AUTOMATED WHOLE BLOOD PROCESSING WITH A PORTABLE MICROFLUIDIC DEVICE FOR POINT-OF-CARE DIAGNOSIS

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ABSTRACT

This paper describes a simple and portable microfluidic device enabling automated whole blood processing for point-of-care (POC) diagnosis. The device integrates blood loading, metering, diluting, fixing, and staining on a chip, which not only avoids the technical expertise requirement and elaborate handling steps needed for traditional peripheral blood smears, but also keeps the advantages of general applicability and clinical versatility. Using this device, two dilutions of whole blood was obtained and two densities of monolayer blood cells were achieved on chip by minimally trained personnel in a rapid, automated, and safe way.

KEYWORDS: Point-of-care diagnosis, portable, blood loading, metering, diluting, fixing, staining.

INTRODUCTION

Peripheral blood smear examination is a common, inexpensive and powerful diagnostic aid, which can provide reliable information about a variety of hematologic disorders [1], and is often used as a follow-up test to abnormal results on a complete blood count. But these techniques require technical expertise and involve elaborate handling steps for sample preparation, which is prone to introducing artifacts [2], and neither is readily adaptable to use in low resource environments by untrained personnel.

Attempts have been made at developing faster, automatic diagnostic methods for more reliable blood analysis, but most of these methods are expensive and focused on specific disease diagnosis, like malaria [3]. To overcome the limitations of current blood smear techniques, we designed and fabricated a portable, automated, and low-cost microfluidic device that integrates blood processing steps needed for performing a manual blood smear on a chip, simplifying the sample preparation procedure and providing the potential to do a variety of clinical diagnostic measurements such as: complete blood count, differential blood analysis, and sickle cell determination.

THEORY AND DESIGN

A typical blood smear requires a series of sample preparation, fixing, and staining steps that must be automated for a standalone microfluidic device to be of value. To implement these steps, a microfluidic device is integrated with seven pneumatic microvalves made from three polydimethylsiloxane (PDMS) layers, as shown in Figure 1. The top valve layer includes two groups of pneumatic valves, A and B, that control the various processing steps. The middle layer is a PDMS membrane (~20μm thick), which can control the opening and closing a valve based on membrane deflection. The bottom channel layer is made from PDMS with an integrated cover glass slide serving as the reaction and fixing surface. The three layers are bonded together using corona discharge. All the features of the device, including the pre-loading chamber, metering, dilution chambers, and reaction chambers are integrated on the channel layer, as shown in Figure 2.

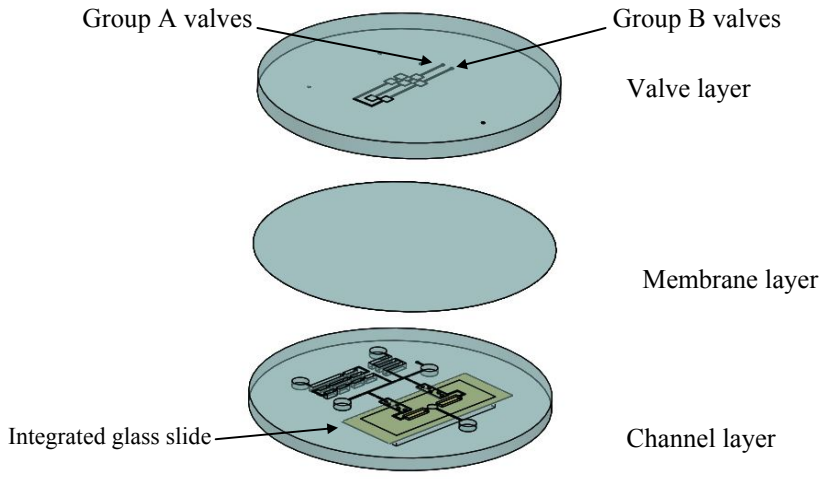


Figure 1: Exploded view of the whole blood processing microfluidic device. The top layer is the valve layer, the middle layer is the membrane layer, and the bottom layer is the flow channel layer.

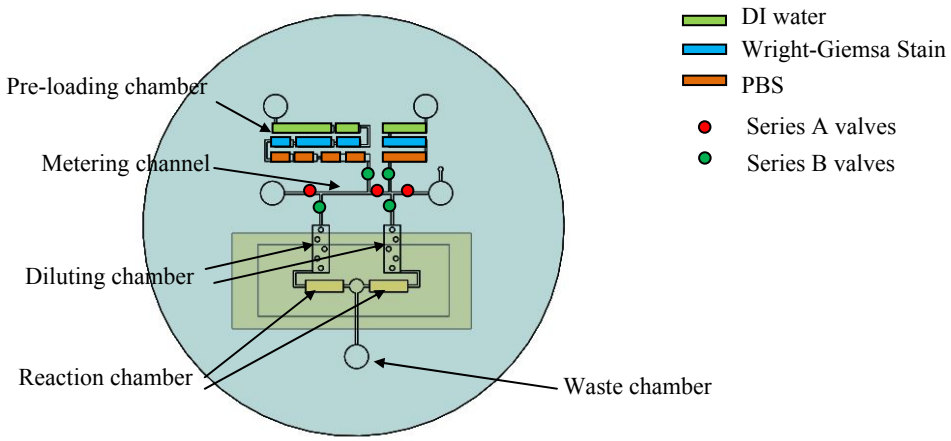
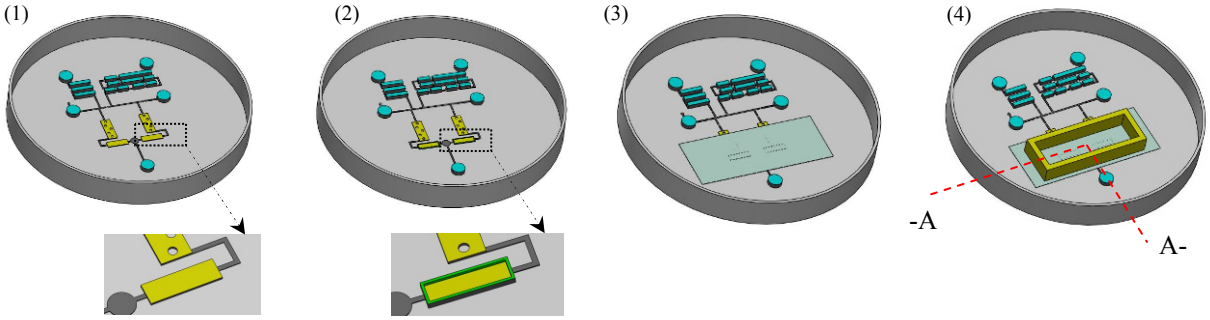
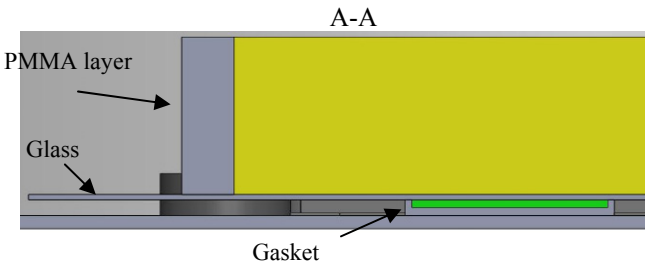


Figure 2: Front side of the channel layer. Phosphate buffered saline (PBS), Wright-Giemsa stain and DI water are pre-loaded in the chamber in sequence. Poly-lysine is pre-coated on the glass reaction surface for cell attachment.

A three-dimensional channel layer mold was made to build the glass integrated channel layer. By stacking layers of double sided tape with varying geometries, the three-dimensional structure of the channel layer can be obtained easily. One piece of cover glass slide is sandwiched between a double-sided tape gasket and the PMMA layer, which creates a space for the microscope to closely approach the glass surface. After the PDMS is fully cured in the mold and the PMMA layer is removed, the cover glass slide is embedded in the PDMS channel layer. One side serves as the reaction surface, and the other side is exposed to the outside environment, making it compatible with high resolution microscopy, as shown in Figure 3.



a. Fabrication process for the channel layer.



b. Cross section of the channel layer mold.

Figure 3: Diagram showing fabrication of the device (a) Fabrication process for the channel layer mold. (1), three layers of tape with varying geometry were patterned and stacked together to create a series of flow channels with different volumes and channel cross sections. (2), a double sided tape gasket was placed on the reaction chamber area to seal around the perimeter in preparation for attaching the glass substrate. (3), a piece of cover glass slide was placed on the double sided tape. (4), a layer of PMMA was placed above the glass slide to keep PDMS from flowing into the central area of the glass. (b) Cross section of the channel layer mold. The cover glass slide is sandwiched between the PMMA layer and the double sided tape gasket.

EXPERIMENTAL

Before exposing the device to any samples, the reaction chamber is pre-coated with poly-lysine. The group A valves are opened and the group B valves are closed, allowing a small amount of whole blood to be injected into the loading chambers. Then, with the group A and B valves reversed, two aliquots of the blood sample are metered in the channel, as shown in Figure 3. Three pre-loaded reagents flow through the channel in order. The two separate metered samples of whole blood are simultaneously diluted by phosphate buffered saline (PBS) in two separate dilution chambers and flowed into two reaction chambers. After the blood cells settle and adhere to the bottom surface, a Wright-Giemsa stain is then flowed into the reaction chambers to stain the blood cells. Finally, DI water washes away the excess stain.

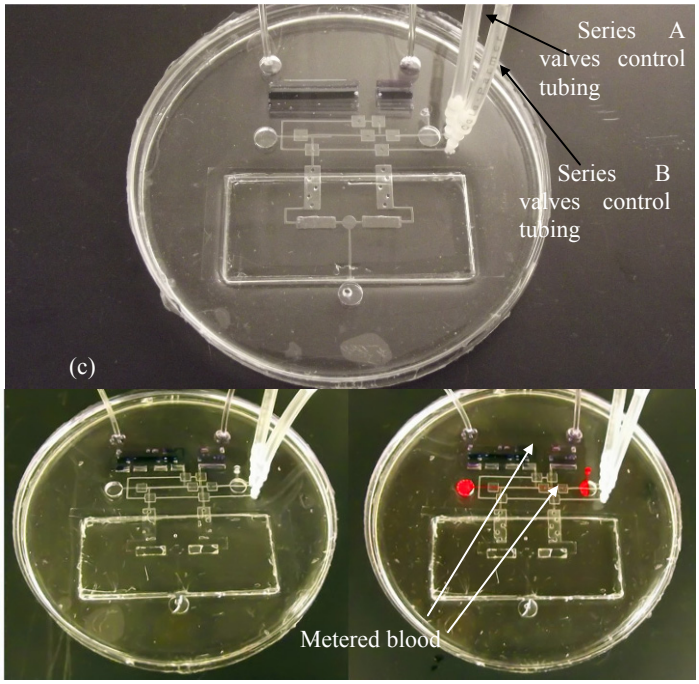


Figure 4: (a) The final microfluidic device; (b) Three reagents are pre-loaded; (c) Two specific amounts of injected whole blood are metered in the channel.

RESULTS

Blood cells attached to the glass slide in the reaction chamber with different densities, which depend on the amount of metered blood and the time washed by DI water, as shown in Figure 5. Cells are spread in a monolayer, allowing easy counting and identification of cell types. Using different dilutions provides internal controls and the ability to focus on either red cells or white cells, depending on the needs of the assay.

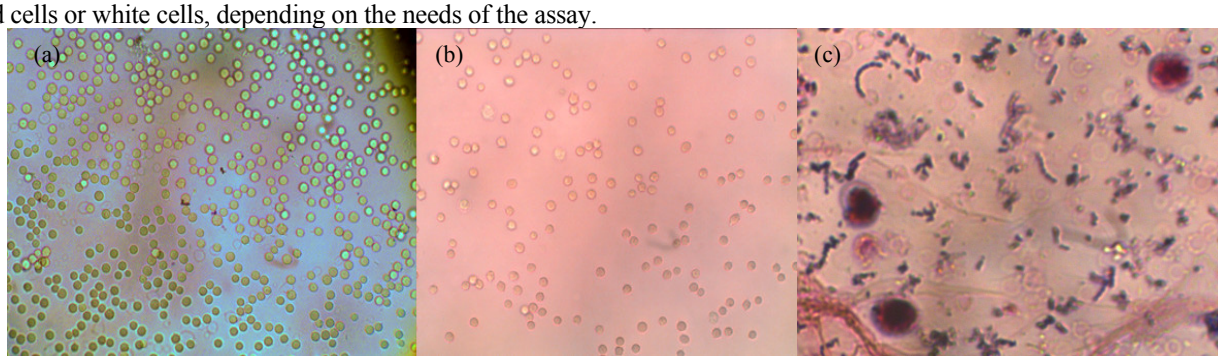


Figure 5: (a) Higher density of blood cells sticking to the glass surface; (b) Lower density of blood cells sticking to the glass surface; (c) White blood cells after staining under 40X microscope objective.

CONCLUSION

A microfluidic device that can automatically process whole blood including loading, metering, diluting, fixing and staining has been presented. Future work will optimize the process and enable this device for use in performing complete blood counts, differential blood analysis, and other cell fixation diagnostics. With these improvements, this device has the potential to be useful for a large variety of POC diagnoses.

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