Hydrogel-based Multi-stimuli Responsive Cilia

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ABSTRACT

Large arrays of multi stimuli responsive, hydrogel based, cilia are produced by means of micro-fabrication techniques. The cilia operate in aqueous solutions and their actuation can be triggerd by pH change, electric and/or magnetic fields. This artificial cilia system can combine, in a single unit, detection/triggered reaction behaviour. An external change in an environment, such as a decrease in pH, triggers collective cilia shrinkage and motile response, to an external time-dependent magnetic field.

Keywords: gels, smart materials, microactuators, cilia

1 INTRODUCTION

Polyelectrolyte gels attract particular attention among smart materials as they can exhibit a discontinuous volume phase transitions triggered by small variations of external parameters such as temperature, electric field, solvent quality or pH[1-4]. We have recently shown that the characteristic response time of macroscopic polyelectrolyte gels to DC electric fields, although very sensitive to electrode/gel configuration, is in general governed by transport of ions and water across the gel[5]. This implies that the response time can be significantly reduced by system miniaturization. However, the fabrication of micrometer-scale soft matter based responsive structures over large areas still remains a challenge.

In nature, many organisms interact and detect changes within their environment by means of various sorts of fibrillar structures. The role of those structures, called cilia, can be mainly divided into two categories: motile cilia that induce movement [6] and stationary cilia that usually act as a chemical sensor to environmental changes [7]. On the other hand, some cilia in nature combine both functions and are able to perform a stimuli-triggered action [8]. In the last years research on microfabricated biomimetic stimuli responsive cilia has achieved significant advances and many artificial systems have been introduced [9]. The response triggering factors can vary and depend mainly on the cilia material used. The artificial cilia can respond to environmental stimuli (pH [10, 11], chemical reaction – Belousov-Zhabotinsky reaction [12], humidity [13, 14], light [11, 15]) or in response to electrical [9, 16]/magnetic [17, 18] signals.

In this paper we present artificial hydrogel cilia systems that can be stimulated environmentally (pH), electrically (electro-chemical reactions occurring at the electrodes) or magnetically (magnetic field). In addition, a system that combines magneto-responsiveness with sensing of environmental pH variations is also fabricated. By means of lithography techniques, we create high-aspect ratio molds on which the polymer solution is cast. The resulting high-aspect ratio hydrogel cilia are stable, highly responsive and their geometry is controlled by varying the mold pattern. When the cilia is environmentally (pH) or electrically stimulated the dimensiones of the individual cilium is signifcantly reduced (up to 89%). In case of magneto-responsive cilia filled with iron particles the maximum cilium end displacement achieved, when exposed to a rotating magnetic field, is around 45 μm – for 10 μm thick and 55 μm long cilium – and is only limited by the single cilium length. In case of the combined multi-stimuli responsive system the hydrogel cilia start to move, under a time-dependent magnetic field, only when triggered by a sudden pH change in the cilia vicinity.

2 MATERIALS & METHODS

Polyacrylamide gel synthesis is performed by free radical polymerization as described elsewhere [5]. Shortly, 2.5 grams of acrylamide solution (GERBU Biotechnik, Acrylamide 40% solution; #1138,1000) and 1.5 grams of bis-acrylamide aqueous solution (GERBU Biotechnik, Bisacrylamide 2% solution; #1110,1000) are mixed with 16 grams of high-purity water (MilliQ, resistivity greater than 18 MΩ cm). The acrylamide/bis-acrylamide ratio used yields hydrogels with the suitable mechanical properties and was optimized by means of rheology measurements. To improve adhesion between the polymer and silicon mold the surface of the mold was modified with the silane layer (Tridecafluoro - 1,1,2,2 - tetrahydrooctyl) trichlorosilane) (AB111444, ABCR). To enhance the mold filling by the monomer solution we add 1wt% of Sodium Dodecyl Sulfate (SDS, Sigma Aldrich, 436143, purity > 99%). The monomer mixture is then cast on the mold. The ammonium persulfate (APS, Sigma Aldrich, A3678, purity > 98%) is then added (1/100 of total volume, 10% APS solution) as a radical initiator and *N,N,N',N'*-tetramethylethylenediamine, (TEMED, Sigma Aldrich, T9281, purity > 99.0%) as an accelerator (1/1000 total volume). The solution is then left overnight to complete polymerization, yielding a polyacrylamide gel.

3 RESULTS AND DISCUSSION

In this section the microfabrication steps yielding high aspect ratio molds used for making large arrays of hydrogel cilia are described. The process of making the molded hydrogel cilia responsive to single and multiple stimuli is also presented.

3.1 Mold microfabrication

The high-aspect ratio silicon mold is fabricated by means of Electron Beam Lithography and Deep Reactive Ion Etching techniques. The detailed description of the mold fabrication is avaiable elsewhere[19]. Shortly, the cilia pattern is transferred by means of electron beam lithography (EBPG 5000+ Leica) to a PMMA spin-coated silicon chip (PMMA,950k – 7% dissolved in Anisol). After developing, three Deep Reactive Ion Etching (DRIE) steps are performed in an Alcatel Microsystems AMS 100 system that transfer the pattern deep into the silicon wafer creating rectangular or cilindrical prisms, as illustrated in Figure 1. In the last fabrication step, a thin oxide layer, that promotes silane adhesion, is added (~70 nm) by means of Plasma Enhanced Chemical Vapour Deposition. The highest cilia aspect ratio achieved is around 50 (1µm thick and 50 µm long cilia). In general this hydrogel molding technique can easily be scaled up to full wafer scale.

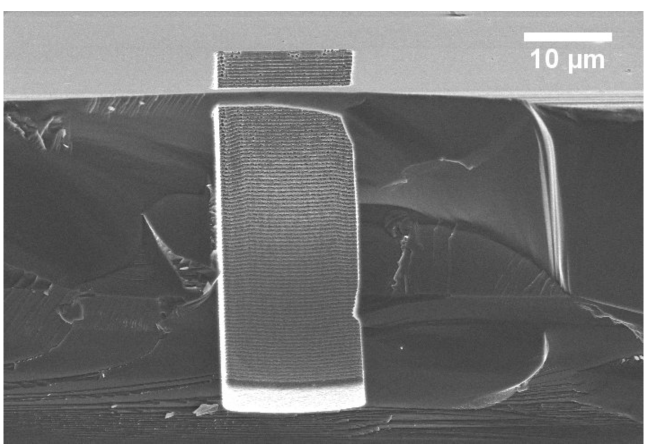

Figure 1 Scanning electron microscope image of a high aspect ratio mold cross section obtained after cleaving the wafer.

The schematic illustration of the hydrogel cilia fabrication with the molding technique is presented in Figure 2. The monomer mixture is cast on the silicon mold (Figure 2a) and the free radical polymerization is initialized and catalyzed with APS and TEMED, respectively (Figure 2b). When the polymerization is complete the hydrogel cilia are released from the mold (Figure 2c). The image of the released cilia, collapsed in air, is illustrated in Figure 2d.

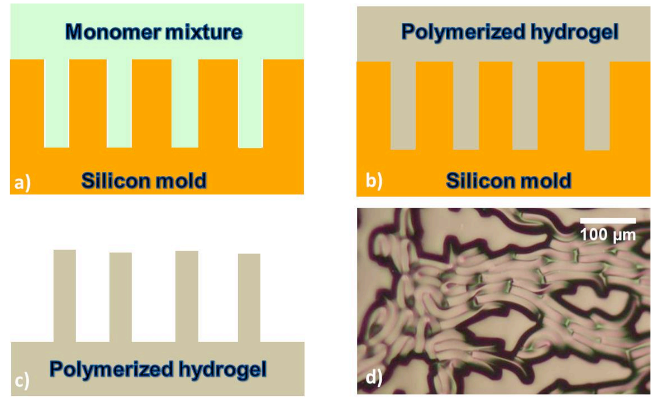

Figure 2 Microfabrication steps of the hydrogel cilia. The monomer mixture is cast on the silicon (a) and the polymerization is initiated (b). When the polymerization is complete, the hydrogel cilia is released from the mold (c). The microscope image of the released cilia in air (d).

3.2 Electro-responsive cilia

To make the molded polyacrylamide cilia electro-responsive, the released gel is hydrolyzed (in a 2M NaOH solution) and then swollen in demineralized and deionized water. Swollen hydrogel cilia capable of electro-actuation are illustrated in Figure 3a.

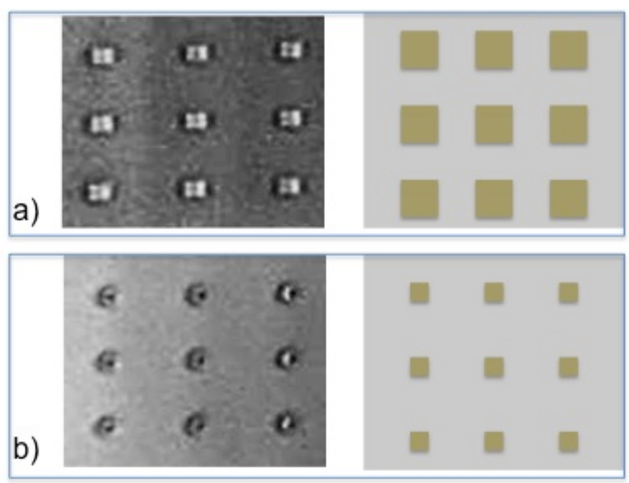

Figure 3 Electroactuation (and its graphical illustration on the right) of the hydrolyzed and swollen polyacrylamide cilia: a) the cilia in a swollen state, immersed in 0.1 M KCl solution, before applying electric potential; b) the shrunken cilia.

To test the electro-responsiveness, the sample is placed in a 0.1 M KCl solution and a platinum wire electrode is positioned next to cilia array by means of a micromanipulators. When the electric potential is applied

(3V), the cilia close to the anode start to shrink and an actuation front propagates from the electrode. Within seconds after the initial actuation starts, the complete array has shrunk as illustrated in Figure 3b. The area reduction of an individual cilium is approximately 80 %. It is important to stress that the contracted cilia swell again when the polarity of the electrode is reversed. No visual mechanical defect of the cilia was observed while changing the electrodes polarity during the experiments.

The electro-actuation is driven by the electrochemical reactions occurring at the electrodes when an electric potential of 3V is applied across Pt electrodes. The pH wave originates from each electrode and propagates with a velocity that is a function of applied voltage. The local pH distortion in the anode vicinity (low pH) changes the gel ionization degree which in turn result in cilia shrinkage. When the polarity of the electrodes is reversed, the opposite process (high pH) ionize the gel again. This induces water uptake (cilia swelling) to balance the electrostatic repulsion between ionized groups attached to the hydrogel polymer network. The detailed description of the actuation mechanism is presented elsewhere [19].

3.3 Magneto-responsive cilia

The artificial electro-responsive cilia described above represent sensing cilia. We will now focus on motile cilia that can rotate when stimulated by a magnetic field. The hydrogel synthesis scheme is the same; however, in order to obtain magneto-responsiveness, we selectively place magnetic iron particles inside the cilia. To achieve this the mold is placed in a container, filled with 1µm iron particles (BASF), and shaken vigorously. The surface of the mold, including prisms, becomes covered with the particles. The mold is then removed from the container and cleaned with adhesive tape. This leaves the particles inside the prisms only. The monomer solution is then cast, followed by addition of APS and TEMED, and left overnight to complete polymerization. To prevent particles migration during casting and gelation, a permanent magnet is placed beneath the mold. It is important to stress that the adhesive tape-cleaning procedure increases the cilia flexibility by slight base depletion.

The magneto-responsiveness is tested by applying a rotating magnetic field in the horizontal plane by means of a self-built setup, mounted on a Nikon Eclipse microscope [19]. Figure 4 shows typical response of hydrogel cilia filled with iron particles when exposed to the rotating magnetic field.

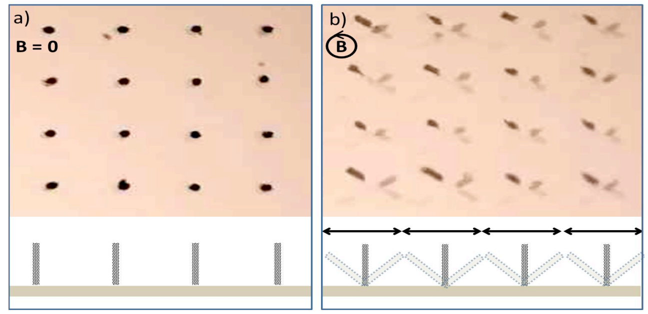

Figure 4 Microscope image of a 55 µm long and 10 µm thick magnetic cilia rotation in response to an external rotating magnetic field (top part) and its schematic illustration (bottom part).

The cilia presented in Figure 4 are 55 µm long and 10 µm thick, an average deflection of around 40 µm is achieved. In fact the maximum deflection seems to be only limited by the single cilium length. By increasing the magnetic field frequency the cilia are able to rotate at more than 160 rpm and no visual defects after thousands of cycles are visible. The single cilium tip positon was also mapped during rotation (over 10 rotating cycles), with the help of ImageJ software, and the cilium movement followed the field rotation, without any visual perturbation, even at high frequencies [19].

3.4 Multi-stimuli responsive cilia

The electro- and magneto- responsive cilia systems introduced in the previous parts can either perform sensing or motile function when activated. In the following part we present a combined multi-stimuli system that reacts with the rotational motion (motility) when trigered by low pH (sensing). The magnetic cilia, prepared as described in the previous section, are hydrolysed and swollen in deionized and demineralized water. This redistribute the magnetic particles in the hydrogel matrix. However, no visual damage to individual cilia is detected. The cilia are then exposed to a rotating magnetic field. In the swollen state, no response from the cilia is observed. However, when the local pH is lowered, by addition of a 20 µl pH 1 droplet, the cilia detect the environmental change (shrinks) and start to rotate. The schematic illustration of a triggered response, including intermediate phases, is illustrated in Figure 5.

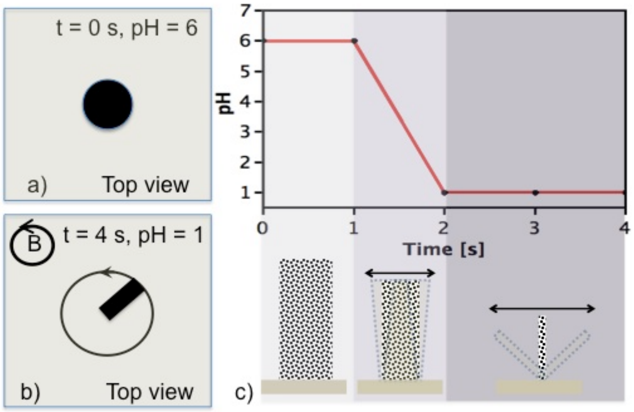

Figure 5 Hydrolyzed and swollen hydrogel cilia, filled with iron particles, do not respond to a magnetic field when immersed in pH 6 solution (a). When the pH of the surrounding solution is lowered the size of the individual cilia is reduced and the array starts to follow the magnetic field rotation (b). Schematic illustration of this triggered response, including intermediate phases (c).

4 CONCLUSIONS

Large arrays of high-aspect-ratio hydrogel based cilia, that can respond to different stimuli, are fabricated by means of a molding technique. By simply tuning the fabrication approach the same hydrogel system can be activated environmentally (pH), electrically (electrochemical reactions at the electrodes) or magnetically (external magnetic field). In addition a combined multi-stimuli artificial cilia system that reacts with the rotational motion (motility function) when trigered by low pH (sensing function) is produced.

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