A pH Sensor Based on a Microcantilever Coated with Intelligent Hydrogel

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ABSTRACT

Hydrogel gels that contain different amounts of amino groups were used to modify microcantilevers for the pH measurements. These microcantilevers deflected upon exposure to various pH solutions due to the swelling and shrinking of the hydrogels. The microcantilever deflection, as a function of pH, is nearly linear in a wide pH range. A significant 1000 nm/pH bending response was observed from a Gel-4 coated microcantilever, which could be used for precise pH measurements. Such hydrogel coated microcantilevers could potentially be used to prepare microcantilever chemical and biological sensors when molecular recognition agents are immobilized into the polymer.

Key Words: pH Sensor; Microcantilever; Hydrogel; Swelling property.

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INTRODUCTION

Extensive investigations have been focused on stimuli-responsive hydrogels, which are also known as intelligent gels. These hydrogels change volume in response to small changes in temperature or pH. By proper design, some hydrogels could respond to changes in ionic strength, solvent, stress, light intensity, electric or magnetic fields. Such behavior has led to efforts to develop gel-based mechanical systems, such as artificial muscles for robotic devices, controlled-release systems for drugs, insulin and other substances, and molecular separation systems.

The reversible swelling property of hydrogels has also been used to develop chemical and biological sensors, such as glucose, Pb²⁺, etc. For chem/bio sensing, a molecular recognition agent that selectively forms a complex with specific molecules could be attached to the polymer chain. The sensor platforms include electrode, fluorescence, crystalline colloidal array reflection, etc.

Recently, another sensor platform-microcantilever sensor has attracted more and more attention because of its extreme sensitivity for chemical species, both in air and solution. Microcantilevers provide an outstanding platform for sensors with improved dynamic response, greatly reduced size, high precision, increased reliability, and integration of micromechanical components with on-chip electronic circuitry. 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 called adsorption-induced surface tension. Microcantilever bending can occur both in air and solution environment. Two approaches have been used to immobilize the molecular recognition agent to the microcantilever surface: self-assembled monolayers have proved successful for the detection of species in water, and polymeric films, such as those developed for surface acoustic wave (SAW) devices, are useful for sensing in the gas phase.

Since intelligent hydrogels swell in response to the concentration of particular analytes and the gel volume is a function of analyte concentration, it is anticipated that the swelling of the gel changes the surface stress of the cantilever and, subsequently, bends the cantilever if the hydrogel is immobilized on one side of a microcantilever. In this paper, we report a microcantilever coated with a hydrogel that contains amino groups for the measurement of pH. Using this hydrogel coated microcantilever, it is possible to develop a miniature, extremely sensitive, microcantilever-based pH meter.

EXPERIMENTAL

Solvent and Materials

We used commercially available silicon microcantilevers (Veeco Instruments, CA) in all of our experiments. The dimensions of the V-shaped microcantilevers 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 is made of silicon with a thin, naturally grown oxide layer.
The chemicals used, such as allytriethoxysilane (ATS), EtOH, 2-dimethylamino ethyl methacrylate (DMEM), acrylamide (AMD), crosslinkers, such as N,N'-methylene-bis-acrylamide (bis-AMD) and a UV photoinitiator, such as diethoxyacetophenone (DEAP) were used as received from Aldrich. High-purity de-ionized water was obtained with a Milli-Q water system (Millipore). For chemical modification, the cantilevers were cleaned in piranha solution [7 : 3 H$_2$SO$_4$ (96%) : H$_2$O$_2$ (30%)] for 30 min, and rinsed with H$_2$O three times and EtOH (two times) before measurement or SAM preparation.

The pH solutions in our experiments were prepared by adjusting pH of a series of 0.01 M phosphate buffer solutions by HCl or NaOH. The pH of the final solution was measured using standard pH meter (Corning).

**Polymerization Procedure**

In order to covalently attach the hydrogels on one surface of a microcantilever, a layer of allyl groups were introduced on the silicon surface through interaction of ATS with the silicon surface. ATS treated microcantilevers were prepared by immersing the cleaned microcantilevers into a 1 M solution of ATS in EtOH : H$_2$O (95 : 5) mixture (one drop of acetic acid is added to catalyze the reaction) overnight and then the microcantilevers were rinsed with EtOH three times.

The precursor solution contained 2.1 mmol of AMD, certain molar ratios of DMEM, 0.072 mmol of bis-AMD, and 0.0072 mmol of DEAP dissolved in 3 mL of water. Table 1 lists the various molar amounts of DMEM in the four hydrogels used in our experiments.

A microcantilever was laid on a 1 cm$^2$ glass microslide, separated from the glass surface by a 150 mm parafilm spacer; the microcantilever beam was placed out of the parafilm spacer above the glass surface so that there was a 150 mm distance between the microcantilever beam and the glass microslide (Sch. 1). The glass microslide was placed in the bottom of a vial that contained a precursor solution. The vial was cooled in an ice bath and then exposed to UV light from a 254 nm mercury lamp to initiate polymerization. After 5 min of exposure, the vial was taken out. The hydrogel attached to the microcantilever silicon surface could be observed with a high resolution microscope. The hydrogel, loosely attached on the gold surface, was mechanically removed using a micromanipulator with observation under a microscope. The resulting hydrogel film bound to the substrate was washed in deionized water for 2 days.

**Deflection Measurement**

All the buffer solutions had the same buffer concentration and ionic strength (0.01 M) with different pH. The deflection experiments were performed in a flow-through glass cell.

**Table 1.** Amount of DMEM in the four hydrogels.

<table>
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<tr>
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<th>Gel 1</th>
<th>Gel 2</th>
<th>Gel 3</th>
<th>Gel 4</th>
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<tbody>
<tr>
<td>Amount of DMEM</td>
<td>0 mol</td>
<td>0.09 mmol</td>
<td>0.27 mmol</td>
<td>0.81 mmol</td>
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(Digital Instruments, CA), such as those used in atomic force microscopy, where the V-shape microcantilever was immersed by phosphate buffer (pH = 7.0). Initially, the buffer solution was circulated through the cell using a syringe pump. A schematic diagram of the apparatus used in this study was previously reported.[25] Since the flow rate can change the cantilever bending, a constant flow rate of 4 mL/hr was maintained during the entire experiment. Experimental solutions were injected directly into the fluid flow, via a low pressure injection port/sample loop arrangement. This arrangement allowed for continuous exposure of the cantilever to the desired solution, without disturbing the flow cell or the flow rate. Since the volume of the glass cell including the tubing was only 0.3 mL, a fast replacement of the liquid in contact with the cantilever was achieved.

The deflection measurements were carried out with an AFM photodiode. The bending of the cantilever was measured by monitoring the position of a laser beam reflected from the cantilever onto a four-quadrant photodiode. The cantilever was immersed in the buffer until a stable base line was obtained, and the voltage of the position sensitive detector was set as background corresponding to 0 nm. In our experiment, we define “bending down” as cantilever bending toward the silicon side while, “bending up” refers to bending toward the gold side.

RESULTS AND DISCUSSION

A 15 μm thick Gel-3 (0.27 mmol DMEM) coated microcantilever was initially exposed to a constant flow (4 mL/hr) of phosphate buffer (pH = 7.0). When various pH solutions were injected into the fluid cell, the microcantilever bent with different amplitudes, as shown in Fig. 1. When the pH was < 7 [Fig. 1(a)], the cantilever bent down towards the gel side (silicon side), the microcantilever deflection increased as the pH decreased, i.e., the solution turned more acidic. When the pH was > 7 [Fig. 1(b)], the microcantilever bent up towards the gold side, the cantilever deflection increased as the pH increased. For each measurement, a 0.5 mL buffer solution was injected into the fluid cell where the microcantilever was held. It took 15 min for the injected buffer solution to flow through the fluid cell, then the original buffer solution at pH = 7.0 was circulated back into the fluid cell. This matches well with our observations, that the deflection of a microcantilever reached a maximum in approximately 15 min after the injection of various pH solutions. Then, the microcantilever deflection gradually returned to its original position, since the solution returned to the original pH.

The maximum deflection amplitude of the microcantilever vs. pH is shown in Fig. 2. In the range of pH = 3–12, the deflection amplitude of the microcantilever, as a function of pH is nearly linear, with a significant sensitivity of approximately 170 nm/pH unit.
pH Sensor Based on Hydrogel Coated Microcantilever

Figure 1. Bending response as a function of time for a 15 µm thick Gel-3 coated silicon microcantilever, upon injection of a 0.01 M phosphate buffer solutions, at various pH (the injection time is indicated with arrows). Inset: bending response as a function of time for a 15 µm Gel-1 coated silicon microcantilever, upon injection of a 0.01 M phosphate buffer solutions, at various pH (in inset (b), the peaks refer the response to pH at 7.48, 8.0, 8.52, 9.19, 10.0, respectively, from the left to the right).
This is much more sensitive than the monolayer modified microcantilevers reported previously. As discussed before, the intelligent hydrogel swells upon interaction of specific charged molecules. The expansion and contraction of gels allow chemical or electrical energy to be converted into mechanical work. Given this, it is readily concluded that the bending response of the microcantilevers activated by pH is generated from the hydrogel swelling and shrinking, depending on the equilibrium between –N(CH₃)₂ and –NH⁺(CH₃)₂ in the hydrogel, as shown in the following reaction:

\[-N(CH₃)₂ + H⁺ \rightleftarrows –NH⁺(CH₃)₂\]

At low pH values, the amino groups of the gel tend to be protonated, and the gel expands because of increased electrostatic repulsion between the cationic chains. The polymer chains become more hydrophilic owing to the increased charge. This leads to increased hydration of the polymer chains. At high pH values, amino groups become neutralized, hydrogen bonds form between them and the carbonyl oxygens on the amide chains. These interpolymer complexes lead to increased hydrophobicity, which causes the gel to shrink. Since most intelligent gels are relatively homogeneous materials that shrink or swell uniformly, with no dramatic change in shape, the microcantilever bending is reversible and reproducible.

Since the hydrogel film is relatively thick (15 µm), it took hours for the whole film to reach the equilibrium in different pH solutions. In order for fast response, for each measurement in our experiments, a 0.5 mL buffer solution was injected into the fluid cell, and it took 15 min for the injected buffer solution to flow through the fluid cell, then the
original buffer solution at pH = 7.0 was circulated back into the fluid cell. In consequence, only part of amino groups in the polymer interacted with H⁺ in the buffer solution, and the maximum response shown in Fig. 1 and 2 did not provide a full picture of the microcantilever’s actual response at equilibrium at each pH.

For comparison, the pH effects on the deflection of a microcantilever coated with a 15 μm thick Gel-1 that does not contain any amino groups, is shown in Figs. 1(a) and 1(b) inset. The y-axis (microcantilever deflection) of Fig. 1 and the inset is drawn in the same scale for a direct comparison. The Gel-1 coated microcantilever slightly bent when exposed to various pH buffer solutions. The slight bending of the microcantilevers might due to the interaction of the network of the hydrogel with H⁺ or OH⁻. It was also noticed in Fig. 1(b) that, before the amino containing Gel-3 coated microcantilever bent up upon exposure to alkaline buffers, it first slightly became bent up; that showed a characteristic pattern of the Gel-1 network interaction with H⁺ (the same response time and the same deflection amplitude). The difference between the deflection response of a Gel-3 coated microcantilever and a Gel-1 coated microcantilever is the net response of amino groups in the hydrogel to the pH.

The maximum deflection amplitudes vs. pH of two other cantilevers coated with Gel-2 and Gel-4, are also shown in Fig. 2. Gel-2 and Gel-4 contains less (0.09 mmol) or more (0.81 mmol) −N(CH₃)₂ groups than Gel-3, respectively. Gel-2 coated microcantilevers were less sensitive to pH, with a linear bending response of approximately 43 nm/pH between pH = 4–10. On the contrary, Gel-4 coated microcantilevers showed a significant 1000 nm/pH bending response between pH 6 and 9. The improved sensitivity of Gel-4 coated microcantilevers could be used for precise pH measurement. The microcantilever bent more in either pH < 6 or pH > 9 environments, however, the bending amplitude is too big to be measured using our current photodiode detection system. From these observations, it is concluded that the hydrogel with more molar ratio of amino groups causes more bending deflection of the microcantilever to pH. However, a polymer precursor mixture that contains more molar ratio of amino groups than that in Gel-4, does not give a solid hydrogel at ambient temperature.

The reproducibility of the sensor responses has been investigated, and the results showed that the reproducibility is extremely good. Three individual Gel-4 coated microcantilevers bent 800, 821, 835 nm, respectively, in response to pH = 3.34 solution. The cantilever deflection variations were within 10%, as determined by replicate measurements.

In summary, we have demonstrated sensing changes in solution pH, using a microcantilever coated with an intelligent hydrogel containing pH-responsive amino groups. Such hydrogel modification methods for microcantilevers could be potentially used to detect many chemical and biological species, when various molecular recognition receptors are embedded in the hydrogel on the microcantilever surface.

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REFERENCES


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27. Lang, H.P.; Baller, M.K.; Berger, R.; Gerber, Ch.; Gimzewski, J.K.; Battiston, F.M.;
    59–65.
    1–6.

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