Capillary-assembled Microchip for the Electrochemical Determination of Glucose

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An electrochemical glucose-sensing chip is developed based on a capillary-assembled microchip (CAs-CHIP) technique, which involves the simple assemblage of square capillaries possessing anti-interferential, glucose-sensing and electrochemical-detecting functions in poly(dimethylsiloxane) micro-channels.

Much attention has recently been focused on miniaturized biosensing system that can provide simple, rapid, and inexpensive on-site measurement and personal use.[1] For the quantitative assay of biological and food samples, integration of various chemical functions (e.g., the recognition of analyte, the elimination of interferants, and signal transduction) onto a microsensor system is required. The patterned immobilization of recognition/elimination elements as well as the fabrication of a miniaturized transducer (e.g., electrode) has been carried out for the fabrication of such a micro-sensor system. Various surface patterning techniques applicable to miniaturized and semiclosed systems involving flow-based patterning,[2a] photochemical,[2b] and electrochemical[2c] methods have been reported.

On the other hand, we have developed a simple and universal methodology for constructing multi-functional chip, called "capillary-assembled microchip" (CAs-CHIP).[3a] In this case, different kinds of chemically functionalized square capillaries are prepared and embedded into the lattice microchannel network fabricated on poly(dimethylsiloxane) (PDMS) having the same channel dimensions as the outer dimensions of the square capillaries. We have prepared CAs-CHIPs for the simultaneous determination of ions,[3b] ions and enzymes,[3c] and proteins[3d] based on the fluorescent signal detection using an optical/fluorescence inverted microscope.

Here, we report the combination of the CAs-CHIP-principle with amperometric signal transduction to fabricate a miniaturized biosensor for the determination of glucose by integrating the interferant-elimination, analyte recognition and signal transduction-functions. The electrochemical detection of the hydrogen peroxide produced through glucose oxidase (GOD)-catalyzed reaction provides highly sensitive, reagentless assay of glucose.[4] We have prepared and assembled two kinds of enzyme capillaries, one used ascorbate oxidase (AsOD) for eliminating L-ascorbic acid and the other used GOD, and combined them with a pair of platinum electrode-supporting capillaries for the amperometric signal transduction.

Square microcapillaries having 300-μm outer widths and 100-μm inner widths were purchased from Polymicro (Phoenix, AZ). The polyimide coating of the capillaries was removed by heating before use. A platinum wire (80-μm diameter, 99.98%) was obtained from Nilaco, Tokyo. A PDMS prepolymer (Sylgard184) was obtained from Dow Corning (Midland, MI). The enzymes used were GOD (EC 1.1.3.4, from *Aspergillus* sp., 180 U mg^{-1}; Toyobo, Osaka) and AsOD (EC 1.10.3.3, from *Cucurbita* sp., 230 U mg^{-1}; Toyobo). F-kits (Roche Diagnostics, Basel) were used for the spectrophotometric measurement of glucose and ascorbic acid. Other reagents used were of analytical reagent grade (Wako, Osaka). Distilled and deionized water was used throughout.

Platinum disc and cylindrical electrodes were prepared as follows. The platinum wire was inserted in the hole of microcapillary, the wire/capillary interstices were filled with the PDMS prepolymer, and finally the prepolymer was cured for 2 h at 70 °C. The disc and cylindrical electrodes were served as working and counter electrodes, respectively. The length of the cylindrical electrode was 200 μm.

GOD- and AsOD-attached capillaries were prepared by immobilizing enzyme molecules to the inner surface by well-known silanization chemistries. Briefly, 3-aminopropyltriethoxysilane was introduced into the microcapillary, and left for 30 min to introduce amino groups. After that, the microcapillary was washed with methanol and dried at 70 °C. An aqueous solution of glutaraldehyde (5%) was then introduced and allowed to react for 120 min to attach aldehyde groups. After washing with water, 50 mM phosphate buffer solution (pH 7) containing corresponding enzyme (20 mg/mL) was introduced and left for 120 min.

A PDMS plate with lattice microchannel network with a 1.3-mm × 3.3-mm pitch was prepared according to the procedure described previously.[3a] The width and depth of the microchannel were 300 μm, which was the same as the microcapillaries' outer dimension. The preparative procedure and the structure of preparing electrochemical CAs-CHIP are shown in Figure 1. The enzyme capillaries which were cut into appropriate lengths, and electrodes were embedded into the lattice microchannel network to prepare a designed microfluidic device. After

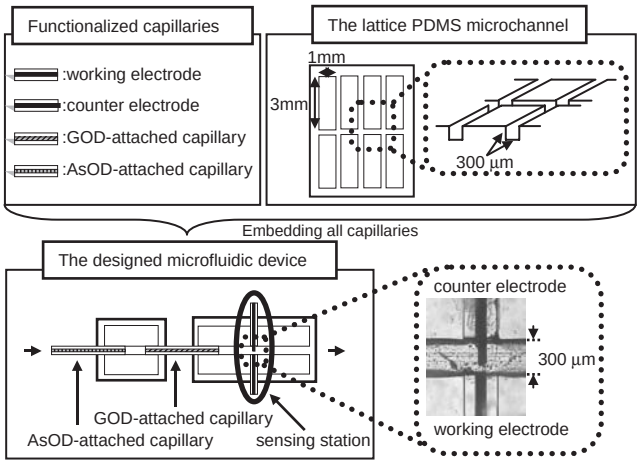

Figure 1. Schematic representation for the fabrication of the glucose-sensing, electrochemical CAs-CHIP.

embedding all the capillaries, a PDMS cover was bounded on top: a spin-coated PDMS prepolymer on an acrylic plate (1-mm thick) was adhered to the capillary-embedded PDMS plate and cured at room temperature for 24 h.

The carrier solution usually used was an air-saturated 0.1 M phosphate buffer solution (pH 7.0). The solution was introduced into the electrochemical CAs-CHIP by a syringe pump (KD Scientific Inc., U.S.A.) with a flow rate of 0.1 μL/min unless otherwise stated. The electrochemical measurements with a two-electrode configuration were carried out by using an electrochemical analyzer (HSV-100; Hokuto Denko, Tokyo).

The present glucose sensing system was based on the anodic determination of hydrogen peroxide. Before embedding the pair of electrodes in the CAs-CHIP system, we examined the current–voltage profile for hydrogen peroxide detection with the working/counter-two electrode system. Both the electrodes were immersed in the phosphate buffer solution (20 mL) placed in a conventional glass cell, and a current–voltage curves were recorded before and after the addition of hydrogen peroxide (0.1–1.0 mM) by sweeping the voltage of the working electrode against the counter electrode from 0 to 0.7 V (sweep rate, 0.1 V/s). For >0.5 V, the current showed a plateau whose magnitude was proportional to the hydrogen peroxide concentration. Therefore, the following experiments on the CAs-CHIP system were performed by setting the voltage of the working electrode at 0.6 V vs. the counter electrode.

Solid circles in Figure 2 denote the current increase upon the injection of glucose into the electrochemical CAs-CHIP using only the GOD-attached capillary (length, 30 mm). The electrode current increases linearly with the increase of the glucose concentration up to 4 mM. The detection limit was 10 μM (signal-to-noise ratio, 3). The relative standard deviation for 5 successive measurement of 3 mM glucose was 2.8%. The electrode response to 1 mM glucose was almost proportional to the cube root of the flow rate (0.05–1 μL/min), suggesting that the current was essentially controlled by the diffusion of hydrogen peroxide. The preparation of a three-electrode system with the appropriate arrangement of the electrodes is under investigation for improving the sensitivity and reproducibility.

On the other hand, the injection of sample containing L-ascorbic acid to the CAs-CHIP caused a discernible increase in the electrode response. Open circles in Figure 2 shows the current response for samples containing different concentrations of glucose and 0.1 mM L-ascorbic acid obtained at the GOD-based system. Such a interferential current increase caused by the oxidizable species coexisted in glucose-containing samples is a serious problem.[4] The interferential response by L-ascorbic acid could successfully reduced by employing the AsOD-attached column (length, 50 mm), as shown open triangles in Figure 2. Thus the CAs-CHIP using the two enzyme capillaries is expected to be useful for the precise determination of glucose in real samples, such as L-ascorbic acid-containing beverages. We have determined glucose in a vitamin C-enriched beverage (the concentration of L-ascorbic acid in the beverage was determined to be 41 mM by using the F-kit for L-ascorbic acid). The beverage was diluted by 100 times with the carrier solution before injection. From the current increase at the two-enzyme-based CAs-CHIP, the glucose concentration in the beverage was determined to be 154 mM, which agreed well with the result obtained by using the F-kit for glucose, 152 mM. The GOD/AsOD-based CAs-CHIP could be used for more than a week.

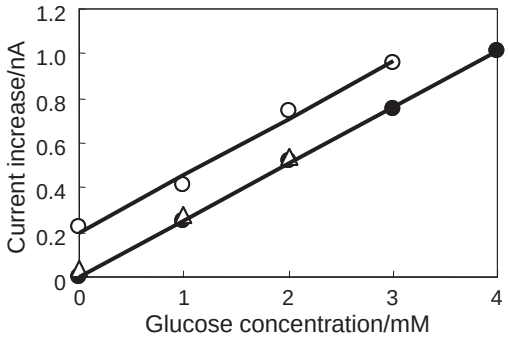

Figure 2. Relationships between the current increase and the glucose concentration: glucose (●) and glucose plus 0.1 mM L-ascorbic acid (○) were injected into the GOD-based CAs-CHIP, respectively, and glucose plus 0.1 mM L-ascorbic acid (△) was injected into the GOD/AsOD-based CAs-CHIP.

The CAs-CHIP technique is highly suitable for the preparation of multi-sensor systems.[3] The fabrication of a chip for the simultaneous determination of a few sugars is now in progress.

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