MEMBRANE-EMBEDDED DISPOSABLE MICROFLUIDIC CHIP FOR CARDIAC BIOMARKER SCREENING

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ABSTRACT

We report a simple and multifunctional microfluidic cartridge with electrochemical (EC) sensing for cardiac biomarker screening including a passive valve, reagent storage and electrochemical sensors from a layer-by-layer rapid prototyping technique. By embedding multiple membranes in the device, immunoassay procedures were performed by controlling fluidic paths and releasing reagents to obtain proper reactions on the electrodes. With the disposable multifunctional cartridge device, we was able to detect as low as 0.25 ng/mL of Creatine Kinase (CK)-Myocardial Band (MB) automatically.
KEYWORDS: electrochemical sensor, passive valve, reagent storage, cardiac biomarker screening

INTRODUCTION

Miniaturized EC sensors have been broadly explored for detection of various biomarkers [1-2]. Especially, cardiac biomarkers need to be assayed quantitatively with high sensitivity and specificity [3]. Microfluidic platform with EC sensor offers simple, rapid, and high sensitivity with selectivity making it attractive tools for such biomarker detection. However, surface of EC sensors can be easily contaminated by co-existing molecules, different pH, and ionic strength in fluidic channel. In this study, we developed a microfluidic EC sensing cartridge for a cardiac biomarker screening. The microfluidic EC cartridge is coupled with multiple functionalized porous membranes which provide controlling fluid flow, storing a dried detergent, and trapping liquid waste in the microfluidic EC chip to remove aforementioned cross-talk issue. By using the multi functioned and disposable form of microfluidic cartridge, we tested CK-MB which has reported as one of the key cardiac biomarkers for indicator of acute myocardial infarction (AMI).

EXPERIMENTAL

A multilayered microfluidic EC cartridge (85 mm × 54 mm × 1.8 mm) with 3-electrode constructed by layer-by-layer assembly [4]. In the cartridge, three membranes indicated as M1, M2 and M3 in figure 1 are embedded for assay automation. Figure 1 shows each step denoting the state of pressure and vacuum pumps to manipulate each path of samples and reagents. When negative pressure is turned on, sample flows through the main channel for immunoreaction while substrate solution is held in the reservoir (Fig 1A). When positive pressure turned on, it generates bubbles at the junction and meniscus force generated from the bubbles removes non-specifically bound targets (Fig 1B). Subsequently, air bubbles are removed by switching off the positive pressure and a fresh substrate solution was delivered over the electrodes (Fig 1C). Finally, by switching off all the pressure sources, an EC signal is measured during an enzymatic reaction (Fig 1D). With the cartridge, we performed a cardiac biomarker screening for 5 different CK-MB concentrations (0, 1, 5, 10, 25 ng/mL). A 50 μL mixture containing an antigen-ALP labeled detection antibody was applied to the sample port of the cartridge, and immunoreaction occurred during sample flow. A 200 μL of substrate solution was then constantly pumped at a flow rate of 5 μL/sec for wash and halted for a 5-min enzymatic reaction. After the reaction, the current responses at the working electrode plotted against the applied potential in LSV. Faradic current contribution was extracted at a fixed potential of 0.15 V to give a peak anodic current.

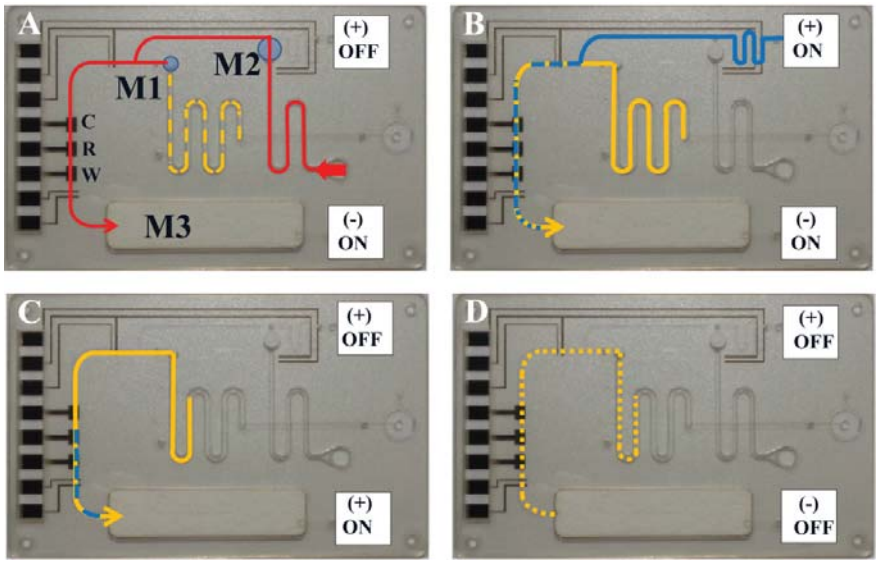

Figure 1: Biomarker assay for each step in membrane embedded microfluidic cartridge. C, R, and W represent the counter, reference and working electrode, respectively (A) Antibody derivatized working electrode (denoted by W) transports the sample. (B) Non-specifically bound target are removed by meniscus force (blue and orange arrows) of bubbles. (C) Air bubbles are removed and a fresh substrate solution was delivered over the electrodes (orange arrow). (D) During enzymatic reaction and EC detection (orange dotted line), the substrate solution is held on the electrode.

RESULTS AND DISCUSSION

A uniqueness of this cartridge is to use multi-functional membranes for immunoassay procedures. The M1 membrane was used to control an air flow through the device. When the M1 was wetted by the sample solution, it functions as a valve to prevent air inside the channel from being discharged to the reservoir via the membrane as shown in figure 2.

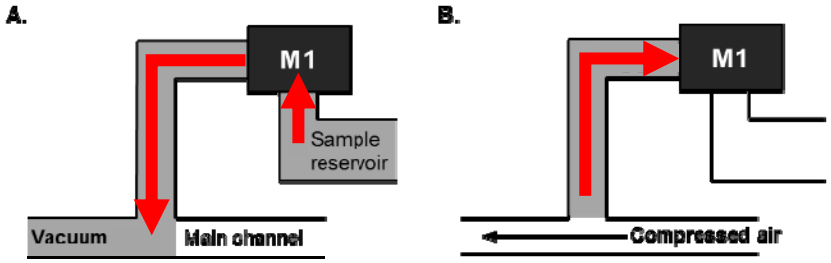

Figure 2. First membrane (M1) operation for valve function. (A) The valve is used in the sample transport step (red arrow) when it's open by using a vacuum pump. (B) A pressure pump applies a force to separate the liquid from the main channel and the valve blocks air flow (red arrow) from leaking into the sample reservoir.

Additionally, M2 membrane was used as a dried chemical storage. Once the M2 membrane is wetted with a buffer solution, we can simply reconstitute to obtain the desired buffer, greatly simplifying an immunoassay design. For the washing step, by passing the solution through a Tween-20 dried membrane, the solution was modified to 1% Tween-20 buffer to remove the non-specifically bound protein on the electrodes. The result shows that the cartridge utilizing the Tween-20 coated membrane yielded a lower background signal than one without Tween-20, demonstrating that the membrane with Tween-20 enhanced washing efficiency.

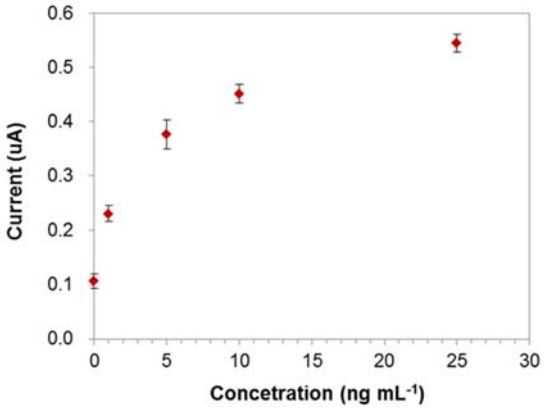

Figure 3. Anodic peak current vs. CK-MB (0, 1, 5, 10 and 25 ng mL-1) input concentration including negative controls calibration plot. For each concentration, the test was repeated three times keeping the background current (with no target) as 0.11 ± 0.014 μA.

Lastly, M3 membrane was used for isolating the waste solutions from both the main channel and the vacuum port. By using M3, we were able to completely remove all cross-contamination chances. As following, the standard curve of the peak anodic current versus the concentration of CK-MB was obtained to demonstrate assay capability of the cartridge. As shown in figure 3, each peak current of LSV enhances as the analyte concentration increases. From a series of immunoassays, we achieved a limit of detection of 0.25 ng/mL in the CK-MB assay. Threshold CK-MB concentration is around 10 ng/mL for early acute AMI) screening assays [5]. Thus, the microfluidic EC cartridge can be used for a cardiac biomarker panel screening by extending its capabilities for other cardiac biomarker assays.

CONCLUSION

We developed a microfluidic EC cartridge for CK-MB cardiac biomarker screening and achieved clinically relevant sensitivity. The cartridge technique eliminates the need for specialized cleanroom equipment, active valving components and on-chip chemical storage, which provides a miniaturized, affordable platform for POC testing.

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