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# A Microfluidic Labchip For Angiogenesis Studies Using Multi-Gradients Generator and Cell Trapping Structures

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**Abstract**

**Recently, the studies of in vitro capillary network using microfluidic devices by generating single concentration gradient have uncovered much biological information of angiogenesis. However, the platform for angiogenesis inhibitors has not been proposed. In this study, we developed a microfluidic device for generating multi gradients to stimuli cancer-induced angiogenesis and fibroblast-induced angiogenesis simultaneously. The formation of multiple gradients is achieved in the interaction chamber through diffusion and sustained by continuous perfusion. The results show that our device is able to generate multiple concentration gradients and trap clusters of cells for further applications.**

## 1. Introduction

Angiogenesis process was considered to play a critical role in tumor expansion [1]. Discovery of angiogenesis inhibitors provides a new weapon for human-being to fight cancer. The angiogenesis inhibitors will not eliminate cancer cells directly but cut off their nutrient supply to suppress tumor growth and expansion. Angiogenesis inhibitors for cancer treatment have several benefits because they have no need to concern about the drug resistance and have fewer side effects than conventional chemotherapeutic. However, development of anti-angiogenic agents still faces the problems such as unexpected side effects and drug ineffective [2]. Therefore, how to make good use of this treatment would be a solution for cancer therapy [3].

Lab-on-chip provides an excellent platform for biological and pharmaceutical study. Chung et al. used their microfluidic to control biochemical gradients within a three dimensional scaffold for building cancer-induced angiogenesis [4]. Yeon et al. showed their novel approach to form perfusable vascular network using endothelial cells and fibroblasts [5]. Studies have shown the capability of forming *in vitro* vascular network on chip but there was no attempt to develop a platform for angiogenesis inhibitor study.

In this paper, we report an *in vitro* model using microfluidic device by forming two types of angiogenesis simultaneously to mimic the *in vivo* environment for angiogenesis inhibitor study. This chip could locate cells of different types respectively around the chamber, and promote the cell secretion to form multiple long-lasting concentration gradients inside the chamber to stimuli the angiogenesis.

## 2. Materials and Methods

*Design Concept*

The microfluidic device features three low-height structures for cell trapping, a chamber for cell interaction and two valves for flow control (Fig. 1). The brief operation flow is shown in Fig. 2. By the flow control using microfluidic valves, cells of different types, including cancer, human umbilical vein endothelial cells (HUVECs) and fibroblasts could be trapped in the low-height structures respectively, Fig. 2(a). After all the cells were located, culture medium was introduced into the device to promote cell secretions from cancer and fibroblast forming gradients individually toward the HUVECs to induce angiogenesis, Fig. 2(b). Finally, the angiogenesis inhibitors could be introduced into the system for drug study. Fig. 3 shows the microfluidic network and corresponding equivalent electrical circuit model. The device was fabricated using standard soft lithography, Fig. 4.

*Finite Element Analysis*

After cancer cells and fibroblasts are trapped at the trapping area, the microfluidic valve will be opened for the fluid passing instead. The main task of the liquid is to flush away all the chemical solute diffusing to the surrounding channel and to maintain the profile of concentration gradient by remaining low concentration of solutes in the surrounding channel. The concentration gradient simulation was made with diffusion coefficient of the chemical substance VEGF, $5.8 \times 10^{-11}\, m^2/s$, which is referring to the other report [6]. Results shown in Fig. 5(a) and Fig. 5(b) are concentration gradient simulation of fibroblasts and cancer cells secreting growth factors at different position. The results indicated that the multi-gradients could be formed simultaneously inside the interaction chamber instead of being flushed away by the flow.

## 3. Results and Discussion

The A549 cell suspension was first injected from the cell loading inlets to the cell trapping outlets using syringe pump. The entire process is observed using microcopy. As soon as the cells are trapped, we halted the pumping and transferred the chips to the incubator for cell culturing. Fig. 6 (right side) shows the (a) A549 cell trapping result and subsequent cell culturing results in the next (b) 8 hours and (c) 20 hours.

Whether the cells could be trapped or not is highly dependent on cell size. A slight shape deformation is suffi

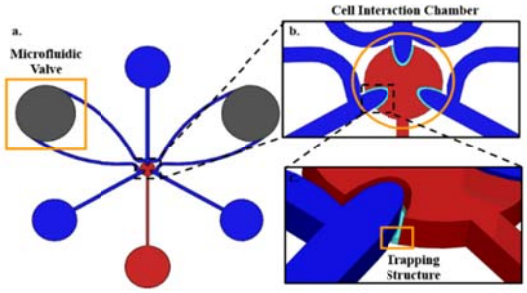


Fig 1 Schematic of the microfluidic chip. The chip features (a) microfluidic valves (b) a cell interaction chamber and (c) low-height trapping structures

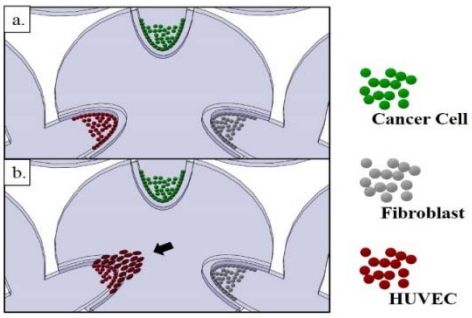


Fig 2 Operation flow for reconstructing the in vivo environment on chip. (a) Locate the fibroblasts, cancer cells and HUVECs at the low-height structures (b) Stimuli the angiogenesis by promoting the cell secretion using culture medium.

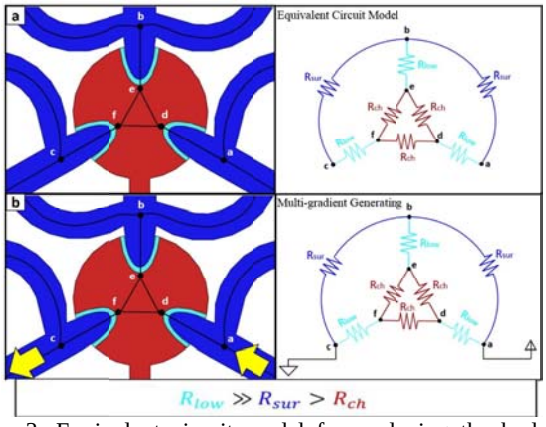


Figure 3: Equivalent circuit model for analyzing the hydraulic resistance of the chip.

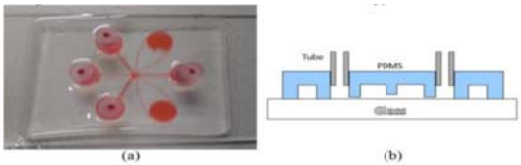


Figure 4: (a) A photograph of the microfluidic chip. (b) A side view of the chip. The chip is composed of a layer of PDMS structure with tube and a layer of glass substrate.

cient for them to pass the low-height channel and cause them to be flushed away by the flow. As a result, height of low-height channel should be adjusted to different kinds of cell, Fig. 4 (left side). The other issue is that A549 cell should be restricted in the trapping area instead of migrating to the interaction chamber, otherwise it will be hard to define the position of secreting growth factor, Fig 4 (c).

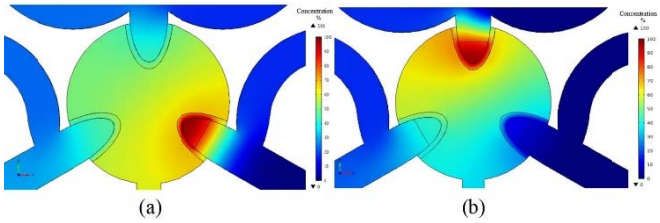


Fig. 5 Distribution of concentration on chip using simulation software, COMSOL. Simulation of molecule gradients formed by (a) fibroblasts and (b) cancer cells.

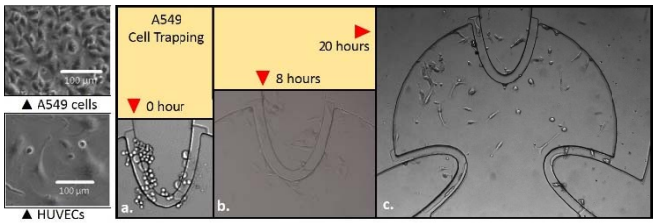


Fig. 6 (Left side) A549 cells and HUVECs cultured in 25T flask. (Right side) Experiment of cell trapping using A549 cells and subsequent culturing after (a) 0 hour (b) 8 hours and (c) 20 hours.

## 4. Conclusions

The problem occurred in the cell trapping mechanism can be solved by changing the channel height of the low-height channel and the practical solution will be developed in the future. In addition, the microfluidic valve will be developed for flow control so that different kinds of cells can be trapped in the different trapping area simultaneously. Finally, the HUVEC, A549 co-culture can be achieved after finishing the development of the microfluidic valve. The HUVEC, A549 co-culture will be the first step to reconstruct the *in vitro* model for angiogenesis study and drug screening.

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