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A general microcantilever surface modification method using a multilayer for biospecific recognition

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The key to microcantilever surface modification technology is to selectively modify one side of a microcantilever surface with molecular recognition layers. In this paper, we report a general microcantilever modification method using a multilayer film.

Introduction

Microcantilever sensors have attracted more and more attention because of their extreme sensitivity to chemical species, both in air and solution.[1–6] Microcantilever chip characterizations offer improved dynamic response, greatly reduced size, high precision, increased reliability and integration of micromechanical components with on-chip electronic circuitry. The sensor technology is based on changes in the deflection or resonance properties of the microcantilever induced by environmental factors in the medium in which a microcantilever is immersed. One unique characteristic of microcantilevers, is that microcantilevers undergo bending due to molecular absorption by confining the adsorption to one side of the cantilever, which is so called adsorption-induced surface tension.[7–9] Microcantilever bending occurs both in air and solution.

Selectivity of the microcantilever sensor is achieved by modifying the microcantilever with coatings for the recognition of specific molecular species. Two approaches have been used to immobilize the molecular recognition agent to the microcantilever surface, with the corresponding method used depending on the final application: self-assembled monolayer (either through silane–silicon or through thiol–Au bond coating), which has proved successful for the detection of species in water,[2–4] and polymeric films, such as those developed for surface acoustic wave (SAW) devices,[10] are useful for sensing in the gas phase.[8–9] However, most molecular-recognition agent containing molecules that could self assemble on the microcantilever surface are not commercially available, consequently tremendous amounts of synthetic work needs to be done to develop each molecular specific microcantilever sensor.

Polymer coatings offer significant bending amplitude, but are generally not selective for a specific vapour species and so far little work has been done on the detection of analytes in water based solutions with polymer-modified microcantilever.

Recently, a nano-assembly layer-by-layer (LBL) technique was introduced, which allows formation of ultrathin organized films on any surface through alternate adsorption of oppositely charged components, such as linear polyions and enzymes.[11–15] The required component could be located at a designed location in the film with nanometer precision.[12] In this paper, we report a general and convenient microcantilever surface modification method through layer-by-layer technology for biochemical recognition. Since microcantilever bending is generated from adsorption-induced surface stress by 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.

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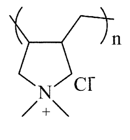
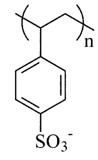
Results and discussion

Our work focused on surface modification of the commercially available silicon microcantilevers (Park Instrument, CA) for such a purpose. The dimensions of the V-shaped microcantilever are 200 µm length, 20 µm width, and 1 µm 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. Poly(diallydimethylammonium chloride) (PDDA) and poly(sulfonatostyrene) (PSS) were used for layer-by-layer modification of the cantilever surface. After a typical multilayer formation procedure, *i.e.* alternate dipping the cantilever into a PDDA and PSS solution,[12] contact angle data show that the formation of PDDA–PSS multilayer films, due to electrostatic attraction, occurs on both sides of the cantilever (Fig. 1). In fact, the formation of polymeric layer-by-layer multilayer films appears on almost any metal or non-metal surfaces,[12–15] therefore, it is difficult to perform LBL assembly only on one side of the cantilever.


PDDA


PSS

It is well known that perfluorocarbons are both hydrophobic and lipophobic. Fluorocarbon and hydrocarbons exhibit pronounced mutual phobicity.[16–17] By immobilizing perfluorocarbon materials on one surface of a microcantilever, we expect that the formation of multilayer film will occur solely on the other surface of the microcantilever due to the unique property of perfluorocarbons. (Tridecafluoro-1,1,2,2-tetrahydrooctyl)-triethoxysilane (TTS) was used to develop a thin perfluorocarbon film on the silicon surface using a typical silicon surface modification procedure.[18]

A TTS treated microcantilever was immersed into a 10^{-2} M solution of PDDA for 20 min, and rinsed with water, the cantilever was then immersed into a 10^{-2} M solution of PSS also for a duration of 20 min and again rinsed with water. This procedure was repeated several times until a desired multilayer film was formed. During the procedure, after rinsing with water, the cantilever was dried in air and the contact angle of water on the surface of cantilever support was measured,[19] the data are shown in Fig. 1b. As expected, the contact angles of water on this TTS treated silicon surface remained at approximately 90°

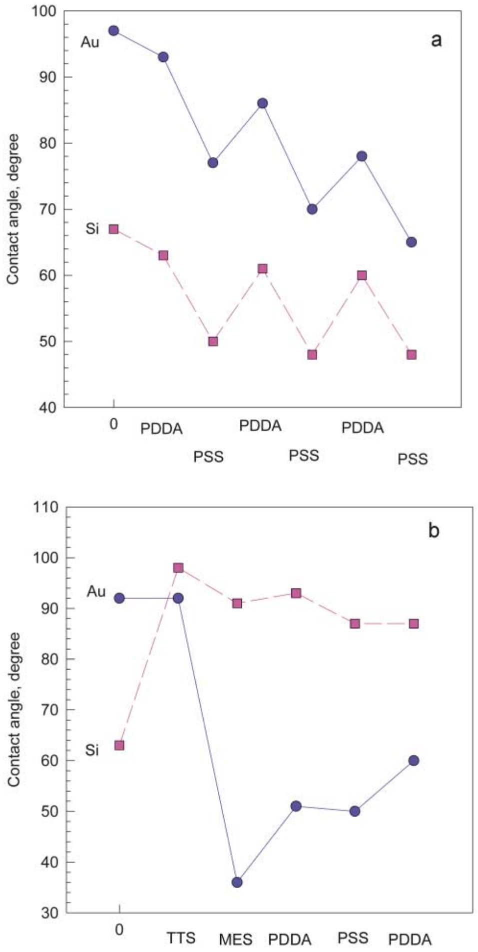


Fig. 1 (a) Contact angles of water on the gold and silicon surface of a microcantilever during the procedure of layer-by-layer formation. (b) Contact angles of water on the TTS treated silicon surface and MES coated gold surface of a microcantilever during the procedure of layer-by-layer formation.

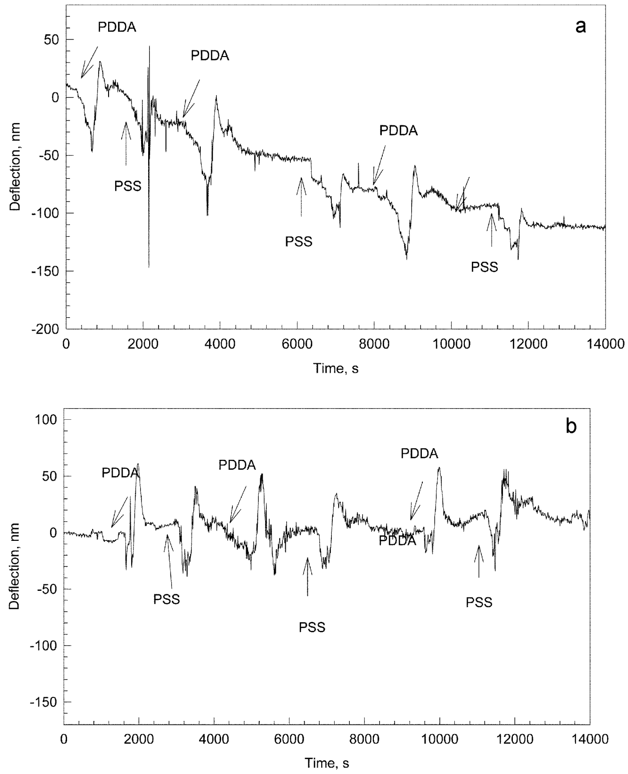


Fig. 2 (a) Bending response as a function of time for a TTS and MES treated silicon microcantilever upon alternate injection of a 10^{-2} M solution of PDDA and PSS in 10^{-2} M PBS buffer (pH = 6.5) at a constant 4 ml h^{-1} flow rate. (b) Bending response as a function of time for a unmodified coated silicon microcantilever upon alternate injection of a 10^{-2} M solution of PDDA and PSS in PBS buffer (pH = 6.5) at a constant 4 ml h^{-1} flow rate.

after a couple of cycles of PDDA–PSS layer-by-layer formation procedure, indicating that no multilayer forms on this surface. (A SEM picture shows that no multilayer sticks on this surface). On the other hand, a monolayer of mercaptoethane sulfonate (MES) was self-assembled on the gold surface in order to increase the adhesion of multilayer film on the gold surface, the contact angles of water on the gold surface upon multilayer formation are also shown in Fig. 1b.

Fig. 2a shows an *in situ* bending deflection of a TTS treated microcantilever when the PDDA and PSS were alternately switched into a fluid cell that holds the cantilever at a constant 4 ml h^{-1} flow rate. The microcantilever was pre-equilibrated in a 0.01 M PBS (pH = 6.5) buffer solution before injection of the polymer solution. Immediately after the injection of the polymer solution (0.01 M polymer in 0.01 M PBS buffer, pH = 6.5), the cantilever bends vigorously down and up as shown in Fig. 2a, which may be due to the electrostatic interaction of the ionic polymer film and its counter-ionic polymer in the solution. Further studies are underway in order to fully explain this phenomenon. After 15 min interaction time, a buffer solution was switched into to cell to flush away the remaining polymers in the solution. At equilibrium, the microcantilever bends down approximately 20 nm after each polymer injection. After three PDDA–PSS cycles, the cantilever bends down approximately 120 nm due to the formation of multilayer on the gold surface of the cantilever. As a comparison, a cantilever without TTS modification does not bend since the formation of layer-by-layer film occurs on both surfaces of the microcantilever. As shown in Fig. 2b, after three PDDA–PSS layers, the neat deflection of the microcantilever is close to 0, suggesting the offset of the surface tensions caused on both sides of the microcantilever.

The step-by-step modification procedure is illustrated in Scheme 1. When a layer of glucose oxidase (GOX) was immobilized on the outmost layer of such a microcantilever surface,[20] the cantilever deflects upon its exposure to glucose as shown in Fig. 3. In comparison, a bare gold coated silicon cantilever or a microcantilever with multilayer formation on both sides does not deflect upon exposure to glucose.

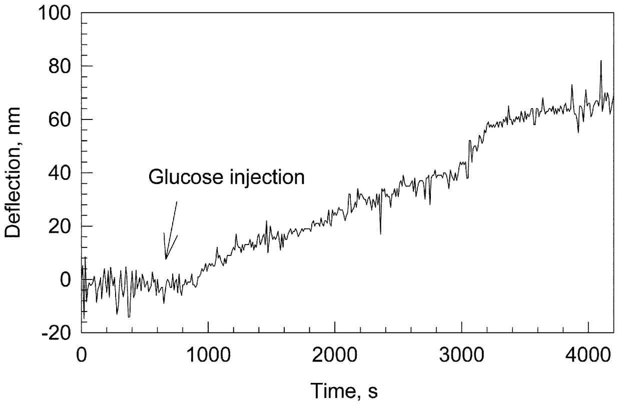
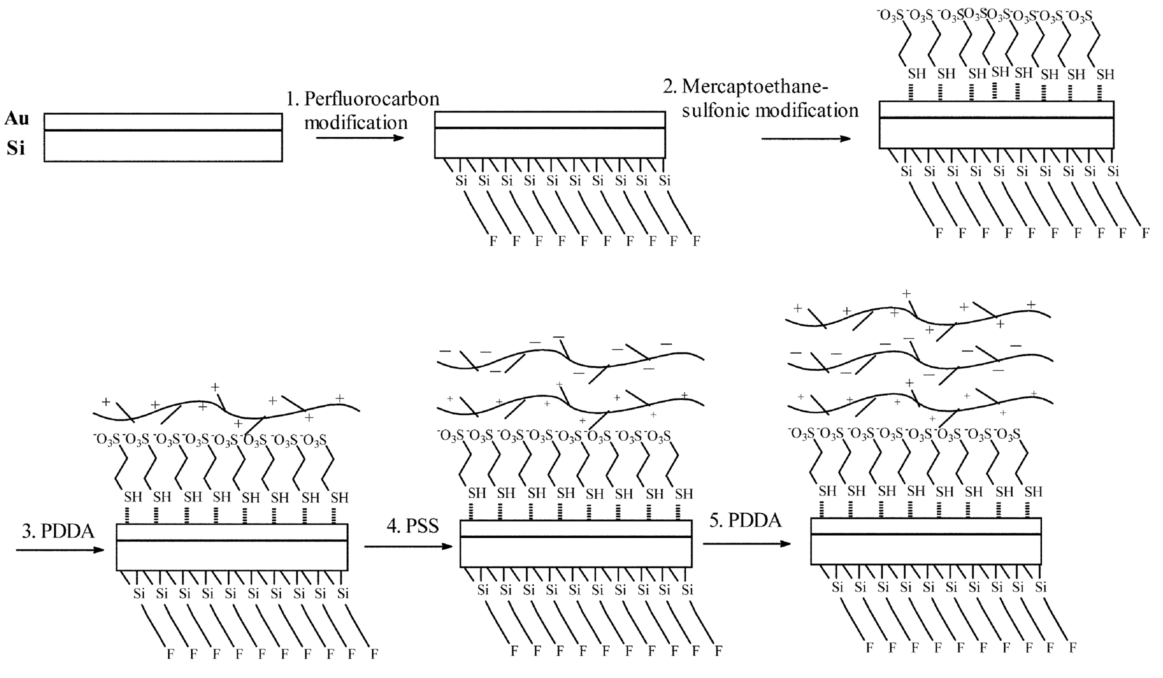


Fig. 3 Bending response as a function of time for a GOX–PDDA–PSS–PDDA–MES–Au–Si–SiO_2–TTS cantilever upon addition of a 10^{-3} M solution of glucose in 10^{-2} M PBS buffer.

In summary, we have developed a general microcantilever modification method using a multilayer film. Such a controllable multilayer modification method could potentially be used to detect chemical and biological species when a molecular



Scheme 1

recognition receptor is embedded in or onto the multilayer. Based on this approach, we are assembling organophosphorus anhydrolase on one side of a cantilever for the detection of organophosphorous agents in the solution.

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19 It is reasonable to believe that the contact angles of water droplets on the cantilever and the cantilever support are the same since they have been through the whole process as one unit.
20 Glucose oxidase (GOX) is a negatively charged polymer. After formation of a PDDA–PSS–PDDA layer, the cantilever with PDDA at the outmost layer was immersed into a solution that contains GOX at pH = 6.5 in TRIS buffer and GOX deposited onto the outmost layer of the film.