A MICROFLUIDIC WOUND-HEALING ASSAY TO STUDY ENDOTHELIAL CELL PROLIFERATION AND MIGRATION UNDER OXYGEN GRADIENTS

Hsiu-Chen Shih[1], Man-Chi Liu[1], Te-Wei Weng[2], Ying-Hua Chen[1], Wei-Hao Liao[1], Yi-Chung Tung[1]

[1]Research Center for Applied Sciences, Academia Sinica, Taipei 11529, Taiwan

[2]Department of Mechanical Engineering, National Taiwan University, Taipei 11607, Taiwan

ABSTRACT

Endothelial cell (EC) plays an important role in various biological phenomena, which are highly regulated by oxygen microenvironments. For instance, tumor angiogenesis initiated by hypoxia microenvironment induces migration of ECs to form blood vessels. Previous studies suggested that hypoxia induces migration of ECs by vascular endothelial growth factor (VEGF) under different oxygen tensions. However, the effect of spatial oxygen gradients on ECs is remained unclear. In this study, we develop a microfluidic device to study EC proliferation and migration under the oxygen gradients based on a wound-healing assay. The experiment results show that not only oxygen tension but oxygen gradient does affect the proliferation and migration of ECs. Consequently, the developed platform in this study provides a promising *in vitro* model to better study the *in vivo* EC behaviors.

KEYWORDS

Wound-Healing Assay, Oxygen Gradient, Endothelial Cell, Microfluidics.

INTRODUCTION

Angiogenesis, which forms the new blood vessels from preexisting vasculature in both normal developmental processes and numerous pathologies, is an essential topic for a number of biomedical researches. Hypoxia is a common feature of tumor and ischemic tissues, alters fundamentally and physiologically intracellular pathways such as vascular endothelial growth factor (VEGF), and has been recognized as a stimulus for angiogenesis. Angiogenesis requires the coordinated growth and migration of endothelial cells (ECs) to maintain the vascular homeostasis. Tumor angiogenesis initiated by hypoxia microenvironment induces migration of ECs to form blood vessels [1]. Hypoxia-inducible factor, HIF-1 plays a central role in regulating cellular response to hypoxia. Furthermore, Previous studies suggested that hypoxia induces migration of ECs by VEGF under different oxygen tensions [2]. However, the effect of spatial oxygen gradients on ECs is remained unclear. Here, we develop a microfluidic device to observe ECs proliferation and migration under the oxygen gradients by wound-healing assay.

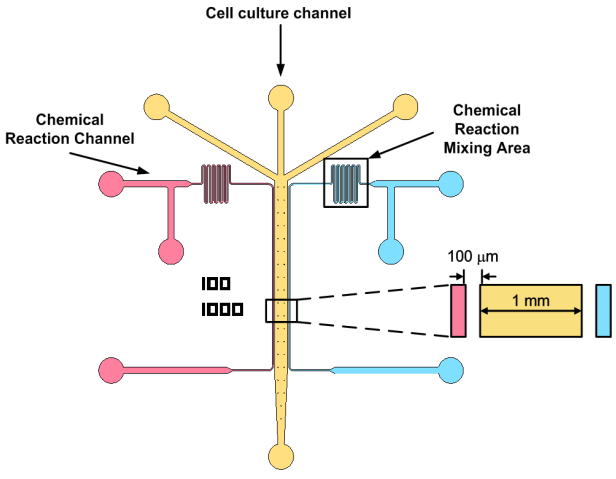

Figure 1. Schematic of the microfluidic device for wound-healing assay with oxygen gradients.

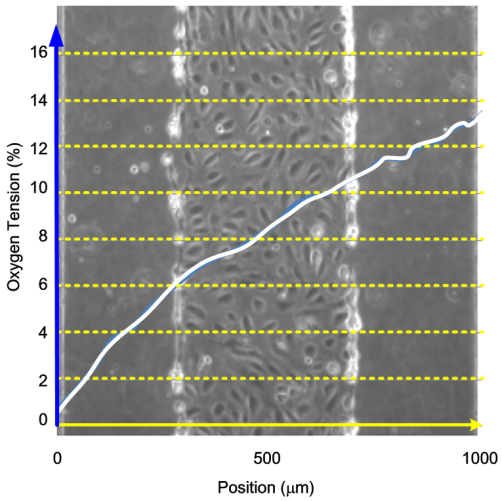

Figure 2. Experimentally characterized oxygen gradient profile, and its relative position to the HUVEC wounds.

EXPERIMENT

Device Design and Characterization

The device is constructed by a single PDMS layer then irreversibly bonded to a glass slide coated with a PDMS thin film. On the PDMS layer, there are three sets of microfluidic channels as shown in Fig. 1. The middle channel with three inlets is exploited for cell culture and wound formation, and two side channels are used to generate oxygen scavenging chemical reactions [3]. The device takes advantage of the oxygen

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

diffusivity through PDMS between the channels. Therefore, the oxygen inside the middle channel can be controlled by the chemical reactions in the side channels. In the experiments, pyrogallol and NaOH are utilized for oxygen scavenging. Figure 2 shows the experimentally characterized oxygen gradient (Left: 0%, Right: 14%) inside the middle channel while performing oxygen-scavenging reaction in the left side channel. The developed microfluidic device possesses several desired advantages, including: elimination of tedious gaseous interconnections, cell incubator compatibility, compact and simple setup.

Cell Experiments

To examine the performance of the developed device for cell culture, Human Umbilical Vein Endothelial Cells (HUVEC) is cultured in the microfluidic device. Figure 3 shows the experimental procedure for wound-healing assay in the microfluidic device: (a) HUVECs are introduced into the middle channel coated with extra cellular matrix protein, fibronectin. (b) After 24-hour cell culture, the cells attached onto the substrate and reach confluence. (c) The wound is created using a laminar flow patterning by introducing 0.25% trypsin from the side inlets (flow rate: 10 µl/ min) and medium from the middle inlet (flow rate: 20 µl/min). To examine the proliferation and migration of ECs under oxygen gradient, we performed wound-healing assay after cell seeding for 24 hours.

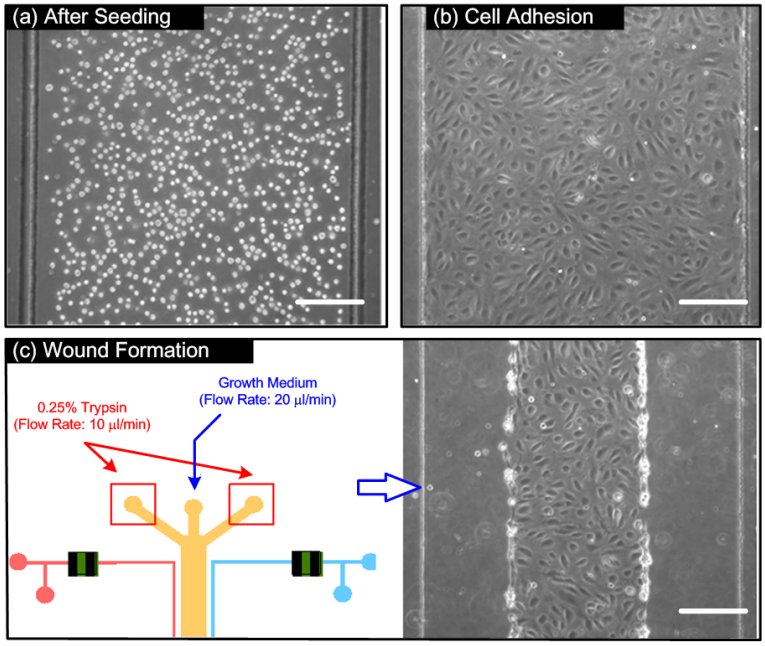

Figure 3. Experimental procedure and microscopic phase images for wound formation within the microfluidic device. (a) Cell seeding. (b) Cell adhesion to the substrate after 24-hr culture. (c) The experimental setup for microfluidic wound formation, and the resulted cell pattern. (Scale bar is 200 µm)

RESULTS AND DISCUSSION

Figure 4 shows the wound healing microscopic photos of HUVECs under 20% oxygen, 1% oxygen, and the oxygen gradient at different time points. The results show the wound healing speed of HUVEC under a low oxygen tension (~7%) with oxygen gradient is faster than that under 20% and 1% oxygen tensions. Furthermore, figure 5 shows wound reduction characterized from the aforementioned photos under 20% oxygen and the oxygen gradient after 16-hour experiments. The wound size reduction at the low oxygen tension with the oxygen gradient (105.5 µm) is faster than that under normoxia (89.9 µm). We found that an oxygen gradient plays an essential role in the proliferation and migration of ECs. Consequently, the platform we established in this study provides an *in vitro* model to study the *in vivo* EC behaviors under oxygen gradients.

CONCLUSION

In conclusion, the developed microfluidic device provides a promising platform to study cell proliferation and migration under oxygen gradients.

ACKNOWLEDGEMENTS

This material is based on work supported by the Taiwan National Science Council (NSC) under the contract

number NSC-100-2221-E-001-002, Taiwan National Health Research Institute (NHRI) Career Development Grant (CDG) under the contract number NHRI-EX101-10021EC, and the Academia Sinica Research Program on Nanoscience and Nanotechnology.

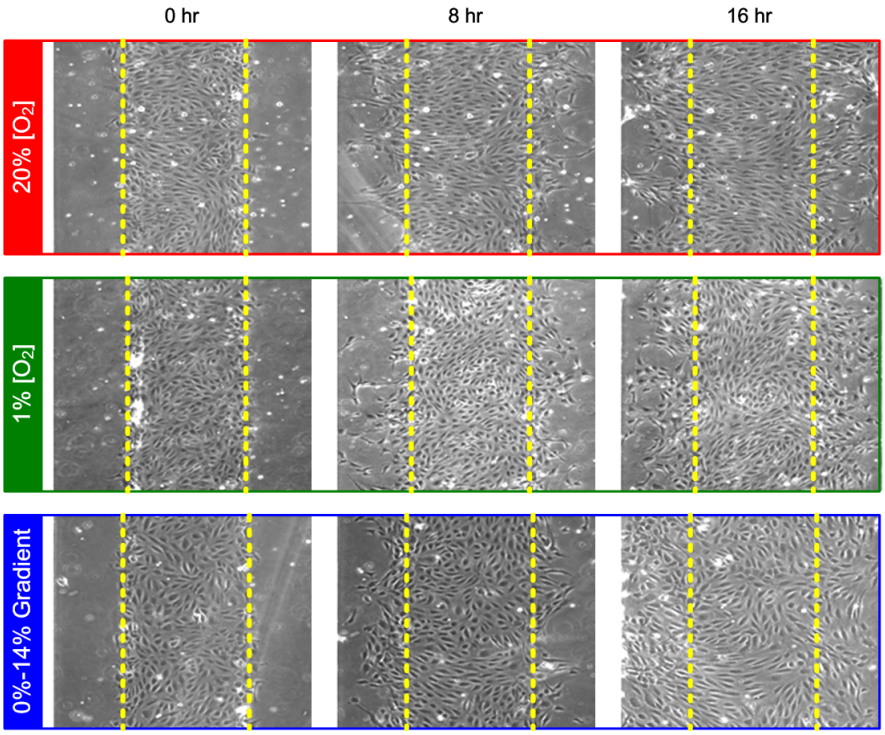

Figure 4. Flow cytometric measurement results of standard fluorescence beads using a device with a 2 mm-diameter sheath flow inlet. (a) Typical time-domain measurement results of 10 μm-diameter beads within a 3 sec-period with core and sheath flow rates of 100 and 400 μl/min, respectively. (b) Histograms of the measured peak fluorescence intensities and the Gaussian fit (black line) under the same flow conditions.

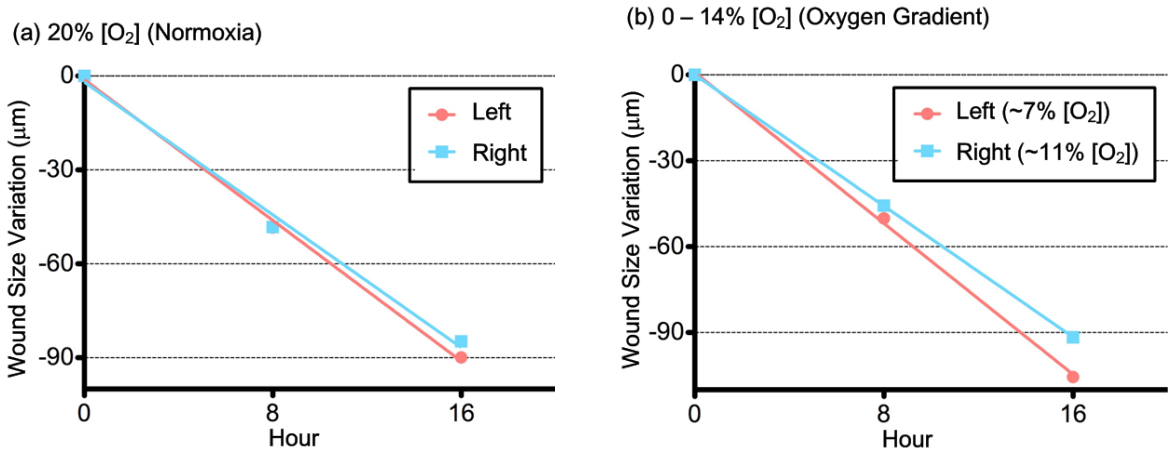

Figure 5. Wound size variation characterized from the experimentally captured phase microscopic images under (a) uniform 20% [O_2] normoxia, and (b) 0 – 14% [O_2] oxygen gradient. The discrepancy in wound size variation between two sides suggests that oxygen gradients do affect HUVEC migration and proliferation.

REFERENCES

[1] X. Mao, S.-C. Lin, C. Dong, and T. J. Huang, *Lab Chip*, 2009, **9**, 1583.
[2] S. L. Anna, N. Bontoux, and H. A. Stoneb, *Appl. Phys. Lett.*, 2003, **82**, 364.
[3] C.-C. Chang, Z.-X. Huang and R.-J. Yang, *J. Micromech. Microeng.*, 2007, **17**, 1479.

CONTACT

Yi-Chung Tung; Tel: +886-2-2-789-8000 ext 67; Fax: +886-2-2782-6680; E-mail: tungy@gate.sinica.edu.tw