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Trapping cells in paper for white blood cell count

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ABSTRACT

White blood cell count is an important indicator of each individual's health condition. An abnormal white blood cell count usually results from an infection, cancer, or other conditions that trigger systemic inflammation responses. White blood cell count also provides predictive information on the incidence of cardiovascular diseases and Type 2 diabetes. Therefore, monitoring white blood cell count on a regular basis can potentially help individuals to take preventive measures and improve healthcare outcomes. Currently, white blood cell count is primarily conducted in centralized laboratories, and it requires specialized equipment and dedicated personnel to perform the test and interpret the results. So far there has been no rapid test that allows white blood cell count in low-resource settings. In this study, we have demonstrated a vertical flow platform that quantifies white blood cells by trapping them in the paper. White blood cells were tagged with gold nanoparticles, and flowed through the paper via a small orifice. The white blood cell count was determined by measuring the colorimetric intensity of gold nanoparticles on the surface of white blood cells that were trapped in the paper mesh. Using this platform, we were able to quantify white blood cells in 15 μL of blood, and visually differentiate the abnormal count of white blood cells from the normal count. The proposed platform enabled rapid white blood cell count in low resource settings with a small sample volume requirement. Its low-cost, instrument-free operations would be attractive for point-of-care applications.

1. Introduction

When one visits the clinic with signs and symptoms such as fever, headache or bruises, doctors usually suggest a blood cell count to help diagnose the causes of these conditions. Blood cell count is likely the most common test in clinical practice. Each day, doctors in big hospitals order thousands of blood cell counts (Tefferi et al., 2005). Blood cell count is one of the earliest parameters established to quantitatively study the blood and aid the diagnosis (Verso, 1964). A complete blood count comprises of detailed analyzes of red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes). It measures the number of these cells, the relative portions of their subpopulations (for white blood cells), and the volume fraction (for red blood cells) among other indices (Tefferi et al., 2005). White blood cell count is an essential aspect of the complete blood count. White blood cells act in response to infections or injuries, and play an important role in our body's defense system. Most white blood cells migrate from the circulating system to the tissues in order to soothe local conditions (Alberts et al., 2002). Other white blood cells trigger adaptive immune system to eliminate pathogens and provide long-lasting protections (Alberts et al., 2002; Tortora and Grabowski, 2002). Such responses would lead to abnormal circulating white blood cell count. The normal range of white blood cells is 5000–10,000/μL (Tortora and Grabowski, 2002). A higher than normal count, a condition known as leukocytosis, is usually caused by bacterial infection, tissue damage, and inflammatory diseases (*e.g.* rheumatoid arthritis or allergy) (Berliner, 2011). A lower count, a condition known as leukopenia, is often associated to bone marrow deficiency, certain viral infection, and severe bacterial infection (Berliner, 2011).

Besides acting as an indicator of current health status, white blood cell count has also been suggested as a predictive and prognostic marker for a number of chronic diseases (Horne et al., 2005; Kannel et al., 1992; Ohshita et al., 2004; Vozarova et al., 2002; Weijenberg et al., 1996). Friedman and co-workers compared the white blood cell counts of patients with myocardial infarctions to those of matched control groups. Results revealed significantly higher white blood cell count in patients that were closely related to the onset of infarction (Friedman et al., 1974). Grimm and colleagues discovered that changes in white blood cell count from the baseline value was “a significant and independent predictor of coronary heart disease” (Grimm et al., 1985). Vozarova and colleagues found that high white blood cell count could predict the development of Type 2 diabetes (Vozarova et al., 2002). The authors speculated that it was due to the chronic activation of

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immune systems in the pathogenesis of the disease (Pickup and Crook, 1998). The predictive values of white blood cell count in cardiovascular diseases and diabetes dictate the need for routine measurements, especially in light of the rapidly ageing and obese society. By establishing individual's baseline value of white blood cell count and closely monitoring it, one could identify his/her risks and presumably take preventive measures to reduce the propensity for disease incidence.

Traditionally, white blood cells were counted under the microscope on a blood smear slide or a hemocytometer. This manual approach is no longer practiced clinically because it is time-consuming and labor-intensive (Hyun et al., 1991). Automated white blood cell count with hematology analyzers now prevails (Buttarello and Plebani, 2008). Hematology analyzers count white blood cells by identifying their unique characteristics in terms of scattering profile (Terstappen et al., 1988), fluorescence-labeled surface markers (Goldmann et al., 2013) or electrical impedance (Graham, 2003). Although automated white blood cell count with hematology analyzer offers high throughput, such tests require the use of bulky equipment, and has to be conducted in centralized facilities by experienced medical technicians. These limitations significantly hinder the potential benefits of measuring white blood cell count on a regular basis for health management, because one cannot perform white blood cell on his own in low-resource settings. It is therefore desirable to develop a simple white blood cell count platform that can be easily adopted by inexperienced users who do not have much medical background. Some groups have incorporated microfluidics for the solution with the hope of bringing the instrument to end users by reducing the footprint of the devices. They had built miniaturized versions of optical or electrical hematology analyzers (Fu et al., 2004; Gwo-Bin et al., 2005; Holmes et al., 2009; Sung-Yi et al., 2006). Many of these microfluidic platforms performed well, but they required external fluidic control systems that are bulky and expensive. A couple of other groups proposed more innovative approaches. Cheng and colleagues captured target cells using antibodies in a microfluidic chamber and counted immobilized cells under the microscope (Cheng et al., 2007). Glynn and colleagues labeled the target cells with relatively large beads and packed the labeled cells into a microchannel. The cell number was estimated by examining the packing volumes under the microscope (Glynn et al., 2014). Although both platforms operate by hand and do not require external fluidic control units, they still rely on bulky equipment such as microscope for cell counting.

Paper-based platforms, on the other hand, offer both pump-free fluidic handling and instrument-free signal readout. They work in the form of lateral flow or vertical flow immunoassays. They are widely adopted both by the general public for personal healthcare management, and by professionals for specialized applications such as pathogen identification (Mao et al., 2009; Ngom et al., 2010). Capillary force and gravity guide the liquid through the designed path through the paper, eliminating the need for external pumping. Antibodies or antigens immobilized on the paper capture the target analytes that are labeled by detector agents. The detector agents frequently employ colloidal particles or enzymes to generate colorimetric readouts that are visible to the unaided eyes. However, the paper-based immunoassay has one disadvantage stemmed from the small pore size of the paper. When paper-based immunoassays are applied to relatively large analytes such as cells, the detector agents form large complexes with the analytes. These complexes have difficulty moving through the small pores of the paper, and are not able to reach test zones where they are expected to generate signals (Esfandiari and Brook 2013). Although probable as demonstrated by a limited number of attempts (Liu et al., 2009; Wu et al., 2013), the paper-based lateral flow platform is not ideal for analyzing large mammalian cells.

The paper-based platforms still have great potential for point-of-care cell analysis by offering pump-free fluidic handling and instrument-free signal readout. In this study, we have overcome the aforementioned issues, and have demonstrated a vertical flow device that traps the cells in the paper for white blood cell count by turning the disadvantage of paper-based platforms to our advantage (Fig. 1). To count the white blood cells, we first stained the cells with antibody-conjugated gold nanoparticles. The stained cells are subsequently flowed through the paper *via* a small orifice.

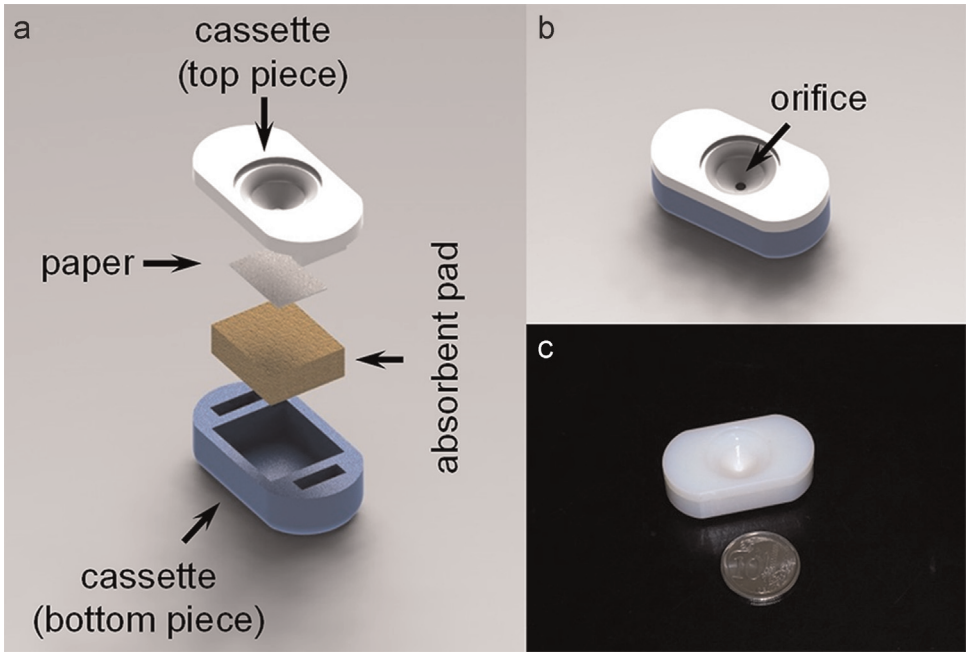

Fig. 1. 3D model of the white blood cell count device. a) The test paper is placed on top of the absorbent pad, and both items are housed in the plastic cassette. b) View of the assembled device. The liquid sample would flow through the device through the small orifice at the sample inlet. c) Photograph of the actual device prototyped by 3D printing.