

A Microfluidic 3D Cell Culture System for Drug Discovery Studies

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Abstract - In this paper, a microfluidic platform with bottom and upper microchannels separated by a polycarbonate membrane is studied for three-dimensional cell culture applications. Microfluidic chips were designed and fabricated using standard soft lithography and micromolding methods. The effect of surface modification by NaOH of polycarbonate membranes on the cell viability was investigated. Mouse fibroblast cells were cultured on modified and non-modified membrane integrated microfluidic chips. On 2nd, 3rd and 4th day of cultivation, cell concentrations were recorded and it was found that the cell concentration on modified membranes was always higher at all time intervals, and after four days, the cell concentration on the modified membrane was about 55 % higher than that of unmodified case.

Keywords: 3D cell culture, microfluidics, PDMS microchannel, PC membrane, surface modification

1. Introduction

It is well proven that two- or three-dimensional static cell culture media does not allow different cell types to communicate with each other and thus, not adequate to mimic a real tissue or an organ. Microfluidic-based organ on-a-chip systems have been studied extensively in the literature as a novel approach to mimic the in-vivo tissue microenvironment that would make pre-clinical animal experiments unnecessary in the foreseeable future [1-5]. The microfluidic system is designed such that the microenvironment is very similar to that in vivo is achieved by allowing cell-to-cell communication on each side of a porous membrane and by supplying required fluid flow into channels that also mimics the shear stress felt in real tissue. Different cells can be seeded on both surfaces of the membrane and flow of different fluids into the upper and lower microchannels can be provided. Various tissues and organs such as lung [6], blood-brain barrier [7], gut [8], liver [9], kidney [10] and bone marrow [11] have been studied.

In this study, mouse fibroblast cells (L929) are seeded on modified and non-modified polycarbonate (PC) membranes integrated in microfluidic chips. It was cultured for four days under static conditions and the vitality of the cells was recorded by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay.

2. Materials and Methods

Polydimethylsiloxane (PDMS) microchannel geometries were designed and fabricated using standard soft lithography and micromolding methods. The SU-8 3050 negative photoresist (MicroChem Corp., USA) was used as a template for forming microchannel cavities. This photoresist allows the production of up to 100µm thick film at a time. Two layers were produced on top of each other and a total thickness of 200µm was obtained on a 4" silicon wafer. PDMS (Slygard 184, Dow Corning, USA) is mixed with 10% by weight of curing agent. After the bubbles were removed under vacuum, the mixture

was poured onto SU-8 molds and allowed to solidify on a 90°C hot plate for 45 minutes. The PDMS microchannels were then cut and the microchannel inputs and outputs were punctured and prepared to be bonded with the membrane.

PC membranes with 0.4µm pore size (Merck KGaA, Darmstadt, Germany) were soaked into 2M NaOH at 70°C for 30 minutes for surface modification, then washed with distilled water until pH was neutral. Modified or non-modified PC membrane was placed between PDMS having microchannels, bonded with O₂ plasma activation as seen in Figure 1 (A-B), and before the test, the microfluidic chip was sterilized in autoclave.

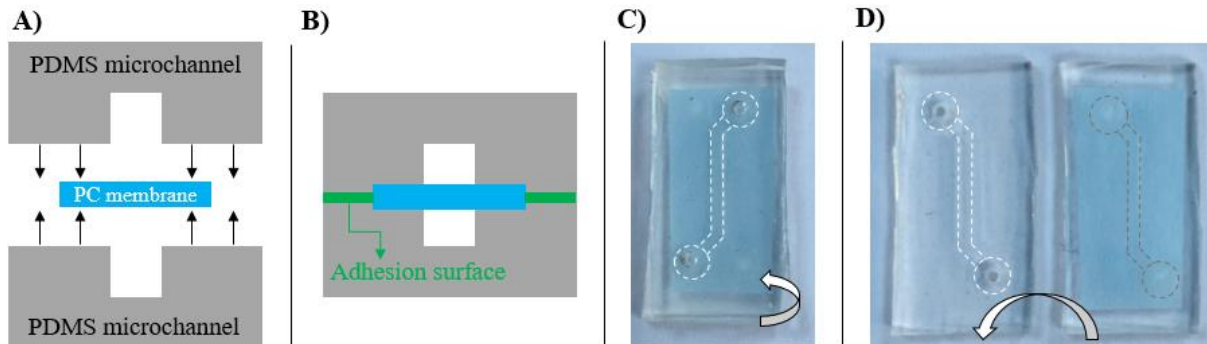


Fig. 1: A) PC membrane is caged between top and bottom PDMS microchannel and B) a tight seal is achieved after O₂ plasma bonding. C) Fabricated microfluidic platform for 3D cell culture. D) Top layers were cut and PC membranes were carefully removed for cell concentration determination.

L929 cells were cultured in RPMI-1640 medium containing 10% FCS at 37°C, 5% carbon dioxide atmosphere, in 25cm² flasks.

L929 cells were harvested with Trypsin-EDTA solution and cells were infused through the upper microchannel and a batch of microfluidic chips were cultured in RPMI-1640 medium containing 10% FCS at 37°C, 5% carbon dioxide atmosphere. After 2nd, 3rd and 4th day of cultivation, MTT assay was applied for cell concentration determination.

3. Results and Discussion

The chips were cut and the membranes were carefully removed as seen in Figure 1 (C-D) and placed in 24-well plates. Membranes were gently washed with PBS and 300µl cell culture medium containing 30µl MTT solution (5mg/ml in PBS) were pipetted onto the membranes. Plate was incubated at 37°C, 5% carbon dioxide atmosphere for 4h. Media were removed in the wells and 300µl isopropanol containing 4M HCl was pipetted onto the PC membranes. Absorbance of dissolved formazan crystals was read at 570nm. Results was given at Figure 2.

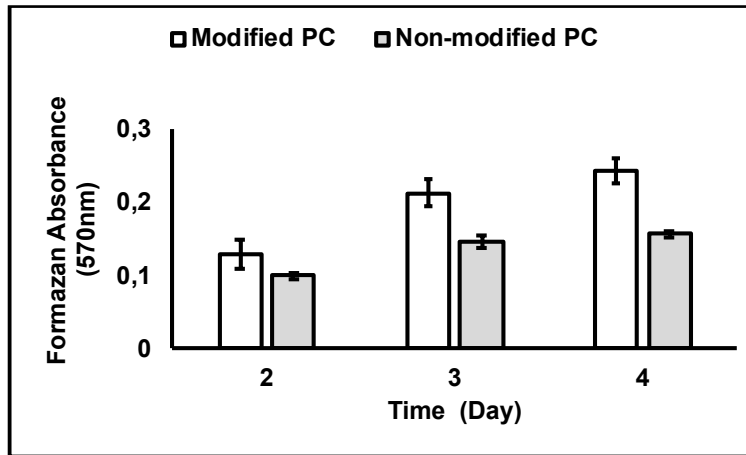


Fig. 2: MTT results of the L929 cells cultured on the modified and non-modified PC membranes.

When the MTT assay results were examined, it was observed that the cells were attached both on modified and non-modified membranes. However, at the end of the fourth day, the cell concentration on the modified membrane was about 55% higher.

4. Conclusion

In this study, mouse fibroblast cells (L929) were seeded on modified and non-modified the polycarbonate (PC) membranes integrated in the microfluidic chip, cultured for four days under static conditions and the vitality of the cells was recorded by MTT assay. It was found that the cell concentration on modified membranes was always higher at all time intervals, and after four days, the cell concentration on the modified membrane was about 55 % higher than that of unmodified case.

The study under dynamic conditions while a continuous fluid flow at different flow rates in the upper and lower channels is being investigated. In addition, human primary renal proximal tubule epithelial cells (RPTEC) are also being tested using the same microfluidic system.

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