MEMS-BASED BIOLOGICAL PLATFORM FOR DYNAMIC CELL-TO-CELL INTERACTION CHARACTERIZATION

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ABSTRACT
MEMS-based biological platforms with the capability of spatiotemporal control of living cells have been demonstrated. The space control is accomplished by microfabrication to have targeted gaps between two adjacent culture areas. The time control is achieved by the electrical activation of corresponding electrode to independently activate the culture area of interest to allow movement/attachment of living cells. Prototype cell co-culture tests with structure of 1×3 array using 3T3 fibroblast cells have been successfully demonstrated through serial surface activations. As such, this MEMS platform could be a basic yet versatile cellular biology tool to characterize transient cell-to-cell interactions.

INTRODUCTION
Interactions between either the same or different types of living cells trigger a variety of unique cellular behaviors, such as growth, migration, differentiation, to name a few. It is desirable to engineer and dynamically characterize in-vitro cell-to-cell interactions to understand in-vivo responses of living cells of multi-types. However, this has not been accomplished in practice due to difficulties in handling and positioning individual cells with good temporal and spatial controls.

Previously, efforts have been introduced for the study of static cell-to-cell interactions (without temporal and spatial control). Typical photolithography processes [1, 2] have been commonly used in cell patterning and co-culture experiments. Researchers have also demonstrated the usage of soft-lithography for easier and reliable cell patterning and cell co-culture [3-5]. These lithography based methods are limited as they allow static patterning without dynamic pattern changes. Microfluidic technology has also been applied in cell co-culture using two different flows to achieve fine pattern of cells [6] and functional surfaces such as thermally responsive polymer (cell adhesive to non-adhesive [7]) has been recently introduced for cell co-culture.

Dynamic cell co-culture processes to mimic in-vivo cell behaviors have been investigated recently. For example, micromechanical control of cell to cell interaction by using MEMS actuators was able to reveal unknown property between cells not discovered by static methods [8]. Electrochemical desorption of SAM (self assembled monolayer) for multi-types of cell co-culture enables cell migration study [9]. Efforts to combine micro contact printing and photoelectroactive surface chemistry have been reported [10]. In this method, cells on selective area can be released while the other cells are remaining in contact with the substrate. The aforementioned methods have advanced further the understanding of cell-to-cell interactions and characterizations.

Here, we propose a MEMS platform to have both temporal and spatial control of cell patterns for the study of cell-to-cell interaction. The important features are, 1) capable of loading multi-types of cells, 2) serial activation of adjacent electrodes to dynamically modify culture areas to study the geometric surface impact to cell migration, and 3) optical transparent for easier biological observations.

![Figure 1. Schematic diagram of MEMS based biological platform and working principle for cell-to-cell spatiotemporal co-culture experiments. (top) Activation of second cell type on the right-side culture area. (center) Activation of the central reaction culture area. (right) Migration and co-culture of cells. The unified control scheme is the selective PEG-silane desorption process controlled by an electrical voltage as shown.](image)
electrode surface is activated by applying a negative voltage of 1.5V (center image), the specific cell culture area is modified from non-adhesive to cell adhesive for the time control. The spatial control is designed by the microfabricated patterns between electrodes as shown.

**EXPERIMENTAL SETUP**

The chemical used for the ITO and Pyrex surface is polyethylene glycol (PEG) silane purchased from Gelest®. PEG is widely used for its non-toxic property with the possibility of versatile functionality. PEG with silane treatment is used here to create strong chemical bonding with ITO surface [12] as illustrated in Figure 2. First, substrate is cleaned with oxygen plasma at 200W for 5 minutes. The substrate is then dipped into 4% v/v of PEG silane dissolved in anhydrous toluene for 15 hours. During this process, the ITO and Pyrex surface is grafted by PEG-silane and covalent bonds are formed between ITO & Pyrex and the PEG silane. A curing process at 100°C is followed to secure the weakly bonded hydrogen to covalent bond by condensation.

The removal of PEG silane from the surface is based on electrochemical desorption [11]. Negative potential on ITO electrodes provides electrons on the top surface to break the covalent bond. The PEG-silane desorption process is conducted in 1×PBS solution for electrical connection. Pyrex surface is dielectric such that only the PEG-silane on electrically-activated ITO surface will be removed.

**Surface Functionalization**

![Surface modification with PEG coating](image)

**Figure 2.** Surface functionalization is based on passivation of PEG silane onto ITO surface and other areas. The PEG-silane desorption process is conducted in 1×PBS solution.

The fabrication process utilizes conventional micromachining techniques as illustrated in Figure 3. Pyrex wafer is chosen as the substrate and 150nm-thick ITO layer is sputtered by DC sputtering and patterned to define connection lines, contact pads and cell culture areas in Fig. 3a. The sheet resistance of the ITO layer is measured as 20-25Ω/□. Afterwards, a layer of 200nm-thick silicon dioxide is deposited at 200°C by PECVD (Plasma Enhanced CVD) and patterned as shown in Fig. 3b. The cell co-culture area is defined by depositing and patterning another 150nm-thick ITO using the lift-off process as illustrated in Figs. 3c and d.

The top figures in Figure 4 show both optical and SEM images of a fabricated device of 1×3 array structure. The primary reason for having three layers structure is to secure precise gap control between cell culture areas and the prototype device has gap distance of less than 1μm. The loading sites as indicated in Fig. 4 are cell culture areas potentially for two different types of cells to be loaded independently. The center area between is designated as co-culture area. The surface profiler measurements in the bottom of Figure 4 provide information on the heights of various parts of the system. It is observed that the highest point is 450nm at the edges of the central cell culture area. However, the height difference of adjacent electrodes is estimated to be less than 200nm such that cells can easily overcome and migrate into the area.

![Device Fabrication](image)

**Figure 3.** Device fabrication is composed of a three-mask process. ITO is sputtered and patterned with lift-off process for interconnection lines. PECVD oxide is performed for insulation. Final layer of ITO is processed to construct the cell co-culture areas.

![Fabricated platform (a 3x1 array is demonstrated here) is optically transparent that maximize observability](image)

**Figure 4.** Fabricated platform (a 3x1 array is demonstrated here) is optically transparent that maximize observability.
SURFACE TREATMENT VERIFICATION

The PEG coating on the surface and desorption of PEG-silane from ITO surface can be verified by various method such as XPS (x-ray photoelectron spectroscopy), AFM (Atomic force microscope), fluorescent imaging method or contact angle measurement. Here, contact angle measurement is used. The initial contact angle without any surface treatment is measured as 23±4° on bare ITO surface. After the PEG silane passivation, the contact angle increases to 64±4°, indicating successful surface modification. After the PEG-silane desorption process, 31±4° contact angle is observed. The angle change between the original ITO surface and after the desorption of PEG-silane layer is not clearly understood. One possible reason is hydroxyl groups contributing hydrophilic properties of ITO surface are consumed and broken away from the surface during electrochemical desorption of PEG-silane. The average measurement result is plotted in Figure 5 with corresponding optical images below. As clearly shown in the optical and fluorescent images, cells are able to attach on the activated (PEG removed) surface while the other areas remained non-cell adhesive due to PEG-silane.

CELL CO-CULTURE PLATFORM TEST

In order to have both temporal and spatial control for cell studies, preliminary experiments are conducted by using a single type of cells, 3T3 fibroblast. Figure 6 shows results on dynamic cell co-culture experiments. For temporal control of the 1st type of cell, the top side electrode is activated and cells are loaded while the other pads still have the PEG-silane coating. A cell flushing process is conducted to remove loosely bonded cells on activated pads and the other areas. The left-side photo in Fig. 6 shows the result after these steps. The second type of cells can be loaded on the bottom electrode after surface activation of that electrode. These cells are not able to go beyond the activated cell culture area as illustrated in the center photo in Fig. 6 which is taken after 15 hours of cell culture. Only 3T3 fibroblast is used for this prototype experiment, both the top and bottom cell culture areas look similar. After the center cell co-culture area is activated, cells on both top and bottom sides migrate and proliferate together. The right-side photo in Fig. 6 is taken 15 hours after the central cell co-culture is activated. Theoretically, multi types of cells can be loaded and co-cultured for the studies of transient cellular interactions.

DISCUSSIONS

The key feature of surface modification is based on strong adhesion between ITO and PEG-silane. Several key processes are crucial to achieve strong adhesion: (1) uniform coating of PEG-silane on the surface, and (2) incubation process in PEG-silane dissolved solution. For example, non-uniform coating and failure of PEG passivation have been found on the ITO surface without enough time for incubation. Curing under proper temperature is also known to help changing hydrogen bond to covalent bond through condensation process.
The structural pattern is crucial to constrain cells on defined electrode areas. Figure 7 shows a typical device which has obtuse angled cells (cells occupy angle over 180°) on the corners. The dotted line is the electrode pattern and cells are able to protrude from the both corners. This phenomenon is rarely observed for corners with acute angles. Therefore, cells that require high degree of confinement are better to have minimal structures with obtuse angles.

Further experiments with two different types of cells (Hepatocyte and 3T3) have been scheduled. It is noted that the feasibility of the two types of cells co-culture is based on the hypothesis that affinity between the adhesion of cell type #1 and cell type #2 is less than the adhesion of cell type #2 to substrate. Otherwise, when the second type of cells is loaded on the substrate after the first type of cells is already loaded, co-culture of cells can happen immediately.

The size of the structure demonstrated in the paper is 50 μm by 50 μm such that around tens of cells can sit on the activated area. It might be feasible to shrink down the size of the structure for possible studies at the single cell level. Proper design could allow a single living cell to sit, to stretch into selected area for sub-cellular level studies.

CONCLUSION

In this work, we present a MEMS based biological platform designed for dynamic cell patterning and multi types of cell co-culture with a simple process. Serial and selective activation, optical transparency and that capability of dynamic cell pattern modifications are combined into a single platform with straightforward procedures. The surface of the device is successfully grafted with PEG-silane to control cell adhesion. It is found that electrochemical desorption of the PEG-silane can effectively restore the ITO surface as supported by the contact angle measurement data. The biological platform has been verified for capability of controlling cell position in time and space based on the single type of cell experiments. Co-culture tests through serial activations have been performed to illustrate the capability of both spatial and temporal management. Based on current results, advanced designed with array structure is expected to allow multi-types of cells co-culture in a single platform and as well as arbitrary cell patterning. It is believed that the proposed biological platform can help exploring and revealing biological insight of transient and dynamic cell to cell interactions.

REFERENCES