Modification of microcantilevers using layer-by-layer nanoassembly film for glucose measurement

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Abstract

Here, we report a new approach of glucose measurement by using a multilayer modified microcantilever. Glucose oxidase (GOx)/polyethyleneimine (PEI) multilayer modified microcantilever underwent bending when it was exposed to glucose solutions. The magnitudes of bending were proportional to the concentrations of glucose. This technique provides a new platform for enzyme functionalized cantilever biosensors.

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Monitoring blood sugar levels is a critical component of diabetes care. Extensive research is still underway to develop new methods and technologies for improved glucose monitoring. Advances in the field of micro/nano-electro-mechanical systems (MEMS and NEMS) now offer unique opportunities in the design of small and ultrasensitive analytical methods. One of the most promising MEMS is microcantilevers, which have been proven to be an outstanding platform for chemical and biological sensors [1–7]. Integrated circuits of microcantilever-based chips have been developed for processing the data and transmit it as radio signals to a remote receiver [7]. The unique characteristic of microcantilevers is their ability to undergo bending due to molecular adsorption or binding-induced change in surface tension. This is achieved by confining the adsorption to one side of the cantilever. The cantilever can be made of any materials that can deflect when force or surface stress is applied on the cantilever surfaces, the deflection amplitude depends on the thickness, the length, and the materials of the cantilevers.

Many electroenzymatic glucose measurement methods are based on the catalytic activity of glucose oxidase (GOx) [8,9]. The foundation of these devices is the oxidation of glucose, which produces gluconic acid, as shown in the following equation.

\[
\beta-D\text{-glucose} + O_2 + H_2O \rightarrow \text{Glucose Oxidase} \rightarrow D\text{-gluconic acid} + H_2O_2
\] (1)

The reaction results in a decrease in pH, which has been monitored as an indirect measurement of glucose concentration. The detection scheme based on the monitoring of GOx-catalyzed oxidation products of glucose is suitable for continuous monitoring of glucose.

Our initial efforts in the development of GOx modified microcantilever glucose sensors focused on conventional surface conjugation chemistry [10]. For instance, aminopropylthiol was used to form a monolayer on the gold surface of a microcantilever, then the microcantilever was treated with 1-[3-(dimethylamino)propyl]3-ethylcarboxodiimide hydrochloride to conjugate GOx on the cantilever surface. However, similar to the work reported recently, [11] only slight microcantilever response (<7 nm deflection for a 200 μm-long silicon microcantilever) was observed upon exposure of these microcanti-
levers to glucose. A novel approach was needed. Recently, a nanoassembly layer-by-layer (LBL) technique was introduced, which allows formation of nanometer scale organized films on any surface through alternate adsorption of oppositely charged components, such as linear polyelectrolytes and enzymes [12–16]. The required component could be located at a designed location in the film with nanometer precision.

Since microcantilever bending is generated from adsorption-induced surface stress from one side of the microcantilever, the key surface modification technology is to control the formation of multilayers on only one surface of the microcantilever by choosing appropriate surface materials. Recently, we found [17] that the charged polymers do not stick on perfluorocarbons surface because perfluorocarbons are both hydrophobic and lipophobic. By immobilizing perfluorocarbon materials on one surface of a microcantilever, formation of multilayer film occurs solely or mainly on the other surface of the microcantilever due to the unique property of perfluorocarbons. In this Letter, we report for the first time layer-by-layer nanoassembly technology can be readily used to modify microcantilevers for biosensor developments. The glucose sensing behaviors of GOx modified microcantilevers are presented.

The deflection experiments were performed in a flow-through glass cell (Digital Instruments, CA) similar to those used in atomic force microscopy (AFM). Cantilever bending was measured by monitoring the position of a laser beam reflected from the gold-coated side of the cantilever onto a four-quadrant AFM photodiode. We focused on surface modification of the commercially available silicon microcantilevers (Park Instrument, CA) in these experiments. The dimensions of the V-shaped microcantilever were 180 μm in length, 20 μm in width, and 1 μm in thickness. One side of the cantilever had a thin film of chromium (3 nm), followed by a 20 nm layer of gold deposited by e-beam evaporation. Another side of the microcantilever was made of silicon with a thin, naturally grown oxide layer. (Tridecafluoro-1,1,2,2-tetrahydrooctyl)triethoxysilane (TTS) and 1H,1H,2H,2H-perfluorodecanethiol (PFDT) were used to develop a thin perfluorocarbon film on the silicon surface or the gold surface using typical surface modification procedures, respectively [18,19].

Cationic polyethylenimine (PEI), anionic poly(sulfonate styrene) (PSS), and GOx (Sigma, 166,500 units/g solid, negative at pH 6.7) were used for modification of the cantilever surface by using layer-by-layer self-assembly technique.

The formation of multilayer on the microcantilever was conducted at pH 6.5 using a modified multilayer formation procedure: A TTS [20] or PFDT treated microcantilever was immersed into a 1.5 mg/mL solution of PEI for 20 min, and rinsed with a stream (~100 ml/min) of water. The cantilever was then immersed into a 3 mg/mL solution of PSS (or 1 mg/mL GOx) also for a duration of 20 min and again rinsed with the water stream. This procedure was repeated several times until a desired multilayer film was formed through interactions between oppositely charged components. In our experiments, the multilayers on both types of microcantilevers were (PEI/PSS)₃, followed by (PEI/GOx)₃. Hereafter, the multilayer modified TTS or PFDT treated cantilevers were named microcantilever-A and microcantilever-B, respectively. Since the glucose level in a human body is between \(2 \times 10^{-3}\) and \(1 \times 10^{-2}\) M (diabetic patients have a glucose level of \(>6 \times 10^{-3}\) M), the cantilever responses to glucose solutions between \(1 \times 10^{-3}\) and \(1 \times 10^{-2}\) M were investigated.

The microcantilevers were initially exposed to a constant flow (4 mL/h) of a 0.01 M solution of NaCl in a fluid cell. The NaCl solution was circulated through the cell using a syringe pump. When a solution containing a \(8 \times 10^{-3}\) M glucose in the 0.01 M NaCl solution was injected into the fluid cell, the microcantilever-A bent down, but the microcantilever-B bent up, respectively, as shown in Fig. 1. This was in accordance with our expectation that the bending direction of microcantilever-A and microcantilever-B should be opposite since the multilayer films were assembled on the opposite sides of the two types of microcantilevers. However, microcantilever-A reached its equilibrium in 5 min after exposure to glucose, but microcantilever-B did not reach its equilibrium even ~30 min after the injection of the glucose solution. For each measurement, a 2.0 mL aliquot of the glucose solution in 0.01 M of NaCl was switched into the fluid cell where the microcantilever was held. It took 30 min for the injected glucose solution to flow through the fluid cell, and at this time, the original 0.01 M NaCl solution was circulated back into the fluid cell. This correlates well with our observations that the deflection of the microcantilever-A quickly returned to its original position ~30 min after the injection of glucose solution. Microcantilever-B, however, bent up first.

![Fig. 1. Bending response for a microcantilever-A and a microcantilever-B to a 8 \times 10^{-3} M glucose solution in 0.01 M NaCl, respectively.](image-url)
then gradually returned to its original position as the solution composition returned to the original 0.01 M NaCl solution. The bending response profile difference between microcantilever-A and B reflected the difference between the multilayer structures on silicon and gold surfaces. These experiments suggested that microcantilever-A could be potentially used for glucose monitoring while microcantilever-B may only be used for glucose measurement.

For the 24 nm maximum deflection of microcantilever-A, the surface stress change was 0.056 N/m according to the following equation [21].

\[
\Delta Z = \left( \frac{3(1-\nu)L^2}{Et} \right) \delta s,
\]

where \(\Delta Z\) is the observed deflection at the end of the cantilever, \(\nu\) and \(E\) are Poisson’s ratio (0.2152) and Young’s modulus (155.8 GPa) for the silicon substrate, respectively, \(t\) is the thickness of the cantilever (1 \(\mu\)m), \(L\) is the length of the cantilever (180 \(\mu\)m), and \(\delta s\) is the differential stress on the cantilever.

Fig. 2 shows the bending response of a microcantilever-A to various concentrations of glucose. The microcantilever deflection increased as the concentrations of glucose increased. Multilayer film stability experiments were conducted on the microcantilever after 1 month of storage in a 0.01 M NaCl solution. The cantilever deflection showed a similar profile and bending amplitude as those in Fig. 2.

Control experiments were performed with an unmodified cantilever and a cantilever with multilayer formation on both sides of the cantilever (i.e., a cantilever without TTS or PFDT treatment). No deflection of the cantilevers was observed upon exposure to a 10^{-2} M glucose solution because the changes in surface stress on the two sides cancelled each other. Further control experiments were performed with a TTS treated cantilever with a multilayer composite of (PEI/PSS)_6. No deflection of the cantilever was observed upon exposure to a 10^{-2} M glucose solution, either. The results given above indicate that the multilayer film swells upon interaction of glucose with the multilayer film. Since glucose molecules are bound, oxidized and released from the GOx’s active sites during the interaction with the enzyme, all of these interactions may contribute to the bending of the microcantilever. Both the catalytic reaction products, H^+ (from gluconic acid produced) and H_2O_2 could affect the multilayer structure as well. Our preliminary calculations suggest that the H_2O_2 and temperature produced from the reactions were not significant enough to cause the deflection. The detailed mechanism study will be reported in due time.

Fig. 3 shows the bending response of a microcantilever-A to a 10^{-2} M glucose solution from a 4 \times 10^{-3} M glucose solution in a 0.01 M NaCl solution.

In summary, our study has shown that a microcantilever modified by GOx containing multilayer responded specifically and quantitatively to glucose in saline, which could be potentially used for glucose monitoring. This work opened a new window for developing microcantilever sensors by using layer-by-layer approach. A controllable multilayer modification method could be used to detect many other chemical and biological species when different enzymes or receptors are embedded in the multilayer.

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**References**

[20] For TTS treated microcantilever, after formation of perfluorocarbon film on the silicon side, the cantilever was immersed in a $10^{-2}$ M mercaptoethanesulfonate solution 20 min to form a negative charged film on gold surface. This negative charge film could strengthen the stability of the multilayer film on the gold surface of the microcantilever.