

SELECTIVE ATTACHMENT OF MULTIPLE CELL TYPES ON THERMALLY RESPONSIVE POLYMER

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ABSTRACT

Programmable surface chemistry has been achieved by depositing a temperature sensitive polymer onto arrays of micro-fabricated metallic heaters. Activating a single heater causes a localized change in the device surface chemistry from non-fouling to fouling in an aqueous environment. Two types of proteins and two types of cells were used to demonstrate localized immobilization of proteins and cells on such surfaces. These experiments show, for the first time, selective cell attachments on thermally responsive polymer controlled by a micro heater array. It suggests a new approach to realize proteomic chips and cell chips.

Keywords: Bio MEMS, Biochips, Protein chips, Cell chips, Heater array, NIPAM, Non-fouling.

INTRODUCTION

Protein and cell chips have become one of the most active areas emerging in biotechnology today. Array fabrication methods include robotic contact printing [1], ink jetting [2], piezoelectric spotting and photolithography [3]. A number of commercial arrays are available as well as manual equipment. Each method has its advantages and inherent limitations, and there are great needs of new technologies to realize biochips inexpensively and efficiently. Here we report a novel approach based on micro heater arrays and thermo responsive surface chemistry to develop biochips.

Surface modification for adsorptive and covalent coupling of biochemical species has been widely exploited for production of hybrid micro arrays [4-5]. Poly(*N*-isopropylacrylamide) (pNIPAM) is a good candidate to realize programmable surface chemistry due to its lower critical solution temperature (LCST) at 32°C in an aqueous environment [6]. When grafted onto a solid substrate, the phase transition of pNIPAM produces a “smart” surface with varying chemical properties controlled by changing the temperature. Below the phase transition temperature, the surface is hydrophilic, swollen and nonfouling. As the temperature is increased above the LCST the grafted polymer chains collapse and the surface becomes hydrophobic and protein or cell retentive.

Benefiting from the current micro fabrication technology the surface can be pre-patterned with an electrically addressable micro heater array, which can be used to switch the local fouling property thereafter to direct site-specific protein and cell attachments.

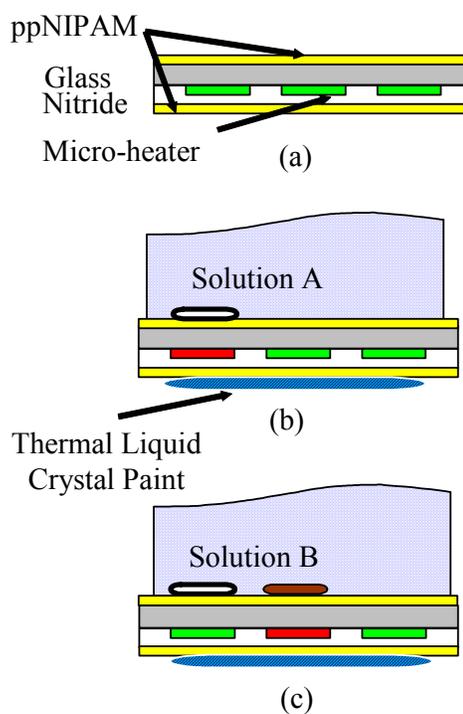


Figure 1. Schematic description of ppNIPAM devices. (a) Devices consist of micro-heaters on a glass slide coated with ppNIPAM. (b) Active heater (dark) turns the surface fouling. Selective protein/cell adsorption occurs exclusively on heated areas. Temperature sensitive paint was used to characterize the temperature profile. (c) A second adsorption step with different protein/cell solution on a different heater.

Figure 1 shows the principles of the proposed method: A metallic micro heater array is patterned on a glass substrate. Temperature sensitive NIPAM is deposited on the device surfaces. When exposed to protein/cell solution, proteins/cells are adsorbed exclusively at heated areas. Repeated adsorption steps form arrays of various

proteins/cells. The main advantage of the presented technique is the ability to perform the entire process in an aqueous environment, which is critical to maintain the integrity of sensitive proteins/cells. An additional advantage is the low power consumption, which enables a compact and portable setup by using low voltage batteries. Overall the capability for parallel processing makes it a high throughput and low cost method.

EXPERIMENTAL

Fabrication

The present work started with finding a suitable substrate which is both micro fabrication compatible and biocompatible. Since fluorescence technique was used to detect proteins and cells the substrate should not have high autofluorescence. The lower thermal conductivity of glass ($1.4\text{Wm}^{-1}\text{K}^{-1}$) compared to silicon ($80\text{-}150\text{Wm}^{-1}\text{K}^{-1}$) makes it easier to achieve localized heating. In addition, glass is transparent which allows for the direct visualization during protein and cell attachment. Micro cover glass (VWR) with a thickness of $170\mu\text{m}$ was selected as the substrate. It is thin and light and thus it can be easily bonded to a 3" silicon wafer using positive photoresist (AZ1512, Clariant) for processing with standard micro fabrication equipment. Soaking in acetone for less than one hour will release it after processing is complete.

A micro heater array chip with three independent resistive heating elements was fabricated by a lift-off process. To clean the glass it was soaked in nano-strip for 10 minutes at the very beginning. After the photoresist was patterned by photolithography, a thin gold film (100nm) with chrome as an adhesion layer (10nm) was thermally evaporated onto the glass. The photoresist was then removed by soaking in acetone for 30 minutes with additional ultrasonic bath for 30 seconds. Silicon nitride (400nm) was sputtered on the top as a passivation layer to increase the stability of the heaters during the experiments. The resistance of each heater ($1\text{mm}\times 1\text{mm}$) was around 100Ω and varied slightly from heater to heater.

Formation of ppNIPAM

The pNIPAM coating was achieved by exposing samples to the NIPAM glow discharge under continuous plasma (plasma polymerized NIPAM or ppNIPAM) [7], which ensures a solvent free, conformal, sterile and pinhole-free coating [8-9]. Since the sample was suspended by a string in the reaction chamber the ppNIPAM was coated on both sides (Figure 1).

Protein and cell attachment experiment

A teflon chamber was built to hold the solutions in contact with the glass (Figure 1b). The local surface temperature was controlled by the power supplied to the individual heaters. Simulation was performed to predict the thermal response of the system. After the series of

protein/cell attachments, a fluorescent microscope was used to detect the proteins and cells.

RESULTS AND DISCUSSION

Temperature control

Precise temperature control is critical for the experiment. As shown in Figure 1, the proteins and cells were attached on the non-heater side of the glass. The main reason is to avoid electrolysis since for the required power the voltage drop on the heater was around 2.2V. An additional advantage is that this side is smoother due to less processing. The temperature of the bottom surface was monitored by applying a layer of thermo chromic liquid crystal paint (Hallcrest). The surface was quickly heated up (after about 1 second) showing circular fringes around the heater (Figure 2), which remained unchanged throughout the experiment. Simulations were performed to obtain the thermal profile of the top surface in contact with the solution.

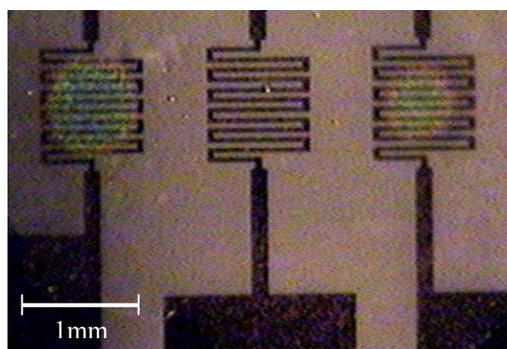


Figure 2. A three-micro-heater array with left and right heaters activated ($V=1.2\text{V}$, $I=7.9\text{mA}$).

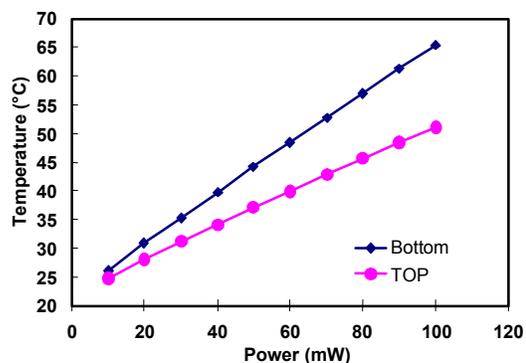


Figure 3. Simulated temperature of top and bottom surfaces at the center of the heater versus electrical power by CoventorWareTM.

CoventorWare™, an integrated MEMS design software, was used to simulate the electrical-thermal properties of the designed heaters (Figure 3). The simulation result provided a guide for applying input power. It showed that in order to achieve the LCST at 32°C a power supply of 34mW is required. Also to keep the cells alive, i.e., at less than 40°C, the input power should be lower than 60mW. In the current experiment an input power of 50mW was applied in order to heat the ppNIPAM surface to 37°C with 400μl buffer in the chamber, which was verified by the experimental result.

Protein patterning

Protein patterning on ppNIPAM was performed using TRITC-labeled anti-bovine serum albumin (BSA) and FITC-labeled goat immunoglobulin G (IgG). The two proteins were added to the chamber sequentially with two different heaters turned on respectively with 50mW power supplied to achieve 37°C on the top surface. Each protein was allowed to adsorb for 30 minutes. Two well-defined protein spots are clearly seen on the heated area under the fluorescent microscope (Figure 4). The protein adsorption on ppNIPAM is irreversible, i.e., after the attachment it stayed on the surface even when the temperature was dropped to 23°C [9].

Cell patterning

In addition to the success in protein patterning, cell patterning was performed using the same setup with bovine aortic endothelial cells (BAEC) and bovine aortic smooth muscle cells (BASC). The procedure is similar to the one above except that the incubation time was 3 hours for each cell type. In contrast to protein adsorption cell attachment on ppNIPAM is reversible, i.e., it would detach from ppNIPAM upon cooling down which is probably due to higher surface tension of cells. In order to prevent detachments the heater for the first batch attachment was kept on all the way through the experiment. After staining, cell patterning was observed under the fluorescent microscope (Figure 5). BASMC showed up only around the top heater even though the bottom heater was kept on during its adsorption. The reason is that the bottom region has already been fully covered with BAEC due to the first step of adsorption. Since there is no cell-cell adhesion between them BASMC could not adsorb on top of BAEC.

For both protein and cell patterning, the shape of each pattern was consistent with the temperature profile of the liquid crystal paint, proving the patterning experiments was driven by the thermal response of ppNIPAM. When the patterning experiments were performed with a micro heater chip without ppNIPAM coating, no site-specific pattern was observed, further supporting the role of ppNIPAM in directing protein and cell attachment.

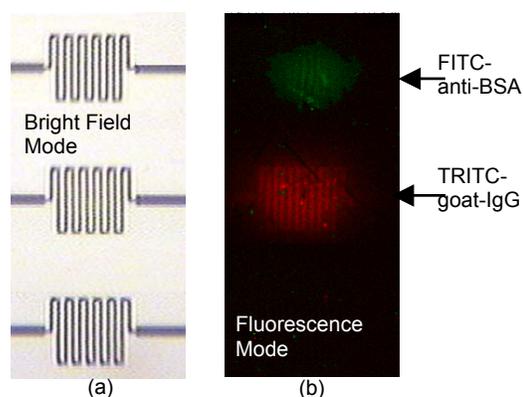


Figure 4: Labeled IgG proteins and TRITC-goat-IgG proteins were immobilized on the top and middle heater respectively. (a) Bright field mode. (b) Fluorescence mode. Fluorescence was false-colored green and red respectively. Because the two fluorophores have no overlapping excitation and emission spectra, we were able to detect them simultaneously.

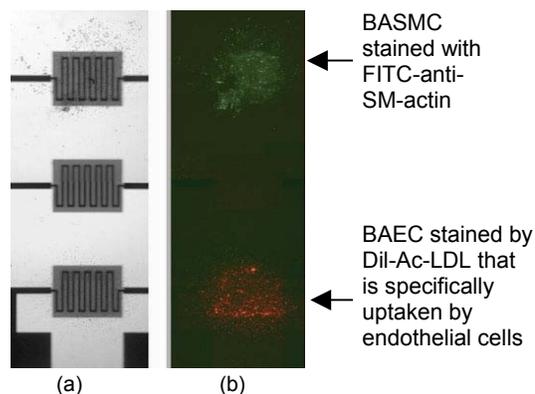


Figure 5: Two steps of cell attachments on the top and bottom heater, respectively. Unlike proteins, cells are visible in both (a) Bright field mode and (b) Fluorescence mode.

CONCLUSIONS AND FUTURE WORK

The technique described in this paper offers a new approach to realize biochips by using programmable surface chemistry devices. The entire process is performed in an aqueous environment, which is critical for protein integrity and cell viability. Using thermal response of ppNIPAM to pre-patterned micro heater arrays makes the process reliable and flexible, excluding complicated alignment and solvent/elastomer contamination common in the conventional methods.

Incorporating standard micro fabrication processes to fabricate the biochips drastically reduces the cost and time. It is desirable to create micro heater arrays with feature sizes of single cell dimensions. Larger scale protein and

cell chips with various proteins and cells can be realized based on this technique. Addressing and automatic temperature control will be needed in such more complicated systems. Thermal isolation will be a major challenge, and micro heaters suspended on thin films might be the only choice in this case.

An alternative approach could replace the micro heaters with optical delivery of the heating energy, via either a scanning laser beam or a digital light processing (DLP) micro mirror array. In such a system, reduced complexity in control electronics is traded off with larger and more costly optics components.

The change in hydrophobicity of NIPAM in aqueous solution in response to relatively small changes in temperature also suggests many other uses, such as, e.g., valves for micro fluid channels. By integrating micro fluid components with addressable protein and/or cell arrays it will be possible to make a compact and portable lab-on-a-chip device. Such a device should be handy and useful in various fields including proteomic and cell chip design, medical diagnostics, biosensors and tissue engineering.

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