Research Proposal

Title: Advanced Applications and Themes in Paper-based microfluidic devices and detection of Metabolic biomarkers .

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Abstract

Microfluidics is the alternative technology for low cost sensing device.Nowadays researchers are going towards paper based sensing devices because of its advantage for developing countries in disease diagnostic.It has various applications such as Point of care,disease diagnostics, environmental monitoring, and food safety assurance etc.Paper based devices are very good for sensing purposes.these are easy to use,affordable and inexpensive.Here some recent applications of microfluidic paper based devices are described and how we can detect metabolic biomarkers through electro-chemical based μPADs.here we describe the detection of glucose, lactate and uric acid in urine using microfludic device.Paper substrate are very useful to make biosensors and cell detection devices.The paper-based biosensor array using electrochemical reader can enable to acquisition of high-density, statistically meaningful diagnostic information at the point of care in a rapid and cost-efficient way.

Introduction

Microfluidics deals with the behavior that fluidic can flow through channel 1mm or smaller.The reynolds number defines the fluidic flow that it is laminar flow or turbulent flow.For microfluidic reynolds number is very low because it is directly proportional to channel length.So it involves the laminar flow that is very good for sensing platform.The main advantage of paper based microfluidic devices is low sample volume and its miniaturization.We can integrate many operations on a single micro-chip using Lab on Chip technology.Paper substrate is very good thing to make sensing device.We can make devices using paper very quickly and simply.Paper has very promising advantages to use for making sensing devices such as it is made by pure cellulose so that it is compatible with the biochemicals/chemicals.These devices are

very affordable,inexpensive,user friendly,robustness. We can have also a disposable kind of system.One of the main the main thing is that we do not require any external source or pump for fluidic flow in paper substrate channel,fluid flow via capillary action in paper based channel.Although Poloydimethylsiloxane(PDMS) based channel are available for sensing devices but they have some drawbacks also.it has transparent property at visible wavelength.It has also a several ben-efits as ease of mass production and bond to glass or other PDMS layers2 through plasma treatment.But drawback to use PDMS as a substrate to make devices.It requires some external sources for fluidic flow but in paper based device liquid flow is based on capillary action it does not require any external force or sources for fluidic flow.PDMS has also a hy-drophobic nature so that it can also absorb small hydrophobic molecules and there may be chances of swelling due to presence of some small hydrocarbon solvent,water can also evaporates through it.Paper based devices are easy to use,user friendly and affordable.we can make disposable kind of system through paper.these are the several things to use paper based rather than PDMS based. One of the very emerging application is biosensor to measured the gluocse level in the body.Blood glucose monitoring has served as a valuable tool for screening, diagnosis, and long-term management of diabetes.

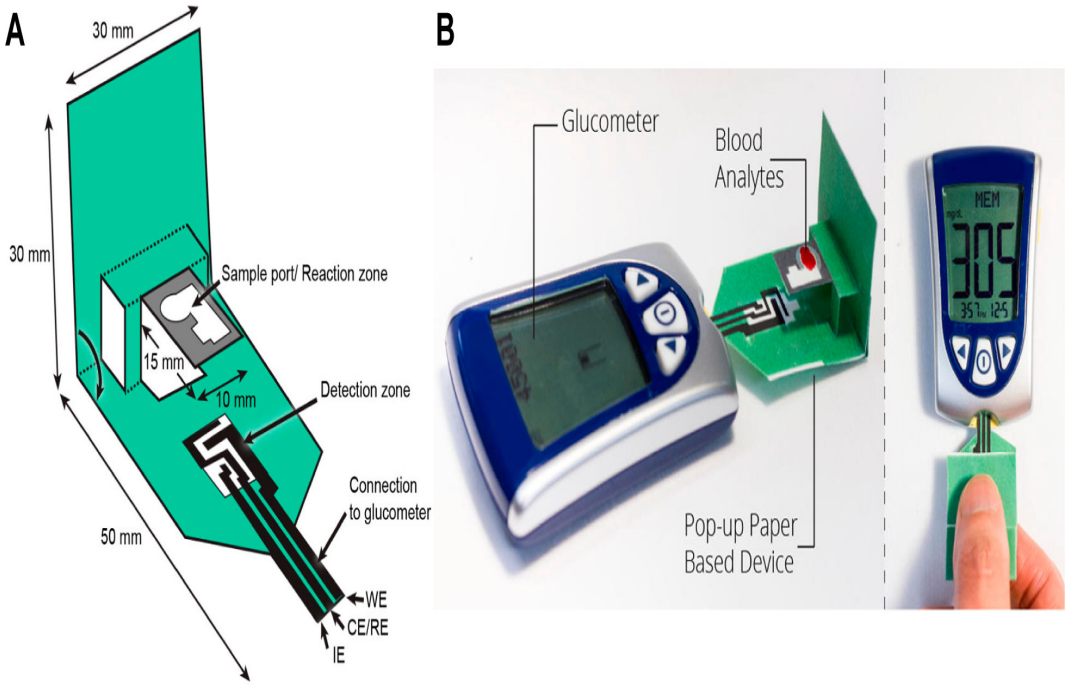

Figure 1. (A) Schematic of the pop-up-ePAD in the open format.(B) Photographs showing the valve capabilities of the pop-up-ePAD when it is used with a glucometer. Reproduced from Wang, C. C.; Hennek, J. W.; Ainla, A.; Kumar, A. A.; Lan, W. J.; Im, J.; Smith, B. S.; Zhao, M. X.; Whitesides, G. M. Anal. Chem. 2016, 88, 63266333 (ref 43). Copyright 2016 American Chemical Society.

Even though glucometers are readily purchased by consumers and patients, it remains a challenge to achieve rapid, accurate, reliable, and sensitive glucose measurements, particularly for the developing world.Glucose oxidase is a major enzyme catalyzing the oxidation of β-d-glucose to gluconic acid and hydrogen peroxide (H_2O_2), is normally immobilized on the surface of electrochemical transducers.The H_2O_2 oxidised and produced a current that measures the glucose level.

Objectives and Work plan

Our main objective here to describe emerging themes and technology to make paper based bio-sensor array and detect the glucose,lactate and uric acid using this device.there are various methods are available to make the paper based channel such as Photo-lithography technique,Ink-jet Printing and Wax printing technology.Here we will describe wax printing to make paper based channel.There are also a various sensing approach for detection.Colorometric,Aperometric and electrochemical sensing etc.Here we used colorometric sensing for detection.

1.Materials

Whatman number 1 paper or chromatography paper,wax printer,Heater,Potassium ferricyanide, d-(+)-glucose, sodium l-lactate, uric acid, glucose oxidase (from Aspergillus niger), lactate oxidase (from Pediococcus sp.), uricase (from Candida).

2.Fabrication

We have chromatogrpahy paper that full hydrophilic and we will have to make the channel .To make the channel we have to create the hydrophobic barrier so that fluidic flow happens only in channel regions and not crossed the barrier.First we make the full design of device in AutoCAD software.We can just easily create this hydrophobic barrier using wax printer.first take the print from the wax printer on chromatography paper and heat it on heater at 150° for 2 minutes so wax will melt and fills the pores through wax and creates the barriers surrounding the hydrophilic channel region.this method is called the wax printing technology and it is widely used.three carbon electrodes (WE: working electrode; CE: counter electrode; and RE: reference electrode) screen-printed on the test zone of the paper channel.The electrodes can be of carbon.We can also make these electrode by conductive ink that is made by any conductive materials such as silver nano-particles and graphene nano-particles etc.We can have paste type solution and we can directly paste it on the paper channel.

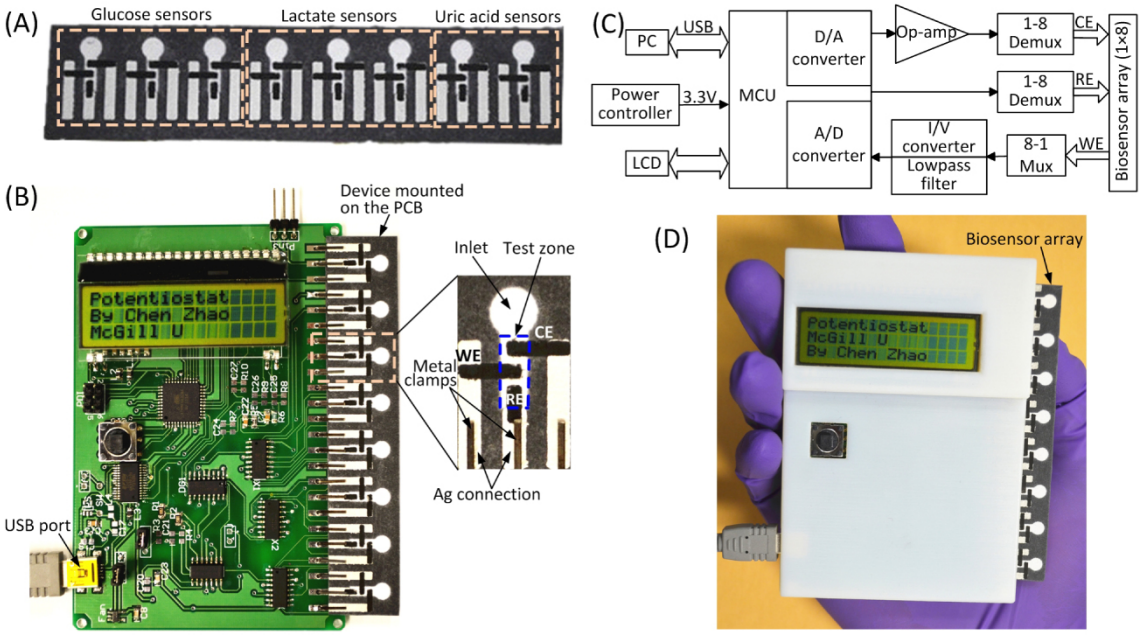

Figure 2.(A) A paper-based electrochemical biosensor array.(B) A microcontroller-based multiplexing potentiostat mounted with a paper-based biosensor array.(C)Potentiostat architecture with eight measurement channels. (D) A photograph of the paper-based biosensor array inserted into the potentiostat.

For the detection of glucose, lactate and uric acid, enzyme-catalyzed reactions were employed and corresponding enzymes and electron-transfer mediators were stored in the test zones to react with the analytes and produce electrical signals. The three electrodes are connected with screen-printed silver strips, and these silver strips serve as contact pads for electrically interfacing with metal clips on the PCB.

3.Sensing Approach

The colorometric is the widely used process for detecting purposes other than any other process.It is very easy process to detect or sense the chemical reaction concentration.When the chemical reaction happens so there will be the change in color and we have a color and concentration trip that on which color what is the concentration and we can easily match the color from the strip and easily get the concentration of chemical reaction.we calculated concentrations of d-glucose, l-lactate and uric acid in AU using chronoamperometry on the biosensor array. Corresponding enzymes (i.e. glucose oxidase, lactate oxidase and uricase) and an electron-transfer mediator (potassium ferricyanide $K_3[Fe(CN)_6]$) were stored in the test zones (figure 1(B)) of the eight biosensing modules in a dry form. To perform a test, a 4 μl drop of spiked AU sample was added to the inlet of each biosensing module, then wicked to the test zone and reacted with the stored reagents. During the reaction, the enzymes GO_X (glucose oxidase), LO_X (lactate oxidase) and UO_X (uricase) catalyzed the oxidation of corresponding analyte into different species (glucose to gluconic acid, lactate to pyruvate and uric acid to allantoin), with a concomitant reduction of $Fe(CN)_6^3$ into $Fe(CN)_6^4$. The generated $Fe(CN)_6^4$ ions were quantified using chronoamperometry:

$$\text{Glucose}+2K_3[Fe(CN)_6+H_2O \xrightarrow{\text{glucose oxidase}} \text{Gluconic acid}+2K_4[Fe(CN)_6$$

$$\text{Lactate}+2K_3[Fe(CN)_6+H_2O \xrightarrow{\text{lactate oxidase}} \text{Pyruvate acid}+2K_4[Fe(CN)_6$$

$$\text{Uric acid}+2K_3[Fe(CN)_6+H_2O \xrightarrow{\text{uricase}} \text{Allaintoin acid}+2K_4[Fe(CN)_6$$

Relevance of the Proposed Study

This is the technology that can has a very promising applications in future.The paper based device are very inexpensive and very easy to use.We need only law sample volume to know any type of disease in our body,not require many tests for disease detection. These type of device are very affordable and disposable kind so that after use it we can destroy them and it can not harm.The emerging applications are nowadays in Point of care diagnostics,Food safety management and environmental monitoring etc.The paper substrate also very good platform to make these device that is compatible for biochemicals/chemicals.The applications of this technology is very useful for the developing country that can not afford the costly diagnostics.

Outcome of the Proposed Study.

In this paper we have shown widely used theme to make paper based microfluidic device and emerging applications and we developed a microfluidic paper-based electrochemical biosensor array (1X8) capable of performing diagnostic tests of multiple biomarkers in a multiplexing and high-throughput fashion. We also designed and developed an inexpensive, compact potentiostat to interface with the paper-based biosensor array for electrochemical signal readout. The paper-based device and the potentiostat formed a portable,affordable, self-contained,user friendly electrochemical biosensing platform, which is promising platform and useful for low-cost, point-of-care diagnostic applications. As a proof of concept, we used the biosensor array for multiplexed detection of three metabolic biomarkers (glucose, lactate and uric acid). The experimental results demonstrated that our device provides analytical performance (LOD and dynamic linear range) sufficient for clinical assays of all the three markers. This is very good paper based sensing platform that will be usefull and an applicable in point of care,disease diagnosis and environmental monitoring and safety.

References

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