ABSTRACT

Blood is the most commonly used human body fluid for preliminary screening of health conditions of any patient in clinical settings. This may be thanks to the fact that a blood sample may provide a plethora of preliminary information that may help a healthcare professional prompt clinical decision-making regarding the further course of actions. Traditionally, pathological tests with blood samples rely on sophisticated laboratories equipped with high-throughput, efficient instrumentation and skilled technicians. However, these gold-standard medical devices suffer from several shortcomings, such as the elevated cost associated with their purchase, maintenance, and routine use; the requirement of large sample and reagent volume; non-portability; and accessibility and affordability to the patients residing in resource-limited settings. Therefore, point-of-care (POC) testing diagnostic technologies are advanced to circumvent these limitations. Microfluidic-based devices have, off-late, ushered great promises to translate the gold standard testing methods to POC settings. The work conducted within this dissertation focuses on the development of simple, inexpensive, and portable methods for detecting hemoglobin, hematocrit, and creatinine using whole human blood exploiting the paper-based and centrifugal microfluidic platforms.

The primary aim of this thesis is to exploit the advantages of paper-based and centrifugal microfluidic platforms for diagnostics and translate the same into prototype development, demonstrating proof of performance. Chapter 1 of the thesis describes the fundamental of paper-based microfluidics and centrifugal microfluidic platforms. In the subsequent sub-sections, the essential physical aspects driving the central theme of the present thesis are accordingly summarized first.

Chapter 2 of the thesis describes the development of a low-cost reagent-free method for hemoglobin estimation on a simple portable device that can be deployed among the under-served population in resources constrained settings without sacrificing the fundamental principle of direct evaluation of hemoglobin extracted from the human blood sample. Exploring the fundamental principle of osmotic hemolysis for extraction of hemoglobin from red blood cells, the method harnesses the dynamics of a blood drop on a rotating platform and simple imaging to come up with results in a turn-around time of about 13 minutes. The efficacy of the device has been justified by validating with established pathological gold standards. These results are likely to pave the pathway of establishing the clinical assay of a first-principle-based yet reagent-free evaluation of blood pathology in health care diagnostics.

Chapter 3 of the thesis describes the development of a low-cost and simply-fabricated μPAD to estimate plasma creatinine from the finger-pricked whole human blood. A simple paper strip is proposed to accurately measure creatinine concentration using just 10 μL of figure-pricked blood. The proposed device mainly involves three steps. The first step is the separation of plasma from the whole blood, the second step is the transportation of plasma to the detection by exploiting the capillary properties of the paper matrix, and the third step is the quantification of the creatinine concentration through a smartphone using an in-house developed app. The creatinine concentration of the same sample is then estimated by a biochemical auto-analyzer and compared with the values obtained from our proposed device. The results obtained by our device show a reasonable accuracy compared to the gold-standard method.

In Chapter 4 of the thesis, a novel paper-based device for simultaneous measurement of hematocrit and hemoglobin is put forth. To determine the hematocrit level,

a 20 μL of the whole human blood is deposited on the detection zone already immobilized with sodium chloride and Ethylenediaminetetraacetic acid (EDTA) solution. However, a 20 μL droplets of the mixture of blood and deionized water are deposited on the hemoglobin detection zone to determine hemoglobin concentration. The resulting stains were digitized with a scanner and analyzed using the freely available software ImageJ. The mean area of the stain is used to quantify the hematocrit level, while the mean grey color intensity is used to quantify hemoglobin concentration. The performance of the paper-based device is compared with an automated hematology analyzer. A high degree of correlation has been observed between the values measured by the paper-based device and automated hematology analyzer (R^2 = 0.9651 for hematocrit level; and R^2 = 0.9701 for hemoglobin concentration). The device provides a simple, fast, disposable, and inexpensive tool to determine the hematocrit level and hemoglobin concentration simultaneously.

Chapter 5 of the thesis describes the development of a simple, affordable, and portable spinning disc for measuring plasma-creatinine concentration with 10 μL of whole human blood. 5 μL of the alkaline picrate solution is loaded into the device and rotated at 1000 rpm to transport this solution to the periphery of the microchannel. Further, 10 μL whole blood is loaded in the same channel and spun at 1300 rpm for 10 minutes. The creatinine in the blood plasma reacts with alkaline picrate (Jaffe reaction), and the color of the mixture changes to yellow-orange color. The resulting color is captured with a smartphone, and creatinine concentration is estimated using an in-house developed app (CREA-SESE). The value of creatinine measured with the present device and the gold standard device is highly correlated (R^2 = 0.998). This study demonstrates the feasibility of a simple, inexpensive, and portable rotating device for measuring creatinine concentration using 10 μL of finger-pricked whole human blood, which can easily be

deployed among underserved population in resource-constrained settings to monitor renal diseases.

Finally, the conclusion of the thesis is presented in chapter 6. The novel contributions from the preceding chapters are summarized, and some possible extensions are also discussed. In a nutshell, our focus is to develop a novel diagnostic delivery model enabled by disruptive technology for better healthcare diagnostics at sufficiently low cost and establish the same through few studies combining the empowerment of underserved population as human resources with niche diagnostic technology such as paper and compact disc-based centrifugal microfluidics.