PMMA BIOSENSOR FOR NUCLEIC ACIDS WITH INTEGRATED MIXER AND ELECTROCHEMICAL DETECTION

S. R. Nugen, P. J. Asiello, J. T. Connelly and A. J. Baeumner
Dept. of Biological & Environmental Engineering, Cornell University, USA

ABSTRACT

This paper discusses the design, microfabrication and use of an electrochemical biosensor based on a polymer substrate for cost effectiveness and disposability. The poly(methyl methacrylate) (PMMA) was surface functionalized to allow adhesion of gold electrodes. Ultraviolet (UV) assisted thermal bonding of the PMMA was used to preserve integrity of the interdigitated ultramicroelectrode array (IDUA). The sensor used potassium ferro/ferrihexacyanide encapsulating liposomes as reporter molecules for electrochemical detection and amplification. The finished device was able to detect the Nucleic Acid Sequence Based Amplification (NASBA) amplicon from a single Cryptosporidium parvum oocyst.

KEYWORDS: polymethylmethacrylate, interdigitated ultramicroelectrode array, surface modification, liposome

INTRODUCTION

The increased use of PMMA as substrate for microfluidic systems has lead surface modification of PMMA to become an emerging area of research. The use of UV irradiation for the carboxylation of PMMA has shown to be a simple and clean surface modification technique.

We have previously demonstrated highly sensitive detection of nucleic acid molecules using interdigitated ultramicroelectrode arrays (IDUA) fabricated on Pyrex® 7740 as a substrate and overlayed polydimethylsiloxane (PDMS) channels [1]. Here, a titanium adhesion layer was used between the gold and substrate surface. In general, gold has poor adhesion properties to most surfaces including PMMA. Thus, an intermediate adhesion layer of another metal such as titanium or chromium is commonly used between the substrate and the gold layer. However, for an electrochemical detection system, a bimetallic system results in a galvanic cell with the less noble of the two metals being solublized [2]. Since it cannot be guaranteed that the adhesion layer is not coming in contact with the solution, it can result in limited lifetime of the electrode.

In this study, cystamine was conjugated to the UV-modified PMMA surface using water soluble carbodiimide chemistries, resulting in a thiolated surface. A liposomal detection system was employed to aid in signal amplification [3]. The liposome was tagged with a DNA probe complimentary to the target RNA. Superparamagnetic beads tagged with a target complimentary capture probe were used to immobilize the target and liposome complex over the IDUA.
EXPERIMENTAL

The device contained a hybridization chamber and a detection channel. A sawtooth mixer [4] was incorporated into the hybridization chamber to aid in mixing during loading and hybridization. The detection channel contained no sawtooth structures but contained the IDUA. The electrode was designed with a 5 µm gap between the fingers and the finger width of 10 µm.

The adhesion of gold electrodes on the PMMA surface was accomplished by thiolating the PMMA surface. The surface thiolation required an initial UV treatment to induce carboxyl formation. Cystamine was then conjugated to the carboxylated surface using water soluble carbodiimide chemistry.

Gold (200 nm) was evaporated on the thiol-functionalized PMMA surface. The gold electrodes were then formed using standard photolithographic methods followed by a gold etch. The final chip measured approximately 10 mm x 40 mm x 3 mm contained two detection channels for dual analyses (Figure 1).

Figure 1. (a) SEM of a gold IDUA formed on a PMMA substrate. (b) The finished chip containing two detection channels.

Figure 2. (a) Electrochemical response following the injection of OG and lysing of immobilized liposomes. The area of the shaded region was determined and used as the assay result. (b) The response from assays using NASBA amplicon from 0, 1, 3 and 5 C. parvum oocysts. Error bars represent the standard deviation of a minimum of three replicates.
Ferro/ferrihexacyanide encapsulating liposomes were prepared using reverse phase evaporation as described by Goral et al. [1]. The reporter probe was incorporated into the lipid bilayer using a cholesterol tag. Streptavidin coated superparamagnetic beads were conjugated with a biotin-tagged capture probe.

For the C. parvum assay, 1 µL of NASBA amplicon was combined with 1 µL of capture beads, 1 µL of liposomes, and 1 µL of a hybridization mixture. The solution was pumped into the channel and allowed to hybridize prior to a washing step. A detergent consisting of 60 mM n-Octyl-ß-D-glucopyranoside (OG) was then injected into the channel to lyse the liposomes and release the encapsulated redox solution which was in turn detected by the IDUA.

A dye assay determined that the initial UV treatment provided a carboxylation density of 8 nmol/cm² of carboxylic acids. Following the conjugation of cystamine the carboxylic acid density dropped to approximately half.

The assay results showed the ability of the IDUA detecting the amplicon from a single C. parvum oocyst (Figure 2). All oocysts concentrations were statistically distinguishable with a P-value below 0.05 when analyzed with a student t-test.

CONCLUSIONS
We have developed a PMMA based microfluidic biosensor with electrochemical detection ability. Although the polymer biosensor was designed to be disposable, the surface modification of the PMMA allowed the IDUA to adhere to the PMMA during repeated use. The use of a liposomal detection system encapsulating potassium ferro/ferrihexacyanide allowed for the detection of amplicon from a single C. parvum oocyst.

ACKNOWLEDGEMENTS
This work was performed in part at the Cornell NanoScale Facility, a member of the National Nanotechnology Infrastructure Network, which is supported by the National Science Foundation (Grant ECS-0335765). This work also made use of STC shared experimental facilities supported by the National Science Foundation under Agreement No. ECS-9876771.

REFERENCES