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Development of an electrochemical biosensor array for quantitative polymerase chain reaction utilizing three-metal printed circuit board technology

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ABSTRACT

A compact biosensing system is presented that contains an array of devices composed of three-microelectrode electrochemical sensors and resistive heaters. The devices are intended for employment in quantitative polymerase chain reactions in multiple chambers that can be controlled via microcontroller. Arrays of sensors and heaters were developed using an inexpensive advanced printed circuit board technology featuring three different metals. The three-microelectrode sensors were fabricated by a new series of photolithographic and electroless plating processes. The surface morphology of the microelectrodes was characterized by several imaging techniques, including scanning electron microscopy and atomic force microscopy. The electrochemical properties of the microelectrodes were studied by cyclic voltammetry in order to estimate the active electrochemical surface area by solving the Randles–Sevcik equation. The on-board thermal cyclers were realized by feedback control embedded in a portable microcontroller. Quantitative polymerase chain reactions with methylene blue as the redox indicator were carried out as an example of biosensing with the proposed devices, and the results indicate that the prototype array is able to serve as an inexpensive, practical platform for mass production of portable point-of-care instrumentation containing arrays of addressable heaters and sensors.

1. Introduction

Miniaturized labs-on-a-chip (LOCs) and micro-total-analysis systems (μ-TAS) have emerged as important tools in the field of bioanalysis for many reasons, including the potential for portable point-of-care diagnostics using small reagent volumes. One important bioanalysis, quantitative polymerase chain reaction (qPCR), is a well-established and standard molecular technique to amplify, detect and quantitatively analyze nucleic acids in biological species of interest through thermal cycling. Microfluidic devices for qPCR have been widely studied, with current trends aiming to develop LOCs and μ-TAS with feasibility for mass production, simplicity of system integration, and low cost per measurement [1,2]. These requirements lead to electrochemical approaches that feature stability, low cost, and the possibility for use in disposable devices. Electrochemistry-based detection methods for qPCR have been widely investigated as options to improve poor portability of bulky fluorescence-based instruments [3–5]. Electrochemical intercalator based methods using redox indicators, such as methylene blue (MB) and ferrocene (Fc), have been suggested in these investigations. Luo et al. also reported a new approaches using specially designed and synthesized ferrocene-labeled peptide nucleic acid probe (Fc-PNA), which was expected to minimize false-positive results [6]. However, prior research has focused primarily on monitoring the interactions of the chosen redox indicators with the PCR product, while still utilizing conventional thermal cyclers that are not portable [3–6]. Alternatively, systems have combined flow-through heaters with micro-fabricated or commercial sensors to form microfluidic systems [7,8]. However, these systems require complicated microfluidic interconnection and apparatus to flow the solution back and forth between the heaters and sensor, leading to inconvenience for multiple samples and/or multiple analyses. Other researchers have proposed a PCR device that utilized a glass-based electrochemical biosensor, and a micro-machined chamber

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on one side of a silicon wafer with a heater that was patterned the other side. The electrochemical sensor and the chamber with the heater were attached and sealed to form a PCR site [9,10]. The device utilized microfabrication techniques on materials such as glass and silicon, which led to problems of integration such as electrical interconnection with instrumentation and liquid control. In order to solve these problems with existing qPCR microfluidics, an integrated and electrochemical biosensor device for qPCR is proposed. The devices feature localized three-electrode voltammeters and thermal cyclers that can be formed easily into large arrays of individually addressable units with convenient system integration.

Electrochemical detection for qPCR is usually performed using three-electrode voltammetry [3–9]. Generally speaking, three-electrode voltammetry consists of a working, an auxiliary, and a reference electrode, and is a universal tool which is able to derive electrochemical information about an analyte in solution by measuring the signals from a redox reaction at the electrode interface as the applied potential is varied [11]. Noble metals, such as gold and platinum layers, are usually used as electrode materials that are deposited on silicon wafers by sputtering and photolithography processes, which may be unavailable in common laboratories and are associated with an increase in cost due to the need for specialized equipment and clean room microfabrication facilities [12]. In addition, metal thin films deposited via microfabrication processes such as sputtering are very vulnerable to scratch. Furthermore, silicon substrates do not allow secure electrical interconnection or facilitate system integration with other microfluidic components. In addition, screen-printed carbon ink has also been widely used as electrodes in devices such as glucose sensors for reasons of low cost; however, the composition of solvent in the ink significantly influences its electrochemical performance and restricts its application in some non-aqueous solutions [13].

Several recent studies have suggested the printed circuit board (PCB) as a new platform to improve practicality of general microfluidics functions, such as liquid control in microfluidic channels and digital droplet manipulation [14,15]. PCB platforms have also been suggested for applications such as cell lysis and nucleic acid extraction [16–19]. Based on the success of this prior work, we have developed an array of integrated and electrochemical biosensor devices for qPCR with localized three-electrode voltammeters and thermal cyclers utilizing three-metal printed circuit board technology. Through the use of electroless plating and photo-patterning, the metallic micro-electrodes on the printed circuit board facilitate electrical interconnection with instrumentation and integration with microfluidic components. The advanced and mature printed circuit board technology ensures reliability, reproducibility, and low cost for mass production of both single device, as well as arrays of devices. In the proposed system, the metal layers were deposited on the board by electroless plating, and the electrochemical properties of the electrodes were investigated. Utilizing the proposed on-board technology, qPCR was demonstrated as an example of complex electrochemical biosensing with integrated thermal cycling. The objective of the work is to develop an array of electrochemical biosensors utilizing printed circuit board technology to facilitate industry-friendly processes that support the possibility in mass manufacturing of miniaturized lab-on-a-chip systems.

2. Materials and methods

The overall system consists of: (1) arrays of three electrodes made of gold and silver on the same planar surface on the front side of the board; (2) arrays of resistive heaters made of copper, which simultaneously act as temperature sensors, on the back side of the board; and (3) double-sided pin holes for electrical interconnection, forming an array of compact devices. The overall device design for a single device is shown in Fig. 1(f). In this section, the fabrication process to realize arrays of these devices, as well as the methodology used to test them, is presented.

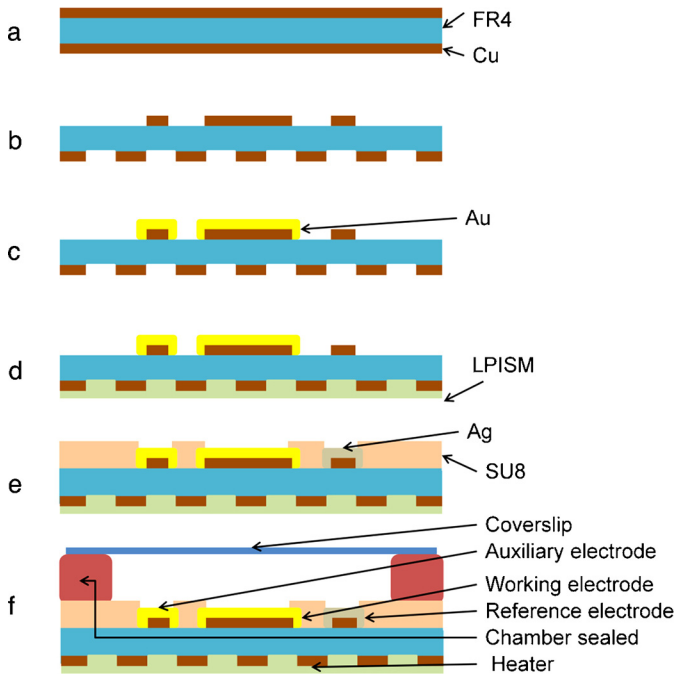

Fig. 1. Fabrication process for arrays of devices containing biosensors and heaters (a single device is shown for simplicity): (a) copper was electrolessly plated on FR4 substrate. (b) Copper layers were patterned to form the base for a three-electrode structure on the front and for a thermal cycler on the back. (c) Gold was selectively plated onto the working and counter electrodes. (d) Soldermask was applied to protect the heater on the back. (e) Silver was electrolessly plated on the reference electrode followed by SU8 coating. (f) The chamber was sealed, attached to the sensor, and covered by a glass slide, forming each biosensor on the board. (The figure is not to scale of the actual dimensions.)

2.1. Fabrication process

The overall fabrication process is shown in Fig. 1. Electroless plating, which is an auto-catalytic chemical technique, was used to deposit layers of metal on 254-μm-thick fiberglass epoxy sheets (FR4, Ventec). The electroless plating process enables a wider range of film thickness and is simpler than sputtering; it was achieved by the process of reactions between a reducing agent with metal ions on the metallic substrate and non-conductive substrates after surface pre-treatment and activation [20–23]. Multiple layers of different metals were formed through processes involving several immersions into chemical baths in an automatic production line. As shown in Fig. 1, after the pin holes were drilled, the FR4 sheet was cleaned with monopersulfate and peroxide based microetch (Oxone® PS-16, DuPont) and immersed in colloidal palladium-tin catalyst (Cataprep™404 and Cataposit™44, Dow). A 2.5-μm-thick copper thin film was deposited on the board by an electroless plating kit that mainly contains copper sulfate and formaldehyde as a reducing agent (Circuposit™3350 and Cuposit™, Dow). The copper thin film was then patterned by dry film photolithography (Riston®, DuPont) and copper etching in ammonium persulfate (High Speed AC-CU guard Plus Replenisher, Phibro-Tech). The copper film on the front side was prepared as a seed layer for gold plating to form the microelectrodes, and the film on the back side was patterned into a serpentine pattern with a wire width of 130 μm to form a resistive heater for each device. To deposit gold only on the working and auxiliary electrodes, a dry film photoresist