Source), optical filters (XF108-2 Cy3/695AF55 CY5M, Omega Optical), an objective lens (UPLFLN10×, Olympus), and a custom-made light-emitting diode (LED). We used two filters to detect the fluorescence signals of CD4 and CD3. These filters were inserted into a round filter container and rotated alternately by an electrical signal so that two images could be obtained from one spot of the sample channel. The device was equipped with z- and x-axes stages and connected to the knobs, in order to manually control the focus on the samples outside the prototype. In addition, we developed an electrical control system and installed it in the prototype. A touch display connected to the electrical board was installed on the prototype device so that several functions such as motor speed control, LED light power on/off, and sample scanning could be adjusted on the monitor. Figure 2 shows the experimental setup and completed prototype. The position of the emission filters for CD4 and CD3 fluorescence detection were switched, and the LED was switched on by a signal generated every time the sample cartridge was rotated by the DC motor. Thus the sample images were obtained automatically.

2.3. Sample Preparation

To obtain clear sample images, blood samples were prepared by dissolving red blood cells in RBC lysing solution. However, the washing process was omitted to minimize leukocyte loss. We collected fresh blood every week from human volunteers under the IRB permission. Note that 200 μL of the RBC lysis solution was dispensed into the sample tube, and 100 μL of blood was added to that tube. Then, the mixture was stirred slowly for 10 min at room temperature, 10 μL each of CD4 and CD3 fluorescent dyes were injected into the sample tube. The tube was incubated in the dark for 10 min at room temperature, accompanied by slow stirring. After incubation, blood conjugated with the fluorescent dyes was injected into the sample cartridge using a 1-cc syringe. The instrument was prepared for operation by inserting the sample cartridge filled with blood into the motor holder.

2.4. Image Processing

The concentration of particles was measured by counting the total number of particles within given sample volume. Two-hundred images were captured from individual samples by using two filters suitable for handling CD4 and CD3 fluorescence. These images were divided into two categories, namely CD4 images and CD3 images, and then analyzed automatically using image-analysis software (ImageJ; http://imagej.nih.gov/ij/) (Cho *et al*., 2011; Burger & Burge, 2016). Each image set was analyzed, and the particles in the images were counted using an image-analysis parameter set suitable for CD4 and CD3 image features. Finally, we counted the number of particles that appeared the same in both CD4 and CD3 images using the "Image calculate" function in the software. A procedure for image processing and analysis for counting CD4+ T-lymphocytes is shown in Figure 3. The images were 0.6 mm in width and 0.49 mm in height, and the channel depth was 0.1 mm. Therefore, one image contained 0.0294 μL of blood. The CD4+ cell-count result obtained using our device was compared with the result obtained using the PIMA analyzer (Pima™ CD4 Analyser, Alere) and flow cytometer (FACSCalibur™, BD).

3. RESULTS & DISCUSSION

We developed a prototype of an optical imaging-based portable CD4+ cell analyzer and measured the CD4+ T-lymphocytes in human blood. The cell count result obtained using *Helios CD4* was compared with PIMA data, which is the most widely used POC CD4+ cell counter, to validate its accuracy. The reproducibility of the device was verified by repeatedly obtaining measured data for the same sample. Figure 4 shows the comparison of CD4+ cell counts obtained by *Helios CD4* and PIMA with 11 blood samples. There was a ±2-20% difference between the results obtained using *Helios CD4* and PIMA, and the deviation in *Helios* data was 1.6 times larger than that in PIMA data in the reproducibility test. *Helios* uses one light source to detect two different fluorescence signals, and CD4 images are twice as bright as CD3 images. This may cause many particles to be lost during image analysis. In addition, the sample volume may be less than the calculated volume if the blood sample does not completely fill the channel, which leads to erroneous counting results. We are currently attempting to further optimize the image analysis parameters for analyzing the CD4 and CD3 images obtained from *Helios CD4*, and improved results are expected as the image quality is expected to be raised by stably fixing the cartridge holder.

4. CONCLUSION

We believe that the quality of human health in resource-confined regions with many HIV/AIDS patients can be enhanced by managing the patients efficiently. This is possible by delivering medical services and health benefits on a larger scale by using low-cost portable equipment. The total cost of POC CD4 test depends on various individual pieces of information (Larson *et al*., 2012). Using the proposed method, a low-cost CD4 monitoring device can be developed in the near future to replace the large and expensive analytical instruments being used currently.

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BIOGRAPHIES

Jung Kyung Kim received a B.Sc. degree in Mechanical Engineering in 1996 and then a M.Sc. and a Ph.D. degrees both in Biomedical Engineering at Seoul National University in 1998 and 2003, respectively. From July 2004 to August 2006, he was a postdoctoral fellow in the Laboratory for Cell and Membrane Biophysics at the University of California, San Francisco, USA. After joining Kookmin University based in Seoul, Republic of Korea in September 2006 as an assistant professor, he has been directing Biomedical Device Lab and now he is an associate professor in School of Mechanical Engineering.

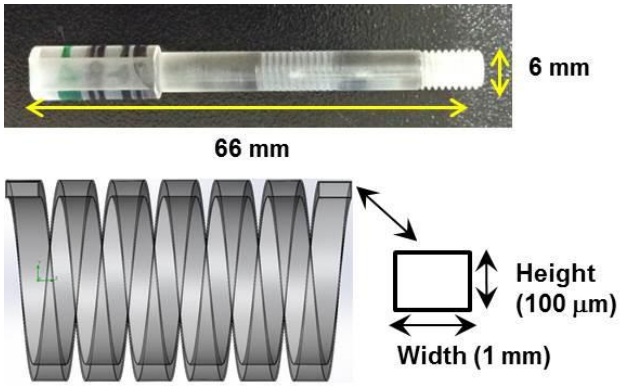

Figure 1. Cylindrical plastic sample cartridge with a helical mini-channel

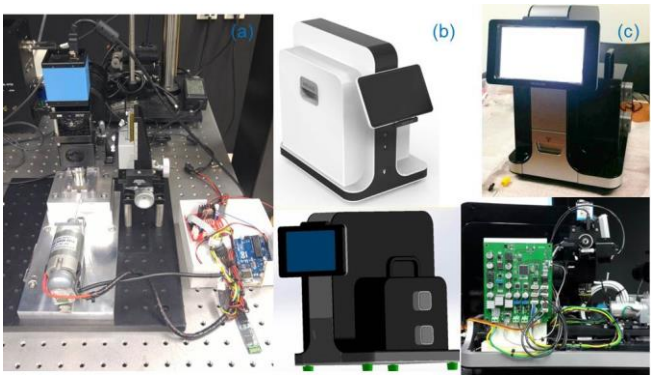

Figure 2. Experimental setup (a), design of the prototype (b) and completed prototype, *Helios CD4* (c)

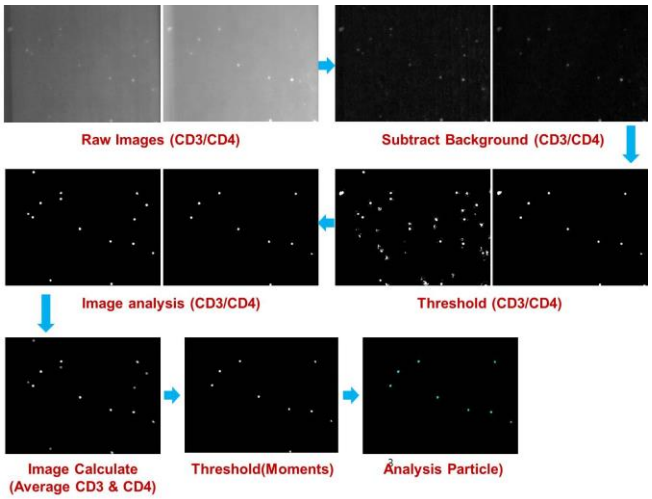

Figure 3. Procedure of image processing and analysis for counting CD4+ T-cells

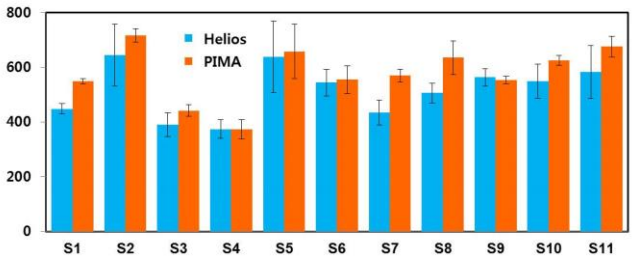

Figure 4. Comparison of the CD4+ blood cell counts obtained by *Helios* and PIMA