OPTOELECTRONIC TWEEZERS FOR LONG-TERM SINGLE CELL CULTURE
Shao Ning Pei*, Tiffany Dai**, Michelle Wu*, Arthur Fan*, Song Li**, and Ming C. Wu*
*Berkeley Sensor & Actuator Center (BSAC), Electrical Engineering and Computer Sciences, University of California, Berkeley, USA
**Bioengineering, University of California, Berkeley, USA
§Both authors contributed equally to this work

ABSTRACT
We report on a new optofluidic platform that combines optoelectronic tweezers (OET) with patterned growth patches coated with extracellular matrix for long-term culture of individual adherent cells. The current device has a 10x10 array of growth patches, each with 0.5mm diameter to allow for expansion of colonies to about 250 cells. The OET platform allows selection of desired single cells before placement in the growth patches.

KEYWORDS: Optoelectronic Tweezers, Optical Trapping, Dielectrophoresis, Single Cell Analysis

INTRODUCTION
There is an increasing interest in microfluidic circuits that can perform single cell analysis for a variety of applications. Several devices have been reported, including flow trapping of cells within wells, surface modification for attachment sites, and water droplet encapsulation of single cells. Most microfluidic single cell studies focused on gene and/or protein expressions. The isolated cells are usually lysed immediately for analysis. To study the proliferation and differentiation of stem cells, there is a need for devices that can effectively culture arrays of single cells into monoclonal colonies. In this paper, we combine the single cell manipulation capability of the optoelectronic tweezers device (OET) with patterned surface modifications to create a new platform for long-term culturing of single proliferative, adherent cells. The current device has a 10x10 array of 0.5-mm-diameter growth patches coated with extracellular matrix to promote cell adherence and proliferation, and eventual formation of clonal populations. The OET platform enables sorting and placement of selected single cell on a patch using projected light patterns. The large growth area allows the cell to expand to a clonal population of 250 cells over two week culture time.

DEVICE FABRICATION AND OPERATION
The OET device fabrication and surface modification processes are shown in Fig. 1. The device comprises a 1-µm-thick photoconductive film of amorphous silicon (α-Si:H) on a indium-tin-oxide (ITO)-coated glass substrate by plasma-enhanced chemical vapor deposition (PECVD). Silane-functionalized polyethylene glycol (PEG) is grafted onto the surface of the chip outside of the growth patches. PEG has been shown to both facilitate cell movement on OET device surfaces and inhibit cell adhesion during cell culture. 2-µm-thick g-line positive photoresist is spun on the surface, followed by a 1 minute soft bake. 10x10 array of 0.5-mm-diameter openings are formed in the photoresist by photolithography. The PEG layer in the opening is removed by CF4-oxygen plasma, which also etches about 100nm of a-Si:H. Extracellular matrix is deposited on the patches by covalently binding collagen I to the substrate through sulfosANPAH, a crosslinker. A fabricated OET patch array is shown in Fig. 3. Collagen I is immunostained to show its selective conjugation within the growth patches. The photoresist layer is subsequently removed by PRS 3000 photoresist remover.

A fluidic chamber is formed between the OET substrate and an ITO-coated cover glass with a 100µm thick spacer (Fig. 2). Cells suspended in low-conductivity electroporation media are introduced to the chamber. An A.C. voltage with 10Vppk and 100kHz frequency is applied between the two ITO layers. Light patterns generated by a projector (Dell 4210X DLP) are focused on the substrate, which locally increase the conductivity of the illuminated a-Si:H by several orders of magnitude, forming "virtual electrodes". The virtual electrodes subsequently impart a dielectrophoretic force on the cells to move cells. Bright-field illumination and a CCD camera (Sony, XCD-X710) are used for visualization and recording.
Fig. 1: Fabrication and surface modification of OET. (a) Start with 1μm thick amorphous Si (a-Si:H) on indium-tin-oxide (ITO)-coated glass substrate and PEG coating on surface. (b) Lithography and CF$_2$-oxygen plasma to remove PEG-silane and 100nm of a-Si:H in growth patches (500μm diameter). (c) Deposit extracellular matrix (ECM) on the growth patches by covalently binding collagen I to the substrate via sulfo-SANPAH. (d) Remove photoresist by PRS 3000 resist stripper.

**SINGLE CELL MANIPULATION AND LONG-TERM CULTURE**

For single cell manipulation, we use OET to sort cells with desired attributes and move them onto the growth patches. Fig. 4 demonstrates the transport of a B16F10 mouse melanoma cell, suspended in electroporation media (Cytoporation Medium T), using a light pattern. The cell is manipulated by light-induced positive dielectrophoresis to a growth patch, where it is isolated. The B16F10 cells adhere to the growth patches within three hours, and the fluid within the chamber is exchanged for cell culture media (Dulbecco’s Modified Eagle Medium with 10% Fetal Bovine Serum and 1% Penicillin and Streptomycin). The extra cells on the PEG surface outside the patch are purged away during the media exchange.

**Fig. 2:** OET operation. An AC bias is applied between the two ITO electrodes. The “virtual electrodes” created by projected light pattern produce nonuniform electric field in the media, causing cells to be trapped through dielectrophoresis (DEP) forces.

**Fig. 3:** Photograph of 10x10 array of ECM-coated patches on OET. Selective ECM deposition within the patches was confirmed with anti-collagen I and Alexa Fluor 488 (GFP) immunostaining. The sulfo-SANPAH crosslinker binds to the substrate and reacts with the amine group of collagen I to covalently coat the device surface with ECM for cell adhesion and culture within the patches.

**Fig. 4:** Manipulation and positioning of a single cell via OET. A B16F10 melanoma cell is transported using a light pattern (light induced dielectrophoresis) to inside the growth patch (large outer circle). The arrows indicates the moving direction of the light pattern. The red circle indicates the reference spot of the final cell position on the patch.
The patterned single cells are placed in an incubator (37°C, 5% CO₂) and observed for an extended period of time in cell culturing media. Fig. 5 shows a long-term proliferation study of single B16F10 cells cultured on two patches. The single cells on day 1 proliferate to 4 and 7 cells on day 4, and finally to 31 and 35 cell colonies on day 7. Under similar growth conditions, the doubling rate of B16F10 cells on common tissue culture dishes is established to be 24.7 hours.

Fig. 5: B16F10 melanoma cell proliferation. A single B16F10 cell was positioned and cultured within a 500-μm-diameter ECM patch. Isolated and well-contained proliferation of a single B16F10 cell was tracked in a patch over the course of 7 days.

CONCLUSION
We have demonstrated an OET platform for the long-term culture of adherent, proliferative single cells. We have used surface modification and microfabrication techniques to create large growth patches on OET device. Cells can be sorted and transported to growth patches where they adhere and proliferate. Long term single cell culture demonstration using B16F10 mouse cells have indicated the platform's efficacy. This platform offers a unique opportunity to study the heterogeneity of cells within the same clone as well as between different clonal populations, thus elucidating the distinct roles of individual cells and providing quantitative answers to questions such as the rate of proliferation and the level of protein expression.

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REFERENCES

CONTACT
Shao Ning Pei; +1-510-909-4522, email: shaoning@eecs.berkeley.edu