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Development of a package-free transparent disposable biosensor chip for simultaneous measurements of blood constituents and investigation of its storage stability

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Abstract

A package-free transparent disposable biosensor chip was developed by a screen-printing technique. The biosensor chip was fabricated by stacking a substrate with two carbon electrodes on its surface, a spacer consisting of a resist layer and an adhesive layer, and a cover. The structure of the chip keeps the interior of the reaction-detecting section airtight until use. The chip is equipped with double electrochemical measuring elements for the simultaneous measurement of multiple items, and the reagent layer was developed in sample-feeding path. The sample-inlet port and air-discharge port are simultaneously opened by longitudinally folding in two biosensor units with a notch as a boundary. Then the shape of the chip is changed to a V-shape. The reaction-detecting section of the chip has a 1.0 μl sample volume for one biosensor unit. Excellent results were obtained with the chip in initial simultaneous chronoamperometric measurements of both glucose ($r = 1.00$) and lactate ($r = 0.998$) in the same samples. The stability of the enzyme sensor signals of the chip was estimated at ambient atmosphere on 8 testing days during a 6-month period. The results were compared with those obtained for an unpackaged chip used as a control. The package-free chip proved to be twice as good as the control chip in terms of the reproducibility of slopes from 16 calibration curves (one calibration curve: 0, 100, 300, 500 mg dl^{-1} glucose; $n = 3$) and 4.6 times better in terms of the reproducibility of correlation coefficients from the 16 calibration curves.

Keywords: Package free; V-shaped transparent disposable biosensor chip; Simultaneous measurements; Blood constituents; SMBG; Glucose

1. Introduction

Many glucose biosensors have been developed in recent years (Cosnier et al., 1999, 2002; Suzuki and Kumagai, 2003; Okuda et al., 2003; Poscia et al., 2003; Muguruma and Kase, 2006; Kudo et al., 2006). In addition, many self-monitoring blood glucose (SMBG) biosensors have been marketed successfully for practical use (Nakamura and Karube, 2003; Newman and Turner, 2005). We have developed several types of SMBG biosensors for practical use, such as disposable sensor chips (Kurusu et al., 2005; Kaimori et al., 2006; Karube et al., 2005; Nakamura et al., 2006a, 2007b) and needle-equipped disposable sensor chips (Nakamura et al., 2006b, 2007a), including those having needles with self-sterilizing effects (Nakamura et al., 2005, 2007). The fact that there are over 170 million diabetics worldwide (WHO, 2004) indicates the need for the further development of SMBG biosensors. In addition, because the need to measure other blood constituents has increased in recent years, many biosensors have been developed for measuring cholesterol (Aravamudhan et al., 2007), urea (Eggenstein et al., 1999), uric acid (Zhang et al., 2007), hemoglobin A_{1c} (Tanaka et al., 2007), and other blood constituents (Nakamura and Karube, 2003). Lactate determination is also required in clinical and medical situations because lactate concentrations increase under many

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pathological conditions; therefore, we selected lactate as another analyte for simultaneous measurement based on the previous study on the development of a lactate sensor (Nakamura et al., 2001).

Methods for packaging a disposable biosensor chip include keeping a number of biosensor chips in a bottle, with a desiccant used to maintain dryness. However, as the bottles are opened and closed repeatedly, the moisture-absorbing ability of the desiccant is reduced, rendering such packaging unsuitable for use in humid conditions.

In another system, each biosensor chip is kept in an individual container with a desiccant, which requires numerous packaging materials (Kushimachi, 2000), resulting in an ineffective use of limited resources and excessive waste.

Another packaging method uses biosensor chips with desiccants sandwiched between two layers of film adhered by thermal compression. Since this method requires the application of heat during packaging and processing, the influence of thermal oxidation originating from this heat could alter the body of the biosensor, degrade chemical materials developed in reagent layers, and alter biomaterials. Further, the influence of heat-derived vapor pressure to maintain a given humidity must be considered. Such packages cannot be easily opened, especially for people with disabilities, the elderly, or children, since the adhered portions, formed by thermal compression, stick together firmly.

The structure of conventional disposable biosensor chips maintains the chip quality. Known mechanisms are used to automatically supply a sample solution to a sensor using a capillary or other type of action (Nankai et al., 1988). A sensor with such a structure was assembled by laminating a spacer and cover on an electrically insulated substrate. An electrode pattern was formed on the substrate, and air holes were opened on the cover to discharge the air necessary for capillary action. The substrate, spacer, and cover formed a sample-inlet port with air holes on one side and a sample-feeding path to provide a given amount of sample solution to the detecting section by capillary action. With these biosensor chips, exposure of the sample-feeding path to open air had to be avoided, and the chips had to be packaged for stable storage.

In this study, a package-free transparent disposable sensor chip with a small sample-feeding path filled by capillary action was prepared by a screen-printing technique (Matthews et al., 1987; Hart et al., 2004). The characteristics of the chip, particularly its double electrochemical measuring elements for the simultaneous measurement of multiple items and its storage stability, were investigated.

2. Experimental

2.1. Materials—reagents

Commercially available reagents were used in this study. Glucose oxidase (GOD; from *Aspergillus niger*; 217 units mg^{-1}) was purchased from Amano Enzyme, Inc. (Nagoya, Japan). Lactate oxidase (LOD; from *Pediococcus* sp.; 49 units mg^{-1}) and Triton X-100^{TM} (Triton) were purchased from Sigma Chemicals (USA). D-Glucose, lithium lactate, potassium ferricyanide, and potassium ferrocyanide, all of analytical-reagent grade, were purchased from Wako Pure Chemicals (Osaka, Japan).

2.2. Chip designs

Two types of biosensor chips are illustrated in Fig. 1. In Fig. 1(a) an unpackaged open-type biosensor control chip is shown. The control chip, flattened to a V-shape with a 90° angle (Fig. 1(a-i)), consists of three parts: a substrate, a spacer

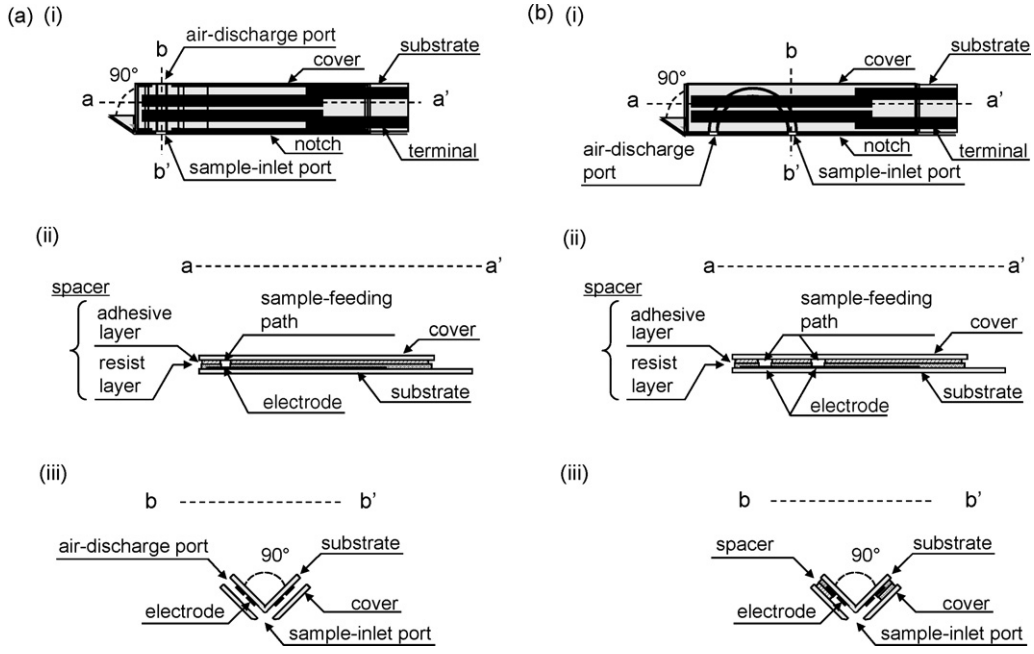

Fig. 1. Assembly illustration of the unpackaged biosensor chip (control). (a) The unpackaged biosensor chip (control) and (b) the package-free biosensor chip (trial). (i) V-shaped for use, (ii) a–a′ cross-section, and (iii) b–b′ cross-section.