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In Vitro Reconstruction of Tumor Microenvironment for Studying Angiogenesis

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Abstract

Angiogenesis is an important mechanism in organ growth and development, oxygen supply, reproduction, wound healing and also in cancer progression. Anti-angiogenic therapy is a topic of interest for cancer cure by inhibiting cancer progression. In this research, we have developed a microfluidic device which integrates a gradient generator and DEP cell patterning technology to reconstruct the micro environment around tumor for the studying angiogenesis and drug screening.

1. Introduction

Angiogenesis, sprouting of blood vessels is an essential component in cancer metastasis. Tumor cells use these vessels as a mode of transfer to locomote from the primary tumor site [1]. Vascular endothelial growth factor (VEGF) plays and important role in angiogenesis and cancer progression (Fig.1). The role of VEGF in endothelial polarization by gradient generation has already been demonstrated on a microfluidic device [1, 2]. Thus, inhibition of angiogenesis by using natural or synthetic angiogenic inhibitors has become a prime investigation strategy for cancer cure. Microfluidic devices with its ability to manipulate and culture cells at micro level [3] provides a useful tool towards investigation of cancer and cancer treatment by reconstructing the cancer microenvironment on chip. Here is in this research we have made an attempt to reconstruct the tumor microenvironment for further investigation of tumor progression and drug screening. The tumor microenvironment is mimicked by culturing fibroblast cells around the tumor cells by using dielectrophoresis (DEP) force. DEP force was used to manipulate, pattern and co culture the cells in desired position.

2. Chip Design

The microfluidic device consists of two main parts, electrode pattern on glass and PMDS microchannel. The electrode pattern was defined on glass wafer by using photolithography process in order to pattern the cancer cells surrounded by fibroblast cells whereas the PDMS microchannels were fabricated by using soft lithography technique. Double layer SU-8 structure was used to create structures of variable height, which allowed stable fluidic system, confinement of cells and extracellular matrix (ECM) in specific channel, and gradient generation. The patterned glass wafer is bonded to the PDMS structure to obtain the complete device.

Microfluidic device comprise of cancer cell patterning region, ECM channel and HUVECs channel (Fig.2). Cancer cells which release the angiogenic growth factors were patterned in the cell patterning region. ECM channel full with ECM provides a 3D space for HUVEC cells to form new blood vessels. HUVEC cells were loaded in HUVEC channel, cultured and further stimulated by angiogenic factors released by cancer cells. Each channel was connected by 4um bridge channel as shown in the cross-sectional view of the chip. In effect of high flow resistance, there is no direct mass transport through the bridged channel whereas the mass transport in these bridge channels takes place through diffusion. Concentration gradient in neighboring channels result in diffusion through the bridged channels.

DEP force generated from the electrode pattern is used to manipulate and pattern the cells at the specific position thus mimicking the tumor microenvironment. By tuning the input frequency, we can select the positive DEP (pDEP) force or negative DEP (nDEP) force to manipulate the cells in designated position and obtain the required cell pattern. pDEP force attract polarized cells of same type towards the region of local electric-field maximum whereas nDEP force repel the cells of same type to the region of local electric-field minimum.

3. Result

The tumor slice of lung cancer (Fig.3) shows the presence of nonparenchymal cells like fibroblasts and macrophages with tumor cells. It is observed that the cancer cells are surrounded by other cells. The DEP force was used to reconstruct the in vitro model of lung cancer microenvironment. The A549 cells (green) and 3T3 cells (red) were manipulated and patterned by using nDEP and pDEP force respectively.

3T3 cells were used for checking the migration phenomena through the bridge channel which connects the ECM channel and HUVECs channel. The 3T3 cells were loaded into HUVECs channel and collagen was injected in the ECM channel. The culture medium with Fetal Bovine Serum (FBS) and without FBS was injected into cell pat-

tering region and HUVECs channel respectively. The generated FBS gradient stimulates the 3T3 cells and induces its migration towards high concentration gradient of FBS i.e. towards of cell patterning region (Fig. 4). After 16 hours, significant migration of the 3T3 cells towards the cell patterning region is observed.

4. Conclusions

Treatment combining traditional techniques with targeted therapy is considered a new hope towards cancer cure. Anti-angiogenic therapy is one of the targeted therapies. In our chip, we can demonstrate some key facts to reconstruct the tumor micro environment and display the phenomena of angiogenesis induced by in vitro tumor. By use of DEP technology we demonstrated the manipulation and patterning of cells to obtain an in vitro model of the lung tumor slice. Whereas the cell migration due to concentration gradient is demonstrated on the chip which will be further observed in case of HUVECs and further utilized for drug screening.

Acknowledgements

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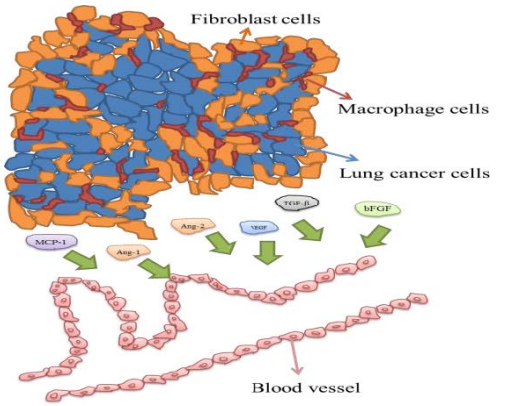

Fig. 1 Schematic Illustration of cancer metastasis. The tumor stroma is composed of different cells like macrophages, fibroblast and lung cancer cells. The angiogenic factors released by tumor leads to tumor angiogenesis.

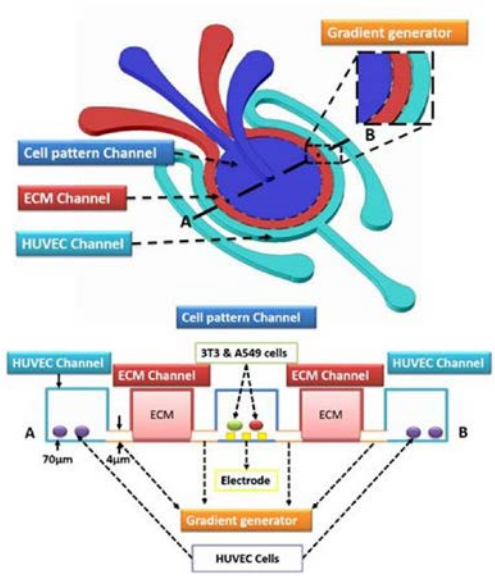

Fig. 2 Schematic illustration of the microfluidic device and the cross-sectional view of the chip. The cross-sectional view shows cell patterning region, ECM channel and HUVEC channel connected by 4μm bridge channel.

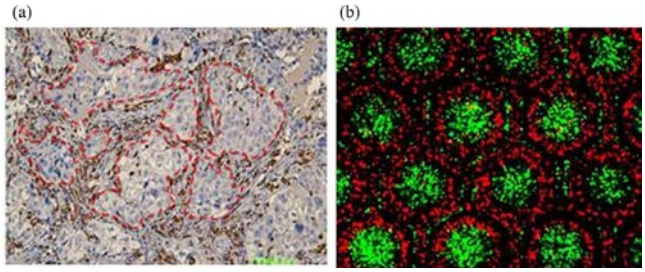

Fig. 3 Mimicking lung cancer tissues on chip by using DEP forcettern. (a) The cross sectional view of the lung cancer tissue slice from human. (b) Lung cancer tissue reconstructed on chip by using DEP force, where A549 cells(green) were fenced by 3T3 cells(red).

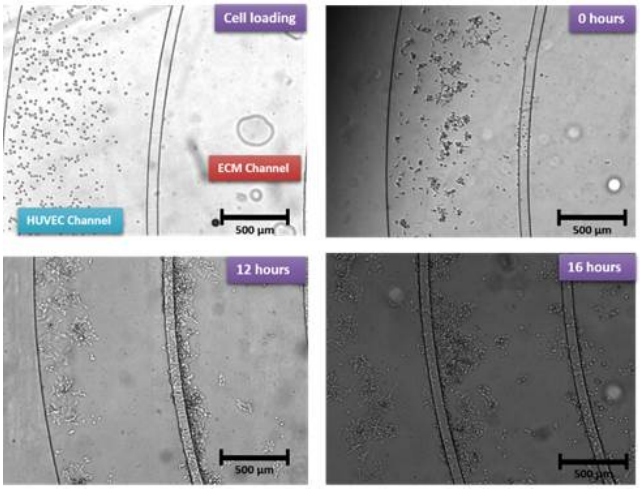

Fig. 4 Cell migration phenomenon on chip. With time, 3T3 cells migrate from the HUVEC channel towards the cell patterning region in response to the FBS gradient.