CHEMICAL-LESS CELL PATTERNING VIA ELECTRICALLY ALTERED ITO SURFACE
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ABSTRACT
A simple and effective cell patterning methodology has been demonstrated without using any chemicals. The flexible cell patterning scheme is based on the selective and electrical modification of ITO (Indium Tin Oxide) thin film to limit cell growth on specific patterns. Under a negative potential around 2.0V for 60 seconds, indium ions in ITO have been found to reduce to indium metal which prohibits cell adhesion as the basic scheme for cell patterning. This methodology has been successfully tested with 3T3 NIH and MDCK cells for up to 96-hour in cell cultures without any chemical treatments.

KEYWORDS: Cell patterning, ITO (Indium Tin Oxide), Chemical-less

INTRODUCTION
Cell patterning is an important procedure in the field of biomedical research as it has been commonly used in cell culture, tissue engineering, and cell-based sensors. Previously, cell patterning has been demonstrated by defining chemical patterns using soft-lithography [1] or micro-contact printing [2] processes which require the usage of chemicals to control preferential areas for the cell adhesion processes. Here, we propose and demonstrate a chemical-less, post-processing technique to modify the surface properties for cell patterning applications. The basic principle is based on surface modification of ITO thin film, which is transparent and has been commonly used in various biomedical studies. It is found that when a negative potential is applied to the ITO thin film, the reduction of indium ions occurs due to the excessive supply of electrons to convert indium oxide to indium [3]. Figure 1 illustrates the basic electron transfer processes where indium ions are converted in sequential steps to pure indium. When observed from the top angle, the ITO film changes it characteristics from transparent to dark brown color and to “mirror-like” silver color viewing from an oblique angle during the conversion process. We found that living mammalian cells such as 3T3 NIH and MDCK won’t attach to the electrically treated ITO surface while they can attach to normal ITO surface. Furthermore, cells attaching to normal ITO and Pyrex surfaces were able to migrate and proliferate. Therefore, it is feasible to activate the pre-patterned ITO thin films in selective patterns to deter the cell adhesion and to achieve the cell patterning process without using any chemicals as shown in Fig. 1.

Figure 1. Various steps showing the reduction of indium ions in ITO thin films to indium by applying negative potential to ITO. Indium surface is found to be non-fouling for cells (cells won’t attach on). Therefore, it is feasible to fabricate lithography defined ITO patterns and selectively activate individual areas to achieve cell patterning without using any chemicals.

The fabrication process is straightforward by constructing a single ITO layer with either wet etching or lift-off process as shown in the top diagram in figure 2. Afterwards, a negative potential is applied in a 1xPBS solution via a reference electrode in the solution and a contact electrode on the specific ITO surface as illustrate in the left-bottom image in figure 2. The right-bottom images in figure 2 are preliminary experimental results. The top photo shows transparent ITO before the surface modification process and the bottom photo shows the “mirror-like” reflecting surface after the electrical activation process for the conversion of ITO thin film to indium thin film.
Figure 2. (top) The proposed ITO surface can be fabricated by lift-off or wet-etching of ITO thin films. (left-bottom) Cell patterning experiment is conducted in 1xPSB solution with a reference electrode and a contact electrode to the ITO thin film. A negative voltage of about 2V is applied for 60 seconds to complete the cell patterning process. (right-bottom) After the application of the surface conversion process, originally transparent ITO changes to “mirror-like” reflecting indium.

CELL EXPERIMENTS

Figure 3 shows cell culture results of 3T3 NIH cells after 18, 36, 72 and 96 hours on electrically treated ITO electrode (right) and non-treated ITO electrode (left) as the control group. It is found that after 18 hours, several cells were visible on the treated electrode without spreading. After 36 hours, no cells can be found on top of the electrically treated ITO electrode while the non-treated electrode on the left side starts to see the attachment of living cells. After 72 hours, full cell growth can be identified on the non-treated electrode. The 96-hour image indicate some cells were able to get into the area of electrically treated ITO electrode, probably only via the connection with the surrounding cells and not with the substrate surface.

Figure 3. Sequential images of the culture results of 3T3 cells for 18, 36, 72 and 96 hours, respectively. Cells were able to adhere to and proliferate on the left transparent ITO electrode which was not electrically treated during the experiment. On the other hand, cells were not able to adhere onto the right side, electrically treated electrode as shown. After 96 hours, some cells penetrated the corners as shown probably floating and not in contact with surface.

Figure 4 shows experimental results on the characterization of the bias voltage for the electrical activation process of ITO surface based on electrode size of 2×2mm². In the test, the initial cell concentration (3T3 NIH) was the same at 5×10⁵ cells/ml. It is observed that 1.7V~1.8V of negative potential is required to effectively modify the surface to prevent the adhesion of cells. When the applied bias voltage is lower than 1.5V, most cells were able to attach to the substrate. On the other hand, when the applied bias voltage is higher than 2V, little cells were able to attach to the substrate. It is desirable that the same characteristics of the surface activation process can be applied to other cell types. MDCK cell lines have been also tested with similar and successful results. Figure 5 shows experimental images after 15, 30, and 60 hours. In these images, the color difference between treated ITO surface and Pyrex area is clearly visible. Similar to the 3T3 test, the cells on treated ITO surface were not able to attach on the surface. Some cell intrusion was observed inside the area of electrically treated
electrode after long hours of culture (60 hours). This is believed that these cells didn’t adhere to the electrically-treated electrode but only proliferated from the neighboring cells. The right-bottom figure is another electrode showing no cell growth on the electrically-treated electrode after 60 hours.

Figure 4. Characterizations of the effectiveness of applied bias voltage. The number of successfully proliferated 3T3 cells after 24 hours of cell culture versus applied negative potential from 0 to 2.1 Volt for 60 seconds.

CONCLUSION
A unique process to pattern cells without using chemical agent has been demonstrated. Electrically reduced indium from ITO is shown to inhibit cell adhesion to make possible this simple and effective cell patterning scheme. Both 3T3 cells and MDCK cell lines have been successfully tested for up to 96hrs of culture studies. As such, the proposed cell patterning method could be an alternative way for cell patterning without using chemicals.

REFERENCES: