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Design, Construction and Testing of a Monolithic pH-Sensitive Hydrogel-Valve for Biochemical and Medical Application

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Abstract. In this work we report on a novel miniaturized pH-sensitive hydrogel-valve for implementation in a microfluidic system. The control of fluid movement represents a critical parameter in the design of these systems. To date, actuation methods for the delivery of sample fluids, such as electromagnetic, piezoelectric or thermopneumatic, require high power consumption. Using a microfluidic valve based on a stimuli-responsive material no external power source is necessary. For this reason smart materials like hydrogels that respond to external stimuli such as the pH-value are qualified. The presented valve was produced using soft-lithographic methods and consists of biocompatible materials. The actuator was fabricated in-situ and the activation of the valve was produced by use of different pH-values. The first investigations were made using a pH 1- and a pH 13-solution successively in order to test the actuator principle. Different times of exposure were used to polymerize the hydrogel and as a result it was discovered that 16 minutes was the most adequate. These tests demonstrated the operation of the hydrogel-valve and its applicability in a microfluidic device.

Keywords—Hydrogels, biocompatibility, in-situ fabrication, soft-lithography, micro-valve, polydimethylsiloxane (PDMS)

I. Introduction

Stimuli-responsive materials are currently being studied for use in microfluidic devices. One such material is hydrogel. Hydrogels are colloidal long-chain polymers that are water insoluble, which absorb large amounts of water as a dispersion medium. The ability to dramatically change their characteristics, such as volume, through external stimuli gives these hydrogels the reputation as “intelligent materials” or “smart gels” [1].

It is well known that “smart gels” like hydrogels have a slow response time, therefore their use in macroscopic systems is not adequate. However, if the size of the actuator is on the order of micrometers, it is possible to acquire a rapid response.

Microfabricated devices are found in a variety of applications in biological and medical sensing. Responsive hydrogel materials allow the combination of multiple functions (e.g., sensing and actuation) in a single component [2].
Stimuli-responsive hydrogels have a significant advantage over conventional microfluidic actuators due to their ability to undergo abrupt volume changes in response to the surrounding environment (via direct chemical to mechanical energy conversion) without the requirement of an external power source. These hydrogels can respond to a variety of inputs such as pH, temperature, electric fields, light, carbohydrates and antigens [3].

In the specific case of pH, the response is triggered due to the presence of ionized functional groups.

When stimulated, hydrogel polymer chains have many similarly charged groups which repel each other and cause the material to expand. Specifically, there are two kinds of pH sensitive materials: ones which have acidic groups (-COOH, -SO$_3$H) and swell in basic pH and others which have basic groups (-NH$_2$) and swell in acidic pH. In this work the first group of hydrogels was used, in which a copolymer acrylic acid becomes ionized with high pH values, causing the swelling of the hydrogel by anion rejection.

The opposite happens when the pH reverses and the functional groups lose their charge, causing the repulsion to stop and the material to return to its former shape [4].

As previously mentioned, there are several types of delivery methods such as electromagnetic, piezoelectric or thermopneumatic actuation [5] that often require high power consumption. Not only is the fabrication process of such microvalves complicated, but the integration into complex microfluidic systems has also proven to be nontrivial [6]. For systems that work with proteins, excessive heat is not acceptable, due to the fact that they become denaturalized, thereby destroying the sample solution. Smart materials such as hydrogels that respond to external stimuli like pH-value and do not generate heat are well qualified [3, 7].

Several groups have explored stimuli-sensitive microvalves using hydrogels. Liu et al. used an in-situ photopolymerization technique to integrate autonomous microvalves into complex microfluidic channel networks [3]. An active hydrogel component was fabricated inside microchannels via direct photopatterning of a liquid phase by Beebe et al. [8]. Eddington et al. presented a microdispensing device using hydrogels, requiring no electronic controls which was able to precisely deliver a given amount of fluid over a specified time [2]. A hydrogel-actuated microvalve that responds to changes in the glucose and pH concentration was fabricated and tested by Baldi et al., in an external liquid environment. In that work a phenylboronic-acid-based hydrogel was used [9].

The approach outline in this paper involves developing a polydimethylsiloxane (PDMS)-valve using a hydrogel as an actuator that varies its volume in response to changes in local pH, and that requires no external energy source. The valve is designed to be integrated in a microfluidic device with other microfluidic components such as a PDMS detection unit [10].

II. Materials and fabrication methods
The presented valve is fabricated by stacking five PDMS layers, by use of soft lithographic methods.

The fabrication process of the microvalve involves two steps: first, a 3-D microfluidic-network is fabricated with PDMS and next the hydrogel actuator is positioned via in-situ photopolymerization.

PDMS is an excellent substrate material for medical and biological application because of its biocompatibility [8]. It is also found to be adequate for the fabrication of microchannel systems for use with biological samples in aqueous solutions, due to its optical transparency, compliant properties, and most importantly, its ability to pattern a relief structure from a mold master. PDMS is also relatively inexpensive, permeable to oxygen and carbon dioxide and cures at low temperatures [11].

The valve is composed of 5 layers. Between the first and the third layer, there is a fluid-network for the sample solution. The fourth layer is 40 µm thick and separates the sample solution network from the hydrogel chamber, in which the pH solutions circulate. The first and third layers have channels with a height and width of 200 µm. The second layer contains a square hole with 200 µm sides and a depth of 200 µm. The circular chamber for the hydrogel actuator, which is located above the 40 µm thick PDMS membrane, has a diameter of 1500 µm.

A schematic view of the pH-sensitive hydrogel-valve is shown in Figure 1.
For the fabrication of the master, negative resist (SU 8, Micro-Chemicals) was spin-coated on 4\" black ceramic wafers. For thin layers SU 8-25 was applied, while SU 8-25 and SU 8-50 were used for the thicker layers.

In order to harden the photoresist, a heating step in which the temperature was ramped from 50°C to 100°C, was implemented. Next, the layer was exposed with UV-light through a mask, leaving a pattern printed onto the master. Then a post-bake at 60°C for 2 min and at 95°C for 3 min was made. Slow temperature ramping during the soft- and post-bake helped to prevent the cracking or delamination of the resist layer [12]. Finally, the masters were developed in 1-Methoxy-2-propyl-acetat (PGMEA) solution.

The layers were fabricated by pouring a 5:1 mixture of the PDMS prepolymer and curing agent (Sylgard 184 silicone elastomer kit, Dow Corning, Midland, MI) onto the master mold. After pouring the prepolymer over the master, all the air-bubbles were degassed in a vacuum bell. To obtain the desired height, the excess PDMS was removed. Next, the PDMS was thermally cured for 1 h on a hot plate at 60°C, and then separated from the mold. All layers were constructed by this method, which is known as replica-molding.

For a permanent bond between each layer, a bonding procedure based on surface treatment in oxygen plasma was used [13]. The PDMS-layers were placed in a barrel etcher and exposed to oxygen plasma. After surface treatment, the layers were aligned with the help of an optic microscope and permanently bonded through thermal curing for 1 h on a hot plate at 40°C.

Figure 2 shows a 3-D view of the hydrogel-valve.

In creating the actuator the hydrogel was prepared by conventional bulk radical polymerization respectively copolymerization in the presence of a crosslinking agent and polymerization initiator.

A mixture of monomers and a photoinitiator were used in the microchannel, and the mixture was exposed to UV-light through a photomask with a 600 µm circular hole positioned above the centre of the hydrogel valve, in which the mask was in direct contact with the valve.

The transparent channels were filled with the hydrogel precursor-solution. This mixture consists of a monomer 2-hydroxyethyl methacrylate (Sigma-Aldrich) and the copolymer acrylic acid (Sigma-Aldrich) in a 4:1 molar ratio. Ethylene glycol dimethacrylate (1 wt%, Sigma-Aldrich) was added as a crosslinker and Irgacure 651 (3 wt%, Sigma-Aldrich) as photoinitiator. Irgacure 651 is the registered name of 2,2-dimethoxy-2-phenyl acetophenone (Ciba Speciality Chemicals). Before polymerization the solution was diluted with DI-water in a 1:1 ratio. A 5 mW UV-source with a wavelength of 366 nm was used for the direct exposure of the hydrogel in the microfluidic system with a variable polymerization time between 8 and 24 min.

Two methods were tested to polymerize the hydrogel. The first was to expose the copolymer through layer 5 (more than 200 µm thick) with the entire valve already bonded (see Figure 3). The second method was to expose through layer 4 (40 µm thick), with only layer 4 and 5 bonded (see Figure 4).
When the polymerization was finished, the channels were washed with ethanol in order to remove any unpolymerized mixture. At this point the valve is in its open form. This method allows direct integration of pH-responsive hydrogels of different shapes and sizes. To verify fluid flow in the device, a flow visualization technique with dye was used.

**III. Results and discussion**

The hydrogel was tested through various means. A 3.4 x 3.4 mm square sample was created in a prefabricated mold and exposed through a PDMS layer with varying polymerization times. The probe was weighed and photographed next to a measuring standard.

The sample was dipped in a pH 13 standard solution for 5 sec and once again weighed and photographed. It was dipped 3 times in pH 13; after which it was immersed 3 times in a pH 1 standard solution. The same process was repeated 5 times. Pictures were taken and the weight was measured after every immersion. As previously mentioned the tested hydrogel expands in high pH and shrinks in low pH.

The tests were conducted with one sample at different times of exposure including 8 min, 16 min and 24 min. Table 1 shows the results of the different experiments.

**Table 1. Deformation of hydrogels at different exposure times.**

<table>
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<tr>
<th>Exposure Time</th>
<th>Deformation (mm)</th>
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<tbody>
<tr>
<td>8 min</td>
<td>1</td>
</tr>
<tr>
<td>16 min</td>
<td>2</td>
</tr>
<tr>
<td>24 min</td>
<td>2</td>
</tr>
</tbody>
</table>

In the first experiment, in which the exposure time was 8 min, the resulting deformation was 1 mm. In the 16 and 24 min tests the difference between the deformation size of the samples did not increase with a longer polymerization time. Figure 5 shows the experiment for 16 min exposure time.
Other tests were also conducted with the valve. Hydrogel was polymerized in-situ through layer 4, with varying times of exposure between 8 and 24 min.

When the valve was exposed for 8 min, incomplete polymerization was found in the internal hydrogel valve. The best resulting time of in-situ polymerization of the hydrogel was 16 min. Figure 6 shows the already polymerized hydrogel inside the chamber. From 18 min on, the hydrogel was completely polymerized, therefore blocking the channels and making the passage of fluids through the fluid-network impossible.

Some difficulties were found when exposing through layer 5 (see Figure 3), with the entire valve bonded. The polymerization was incomplete. A good result was achieved when illuminating through layer 4 (see Figure 4), where layers 4 and 5 were bonded. The subsequent layers were bonded afterwards.

In order to prove the performance of the microvalve, a simple flow test was performed. Different pH solutions were injected for valve testing. In order to close the microvalve, a pH 13 standard solution was used. For opening a pH 1 standard solution was injected. Simultaneously, a dye was injected into the fluid network to verify its correct aperture and closure.

Figure 7 shows the closed and opened hydrogel-valve with a sample solution.

Conclusions
A valve has been developed that can be integrated into a microfluidic system. This device incorporates a responsive hydrogel that has the ability to convert a chemical signal into mechanical energy and can be activated with different pH solutions without the use of an external energy source. The hydrogel swells and deflects a membrane blocking the fluid flow. Due to the small size of the actuator, the response time was 2.5 seg. A biocompatible material was used for the fluid-network. Conclusively, the function of the valve was tested, demonstrating its proper functionality and application. Future work will focus on the integration in a system with a sensor for protein detection.

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