

Developing a Paper-Based Sensor for Cholesterol Measurement and Processing Images Using ImageJ

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Abstract – Cholesterol is an important fatty molecule that functions in maintaining the structural integrity and fluidity of the cell membrane. It is widely found throughout the human body, and the body uses cholesterol to produce vitamin D and bile acids that digest fats. Excess cholesterol in the blood causes cholesterol to accumulate in the blood vessels and thus narrowing and hardening them. Accurate determination of free cholesterol levels in blood serum and food is very important for diagnosis, treatment and prevention of related conditions. Microfluidic paper-based analytical devices (μ PAD) offer different advantages such as low cost, ease of use and disposable (disposable) compared to other measurement methods. Here, a μ PAD was developed using cholesterol oxidase, 3,3',5,5'-tetramethylbenzidine (TMB) and horseradish peroxidase (HRP) for cholesterol measurement. The sensors were tested with solutions containing cholesterol at different concentrations, and color changes in the detection areas were imaged and analyzed using ImageJ. The results showed that the sensor had a low detection limit for cholesterol. The sensor has great potential to be used in different fields from clinic to sports medicine.

Keywords – μ PAD, Cholesterol, TMB, Colorimetric Sensor

I. INTRODUCTION

Cholesterol is an essential molecule to maintain the structural integrity and fluidity of the membrane in the physiological temperature range. Considering its necessity for life, all cells have the ability to synthesize it from simpler molecules. The body uses cholesterol to produce hormones, vitamin D, and bile acids that are critical for digestion and absorption of fats [1]. In order for cholesterol, which is insoluble in the blood, to circulate freely in the blood, it needs to combine with some proteins in the liver for the production of lipoproteins such as HDL and LDL. While a very small amount of cholesterol in the blood is sufficient for related metabolic activities, excess levels cause cholesterol to accumulate in blood vessels, which results in these vessels to be narrow and hardened [2]. Accurate determination of free cholesterol levels in blood serum and food is very important for diagnosis and treatment of various diseases such as stroke,

atherosclerosis, Huntington disease etc. Paper-based sensors have great potential for use in fields ranging from environmental monitoring to clinical and point-of-care testing. These sensors have many advantages such as being disposable, practical, cost-effective and user-friendly [4]. In this study, a microfluidic paper based analytical device (μ PAD) was developed for colorimetric cholesterol measurement. First, a design for the μ PAD was made using PowerPoint. Afterwards, the design was printed on filter paper with a wax printer and hydrophobic channels were formed by thermal treatment. Then, the production of the μ PADs was carried out by transferring cholesterol oxidase, 3,3',5,5'-tetramethylbenzidine (TMB) and horseradish peroxidase (HRP) to the detection areas of the sensors. The results showed that the sensor can successfully measure cholesterol at low levels.

II. MATERIALS AND METHOD

A. Design and Fabrication of μ PADs

First, a design with a single testing area for the printing of μ PADs was made using Microsoft

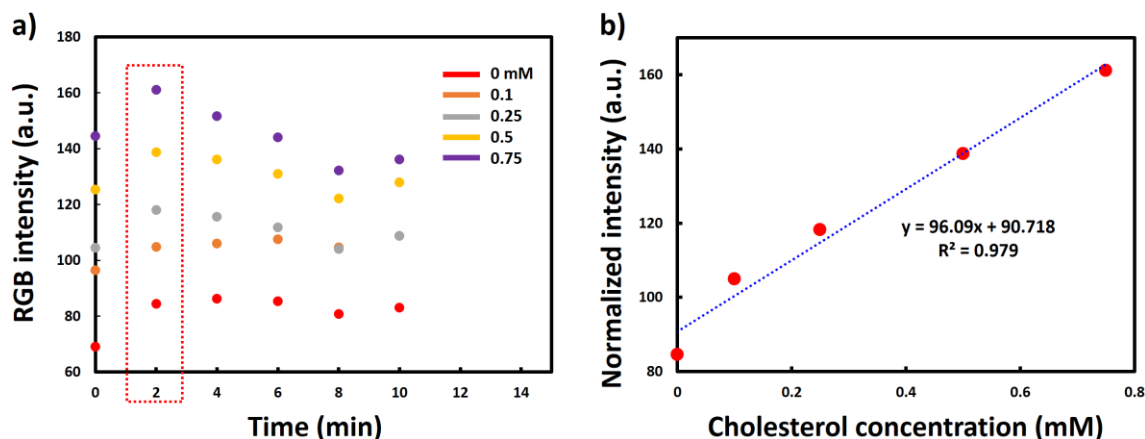


Fig. 2. Color change intensity at different time points (a) along with a calibration curve (b).

Powerpoint. Next, the design was printed on a Whatman filter paper via a wax printer (Xerox 8900) [5-8]. Hydrophobic barriers were formed through heat treatment, in which case the printed papers were placed on a heater (WiseStir MSH-20A) at 120 C and kept there for 5 min to allow solid ink to melt and cross to the other side by passing through the pores of the paper. The heater is covered with aluminum foil and a weight was placed on the filter paper to achieve an equal heat transfer. Afterwards, 0.8 μ l of TMB, 0.5 μ l of HRP and 0.5 μ l of ChOx were added to the test area and allowed to dry for 10 min at ambient temperature prior to testing.

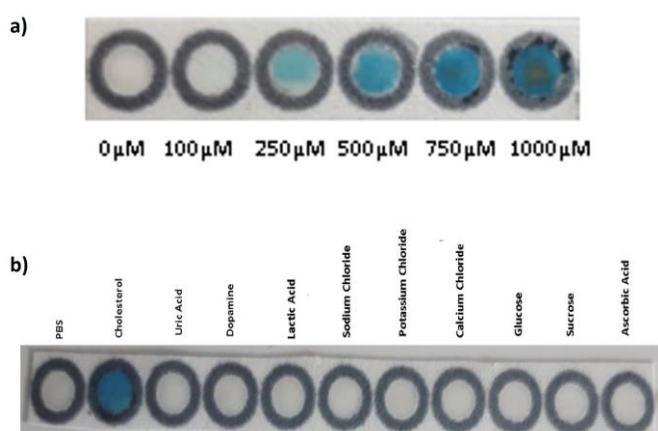


Fig. 1. Color change observed in the detection areas of μ PADs at different concentrations of cholesterol (a) and selectivity test result (b).

B. Colorimetric Detection of Cholesterol and Selectivity Test

Cholesterol was dissolved in a mixture of isopropanol and Triton X-100 (1:1) (49) to prepare a stock solution. In order to ease the dissolution

process, the mixture was kept in an ultrasonic bath for 20 min. Solutions of different concentrations (0, 100, 250, 500, 750 and 1000 μ M) were prepared for testing through dilution with phosphate buffer solution and stored at 4 $^{\circ}$ C. Next, TMB was dissolved in ethanol in an ultrasonic bath for 20 min. μ PADs were prepared by adding first 0.8 μ L of TMB and then 1 μ L of an enzyme mixture containing HRP and ChOx at a ratio of 1:1. In each step, the solution was allowed to dry at ambient temperature. For testing, 2 μ L of cholesterol at different concentrations were added to the detection area of μ PADs and color changes were recorded using a cell phone camera at different time points (0, 2, 4, 6, 8 and 10 min). The images were then analyzed based on RGB data (weighted) using ImageJ. To demonstrate the specificity of μ PADs for cholesterol, a selectivity testing was also performed using different interfering molecules (NaCl, glucose, sucrose, ascorbic acid, uric acid, dopamine, lactic acid, KCl, CaCl₂).

III. RESULTS & DISCUSSION

μ PADs were successfully fabricated using the present fabrication strategy. The hydrophobic barriers resulted in no liquid leakage and thus ensured successful sample containment to detection areas for colorimetric analysis. Given the very low amount of reagents required for testing, the cost of analysis was considerably low. The μ PADs were tested with cholesterol at varying concentrations.

Color change is formed in two steps, each involving a different enzymatic reaction. Briefly, in the first step, ChOx catalyzes the conversion of cholesterol to cholesterol-4-en-3-one, meanwhile H_2O_2 is produced as a byproduct. In the second one, H_2O_2 is used by HRP to oxidize TMB to ox-TMB which has a bluish color. As can be seen in Fig. 1, the color change was formed accordingly with respect to the level of cholesterol. The color change was imaged at different time points and color intensity (RGB intensity (weighted)) data was analyzed using ImageJ. The results showed that the highest intensity was obtained at 2 min, which was selected as the optimum duration for the following experiments. Subsequently, the RGB intensity data of all cholesterol solutions were used to plot a calibration curve. As shown in Fig 2b, the relationship between the cholesterol concentration and RGB intensity data was linear in the range of 100 to 1000 μM with an R^2 value of 0.979. The limit of detection value (LOD) of the μPAD was calculated as 97.9 μM using the formula of $LOD = 3.3 \sigma / \text{slope}$. Addition, a selectivity test was conducted and only in the case of cholesterol, a noticeable color change was observed, demonstrating the high specificity of the μPAD for cholesterol detection.

IV. CONCLUSION

Although cholesterol is an essential molecule in the body, its abnormal levels have been associated with various conditions. Therefore, its sensitive and selective detection is critical in medicine. Here, an easy, disposable, rapid and low-cost paper-based colorimetric sensor was developed for the detection of cholesterol. The sensor displayed an excellent performance in sensitive and selective detection of cholesterol in just 2 min. The sensor can be easily disposed by burning. Therefore, the sensor has a great potential to be applied to real samples for medical purposes.

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