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Integrated 3D Microfluidic System for Stromal Cell Culture

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Abstract

This study reports a 3D biomimetic microfluidic system which mimics the human uterus. This chip integrates a gradient generator and a continuous diffusion system for embryo coculture with stromal cells. We believe that this device can prove an efficient platform for in-vitro fertilization.

1. Introduction

Precise controls over the fluids in microfluidic devices give an added advantage over the traditional devices for coculture methods where it is important to continuously provide the cells with fresh culture medium. This phenomenon of continuously providing fresh medium to the cells and eliminating the waste can be easily carried out on a microfluidic device thus mimicking the in vivo function of blood vessels [1]. Recently, microfluidic devices [2] have been used to establish linear chemical concentration gradients [3, 4] and have been demonstrated for studying chemotaxis. Thus we fabricated a 3D microfluidic chip which can be used to mimic the uterus for embryo coculture and an integrated continuous perfusion system for providing nutrients to the cells.

2. General Instructions

Fabrication

For fabricating the porous PDMS membrane, a SU8 mold with an array of columns, 40µm in height and 13µm diameter were defined on the wafer. As shown in Fig.1d, thin layer of PDMS is spin coated on the mold. The PDMS not only gets distributed in between the columns but it also sticks on the top of the column. In this manner the PDMS gets completely covered on the structure and the mold. Thus it becomes obstacle in molding a porous PDMS membrane. To resolve this, the upper channel structure and bottom layer is bonded together to obtain a closed microdevice. Tetra-butylammonium fluoride (TBAF) (75 wt% in H2O, Sigma-Aldrich, St Louis, MO) is used to etch the extra PDMS in order to obtain a porous PDMS membrane. The membrane is then peeled off and bonded to lower PDMS channel to obtain the complete chip.

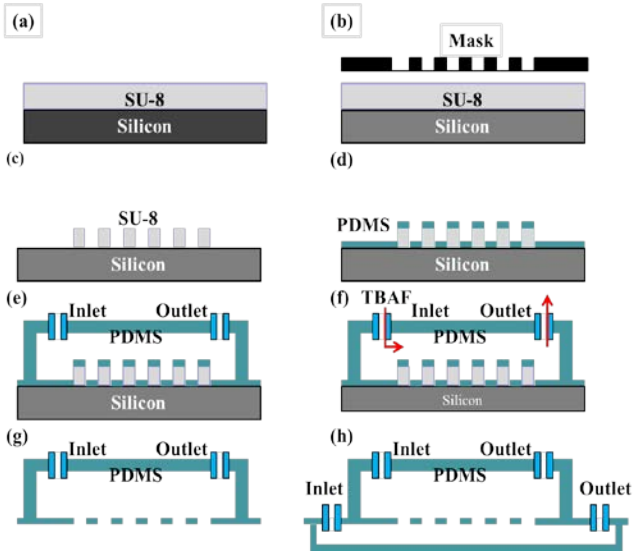

Fig.1 Microfabrication process of the biomimetic chip. (a) SU-8 negative photoresist is spin coated on Si wafer. (b)~(c) By photolithography process cylindrical structures of SU-8, 13~15 µm in diameter are defined on the Si wafer. (d) Thin layer of PDMS is spin coated on the Si-wafer. (e) PDMS structure is bonded to the thin layer to create the upper channel of the chip. (f) TBAF is injected in the upper channel for etching the PDMS in order to create a porous PDMS membrane. (g)~(h) The membrane is then peeled off and bonded to PDMS channel to create the complete chip.

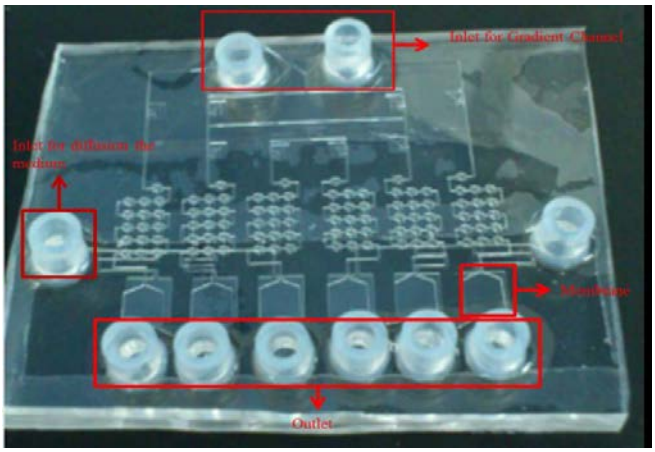

Fig.2. Photograph of the microfluidic chip fabricated by soft lithography techniques.

Simulation

For the concentration gradient design in the chip, we used the CFDRC software to simulate the results. Fig. 3 shows the simulation results for the concentration gradient design. By different water and dye, it is easier to observe different concentrations that are obtained. Fig. 4 shows the chambers of the end of the gradient channel with dye. The results closely agree with the result simulated by simulation.

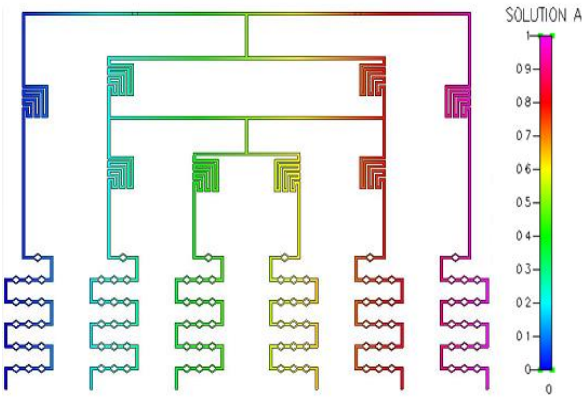

Fig.3. Simulation results for the concentration gradient design.

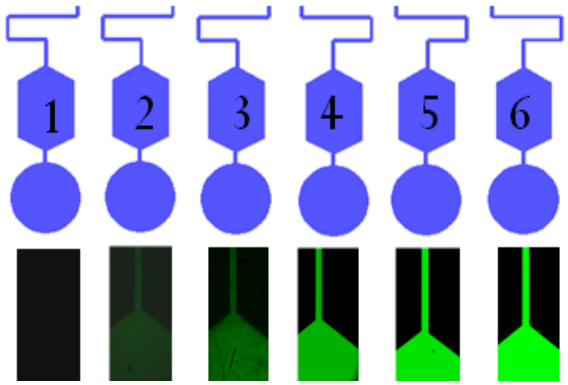

Fig.4. Microscopic images of the chip section which shows concentration gradient by using dye.

Experiments

In a petri dish, the culture medium is stagnant, whereas in the microfluidic chip, the cells receive continuous medium through porous PDMS membrane. Fig.5 shows stromal cell culture in two different conditions. With the increase in hormone concentration the cell proliferation rate in the chip increases, as shown in Fig.6. , providing a better environment for cell culture in bio-chip.

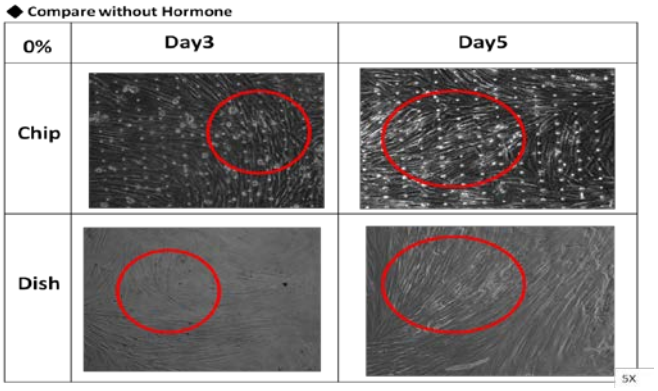

Fig.5 Stromal cell culture in two different conditions.

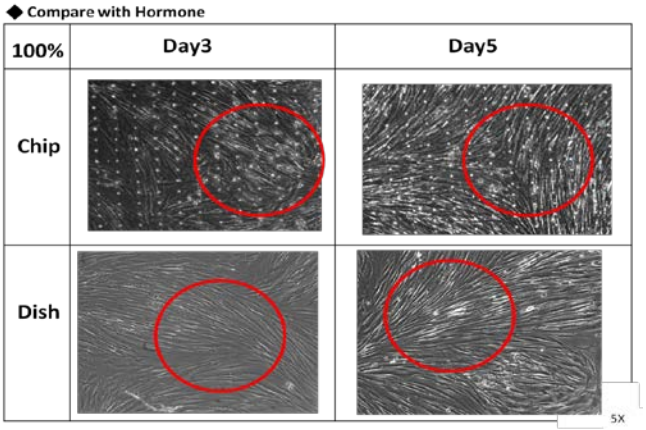

Fig.6 Cell proliferation increases with the increase in hormone concentration. Encircled area shows the cell proliferation.

3. Conclusions

The 3D chip is an efficient diffusion model for culture of stromal cells which can be further extended for coculture with embryo. The concentration gradient design can be successfully used for monitoring cultured stromal cells at different concentration of growth hormones.

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