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Ultrasensitive Biomems Sensors Based on Microcantilevers Patterned with Environmentally Responsive Hydrogels

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Abstract. An innovative platform was developed for ultrasensitive microsensors based on microcantilevers patterned with crosslinked copolymeric hydrogels. A novel UV free-radical photolithography process was utilized to precisely align and pattern environmentally responsive hydrogels onto silicon microcantilevers, after microcantilevers were fabricated and released. Specifically, a crosslinked poly(methacrylic acid) network containing high amounts of poly(ethylene glycol) dimethacrylate was prepared and investigated. Hydrogels were patterned onto the silicon microcantilevers utilizing a mask aligner to allow for precise positioning. The silicon surface was modified with γ-methacryloxypropyl trimethoxysilane to gain covalent adhesion between the polymer and the silicon. The hydrogels sensed and responded to changes in environmental pH resulting in a variation in surface stress that deflected the microcantilever. The bending response of patterned cantilevers with a change in environmental pH was observed, showing the possibility to construct MEMS/BioMEMS sensors based on microcantilevers patterned with environmentally responsive hydrogels. An extraordinary maximum sensitivity of $1 \, \mathrm{nm}/5 \times 10^{-5} \Delta \mathrm{pH}$ was observed, demonstrating the ultrasensitivity of this microsensor platform.

Key Words. free-radical photopolymerization, micropatterning, microcantilever, hydrogel, BioMEMS, MEMS, microsensor, pH sensor

1. Introduction

In recent years, researchers have demonstrated that microcantilever-based atomic force microscope systems are capable of measuring molecular forces, such as avidin-biotin binding (Florin et al., 1994), antigen-antibody interaction (Hinterdorfer et al., 1996), and hybridization of complementary DNA strands interac- tions (Lee et al., 1994). As a result of these achievements, extensive research has been focused on designing and fabricating microsensors based on the ultrasensitive detection abilities of microcantilevers. For applications as microsensors, the surface of the microcantilever needs to be functionalized to provide for selective detection of an analyte or environmental condition. The methods for functionalization of the microcantilever surface include treatment with self-assembled monolayers (SAMs), coating with polymers, coating with metals, and adsorption of receptor molecules. For example, in the work of Wu et al. (2001) single-stranded DNA were modified with thiol groups allowing for formation of a SAM on a gold coated microcantilever, and then these modified microcantilevers were used to detect complementary DNA strands. In other work, polymer-coated microcantilevers were used to successfully detect the selective absorption of organic solvents (Hagleitner et al., 2001).

In this work, intelligent hydrogels were utilized to functionalize the surface of microcantilevers. Hydrogels are mainly hydrophilic polymer networks that swell to a high degree due to an extremely high affinity for water, yet are insoluble because of the incorporation of chemical or physical crosslinks. Due to their high water content and the corresponding rubbery nature, hydrogels are similar to a variety of natural living tissues, leading to their widespread application as biomaterials,

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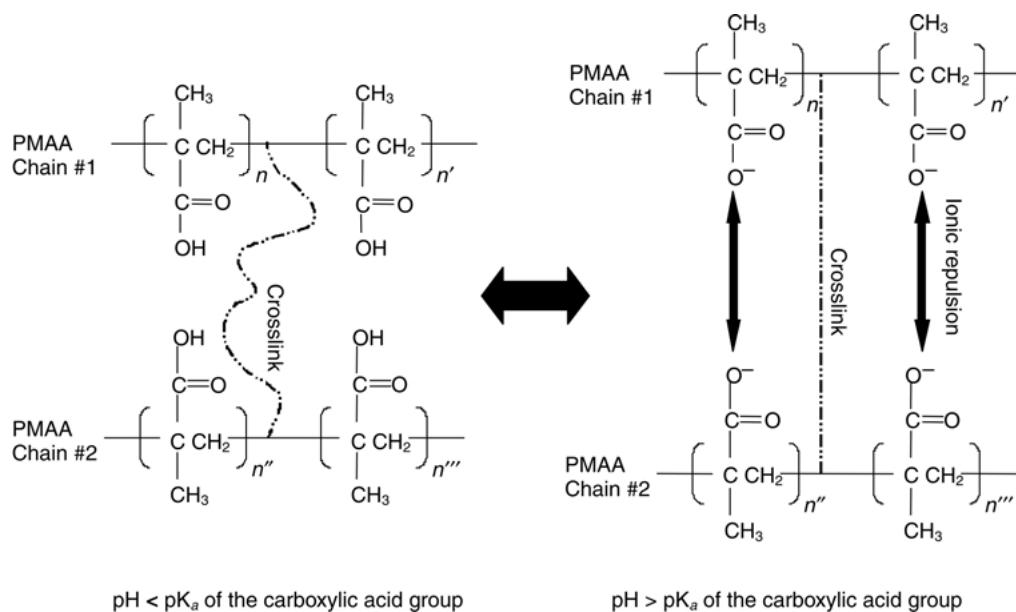
Fig. 1. *Schematic of the pH dependent swelling process of an anionic hydrogel: specifically, a crosslinked poly(methacrylic acid) (PMAA) is illustrated.*

such as in contact lenses, sutures, dental materials, and controlled drug delivery devices (Peppas, 1986, 1997, 2001).

By selecting the functional groups along the polymer chains, hydrogels can be made sensitive to the conditions of the surrounding environment, such as temperature, pH, electric field, or ionic strength (Peppas, 1991). Extensive research has focused on developing new and applying current environmentally responsive hydrogels (Byrne et al., 2002a; Peppas et al., 2002, 2000; Oral et al., 2000), specifically those sensitive to temperature, pH, and specific analytes. The environmentally responsive hydrogels that have received the most attention are those based on ionic networks that demonstrate a reversible pH-dependent swelling behavior. Anionic networks contain acidic pendant groups (Bures et al., 1999; Scott et al., 1999a), such as carboxylic acid, with a characteristic pK_a, while cationic networks contain basic pendant groups, such as amine groups, with a characteristic pK_b. In the case of anionic networks, ionization of these acid groups will occur once the pH of the environment is above the acid group's characteristic pK_a. With deprotonation of the acid groups, the network exhibits fixed charges on its chains resulting in an electrostatic repulsion between the chains and, in addition, an increased hydrophilicity of the network. Because of these alterations in the network, water is absorbed into the polymer to a greater degree causing swelling (Scott et al., 1999a, 1999c, 2000). This actuation process is shown in Figure 1.

In recent work, environmentally responsive hydrogels have been utilized in micro-scale applications as sensing and actuating components. Beebe et al. (2000a, 2000b) micropatterned pH-sensitive hydrogels inside microfluidic channels to create micro-valves that could sense the environmental conditions and rapidly actuate in response. Similarly, Madou and coworkers (Low et al., 2000) utilized a blend of a hydrogel and a redox polymer to create an ''artificial muscle'' that could act as an electro-actuated microvalve for possible application in controlled drug delivery.

In previous work in our laboratory, processes were developed for integrating environmentally responsive hydrogels into micro-scale applications (Ward et al., 2000, 2001; Byrne et al., 2002b; Byrne et al., 2002c; Hilt et al., 2002; Bashir et al., 2002). The objective of this research was to build on this previous work for the development of a MEMS sensor platform based on ultrasensitivity of microcantilevers and the responsive actuation abilities of environmentally responsive hydrogels, as is schematically shown in Figure 2. Specifically, pH-sensitive hydrogels were precisely lithographically patterned onto microcantilevers to create an ultrasensitive pH microsensor. The photolithographic process also allowed for multiple applications of different polymers on different cantilevers on the same chip. When compared with other micro-scale pH sensing techniques such as ion-sensitive field-effect transistors (ISFETs), potentiometric metal oxide electrodes, and light addressable potentiometric sensors (LAPS), the sensitivity of
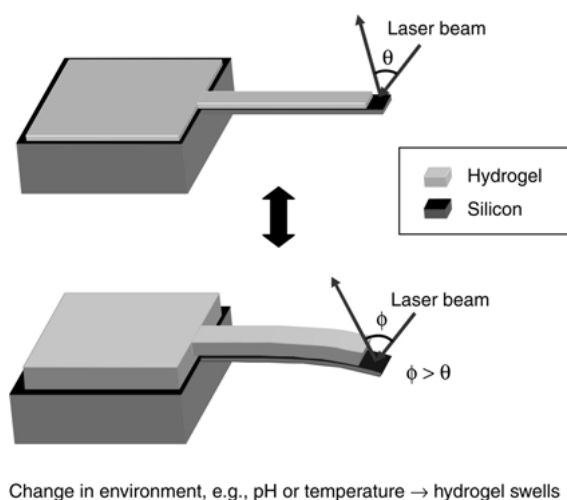
Fig. 2. *Schematic of the MEMS sensor platform based on a microcantilever patterned with an environmentally responsive hydrogel.*

this pH microsensor was at least two orders of magnitude greater. This ultrasensitive microsensor platform will contribute to the creation of innovative microdevices, such as an implantable chip that could monitor a physiological condition with necessary ultrasensitivity, as a key component of a therapeutic micro-device.

2. Experimental Procedure

2.1. Preparation of micropatterned pH-responsive hydrogel

The monomers studied were vacuum-distilled methacrylic acid (MAA) and poly(ethylene glycol) dimethacrylate (designated as PEGnDMA, where *n* is the average molecular weight of the PEG chain). The initiator used for the UV free radical polymerization was 2,2-dimethoxy-2-phenyl acetophenone (DMPA), and adhesion was gained between the silicon substrate and the polymer using an organosilane coupling agent, γ-methacryloxypropyl trimethoxysilane (γ-MPS). The MAA, DMPA, and γ-MPS were purchased from Aldrich (Milwaukee, WI). PEGnDMA, with $n = 200$, was obtained from Polysciences, Inc. (Warrington, PA).

Crosslinked poly(methacrylic acid) (PMAA) networks were prepared by reacting MAA with substantial amounts of PEG200DMA. The kinetics of this type of copolymerization have been discussed in detail (Lowman et al., 1997; Ward et al., 2002; Scott et al., 1999b). Photolithography was utilized to pattern these networks onto silicon wafers. Silicon wafers were cleaved into pieces that were approximately 2 cm by 2 cm, and then cleaned utilizing a standard Piranha clean. To promote covalent adhesion between the silicon

surface and the polymer, the silicon pieces were soaked in a 10 wt % solution of γ-MPS in acetone for more than 2 hours. Then, these were rinsed in acetone followed by ethanol, and then air-dried. The organosilane coupling agent formed a self-assembled monolayer on the silicon dioxide surface and presented methacrylate pendant groups that reacted and covalently bonded the silicon surface with the polymer film.

The monomer mixtures were prepared with a mole ratio of 80:20 MAA:PEG200DMA and containing 10 wt % DMPA. The monomer mixture was spin-coated onto the silicon pieces at 2,000 rpm for 30 seconds. Polymer micropatterns were created by UV free-radical polymerization using a Karl Suss MJB3 UV400 mask aligner, enabling for alignment accuracies of 0.1 microns. After bringing the sample into contact with the mask, it was exposed to UV light with intensity of 23.0 mW/cm^2 for exposure times of 2 minutes. The pieces were then removed and allowed to soak in deionized distilled water for greater than 24 hours to remove any unreacted monomer.

2.2. Micropatterning pH-responsive hydrogels onto microcantilevers

Surface micro-machined cantilevers were fabricated using commercially available SOI wafers with a 2.5 μm silicon layer and 1 μm oxide layer. A 0.3 μm oxide was grown (Figure 3(a)) and photoresist mask was used to anisotropically etch the oxide, silicon, and the buried oxide layers (Figure 3(b)). A 0.1 μm oxide was grown on the sidewall of the SOI layer (Figure 3(c)) and a dry anisotropic etch was used to remove the oxide from the substrate exposed silicon surface while leaving it on the sidewalls of the SOI layer. Tetramethylammonium hydroxide was used to etch the silicon substrate and to release the cantilever/oxide composite structure (Figure 3(d)). The wafers were immersed in buffered hydrofluoric acid to etch off all the oxide and release the silicon cantilevers (Figure 3(e)). The cantilevers all had thicknesses of approximately 2.5 μm.

These silicon samples containing the microcantilevers were treated with an organosilane agent, γ-MPS, to gain bonding between the silicon and polymer. For this treatment, the silicon pieces containing microcantilevers were soaked in a 10 wt % solution of γ-MPS in acetone for more than 2 hours. Then, these were rinsed in acetone followed by methanol, and then air-dried. Monomer mixtures, containing a mole ratio of 80:20 MAA:PEG200DMA and 10 wt % DMPA as an initiator, were spin-coated for 30 seconds onto the silicon samples containing microcantilevers (Figure 3(f)). Polymer micropatterns were created by UV free-radical polymerization using a Karl Suss MJB3 UV400 mask aligner. The 0.1 micron alignment accuracy of the mask aligner
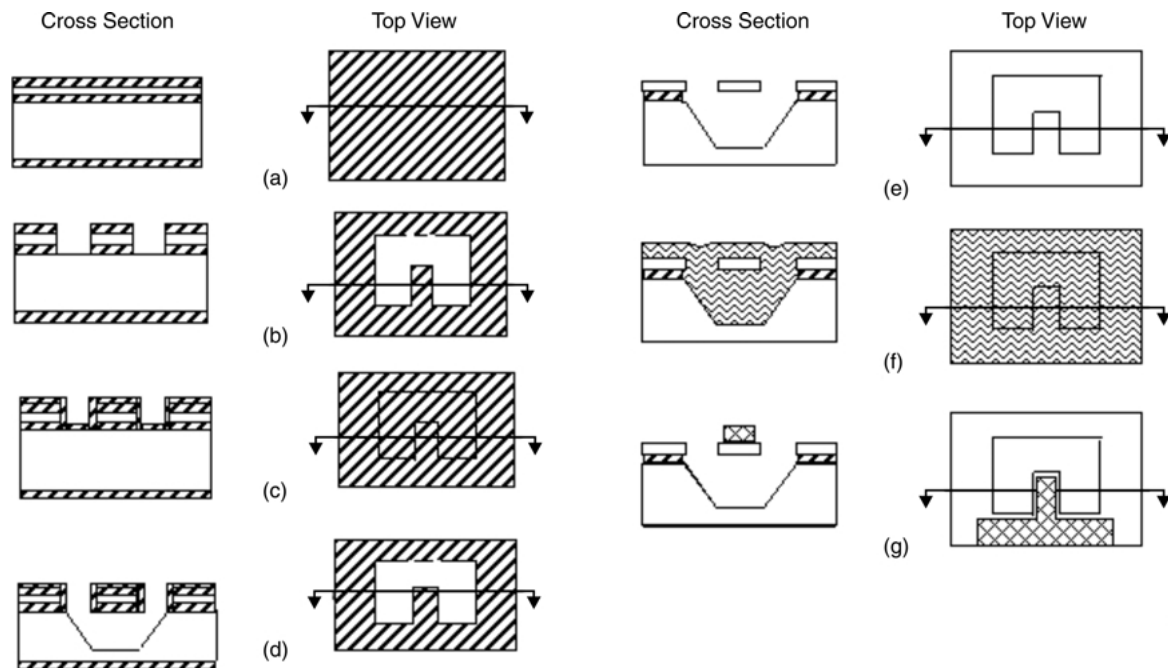
Fig. 3. *The fabrication process-flow for the fabrication of the microcantilever sensor.*

was critical for the precise patterning of the polymer onto the microcantilever. After bringing the sample into contact with the photomask, the samples were exposed to UV light with intensity of 23.0 mW/cm^2 for exposure time of 2 minutes. The samples were then removed and allowed to soak in deionized distilled water (Figure 3(g)) for greater than 24 hours to remove any unreacted monomer.

2.3. *Characterization of patterned microcantilevers*

A microscope (Nikon Eclipse L150, Nikon, Tokyo, Japan) equipped with an insight digital camera with KAI-2000 Mosaic CCD capable of obtaining images with 50×, 100×, 200×, 500×, and 1,000× magnification and another microscope (Nikon Eclipse E600, Nikon, Tokyo, Japan) equipped with a 60× water immersion objective were used to observe the samples. In addition, a profilometer (alpha-step 200, Tencor Instruments, San Jose, CA) was used to determine the thickness and profile of the pattern.

In a typical deflection study, the silicon wafer containing the microcantilevers patterned with polymer was placed in a Petri dish that was filled with a constant ionic strength ($I = 0.5$ M) buffer solution composed of a mixture of citric acid, disodiumphosphate, and potassium chloride. Then, each Petri dish with sample was placed onto the stage of the Nikon Eclipse E600 equipped with a 60× water immersion objective. The sample was allowed to equilibrate at the pH for 30 minutes, and the deflection of the cantilever was measured every 5 minutes by

manually adjusting the focus plane from the edge of the cantilever well to the tip of the cantilever and recording the change in focus values from the *z*-axis fine focus knob. These changes in focus values were converted to vertical distance in micrometers via calibrating a change in focus measurement with a profilometry measurement of an etched well. Subsequently, the sample was removed from the buffer solution, rinsed with deionized distilled water, and placed into the next buffer solution. The pH and temperature of the buffer solutions were measured using a micro pH probe (IQ Scientific Instruments, Inc., San Diego, CA).

3. **Results and Discussion**

3.1. *Micropatterning environmentally responsive hydrogels onto silicon microcantilevers*

In these experiments, micropatterns were prepared by spin coating at 2,000 rpm and then brought in contact with the mask and exposed to UV light for 2 minutes. Using the aforementioned methods, sharp micropatterns of environmentally responsive hydrogels were created on silicon wafers, as shown in Figure 4(a) and (b). Then, these processes were applied to successfully pattern environmentally responsive hydrogels onto silicon microcantilevers (see Figure 5). The optical images in Figure 5 demonstrate the ability to precisely pattern environmentally responsive hydrogels onto microcanti-levers. The thickness of the polymer films patterned on
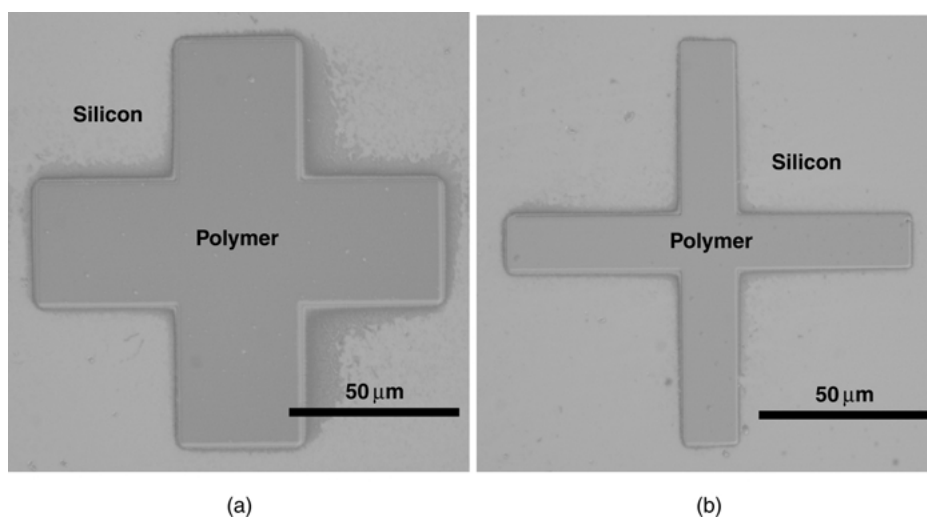
Fig. 4. *Top view images obtained utilizing an optical microscope in Nomarski mode showing micropatterns of enivironmentally responsive hydrogels on silicon substrates. In (A) and (B), the micropatterned hydrogel has a thickness of 455 nm and 400 nm, respectively, as measured by profilometry.*
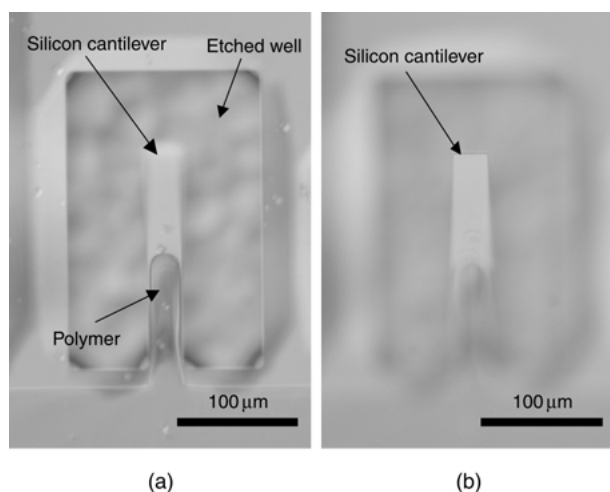
Fig. 5. *Top view images obtained utilizing an optical microscope in Nomarski mode showing a silicon microcantilever patterned with an environmentally responsive hydrogel. In (A) the focus is on the substrate, while in (B) the focus is on the microcantilever tip. Profilometry indicated that the thickness of the patterned hydrogel is approximately 2.2 μm.*

the microcantilevers was determined to be approximately 2.2 μm using profilometry.

During the polymerization to form the PMAA crosslinked with PEG200DMA, the volume of the polymer system shrunk due to densification, which occurs with the transition from monomer to polymer. As the hydrogel film polymerized, it adhered to the silicon substrate via the organosilane coupling agent, but the film was inhibited from shrinking where it was bonded to the cantilever surface. This resulted in stress

being generated within the film. The patterned microcantilever (Figure 5) was bent up due to this stress within the polymer film on the surface of the microcantilever. The magnitude of this bending in the dry state was measured to be 34.5 μm using an optical microscope.

3.2. Microcantilever bending with pH variation

The dynamic and equilibrium deflection characteristics of the patterned microcantilevers were examined in various pH solutions at $18 \pm 0.5\,°C$. The patterned microcantilever shown in Figure 5 was monitored while exposed to constant ionic strength $(I = 0.5\,M)$ buffer solutions of varying pH. The rapid dynamic response of the patterned microcantilevers to the change in pH is demonstrated in Figure 6, where it is shown that the system equilibrates within a few minutes. The equilibrium bending of the cantilever was measured and is included in Figure 7. This result demonstrated that the micropatterned hydrogel film was capable of sensing the change in environmental pH, and then swelling in response resulting in actuation of the microcantilever.

More detailed analysis of the equilibrium deflection data is shown in Figure 8. A linear fit of the deflection response at the region of highest sensitivity demonstrated a sensitivity of about 20.3 μm/pH unit. With the detection abilities of optical-based laser detection set-ups known to easily resolve a 1 nm deflection, this sensitivity can be translated to $5 \times 10^{-5}\,\Delta pH$ for 1 nm bending resolution. This ultrasensitivity is unique to this device and enables for novel applications as a pH sensor in micro-environments. The slight hysteresis observed in the measurement is a result of the relaxation of the polymer network. After the network is expanded at a high pH and
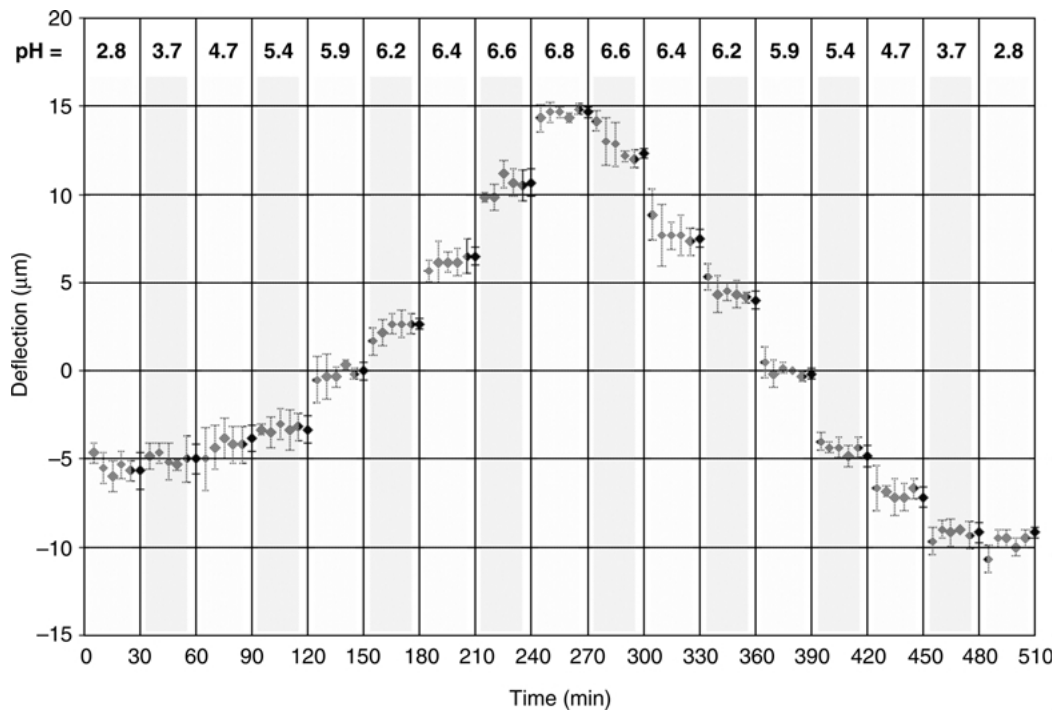
Fig. 6. Plot of the dynamic swelling response versus pH (constant ionic strength of 0.5 M) and time for the patterned microcantilever shown in Figure 5 (mean ± SD, n = 3).
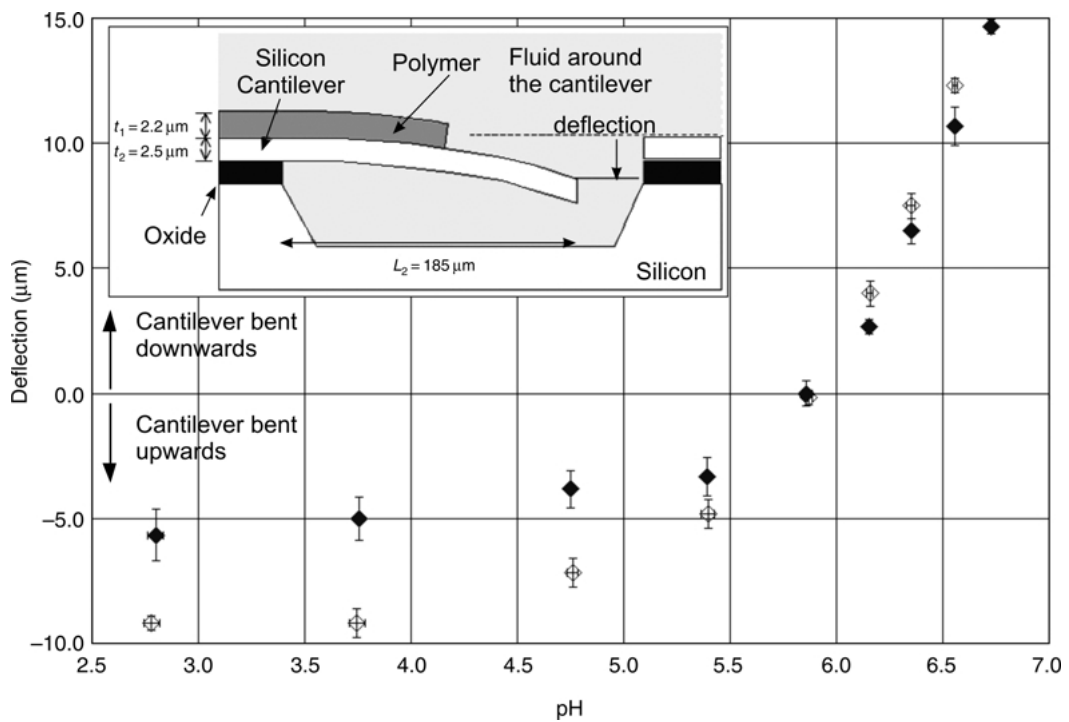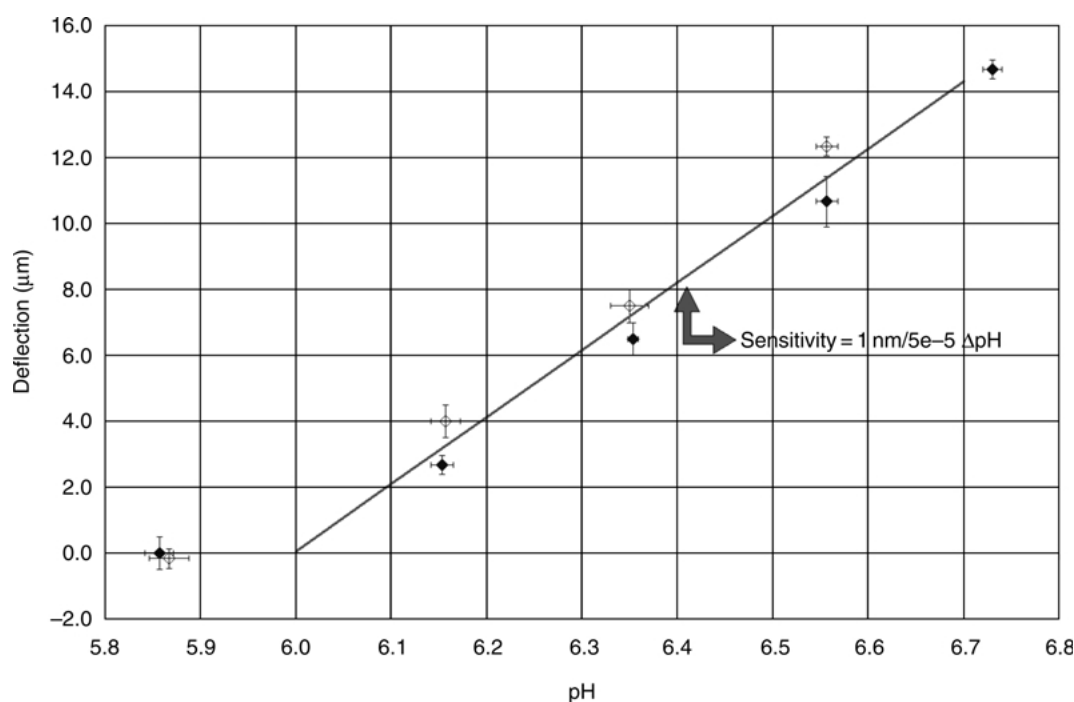

Fig. 7. Equilibrium bending data versus pH (constant ionic strength of 0.5 M) for patterned microcantilever shown in Figure 5. Solid diamonds are for the increasing pH path, while hollow diamonds are for the decreasing pH path (mean ± SD, n = 3).

Fig. 8. *Detailed examination of the equilibrium bending data versus pH (constant ionic strength of 0.5 M) shown in Figure 7. Solid diamonds are for the increasing pH path, while hollow diamonds are for the decreasing pH path. Linear fit shown for region of highest sensitivity (mean ± SD, n = 3).*

then brought back to a collapsed state at a low pH, the network is able to relax into a more collapsed state than its initial collapsed state. This effect is observed in both the dynamic and equilibrium data of Figures 6 and 7.

4. Conclusions

An ultrasensitive microsensor platform was fabricated by patterning a crosslinked copolymeric hydrogel onto a microcantilever. A novel UV free-radical photolithography process was utilized to precisely align and pattern environmentally responsive hydrogels onto silicon microcantilevers, after microcantilevers were fabricated and released. Specifically, a crosslinked poly(methacrylic acid) network containing significant amounts of poly(ethylene glycol) dimethacrylate was studied. This environmentally responsive hydrogel exhibits a rapid swelling response dependent on the environmental pH. By observing the bending of the patterned microcantilever with pH, it was demonstrated that a bioMEMS/MEMS sensor could be fabricated by precisely patterning a microcantilever with an environmentally responsive hydrogel. An extraordinary maximum sensitivity of $1 \, \text{nm}/5 \times 10^{-5} \Delta\text{pH}$ was observed, which demonstrates the ultrasensitivity and uniqueness of this microsensor platform. The methods developed here can be extended to other environmentally

responsive hydrogels enabling the fabrication of a variety of bioMEMS/MEMS sensors.

Another powerful feature of this system is that the response can be tailored via a number of ways, including varying the swelling properties of the polymer and changing the dimensions of the cantilever. For instance, some examples of how the swelling properties of the polymer can be tailored include adjusting the amount of crosslinking, the length of the crosslinker, or changing the monomers used. In addition, the response time of the system can be controlled by increasing the porosity of the polymer network or decreasing the diffusion length by decreasing the polymer film thickness. The aforementioned parameters allow for the sensing response to be tailored for the desired operating range and sensitivity.

To further expand the capabilities of the sensor, other environmentally responsive hydrogels, such as analyte specific, can be patterned onto silicon cantilevers enabling for the detection of other environmental conditions. For example, using hydrogels that have been made responsive to a specific analyte via molecular imprinting techniques, a highly sensitive microsensor can be created for the detection of any analyte, in theory. Furthermore, to enable the integration of this microsensor into a single chip device, a piezoresistor can be implanted into the base of the microcantilever. This enables for the deflection detection of the cantilever to be

made without the need for any off-chip components, and this on-chip detection will easily facilitate the integration of these microsensors into implantable or hand-held devices.

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