A PAPER-BASED MICROFLUIDIC BIOSENSOR FOR LOW-COST, ON-SITE DIAGNOSIS OF URINARY TRACT INFECTIONS ON A SMARTPHONE

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ABSTRACT

A smartphone-integrated paper-based microfluidic (SiPM) platform is presented for low-cost filtration, imaging, and image analysis of fluorescent dyed leukocytes present in urine samples for on-site diagnosis of urinary tracts infections (UTIs). Experimental demonstrations verified the SiPM's capabilities for (1) low-cost, paper-based filtration to isolate, selectively dye, and evenly distribute leukocytes on a capture interface and (2) quantification of urine leukocyte counts through a smartphone-integrated fluorescence microscope at different concentrations. The SiPM system offers a low-cost, portable, smartphone-integrated method for on-site detection of excessive leukocytes in urine without sophisticated lab equipment or skilled personnel.

KEYWORDS: Smartphone detection, Paper-based filter, Leukocyte, Urinary tract infections (UTIs)

INTRODUCTION

The morbidity of UTIs is associated with an excessive number of leukocytes in urine, and is significantly greater in developing countries due to lacking of adequate healthcare resources [1]. Conventional urine culturing methods are laborious and timing-consuming, requiring bulky and expensive equipment and trained personnel [2]. There is a strong need for a low-cost, portable system to precisely diagnose UTIs according to urine leukocyte concentration.

OVERVIEW OF THE SiPM PLATFORM

Figure 1 shows a schematic of the SiPM system for point-of-care detection of leukocytes, or white blood cells (WBCs), in urine samples. Urine solutions collected from UTI patients typically include a variety of cell types, so paper-based devices with three layers with different pore sizes were cost-effectively fabricated to offer an on-site, high-selectivity WBC filtration method (Figure 1A). A 20 µL urine sample is loaded onto a paper-based filter and flows downward through the layers via capillary action. The target WBCs in the solution are stained with sodium fluorescein dye preloaded in Layer 1, and are further isolated by size in Layer 2 made of borosilicate membrane with 9 µm pores. All other cell types smaller than 9 µm go to the Layer 3 wicking pad with 3 µm pores. This paper-based separation process offers a low-cost filtration method to selectively isolate the target WBCs in urine solutions without any bulky and expensive equipment such as flow cytometers and centrifugal machines.

The Layer 2 WBC capture interface is then disassembled from the paper-based device and placed below the smartphone-integrated fluorescent microscope for on-site diagnosis of UTIs by WBC count (Figure 1B). Several extra optical components are assembled below the smartphone's rear camera to create a

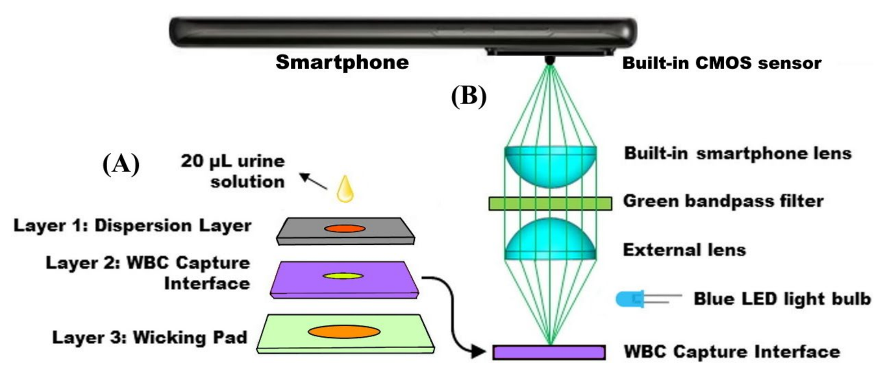


Figure 1: A schematic of the SiPM platform as a low-cost and portable tool for on-site diagnosis of UTI-related diseases. (A) *A paper-based filtration device simply consists of three layers of the papers with different pore sizes. A urine sample collected from UTI patients is loaded onto the center of device in Layer 1, where sodium fluorescein dye is preloaded to selectively attach to target leukocyte cells. The urine leukocyte cells with a 10 ~ 15 µm diameter range are collected in Layer 2, while smaller sizes of the cells are further filtered through to Layer 3. After completing the separation process of the target leukocytes, the filter device is disassembled to remove the WBC capture interface of Layer 2.* ***(B)*** *It is then placed below a smartphone-integrated fluorescent microscope for quantitative analysis of UTIs. A smartphone's rear camera is used to capture fluorescence images of the target WBCs isolated in Layer 2. Using a smartphone's image processing app, the populations of the collected WBCs can be quantified to diagnose UTI-related diseases. Additionally, a smartphone is equipped with various functions such as wireless communications and GPS positioning. These capabilities allow the captured data (e.g., the number of the target WBCs, time of test conducted, etc.) to be shared instantly and wirelessly with a healthcare team for real-time monitoring and further health management of UTI patients.*

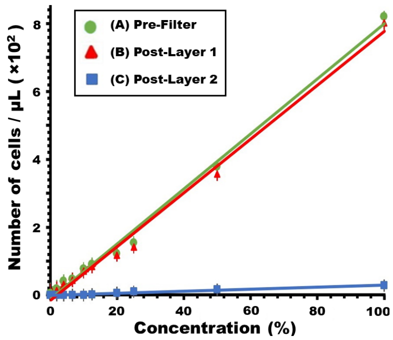
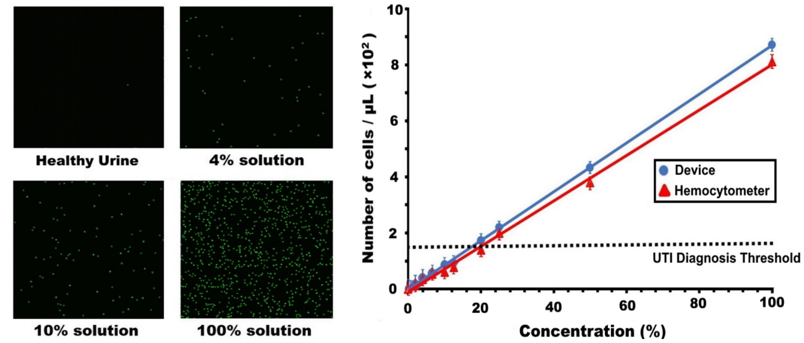


Figure 2: ***Filtration capability of the paper-based device.*** *A graph shows the numbers of the WBCs measured by a hemocytometer at various concentrations* ***(A)*** *before being input into the paper filter,* ***(B)*** *after passing through Layer 1, and* ***(C)*** *after passing through Layer 2. The majority (more than 96%) of WBCs in urine solutions can be cost-effectively collected in Layer 2 without any bulky and expensive laboratory equipment.*



(A) (B)

Figure 3: ***On-chip diagnosis of UTI-related diseases using the SiPM. (A)*** *Fluorescent images of the WBCs in urine solutions are taken by the SiPM platform at various concentrations. Based on these fluorescent images, the populations of the cells were counted using a smartphone's image processing app.* ***(B)*** *A graph shows the comparison of the WBCs counts measured by the SiPM device to the ones by a hemocytometer. The device can accurately diagnose WBC concentrations of urine solutions, matching the results of a traditional hemocytometer cell counting method with no significant difference.*

reversed smartphone lens microscope composed of two identical smartphone lens modules with perfectly matched angular views separated by a green bandpass filter. A blue LED is shined on the solutions for fluorescent excitation, and the emission signals from the target stained WBCs are detected by the smartphone's built-in CMOS sensor after passing through the reversed lens microscope that allows 14.4× magnification over a field of view of ~ 12.25 mm^2.

EXPERIMENTAL DEMONSTRATIONS

The filtration capability of the paper-based device has been first investigated to understand how effectively the target WBCs can be separated from other types of cells in a urine solution collected from UTI patients. Figure 2 shows the populations of the WBCs in a urine solution at various concentrations measured by a conventional hemocytometer method. It is observed that the WBC populations counted at the Pre-Filter (Figure 2A) and Post-Layer 1 (Figure 2B) have no significant difference, while almost negligible numbers of the cells can be found at the Post-Layer 2 (Figure 2C). This indicates the paper-based filter can cost-effectively collect more than 96% of the WBCs in the urine solution without any bulky and expensive laboratory equipment. The next study was conducted for leukopak-urine solutions at various WBC concentrations to simulate urine samples with varying levels of pyuria. Each solution was filtered in the paper-based device, then the Layer 2 WBC capture interface was detached and placed below a smartphone-integrated microscope (see Figure 1) to quantify the WBC concentrations using a smartphone's image processing app. Figure 3(A) show fluorescent images of the stained WBCS in urine solutions. Using a smartphone's image processing app, the numbers of the WBCs were quantified on the SiPM platform and further compared with a hemocytometer (Figure 3B). There is a positive linear correlation between expected and measured concentrations and no significant difference between the SiPM device and hemocytometer measured concentrations. The three highest concentration solutions tested above the UTI diagnosis threshold of 153 WBCs/µL and would be diagnosed as UTI-positive if matching leukocyte concentrations were measured in real urine samples.

CONCLUSION

We have presented a point-of-care SiPM platform that integrates a paper-based filter and a smartphone-integrated microscopic imaging device into a low-cost, portable system for on-site urine leukocyte counting and subsequent diagnosis of UTIs in resource-limited setting without sophisticated equipment or skilled personnel.

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