VAPOR-TIGHT ICE-VALVING IN CENTRIFUGAL MICROFLUIDICS FOR PCR APPLICATIONS

M. Amasia\textsuperscript{1,2}, M. Cozzens\textsuperscript{1}, and M. Madou\textsuperscript{1,2}

\textsuperscript{1}University of California, Irvine, USA and
\textsuperscript{2}World-Class University (WCU), Ulsan National Institute of Science and Technology, REPUBLIC OF KOREA

ABSTRACT

This paper reports a novel application of ice-valving in a centrifugal platform and its utilization for PCR amplification. These ice-valves form as a result of freezing small plugs of fluid in the channels directly above cooled thermoelectric modules. A reversible, vapor-tight seal, strong enough to withstand the sustained high temperatures due to PCR thermocycling can be formed in as little as 5 seconds. In order to demonstrate the effectiveness of these vapor-tight valves, PCR thermocycling was performed on a stationary centrifugal disc (CD), with no appreciable sample fluid loss.

KEYWORDS: Centrifugal Microfluidics, Valving, Polymerase Chain Reaction

INTRODUCTION

Polymerase chain reaction (PCR) is an important step for the detection of biological samples with low target concentrations. PCR enables the detection of pathogenic species by amplifying the target DNA into quantities that can be detected by fluorescence or electrochemical methods [1][2]. The centrifugal microfluidic platform has been utilized for many biomedical applications, including sample preparation, immunoassays, biochemical analysis, and PCR amplification [3]. Centrifugal microfluidics often utilize an intrinsic, non-contact pumping and valving mechanism, which are ideal for liquid handling. However, the handling of liquid/vapor PCR samples in microfluidic devices is not a simple task when confronted with the high temperatures and pressures created within the PCR chamber region. In this work, we demonstrate a novel application of ice-valving in a centrifugal device and its utilization in a PCR amplification process.

EXPERIMENTAL

In order to demonstrate the effectiveness of these vapor-tight valves, a PCR sample was pumped into an outer chamber, and then PCR thermocycling was performed while the CD was stationary. An exploded view of the microfluidic CD is shown in Figure 1. The plastics fabrication process for this multi-layer CD included a thermal welding step to create robust PCR chambers that resist fluid leakage at high temperatures and pressures. The integrated centrifugal system consisted of the centrifugal pumping hardware and the stationary PCR heating system, with all systems fully automated through LabView software. In this work, thermoelectric modules (TEs) were utilized for both thermocycling heating and valving processes. Thermoelectric modules exhibit fast ramping rates and have simple temperature control through modulation of the input current magnitude and polarity [4]. In addition, by utilizing TEs for both heating and valving, the hardware infrastructure complexity is minimized.

Figure 1: Exploded view of multi-layer fabricated centrifugal device. Layers of stock polycarbonate sheets and thin films were thermally-welded to obtain a robust bond in the outer PCR region of the CD.
The ice-valves work in the following way: fluid is pumped into the PCR chamber, with a crucial feature that both the inlet and outlet microchannels are filled with fluid. Next, the thermocycling and ice-valve TEs are brought into contact with the bottom surface of the CD. Then, the ice-valve thermoelectrics (Figure 2) are actuated, and small plugs of the sample fluid are frozen in the inlet and outlet channels directly above the ice-valve TE locations. With the valves actuated, thermocycling can be performed on the central chamber region (Figure 2). When the thermocycling process is complete, the ice-valve TEs are turned off, and the ice-plugs melt: a fully reversible process. The PCR sample can then be pumped into other regions of the disc, or removed via manual pipetting.

![Figure 2: Schematic showing the details and process order for integrated ice-valving in a centrifugal system. Thermodlectric modules are shown as orange shaded square features. The PCR reaction mixture (blue fluid) is first input into the sample inlet chamber (T1) and the fluid is pumped into the PCR chamber region. Once the chamber is fully filled (T2), the ice-valve thermoelectrics are actuated, sealing off the central chamber region by freezing small plugs of fluid (T3a). When the thermocycling process is complete, the ice-valves can be turned off to release the fluid. In T3b, a photo of the integrated ice-valves is shown in the frozen state.](image)

**RESULTS AND DISCUSSION**

To validate the efficacy of ice-valving in a centrifugal system, PCR amplification was performed on the reaction mixture enclosed between the two ice-valves. Twenty microliters of PCR assay mixture (containing template DNA, primers, probes and Taq polymerase) was subjected to 35 cycles of a thermocycling profile of 95-55-72°C. The on-CD PCR amplified samples were then compared to a traditional heating block system to verify amplification.

Shown in Figure 3, the gel electrophoresis demonstrated the positive amplification of template DNA on a CD, resulting in a strong band at the expected 87 base-pair. The band produced using the automated CD system is slightly less bright when compared to the heating block, although further optimization of the system should lessen this discrepancy. For instance, more thorough testing will be performed on the complex temperature gradients that result from the interactions between the three thermoelectrics and the disposable disc. Additionally, since TE modules have relatively fast ramping rates, PCR performed with thermoelectric heating will result in decreased total analysis time compared to systems such as the traditional heating block system.
In this system, the time required for ice-valve formation varies between 5 seconds and 20 seconds. This activation time was dependent on the power supplied to the thermoelectric and the dimensional features of the centrifugal disc and heating system. The ice-valve TEs used in this system were one centimeter square two-stage thermoelectrics, chosen for their heat pumping capacity and surface area required to encompass two PCR channel inlets and outlets. In other microfluidic valving applications, the surface area dimensions, power rating, and temperature operating range parameters of the thermoelectric modules will play an important role in the successful operation of the ice-valves.

CONCLUSION
We have successfully demonstrated the design of a novel ice-valving TE system and its utilization in performing PCR amplification within a centrifugal disc platform. The ice-valving system developed in this work can also be employed in other applications that require vapor-tight valving in centrifugal and other microfluidics platforms, such as sample preparation processes that require heating and containment of the biological sample.

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REFERENCES

CONTACT
*M. Amasia, tel: +1-949-824-1125; mamasia@uci.edu