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Fabrication of a Glucose Biosensor Based on Inserted Barrel Plating Gold Electrodes

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We demonstrate here the application of barrel plating gold electrodes for fabricating a new type of disposable amperometric glucose biosensor. It is prepared by inserting two barrel plating gold electrodes onto an injection molding plastic base followed by immobilizing with a bioreagent layer and membrane on the electrode surface. The primary function of barrel plating is to provide an economical way to electroplate manufactured parts. The manufacture procedure is simple and can increase the fabrication precision for automation in mass production. At the two-electrode system, the detection of glucose is linear up to 800 mg/dL (i.e., 44.5 mM, $r^2 > 0.99$) in pH 7.4 PBS with a sensitivity of 0.71 μA/mM. Excellent sensor-to-sensor reproducibility shows coefficients of variation of only 0.8−1.4% for the detection of 56.5−561.0 mg/dL glucose. In laboratory trials 176 capillary blood samples with a range of 30−572 mg/dL glucose are used to evaluate the clinical application of the biosensor. A good linear correlation is observed between the measured values of the proposed biosensor and laboratory reference. Error grid analysis verifies that the proposed technique is promising in fabricating biosensor strips on a mass scale. As successfully demonstrated by using whole blood glucose as a model analyte, the fabrication technique can extend into other barrel plating noble metal electrodes for various applications.

The development of various type of disposable sensing strips is a continuous interest for the determination of biomolecules in physiological fluids.[1−7] The microfabrication of enzyme-based electrochemical biosensors is commonly based on screen-printed carbon electrodes (SPCEs) with suitable conducting modifiers on the substrate and then immobilizing a bioreagent layer (i.e., enzyme, mediator, stabilizer, surfactant, linking, and binding agents) and membrane on the electrode surface.[8−13] An accurate measurement of glucose level in blood has long been recognized as an important clinical test for diagnosing diabetes mellitus.[14−20] Indeed, amperometric type self-monitoring blood glucose (SMBG) biosensors using the disposable-type screen-printed sensor strips have gained a large market share.[21−25] Such single-use devices eliminate problems of carry over, cross contamination, or drift and have been investigated extensively. Many of these sensors rely on detection of hydrogen peroxide generated by the glucose oxidase (GOx)-catalyzed reaction. However, direct amperometric detection of hydrogen peroxide requires a relatively high oxidation potential (i.e., > +0.7 V versus Ag/AgCl) and thus may suffer severe interference from readily oxidizable species present in physiological sample. Electrocatalytic mediators are often used to modify the electrode in order to accelerate the electron transfer kinetics and also a decrease of the overpotential.[24−30] In order to function effectively, the mediator should react rapidly with the

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reduced enzyme, possess good electrochemical properties, and have low solubility in aqueous medium. As a result of using these electron-carrying mediators, measurements become largely independent of oxygen partial pressure and can be carried out at lower potentials that do not provoke interfering reactions from coexisting electroactive species. The oxygen competition can be minimized if the rate of electron transfer via the mediator is high compared to the rate of the enzyme reaction with oxygen. Commercial blood glucose self-testing meters generally rely on the use of ferricyanide or ferrocene mediators. Nevertheless, despite their low cost and mass production, such sensor strips are based on a high degree of sophistication essential for ensuring high clinical accuracy, uniform sample coverage, and separation of the blood cells. Furthermore, since the printing ink for preparing SPCEs must contain adhesive and insulating polymer to improve the adhesion onto the substrate, the ink might then result in inconsistency of an electrochemically active area with low precision in analysis.

We report here an engineering process of barrel platting technology for mass production of disposable-type electrodes. The primary function of barrel plating is to provide an economical way to electroplate manufactured parts that meet specific finishing requirements.[31,32] Our previous studies have successfully used Rh- and Ni-barrel platting electrodes (BPEs) for analytical applications.[33−36] Such an electrode manufacturing technique holds many advantages, such as consistency of electroactive area, elimination of toxic organic solvents used in the screen printing process, and improvement of electron transfer at noble metal electrodes. To validate the proposed technique, the determination of whole blood glucose was used as a model analyte in this study. The glucose biosensor is fabricated mainly by inserting two Au-BPEs onto an injection molding plastic base. Note that the Au electrode was reported to firmly immobilize GOx on the biosensing interface with excellent conductivity.[37] The strip with a U-shaped notch in its side can allow the correct volume of blood to be drawn into the reactive area of the strip by capillary action when the side comes into contact with a blood drop. Since ferricyanide exhibits an excellent electrochemical activity as electron transfer mediator at the Au-BPE, glucose oxidized by GOx and the electrons involved in the redox reaction can be relayed through the mediator to the electrode, resulting in electric currents proportional to the level of glucose in sample solutions. Overall, the aim of this work is to demonstrate the analytical utility of such amperometric sensors. The electroanalytical properties and analytical performance of the biosensors were reported to exploit the advantages of the proposed system in this contribution.

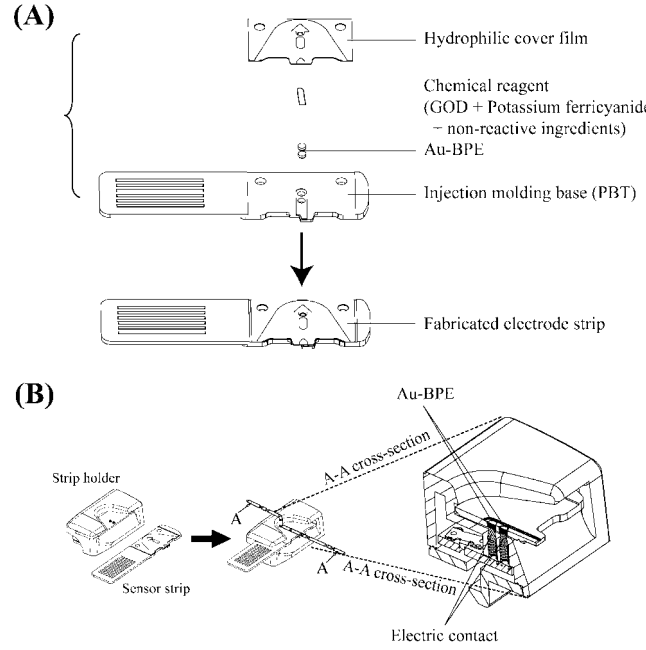

Figure 1. (A) Fabrication of the injection molding plastic strip with two inserted Au-BPEs. (B) Schematic representation of the electric connector for testing the sensor strips.

EXPERIMENTAL SECTION

Materials and Apparatus. GOx (EC 1.1.3.4, >180 U/mg from *Aspergillus niger*) was purchased from Amano Enzyme Inc. (Nagoya, Japan). Potassium ferricyanide and β-D(+)-glucose were bought from MP Biomedicals (Solon, OH). All other chemicals were of analytical grade and used without further purification. All solutions were made using deionized water (18 MΩ cm resistivity) from a Barnstead Easy Pure LF system. Glucose solutions were prepared before use and stored at 4 °C. The 0.1 M, pH 7.4 phosphate buffer solution (PBS) was prepared by stock standard solutions of NaH_2PO_4 and adjusting the pH with 0.5 M NaOH. The electrode surface was examined by using an Olympus STM6 microscope. The electrochemical measurement and cyclic voltammetric experiments were carried out at a CHI1221 electrochemical analyzer (CH Instruments, Austin, TX). A two-electrode configuration was used in which one Au-BPE served as the working electrode and the other Au-BPE as the reference/counter electrode. The Au-BPE with a diameter of 1.145 mm was dried at room temperature (25 ± 2 °C) to 50% relative humidity before use. All applied potentials were versus the reference/counter electrode and carried out at room temperature. Reference values measured by using the YSI 2300 STAT Blood Glucose Analyzer (YSI, Yellow Springs, OH) were used to estimate the accuracy of capillary blood glucose.

Electrode Strip. Figure 1 clearly demonstrates the feasibility of fabricating such sensors with the stepwise fabrication process of the proposed electrode strips. Compared to the multiple-step procedure required by screen printing technology, the fabrication steps are largely reduced. Barrel plating of gold was performed by immersing the electrodes into a 2 g/L $KAu(CN)_2$ solution in

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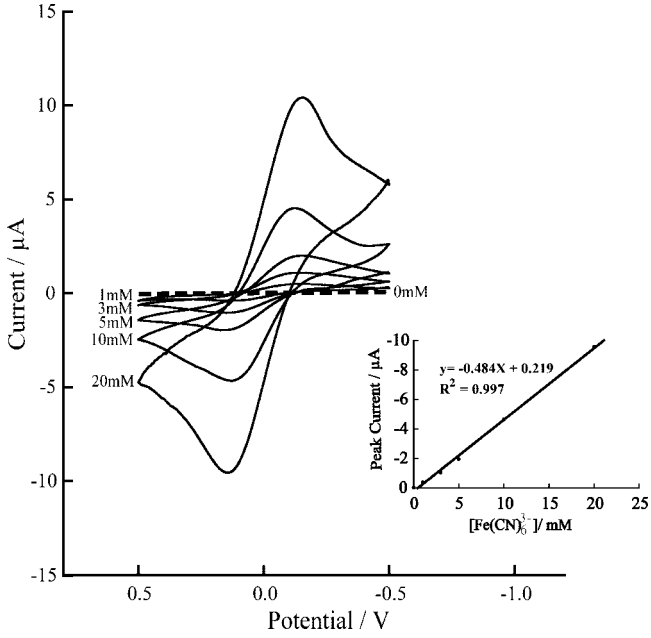

Figure 2. Cyclic voltammograms for various concentration of potassium ferricyanide (0−20 mM) in 0.1 M KCl measured by the Au-BPE at a scan rate of 20 mV/s. Inset shows the obtained calibration curve.

a hexagonal plating barrel and applying a potential in the range of 6−8 V for 50 min. The final diameter of the Au-BPE was ~1.145 mm. The electrodes are plugged into the hollow holes physically without the use of adhesion to connect the electrodes with the base. The key is that the aperture of the holes should be smaller than the diameter of the Au-BPE. Large number of electrodes, i.e., 0.8 million electrodes/per lot, can be processed in our barrel plating manufacture process. Then chemical reagents containing 48.5% GOx, 8.5% potassium ferricyanide, and 43% nonreactive ingredients are immobilized on the surface of the Au-BPEs. The final step is to place a hydrophilic film (3M Company) around the rectangle area of integrated reagent/blood separation film to finish fabricating the electrode strips. Glucose can be detected as the blood is drawn gently into the strip by capillary action.

Sensor Evaluation. Cyclic voltammetric experiments of ferricyanide were carried out in 0.1 M KCl supporting electrolyte at a scan rate of 20 mV/s. The electrochemical measurement of blood constituents was accomplished by a mediator-detecting system using potassium ferricyanide as an electron acceptor. The amperometric current response of glucose was measured at a fixed potential of +0.2 V versus the reference/counter electrode and collected with a 2 s delay time. The anodic steady-state currents (measured in 0.1 M, pH 7.4 PBS at 15 s) were used to plot the calibration curves. Clinical performance was evaluated and compared to the plasma values from the laboratory reference for 176 capillary blood samples. The laboratory results were analyzed by linearity test and Clarke error grid analysis.

RESULTS AND DISCUSSION

The use of ferricyanide as a redox mediator to lower the applied potential to discriminate from interfering species is first evaluated at the proposed sensor. Figure 2 shows the typical cyclic voltammetric responses of ferricyanide at the Au-BPE. As can be

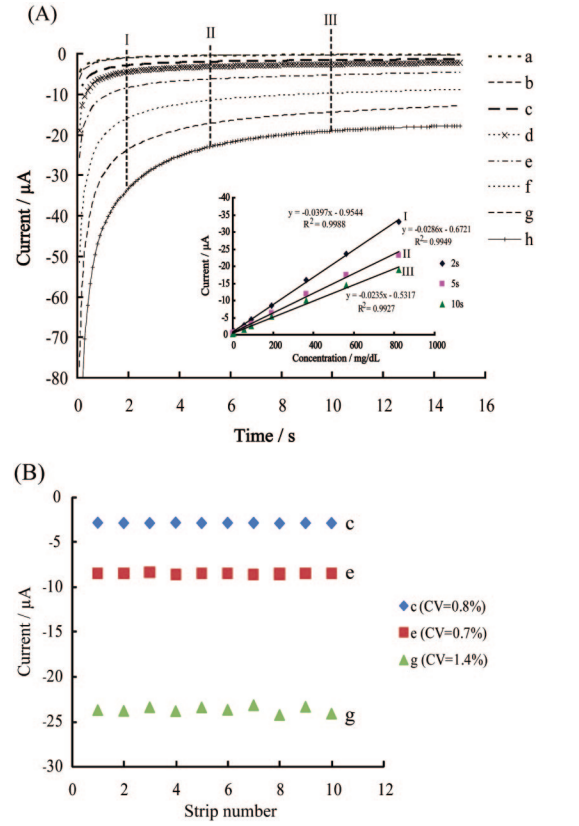

Figure 3. (A) Amperometric dynamic responses of the proposed sensor system. Ten sensor strips were used for glucose concentration of (a) 0, (b) 5, (c) 56.5, (d) 91.9, (e) 191, (f) 364, (g) 561, and (h) 822 mg/dL in pH 7.4 PBS. (B) The data of 2 s recording time at a constant potential of 0.2 V for calculating CVs.

seen, well-defined and reversible one-electron transfer voltammograms up to 20 mM ferricyanide with a good linear calibration plot for the measured oxidation current with respect to the concentration of ferricyanide (slope = −0.484 μA/mM, $r^2 = 0.997$) are observed. Since ferricyanide exhibits an excellent electrochemical activity as an electron transfer mediator at the Au-BPE, glucose oxidized by GOx and the electrons involved in the redox reaction can be relayed through the mediator to the electrode. The resulting current responses proportional to the level of glucose in sample solutions can thus be used for quantification.

As mentioned in the Experimental Section, the strip structure was designed for mass production to ensure sample introduction by capillary action for simple and universal usage. To verify this, 10 sensor strips were used for the detection of each glucose concentration by measuring the chronoamperometric responses with varying concentration of glucose at various times of 2, 5, and 10 s. The observed representative dynamic responses of the sensors toward each concentration are shown in Figure 3. As can be seen, a good correlation of current signal versus the concentration of glucose in pH 7.4 PBS is observed. Most importantly, in the proposed strip, a 2 s delay time is enough for the sample to reach the working electrode for good correlation between the response current and glucose concentration (slope = −0.0397 μA/mM, $r^2 = 0.999$). Compared to the most known glucose sensor, this is a fast-response sensor with good linearity in the measurement of glucose concentration particularly for clinical

use in diabetic patients.[1] As to the electrode-to-electrode reproducibility, the CV of <2% (n = 10) is better than most commercially marketed glucose sensors (CV: 3−6%).[1,5,38] Overall, the sensor-to-sensor reproducibility, sensitivity, and linearity obtained with the proposed sensor system appear to be adequate for glucose measurements in physiological samples.

Good reproducibility of the electrochemical response to glucose of the proposed biosensor can be accounted from the difference in surface morphology between SPCE and Au-BPE (Figure S-1 in the Supporting Information). A rough and uneven plane with randomly distributed carbon particles was observed on the surface of SPCE. The high and low plane was measured as ~2.4 μm at a bare SPCE compared to ~0.6 μm at the Au-BPE. Obviously the surface is much smoother at the Au-BPE. Furthermore, since the SPCE contains adhesive and insulating polymer to improve the adhesion onto the substrate, this might also result in inconsistency of the electrochemically active area. In general, only a better electrode-to-electrode reproducibility can result in high precision in analysis and this is indeed in the case at the Au-BPE. The long-term stability of the devices was further tested. The sensor response up to 5 mM glucose was recorded once every 7 days. Note that the sensor was stored under dry conditions at 25, 40, and 50 °C when not in use. The oxidation current was found to remain almost unchanged for 90 days with a bias versus day 1 of less than 5%. Most importantly, this result shows the stability of the Au film to immobilize the enzyme and not to denature the enzyme.

Our ultimate goal is to devise an amperometric sensor for use in diabetes mellitus. It is well-known that the coefficients of variation (CVs) obtained from the repeated measurement values for the same blood and the CV between the measured values with the sensor and those with the high-end sensor are often used as an index of the sensor's accuracy. In laboratory trials, we used 176 capillary blood samples to evaluate the performance of the amperometric sensors. As can be seen in Figure 4, the Clarke-type error grid analysis of the data shows that 100% of the points are in the clinically acceptable zone A (96.6%) and B (3.4%). Furthermore, a good correlation between the measured values of the amperometric sensors and plasma values from laboratory reference was observed with slope = 1.03 μA/mM and r^2 = 0.982.

CONCLUSION

In this report, we have described a new technique for fabricating disposable amperometric sensor strips by inserting barrel plating gold electrode (Au-BPE) into an injection molding base. The glucose sensor system is shown to work at low working potential with good reproducibility. It is simple to construct and easy to operate. Given the promising results, it is likely that the amperometric sensors fabricated by such a method will offer a relatively straightforward method to manufacture amperometric sensor strips on a mass scale for different kinds of biosensors by using different barrel plating noble metal electrodes. We conclude that the Au-BPE system offers a useful platform for the development of a highly precise glucose sensor. Current efforts target the fabrication of microsensor based on this approach and methods for the routine analysis of other blood constituents, such as cholesterol, urea, uric acid, and hemoglobin A1c.[39−42]

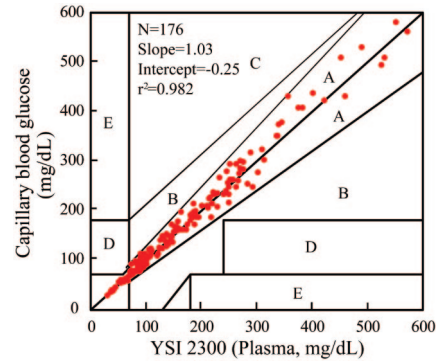

Figure 4. Error grid analysis (EGA) for capillary blood glucose measured by the proposed biosensor and venous plasma glucose values obtained from YSI 2300. Plasma glucose: 30−572 mg/dL, n = 176.

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SUPPORTING INFORMATION AVAILABLE

Microscopic images (5400-fold) of a bare SPCE and the Au-BPE. This material is available free of charge via the Internet at http://pubs.acs.org.

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