

FULL INTEGRATION AND AUTOMATION OF IMMUNOASSAY PROTOCOLS BY ROTATIONALLY ACTUATED DISSOLVABLE FILM VALVES

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ABSTRACT

Here we describe a fully integrated and automated, multi-step immunoassay protocol utilizing our water-dissolvable films (DFs) actuated, centrifugo-pneumatic valving scheme [1]. This technique establishes a sequence of laboratory unit operations comprising of blood separation, metering, mixing/incubation, and liquid reagent release from on-board reservoirs for washing and detection by mere control of the rotational frequency. During storage at rest and at low rotational frequencies, the sacrificial DF valves provide permanent liquid and vapor barriers. However, beyond a critical burst frequency ranging up to 3500 rotations per minute (rpm), these valves yield by inverting a metastable gas-liquid layer to trigger the wetting and thus dissolution of the DFs.

KEYWORDS: Centrifugal, Microfluidics, Dissolvable film, valving, Integration, Sacrificial valve, Burst frequency, Vapor barrier

INTRODUCTION

Interest in sacrificial valves is due to their ability to create vapor barriers and to precisely gate liquid flows at high rotational frequencies which traditional passive valves (e.g. hydrophobic patches) would not be able to sustain. Due to the vapor barriers created by these sacrificial valves, they can be used for on-board reagent storage. While some sacrificial valves such as wax and optofluidic valves have previously been reported, they require external actuators such as laser diodes in order to be activated [2, 3]. The use of DFs as sacrificial valves goes well beyond the state-of-the-art, eliminating the need for external actuators other than the spindle motor intrinsic to centrifugal platforms.

We here consider for the first time the advantageous use of DF-based valving on a centrifugal microfluidic platform [4, 5]. In DF-based centrifugo-pneumatic valving, a gas pocket is pressurized by the centrifugal field acting on the confining liquid plug (see Fig. 1). Beyond a critical spinning frequency, the inverted liquid-gas stack destabilizes and the liquid wets the film, thus dissolving the sacrificial membrane. A novel, multi-material fabrication method was developed for facilitating the system assembly [1].

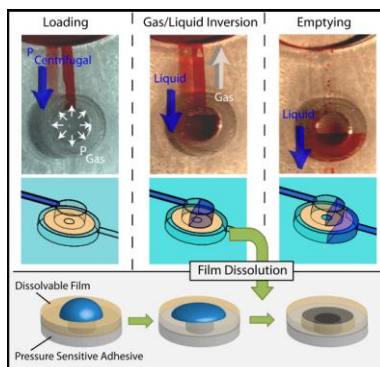


Figure 1: Frame sequence and schematic representation of the trapped air ballast holding back the liquid. When burst frequency is reached, gas/liquid inversion occurs; liquid enters the pneumatic chamber and dissolves the DF, thus opening the valve.

RESULTS AND DISCUSSION

Multi – Step Immunoassay (IA) protocol - Figure 2 provides an illustration of a proposed design for a fully integrated and automated multi-step assay protocol, involving blood separation, metering and mixing. In this design, liquid reagents are pre-loaded in the blood separation, wash and mixing chambers respectively.

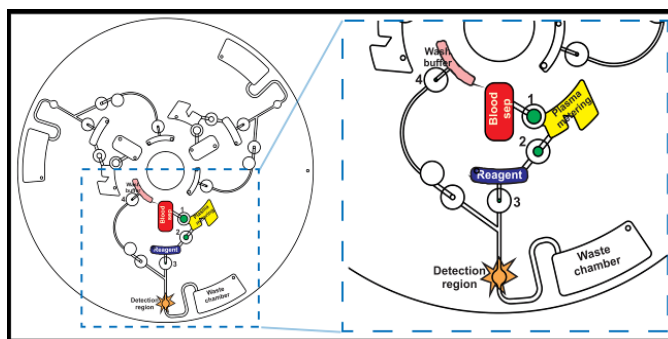


Figure 2: Schematic representation of the full disc and a cut-out section detailing the complete process from blood separation to detection utilizing only DF valves. Four DF tabs are embedded on the disc at positions 1 to 4.

DF valves are embedded on the disc at positions 1 to 4. Initially, the valves are in a closed state. The disc is spun at 2500 rpm for 5 min, in order to separate plasma from whole blood (Fig. 3).

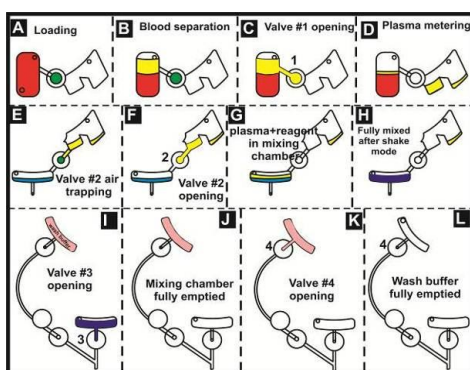


Figure 3: Schematic representation of the fluidic process and sequential opening of the valves. Valve 1 opens after blood separation to aliquot the plasma. Valve 2 opens to deliver the aliquoted plasma to mix with a preloaded reagent. Valve 3 opens to deliver the mixture to the conjugation/detection chamber. Valve 4 opens lastly to deliver the wash buffer.

After processing, rotational speeds are increased to 3000 rpm, in order to invert the plasma/air interface, causing the plasma to enter the pneumatic chamber and dissolve the DF. As the valve opens, plasma enters the metering chamber and aliquots in the appropriate structure. In a similar mechanism, increasing the rotational frequency causes the metered plasma to clear valve 2 and enter the mixing / incubation chamber, which was pre-loaded with a reagent. By a zig-zag “shake-mode” protocol oscillating between ± 600 rpm, the reagent and plasma mix efficiently. It should be noted here that there is no leakage of fluid from the mixing chamber, since valve 3 remains in a closed state throughout mixing/incubation. By increasing the rotational speeds to 2500 rpm, the mixed liquid exits the chamber. Additional valving structures allow for directing the mixture and wash buffer to a conjugation/detection chamber by triggering valves 3 and 4, respectively.

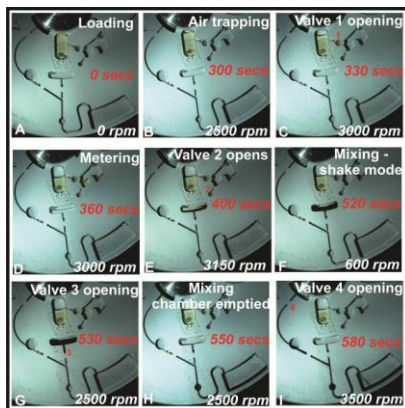


Figure 4: Frame sequence obtained using a color-changing liquid. The images show sequential valving as the DF valves opens from 1 to 4 in sequence depending only on the spinning of the disc.

It should be noted that valve 3 opened at a lower rotational speed than valves 1 and 2. This is because of its radially outward location on the disc, where the centrifugal force acting on the liquid plug is higher, thus requiring lower resistance to yield. The channel and pneumatic chamber geometries can be tailored to enable the positioning of the valves at any location on the disc. Figure 4 shows the full frame sequence of the process. In order to demonstrate the sequential release of reagents and the mixing process, potassium thiocyanate and Iron nitrate solutions are used. The former, which is a colourless solution, was pre-loaded in the mixing chamber, while the iron nitrate was loaded in the blood separation chamber. It is expected that when these reagents are properly mixed, a deep brown solution is formed. This colour was observed in Figure 4F, thus demonstrating efficient mixing while valve 3 is still fully intact.

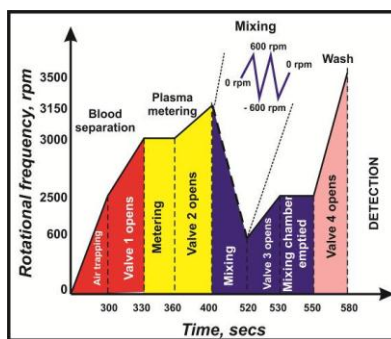


Figure 5: A graphical representation of the fully integrated disc showing the different stages and the variation in the disc rotational frequency. Extract from the stage shows the zig-zag shake mode process that facilitates efficient mixing/incubation

Figure 5 details the protocol of the spinning frequency as a function of time along the integrated process from whole blood separation to detection. This figure shows the pneumatic compression as the rotational speed is increased, leading to the opening of valve 1 as the air/liquid interface is inverted. Plasma metering and opening of valve 2, mixing using the shake mode, by moving the disc in a clockwise and anti-clockwise direction, delivery of liquid from the mixing chamber to the detection region and subsequent release of the washing buffer, after which detection can be performed.

CONCLUSION

We for the first time demonstrate complete integration and automation of a multi-step IA protocol which is merely based on rotationally actuated, Immunoassay compatible DF valves. The high burst frequencies of up to 3500 rpm, which is about 3-fold higher than capillary valves, permit rapid blood sedimentation while the vapor-barrier properties of the sacrificial material allow on-board storage and controlled release of liquid reagents. The technology will apparently lend itself to the facile implementation of different types of assays on instrumentation as simple and cheap as commodity optical disc drives like CD players.

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