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Synergistic effects of micro/nano modifications on electrodes for microfluidic electrochemical ELISA

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ABSTRACT

Microfluidic electrochemical sensing has been considered to be highly efficient. However, we showed, by using numerical simulations in this study, that a planar electrode formed on the bottom of a microchannel is exposed to only a small fraction of analytes in amperometric detection. We also showed that three-dimensional (3D) micropillar electrodes significantly improve the detection current. The practical performance was evaluated using 3D micropillar electrodes fabricated by photolithography. The output current increased as the diameters of the micropillars decreased, as predicted by the simulations. It is noteworthy that the current enhancements obtained with the 3D electrodes were larger than those expected from an increase in the surface area. Further increase in current was achieved by electrical deposition of nanoporous gold-black onto the surface of the 3D electrode: when a 3D electrode with micropillars 30 μm in diameter was used, the output current was approximately 20 times that obtained with a 2D electrode without modification. The applicability of the micropillar electrodes was demonstrated in electrochemical enzyme-linked immunosorbent assay (ELISA) of bone metabolic marker proteins. Although an increase in the surface area of the electrode leads to more noise in general, there is no significant difference in the signal-to-noise ratio between the modified 3D electrode and the 2D electrode without modification in the ELISA experiments. This nanoporous micropillar electrode could potentially be a useful component for the development of on-site diagnosis systems.

1. Introduction

There has been a great deal of interest in the miniaturization of analytical systems for bio/chemical sensing applications, which has led to the development of devices commonly known as a micro total analysis system or a lab-on-a-chip [1,2]. Miniaturized systems offer many potential advantages over conventional assay platforms, including small sample volumes, low cost, short assay time, high throughput, and automation [3,4]. Systems with integrated electrochemical sensors, in particular, could provide new opportunities in bedside diagnosis and assays for use at home, because of their simplicity and ability to function without expensive equipment [5]. Electrochemical sensors have already been exploited in several diagnostic products, including portable systems for self-monitoring of blood glucose in diabetic patients [6]. Despite such favorable characteristics, a critical issue that arises from the miniaturization of such systems is that they must use a relatively low amount of detection current, which sometimes necessitates the use of laboratory-grade detection instruments for performing reliable measurements.

Electrodes in a typical miniaturized system have a two-dimensional (2D) planar form and are often directly patterned inside a microchannel [7]. Although electrochemical sensing in a microchannel has generally been considered to be highly efficient, a large proportion of analyte molecules pass over the electrode even in a microchannel [8]. Therefore, most of the analyte does not come into contact with the electrodes and is wasted. This implies that the detection current can be increased by increasing the collection efficiency, which may lead to high sensitivity.

Many nanotechnology-based approaches have been attempted to improve the detection sensitivity. These include modifications of the surface of an electrode with platinum black [9], carbon nanotubes [10], zinc oxide nanorods [11], and other nanostructures [12,13]. These modifications increase the surface area of the electrode and, in some cases, provide efficient electron transfer, such as in enzymatic sensors. The detection sensitivity is often

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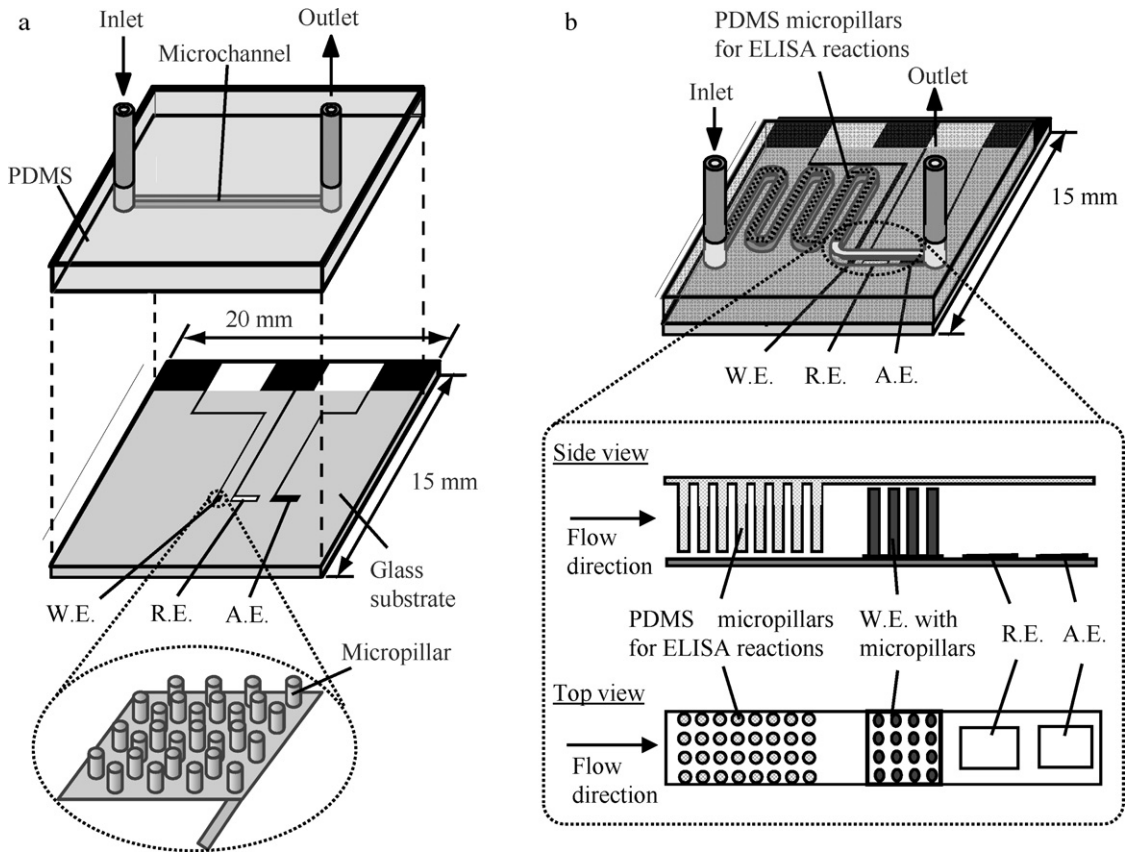

Fig. 1. Schematic of microdevice containing 3D micropillar electrodes and a microchannel. (a) Device used to characterize the 3D electrodes with L-ascorbic acid in numerical simulation and experiments. (b) Device used for electrochemical ELISA. Reactions for ELISA were carried out upstream on the surface of the PDMS micropillars and the enzymatically generated electroactive analytes were detected downstream by the micropillar electrodes. W.E., working electrode (Au); R.E., reference electrode (Ag/AgCl); A.E., auxiliary electrode (Au).

defined as the signal-to-noise (s/n) ratio, in which the detection current represents the signal and the corresponding standard deviation represents the noise. In general, greater the electrode surface, greater is the noise. Therefore, to improve sensitivity and reduce noise, the use of a microelectrode or nanoelectrode array has been proposed [9,14]. The effects of the geometry of the microelectrode or nanoelectrode on the detection sensitivity have been systematically investigated. Although some sophisticated sensing systems have been proposed, modifications have mostly been made to the 2D planar electrodes; therefore, the modifications themselves have not necessarily resulted in the collection of a greater proportion of the analyte molecules that pass over the electrode.

Although microfabrication technologies have been widely used in analytical chemistry, there are only a few studies on an electrode that has 3D microstructures for microfluidic analysis; moreover, no electrodes with 3D microstructures have been developed for the enzyme-linked immunosorbent assay (ELISA). In one such study, an array of micropillar electrodes that were 20 μm in height and 20 μm in diameter were used to eliminate electroactive interferents such as L-ascorbic acid upstream of the detection electrode in the microchannel [15]. In addition, square pillars (~18 μm in height and 20 μm in width) were placed at the end of an electrophoresis microchannel for complete preconcentration and removal of interference in neurotransmitter detection [16]. These studies have clearly demonstrated the efficacy of 3D electrodes in microfluidic bioanalysis.

In this paper, we emphasize the practical applicability of 3D micropillar electrodes further modified with nanoporous structures by electroplating. The efficacy of these 3D electrodes in ELISA is discussed. Micro/nano modifications of electrodes could potentially provide a versatile and fundamental component for the development of portable diagnostic systems with broad applicability.

2. Experimental

2.1. Reagents and materials

Materials used for the fabrication of the 3D micropillar electrodes were obtained from the following commercial sources: Glass wafers (#7740, 3 inch, 500-μm thick), from Corning Japan (Tokyo, Japan); thick film photoresist (SU-8), from Microchem (MA, USA); dry-film photoresist (ME-1048 EA), from Hitachi Chemical Company (Tokyo, Japan); polyimide precursor solution (SP-341), from Toray Industries (Tokyo, Japan); and poly(dimethylsiloxane) (PDMS, KE-1300T), from Shin-Etsu Chemical (Tokyo, Japan).

Reagents used for the characterization of the 3D micropillar electrodes were obtained from the following commercial sources: Human bone alkaline phosphatase (BAP), monoclonal sheep antibodies against human BAP for primary antibody, and biotinylated polyclonal sheep antibody against human BAP for secondary antibodies, from Abcam (MA, USA); human tartrate-resistant acid phosphatase-5b (TRACP-5b), polyclonal antibodies against human TRACP-5b, and biotinylated polyclonal sheep antibodies against human TRACP-5b for secondary antibody, from Abcam (MA, USA); β-galactosidase-streptavidin complex, from Vector Laboratories (Geneva, Switzerland); a blocking solution (Block Ace), from Dainippon Sumitomo Pharmaceutical (Osaka, Japan); and *p*-aminophenyl-β-D-galactopyranoside, from Sigma–Aldrich (MO, USA). All other reagents were purchased from Wako Pure Chemicals