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Development of an integrated a-Si:H photodiode detector and its evaluation for chemical and biochemical microfluidic analysis

T. Kamei[1,3], B. M. Paegel[1], E. T. Lagally[1], A. M. Skelley[1], J. R. Scherer[1], R. A. Street[2] and R. A. Mathies[1]

[1]Department of Chemistry, University of California at Berkeley
307 Lewis Hall, Berkeley, CA 94720, USA
Phone: +1-510-643-1321 E-mail: toshi-kamei@aist.go.jp
[2]Palo Alto Research Center, Palo Alto, CA 94304, USA
[3]On leave from National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba 305-8568, Japan

1. Introduction

The micron-scale geometric definition afforded by photolithographic patterning enables exquisite manipulation of nano- and picoliter quantities of sample, drastically reducing analysis times. The goal of such a microfluidic bioanalytical system is to achieve a high degree of automation through integrating all bioanalytical processes onto a single microchip, the so-called "lab-on-a-chip" (LOC) concept. However, in order to exploit potential point-of-care and point-of-analysis benefits of microfluidic LOC devices, the fluorescence detection system has to be miniaturized. Plasma-deposited hydrogenated amorphous silicon (a-Si:H) is an ideal choice of material for integrating fluorescence detectors on glass- and plastic-based microfluidic LOC devices for a variety of reasons: 1) it exhibits high sensitivity at the emission wavelength of most practical labeling dyes such as green fluorescence protein, TOTO, ethidium bromide, and fluorescein, 2) it exhibits low dark current, 3) it is compatible with standard glass and plastic LOC device fabrication processes, and 4) manufacturing is inexpensive [1]. In this study, we have developed an integrated a-Si:H PIN photodiode detector to perform microfluidic genetic assay and amino acid analysis.

2. Experimental

Microfluidic biochip fabrication. The microfluidic capillary electrophoresis (CE) device was fabricated according to previously published procedures [2]. Briefly, Borofloat glass wafers were micromachined using HF wet chemical etching to produce channels. Access holes were drilled for the sample, waste, cathode, and anode reservoirs and the etched and drilled plate was then thermally bonded to a blank glass plate.

An integrated a-Si:H detection platform. The design of the integrated a-Si:H detection system for microfluidic CE analysis is shown in Figure 1. A half-ball lens, a ZnS/YF_3 multilayer optical interference filter with a drilled pinhole, and an annular a-Si:H photodiode were assembled and supported by black anodized aluminum, forming a compact platform for attachment to a microfluidic CE device. The microfluidic CE device was optically coupled to the integrated detector platform with index-matching fluid. Incident laser light (Ar^+, 488 nm) was introduced normal to the microfluidic CE device through the detector to irradiate the CE channel. The annular a-Si:H photodiode and transparent glass substrate allowed vertical laser excitation while avoiding direct incidence of excitation light on the photodiode. The bottom Cr electrode layer of the a-Si:H photodiode acted as an aperture for the incident laser light. Fluorescence was collected and approximately collimated by the half-ball lens and transmitted by the interference filter, which simultaneously eliminated the excitation light. The photocurrent synchronized to chopped laser light was detected by a lock-in amplifier to reduce background.

3. Results and Discussion

Genetic analysis. Portable genetic analyzers will find various applications ranging from pathogen and infectious disease detection to clinical genetic analysis in emergency medical response. Microfluidic polymerase chain reaction (PCR) coupled with CE will form the basic platform in these devices [2], taking advantage of exponential PCR amplification to synthesize detectable levels of DNA, followed by CE analysis to confirm the amplicon size. Here, the microfluidic CE separation and detection of PCR products generated from *Staphylococcus aureus* are performed with the integrated a-Si:H detector to demonstrate the feasibility of using this detection technology in a portable pathogen detection system. Figure 2 shows high-sensitivity detection of the multiplex PCR products from methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) in comparison with a *Hae*III digest of ϕX174 bacteriophage DNA ladder. Two sets of primers were utilized to amplify either *FecA* (219 bp) or *MecA* (310 bp) genes. *FecA* is unique to *Staphylococcus aureus* and common to both MRSA and MSSA, while *MecA* (310 bp) confers antibiotic resistance. As shown in Figure 2, MSSA exhibits only *FecA* amplification while MRSA produces amplicons indicative of both *FecA* and *MecA*. This result is informative about the different resistance of MRSA and MSSA to antibiotics. It should be noted that the entire CE analysis is completed in 2 min. PCR was performed in a conventional thermal cycler and the total run time for 42 cycles was around 3 hours. However, this should be reduced significantly in a microfluidic PCR device due to its rapid thermal

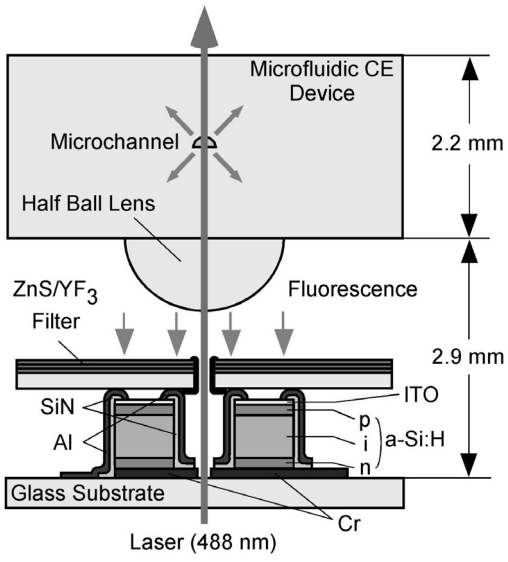

Fig. 1. Schematic cross-sectional view of the hybrid integrated a-Si:H fluorescence detector with a microfluidic CE device.

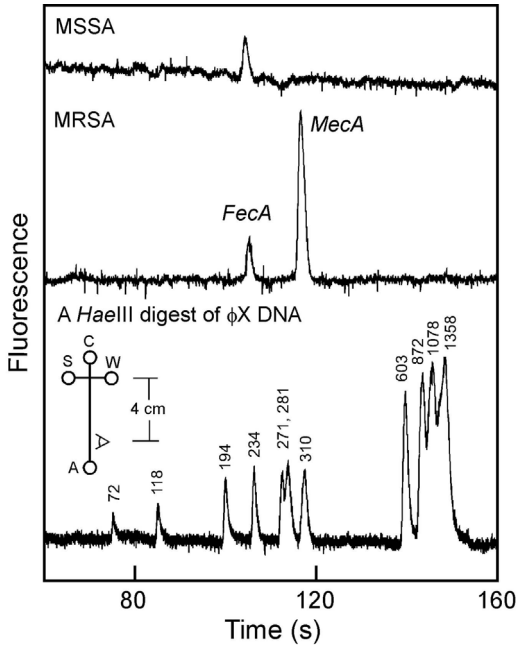

Fig. 2. Analysis of PCR products generated from MRSA and MSSA DNA using the integrated detector. For reference, an electropherogram of a *Hae*III digest of ϕX174 bacteriophage DNA (100 ng/μL) is also shown. CE analysis was performed in a standard sieving matrix (1.4 % w/v hydroxy-ethylcellulose, 50 mM Tris/50 mM acetate/2 mM EDTA) at 250 V/cm. On-column fluorescent labeling was accomplished with the DNA intercalating dye oxyazole yellow (YO, 1 μM).

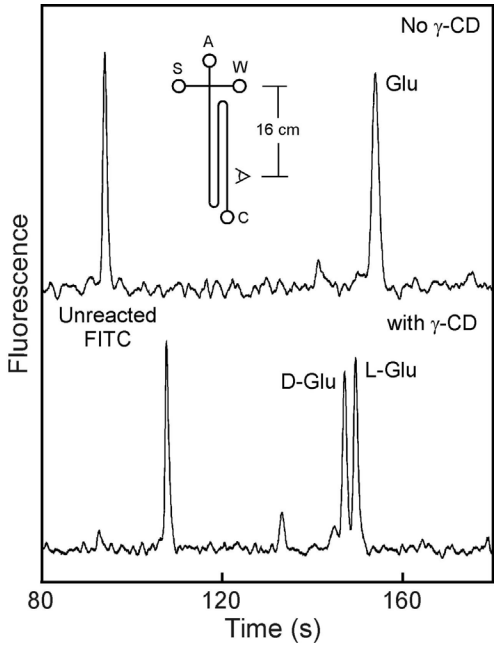

Fig. 3. Separation and detection of FITC-labeled glutamic acid with the integrated a-Si:H detector. The concentration of the derivatized glutamic acid was 23 μM for both the D- and L-isomers. (Upper) Achiral separation of Glu in the running buffer. (Lower) Chiral separation of Glu isomers with 40 mM γ-CD in the running buffer.

cycling capability [3]. Therefore, these results demonstrate that once the integrated a-Si:H detector is coupled with microfluidic PCR-CE the combined system should be capable of high speed pathogen detection and serotyping.

Amino Acid Analysis. Integrated a-Si:H detectors will also be valuable in the development of portable, *in situ* chemical analysis devices. We are developing these systems for biosignature detection in extraterrestrial environments. Assuming that biogenic amino acids are homochiral, an enantiomeric excess of any given amino acid may confirm the presence of extinct or extant extraterrestrial life. *In situ* analysis on Mars is required to exclude the possibility of terrestrial contamination [4]. Light-weight microfluidic chemical analysis systems incorporating high-sensitivity integrated detection are imperative for the success of these missions. Hence, a microfluidic CE device with an integrated a-Si:H detector was used to analyze a racemic glutamic acid (Glu) mixture labeled with fluorescein isothiocyanate (FITC) to demonstrate the potential of the a-Si:H detector for *in situ* chemical analysis. The concentration of the derivatized glutamic acids was 23 μM both for D- and L-Glu. For the microfluidic CE analysis, enantiomeric resolution of the D- and L-Glu was achieved by including a chiral selector agent γ-cyclodextrin (γ-CD) in the running buffer (10 mM carbonate, 12 mM SDS, pH=10). The CE separation was performed at 600 V/cm. Figure 3 shows a single peak characteristic of racemic Glu in addition to an unreacted FITC peak in the absence of γ-CD. The D and L enantiomers are well resolved with excellent S/N by including 40 mM γ-CD in the running buffer and by using a ~16-cm long, 20 μm deep channel. Each peak in the chiral separation is almost baseline-resolved (resolution = 1.35). In addition to the robust integrated construction, the radiation hardness of the a-Si:H photodiode makes it ideal for performing amino acid composition and enantiomeric analysis in harsh extraterrestrial environments.

4. Conclusion

This work is the first demonstration of a high-sensitivity glass- and plastic-compatible phototransducer capable of fluorescence sensing of relevant compounds in a microfluidic channel. An important aspect of the present device is vertical laser excitation through the detector. When combined with VCSEL technology, the integrated a-Si:H detector should facilitate the construction of a coaxial excitation-detection module array for highly parallel monitoring of, for example, capillary and biochemical reactor arrays. The diverse applications for such devices include point-of-care clinical genetic and pathogen analysis, biological warfare detection, and *in situ* remote chemical analysis.

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