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Micro-patterning of mammalian cells on suspended MEMS resonant sensors for long-term growth measurements

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MEMS resonant mass sensors can measure the mass of individual cells, though long-term growth measurements are limited by the movement of cells off the sensor area. Micro-patterning techniques are a powerful approach to control the placement of individual cells in an arrayed format. In this work we present a method for micro-patterning cells on fully suspended resonant sensors through select functionalization and passivation of the chip surface. This method combines high-resolution photolithography with a blanket transfer technique for applying photoresist to avoid damaging the sensors. Cells are constrained to the patterned collagen area on the sensor by pluronic acting as a cell adhesion blocker. This micro-patterning method enables long-term growth measurements, which is demonstrated by a measurement of the change in mass of a human breast cancer cell over 18 h.

Introduction

The use of microelectromechanical systems (MEMS) resonant sensors to study the growth of individual cells is a developing area of research.[1–5] Investigations of growth over the cell cycle require long-term measurements over many hours and are challenged by the movement of cells during the experiment. Cells that are highly motile, such as metastatic cancer cells, will move off the sensor thus ending the measurement. Even cells that are considered non-motile remain active, exhibiting spatial movements that permit cells to escape from the sensor.[6] Previous studies have used fluidic traps[5] and dielectrophoresis (DEP)[1] to control the positioning of cells on MEMS resonant sensors, though these methods require complicated on-chip systems and do not trap the cells for long periods of time. Methods for improving the retention of cells on MEMS resonant sensors for hours, or days, are needed to enable studies into the long-term growth dynamics of cancer cells.

Micro-patterned surfaces enable the capture and confinement of single cells or large populations.[7–12] Cell micro-patterning is an extremely powerful tool that promotes selective attachment and confinement of single cells through surface chemistry.[13,14] Microcontact printing is one of the most popular laboratory techniques for the fabrication of chemical or protein micro-patterns.[13] The printing approach easily transfers protein patterns from a substrate acting as a stamp onto a surface; however, this technique is ideal for surfaces that can withstand the necessary stamping pressure and peeling force. Because MEMS resonant sensors have micron-scale features that are fragile they are incompatible with microcontact stamping process. A micro-patterning technique that can be integrated with suspended MEMS resonant devices offers an attractive solution for long-term measurement, while improving cell retention.

In this paper, we demonstrate a robust technique for selective surface micro-patterning on fully-suspended MEMS resonant mass sensors that overcomes the challenge of patterning on suspended devices by implementing a photoresist blanket transfer technique combined with high-resolution photolithography.[15] Patterning proteins against a background of cell adhesion blocker constrains cells to the viable sensor area of pedestal devices. This improves cell retention and enables growth measurements of even motile cells. We use the micro-patterned sensors to measure the change in mass of a human breast adenocarcinoma cell over eighteen hours, demonstrating the ability to study the long-term growth dynamics of cancer cells.

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Materials and methods

The MEMS resonant sensors used in this work consist of 60 × 60 μm^2 pedestals suspended by four beam springs over a shallow pit.[2] The sensor array consists of 81 individual sensors arrayed in a 9 × 9 format and is fabricated from a silicon-on-insulator wafer with the final step depositing a silicon oxide insulation layer. Fig. 1A shows the device architecture, and full details of sensor fabrication are provided in previous works.[2,16] The remainder of this section describes the procedure for micro-patterning of collagen onto the pedestal sensors following the schematic presented in Fig. 1B.

Hydrophobic surface modification

The chip surfaces were treated by oxygen plasma exposure for 5 min at 200 W. The chips were then placed in small groups in 100 mL glass jars. Hexamethyldisilizane (HMDS) was pipetted into each jar and the jars were sealed and heated at 80 °C for 1 h.[17] After vapor deposition of HMDS on the surface, the samples were gently rinsed with acetone, isopropanol, and DI water and allowed to dry. Fig. 2A depicts the vaporization and self-assembly of the silane that caused pendant methyl groups to form a hydrophobic surface. We verified surface modification by observing water droplets easily rolling off.

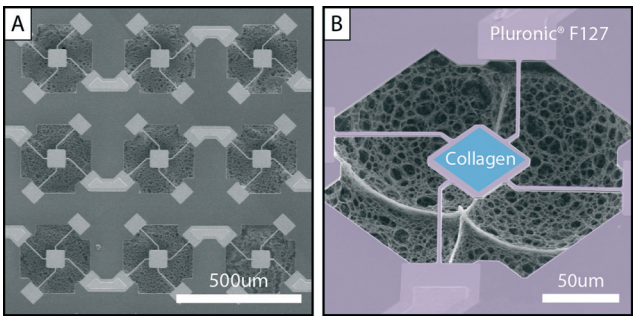

Fig. 1 (A) Scanning electron micrograph (SEM) of a MEMS mass resonant sensor array. The chip design consists of 81 sensors fabricated in a 9 × 9 array. (B) SEM of a single sensor with an overlaid schematic of the selective functionalization and passivation technique where the center of the mass sensor is coated with collagen (represented by the blue square). Remaining areas of the sensor are backfilled with pluronic (a tri-blocking copolymer) to prevent protein and cell adhesion outside the sensor platform region.

Blanket transfer and lithography

Fig. 2B depicts the process of transferring photoresist to the chip as a blanket for lithography.[15] Photoresist AZ 9260 (AZ Electronic Materials) was spin-coated onto a at polydimethylsiloxane (PDMS) stamp and baked for 2 min at 50 °C. Next, the resist is brought into conformal contact with the chip and baked at 50 °C for another 2 min. The chip was then rapidly cooled to 4 °C and the PDMS was quickly peeled off the chip leaving the photoresist behind as a membrane over the sensors and the pit below.[15] After baking the photoresist-coated chip at 50 °C for 2 h to avoid bubble formation, the photoresist was patterned in a Karl Suss i-line mask aligner (SUSS MicroTec Group) with 10 mW cm^{-2} intensity to open the area above each pedestal sensor (Fig. 2C).

Collagen functionalization and pluronic passivation

Following patterning, the chip was exposed to oxygen plasma at 300 W for 10 min to remove HMDS from the regions unprotected by photoresist. We then placed a PDMS chamber over the sensor area for functionalizing with a type I collagen solution in PBS (100 μg mL^{-1}) for 1 h at 37 °C. Following collagen deposition, the chip was rinsed with PBS and dried with a stream of nitrogen. This resulted in collagen deposition over both the patterned hole and the remaining photoresist surface. After removing the PDMS chamber, the chip

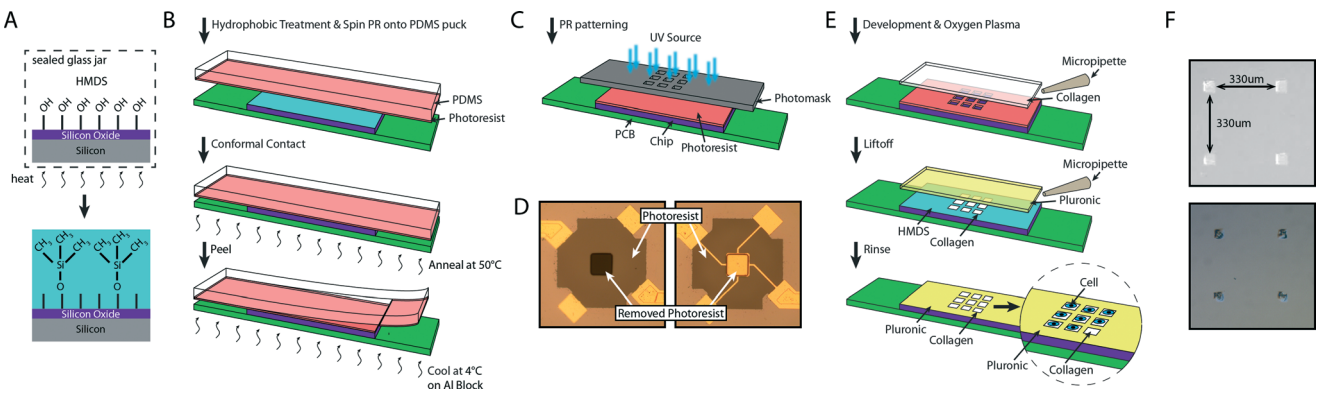

Fig. 2 Overview of micro-patterning process for selective functionalization and passivation on MEMS resonant pedestal sensors. (A) First, the surface is treated with HMDS to promote hydrophobicity for easy attachment of the pluronic at the end of the entire process. (B) Photoresist is spun on a PDMS puck that is larger than the chip and baked, then stamped onto the chip and annealed. Finally, the chip and PDMS are rapidly cooled and the PDMS is peeled off, leaving the PR on the surface in a blanket coat. (C) The PR can then be patterned using a typical UV mask aligner and developed. (D) The square patterns on a broken sensor (left) and functional sensor (right), clearly showing the blanketing of the photoresist and the opening after development. (E) The developed pattern is treated in an oxygen plasma system to remove the HMDS selectively on the surface. After collagen functionalization, the photoresist is removed along with the excess collagen above in a liftoff process. Finally, pluronic is backfilled onto the exposed HMDS to act as a cell adhesion blocker. (F) DIC images of micro-patterned collagen on a plain silicon surface (top) and with cells attached (bottom).

was soaked in acetone upside down to lift-off the remaining photoresist from the surface. The lift-off procedure leaves geometrically defined collagen patterns selectively deposited on the sensor pedestals, while retaining a background of HMDS on the remaining surfaces.

After rinsing with DI water and drying with nitrogen, the chip was attached to a printed circuit board and wire-bonded at room temperature. Finally, Pluronic® F127 (Sigma Aldrich) was deposited by backfilling the chip with a 1% solution in PBS at room temperature for 2 h. Fig. 2E presents the entire functionalization and passivation procedure.

Cell culture and preparation

Human breast adenocarcinoma cells (MCF-7 ATCC # HTB-22) were cultured in Dulbecco's Modified Eagle Medium (Gibco) with 10% fetal bovine serum and 1% penicillin–streptomycin. Human breast cells (MCF-10A ATCC # CRL-10317) were cultured in Dulbecco's Modified Eagle Medium/Ham's F-12 (Gibco) with 5% horse serum, 20 ng mL^{-1} EGF, 0.5 mg mL^{-1} hydrocortisone, 100 ng mL^{-1} cholera toxin, 10 μg mL^{-1} insulin, and 1% penicillin streptomycin. Cells were treated with 0.05% trypsin EDTA (Gibco) for seeding on the sensor through a 100 μL well at a density of 9000 cells per 100 μL.

Results and discussion

Fig. 1 shows an overview of the MEMS resonant sensors and the selective functionalization and passivation process. As a result, this process produces micro-patterned proteins on pedestal surfaces and deposits a protein and cell blocker on all other surfaces. The procedure for fabrication of these micro-patterns is summarized in Fig. 2 and includes surface modification through vapor deposition of a self-assembled HMDS monolayer; blanket transfer of photoresist and high-resolution photolithography to define the pattern for collagen; and collagen deposition and backfilling of pluronic. To our knowledge, this is the first report of a method for micro-patterning cells on fully-suspended MEMS resonant sensors.

The challenge in patterning collagen on the surface of MEMS resonant sensors with lithography is the need for a method to consistently, and uniformly, deposit photoresist on the suspended devices. The devices are fully suspended by four beam springs, thus making them too fragile for spinning photoresist across the surface. To yield a more uniform and consistent photoresist deposition, we employed a transfer technique where photoresist is applied as a membrane, or blanket, covering both the sensors and pits beneath the sensors. Blanket lithography uses a PDMS substrate to spin a flat piece of photoresist for transfer to the chip. After soft baking the photoresist in contact with the chip, the chip is rapidly cooled. This heating–cooling process changes the fracture energy of the photoresist layer and allows for the PDMS substrate to be easily removed, leaving a uniform blanket of photoresist on the chip.[15]

Once the photoresist is blanketed on the surface, standard photoresist patterning techniques maybe used (Fig. 2C), with the note that soft or separation contact would be ideal for delicate samples like this. In this case, we exposed 50 × 50 μm^2 square holes centered on each pedestal according to the pattern represented in Fig. 1B. Fig. 2D clearly shows the blanketing of the PR and the developed patterns over a pit with a removed sensor (left) and an intact sensor (right). In general, the size and shape of this pattern can be adjusted depending on sensor type and application. Oxygen plasma removes HMDS from the surface at the patterned openings in the photoresist to allow for functionalization with collagen. Fig. 2F shows results of the patterning and blocking process, the square collagen deposits retain adherent cells that conform to the printed pattern.

Pluronic is a tri-block copolymer consisting of two polyethylene oxide (PEO) groups and a polypropylene oxide (PPO), and has been shown to be an effective blocking agent to deter protein adsorption[18] and cell adhesion.[19–21] The longevity of the pluronic non-adhesive coating has been characterized and cell are retained in clean patterns for approximately 3 days.[20] Pluronic can be applied to a surface in two ways: pancake or brush-like. The brush-like configuration is ideal for effectively blocking protein and cell adhesion. To achieve this conformation, devices are silanized with HMDS in order to achieve a highly hydrophobic surface with contact angles of about 80°. The PPO portion of the copolymer anchors to the surface and the PEO portions are dangling in a brush-like formation.[22]

Fig. 3A presents examples of captured MCF-10A cells on the platform sensor within the patterned area and not on the

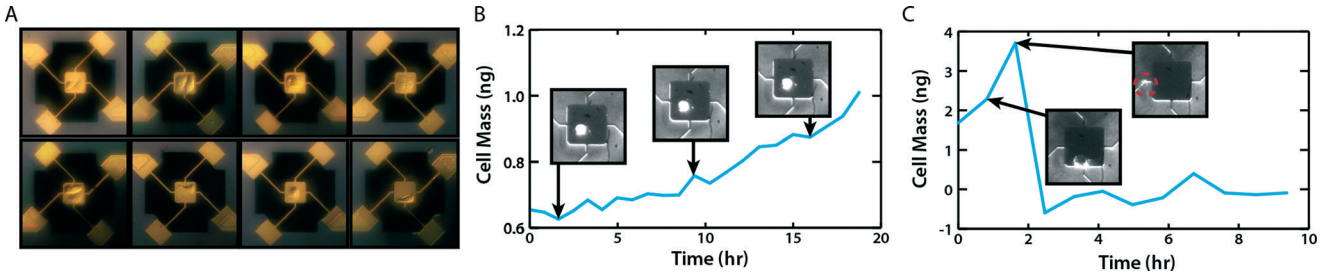

Fig. 3 (A) DIC images of MCF-10A cells attached to the sensor area after collagen patterning and pluronic backfilling, with all adherent cells being within the patterned area. (B) Growth profile of a MCF-7 human breast cancer cell measured over 18 h, demonstrating the ability to capture and retain cells on the sensor surface for long-term growth measurements. (C) Growth profile of a MCF-7 human breast cancer cell measured over 9.5 h, showing the migration of cell starting on the platform, moving onto a spring causing an erroneous mass measurement and finally falling off the sensor entirely.

springs or around the sensor. Our MEMS sensors have been previously used for applications of mass sensing,[16,23] including cell growth measurements.[2] Mass measurements with these resonant sensors use a previously published method that utilizes the change in resonant frequency shift of the sensor after a mass is added.[2,23] Cellular growth measurements require the monitoring of changes in sensor resonant frequency over time. Integrating this micro-patterning technique further enables long-term growth measurements of dynamic and migratory cells. Fig. 3B shows the measured mass of an MCF-7 cell over the course of 18 h. This cell is confined to the pedestal surface as a result of the selective functionalization and passivation process, thus allowing for a long-term growth measurement. This is in contrast to a measurement on an MCF-7 cell using a sensor without patterning seen in Fig. 3C. The cell remained on the sensor platform for only two measurement covering less than an hour before migrating onto the spring thus invalidating the measurement and finally disappearing entirely from the sensor. It is not expected that the confinement of the cells to the sensor platform will affect cellular growth, as the cells are only slightly more constrained than without the passivation.

Conclusions

Here we present a novel method for micro-patterning of single cells directly on MEMS resonant platform sensors to enable long-term growth measurements of motile cells. We describe the use of a blanket photoresist transfer technique with standard photolithographic practices to achieve the basic patterning of proteins on the surface of the sensor. This selective functionalization, along with passivation of the remaining chip surface with pluronic, an effective cell and protein adhesion blocker, traps captured cells to the pedestal sensor area. The ability for long-term growth measurements with MEMS resonant sensors and micro-patterned surfaces is demonstrated by mass measurement of a breast cancer cell over an eighteen-hour period. Future studies can use this technique to investigate metastatic cancer cells and other highly motile cell lines.

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