IN VITRO WOUND-HEALING ANALYTICAL SYSTEM COMPOSED OF A MICRO AUTOMATED SCRATCHER AND OXYGEN GRADIENT CHAMBER

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ABSTRACT

This study described an *in-vitro* wound-healing analytical system composed of a micro automated scratcher and an oxygen gradient micro-chamber. Cell migration assay using sharps has been used for investigating wound healing process, tumor metastasis, and angiogenesis formation. However, the technique is unable to control the physical size of scratching, because scratching is created manually by handy sharps, such as a pipette yet. For obtaining scratches having a contact physical dimension, this study attempted to develop a highly accurate cell-scratching instrument and demonstrated more efficient and precise physiologically investigation on cell migration in a gas gradient micro-chamber to be performed. A micro automated scratcher, composed of X, Y, Z, and θ-axes linear actuators, a pressure sensor, and a micro comb fabricated by a three-dimensional (3D) printer, was developed. Actuators controlled X, Y, Z, and θ directions, and a pressure sensor detected the contact of comb and the bottom of cell culture chamber. This scratcher was able to create of scratches with a 150 μm-scale width at a standard deviation of less than 13%. This high controllability and reproducibility were able to provide a well-controlled cell scratching in an oxygen gas gradient chamber, which allowed cell migration to be analyzed under various oxygen tensions (0-150mmHg) working as a function of wound healing process in one test. As a result, a hypoxia condition from 4 to 10 mmHg was found to promote the closing of cell scratching caused by cell migrations. The proposed scratching system, which had a precise cell-scratching capability and an oxygen gradient micro device, would offer an efficient experimental platform for the future cell migration studies, which would contribute to drug screening, tumor translational research, and regenerative medicine.

KEYWORDS

Nulla, feugiat, pulvinar, diam.

INTRODUCTION

After a part of human body is wounded, endothelial cells and fibroblasts migrate to the wounded area and create capillaries after hemostasis in wound-healing process [1]. Oxygen is crucial for cell migration, and the oxygen tension is known to work as a function of the healing process. To investigate the kinetics of wound-healing process and screening drugs, cultured-cell surface scratching is used as a wound-healing model [2][3]. However, the technique is unable to control the width of scratching, because scratching is created manually by a pipette, etc. No efficient device, which can investigate the effects of oxygen to cells, is available. This study developed a system composed of a micro-scratching designer and oxygen gradient chamber using PDMS and confirmed its controllable and reproducible cell-scratching and cell-culturing abilities under an oxygen gradient.

EXPERIMENT

Figure 1 shows a micro automated scratcher composed of X, Y, Z, and θ-axes linear actuators, a pressure sensor, and a micro-comb fabricated by 3-D printer. X, Y, Z, and θ directions were controlled by the actuators, and the contact between comb and the bottom of chamber was monitored detected by the pressure sensors. Using this system, scratches having a 150 μm-scale width were created successfully (Fig. 2 and 3). The standard deviation of the widths was less than 13 %. The oxygen gradient chamber was made of PDMS by photolithography.

Oxygen diffused through PDMS from the channel to the chamber, and oxygen-gradient was appeared in the chamber under a hypoxia condition (Fig. 4). Oxygen-tension measurement showed that oxygen gradient was able to be controlled by altering depending on the width of channel (Fig. 4). In order to confirm that cells were exposed to an oxygen gradient condition, Hypoxyprobe™ was applied to HeLa cells. Immnofluorescent intensity of cells stained with Hypoxyprobe® increased gradationally with increasing the distance from the channel (Fig. 5), confirming an oxygen-tension gradient on the surface of chamber. When the chamber has 100-, 250-, and 500-μm channels, fluorescent intensity saturated approximately at 8.2, 13.5, and 16.5 mm from the channel, respectively. In addition, distances between the channel and fluorescence points were found to be in proportion to the width of the channel.

Using the system, the scratching of endothelial cells transfected with GFP and fibroblasts was created. After being created the control area closed within 12 h in Fig. 5. Using the cell scratching system and oxygen gradient chamber, the measurement of wound healing process at various oxygen tensions was performed. Figure 5 shows that cell scratching was closed time-dependently on the oxygen gradient chamber. Oxygen affected the width of cell-scratching in various oxygen tension at 12 and 24 h. The closing of cell scratching in hypoxia condition was found to be more quickly than that of normaxia condition. On the other hands, anoxia condition prevented the

closing of cell scratching. Therefore, hypoxia condition from 4 to 10 mmHg oxygen tension promoted the closing most effectively.

The cell migration to the scratched area was affected by oxygen tension, and hypoxia promoted the closing of area. The *in-vitro* wound-healing analytical system was able to work as an experimental platform producing width-controlled and reproducible cell-scratching, and allowing culture cells to grow under an oxygen-gradient condition. Wound-healing process was found to be a function of oxygen tension. Therefore, the *in-vitro* wound-healing analysis system was able to be readily applicable to efficient and precise studies investigating wound-healing process and drug screening.

REFERENCES

[1] Gurtner GC et al., Nature, 15: 314 (2008).
[2] Pan Q et al., Invest Ophthalmol Vis Sci.25: 1723-34 (2011)
[3] Mogford JE et al., J Cell Physiol, 190:259–265 (2002)

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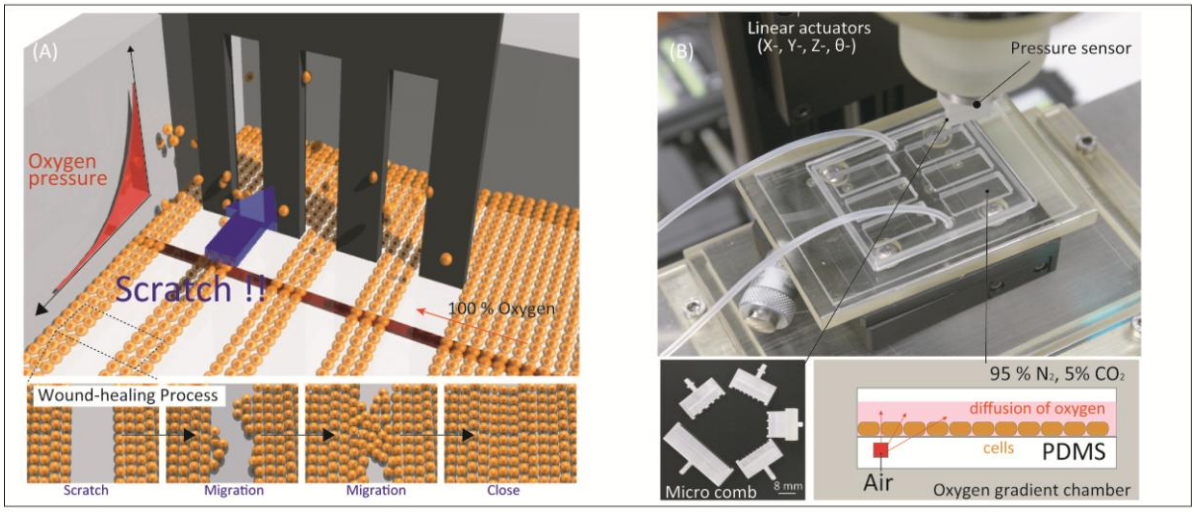


Figure 1 In-vitro wound-healing analytical system composed of a micro automated scratcher and oxygen gradient chamber. (A) Schematic diagram of micro-scratching on the oxygen gradient chamber. The width and location of cell-scratching were controlled by (B) a micro automated scratcher composed of X-, Y-, Z-, and θ-axes linear actuators, pressure sensor, and a micro-comb. Various designs of micro-combs in which the widths of teeth were more than 150 μm were able to be created. Oxygen-gradient chamber gave an oxygen gradient field on the surface of culture well.

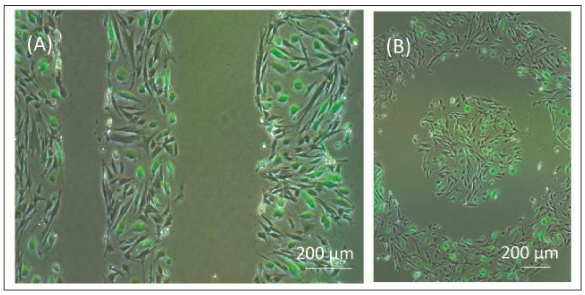


Figure 2 Cell-scratching on endothelial cells transfected with GFP on a 35-mm dish. The widths of tooth of a micro-comb were 150 and 300 μm. Contact between the comb and the bottom of the culture dish was confirmed by the pressure sensor. (A) Straight cell-scratching was created by the scratcher. (B) θ-Axes linear actuator could form a circle shape scratching.

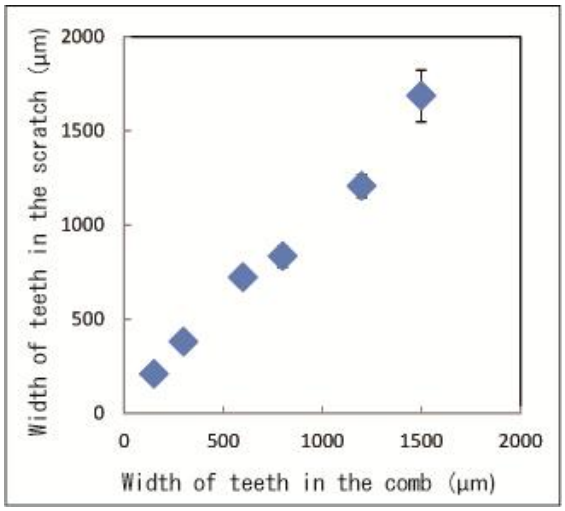
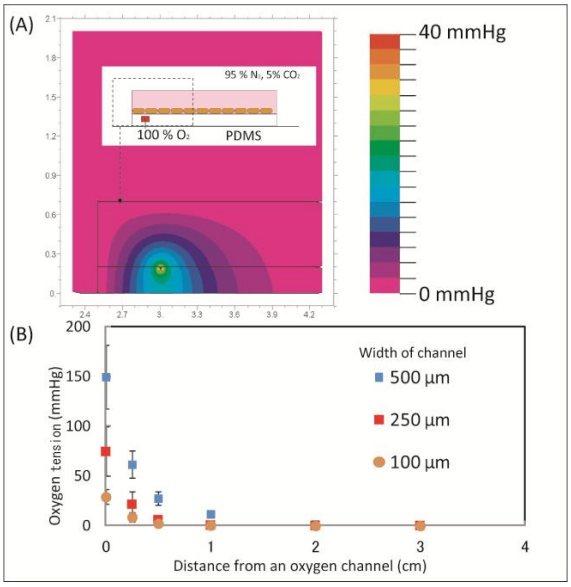


Figure 3 The width of cell scratching by the teeth width of a comb. The width of scratching was able to be controlled by altering tooth the width. The standard deviation of the widths was less than 13 %.



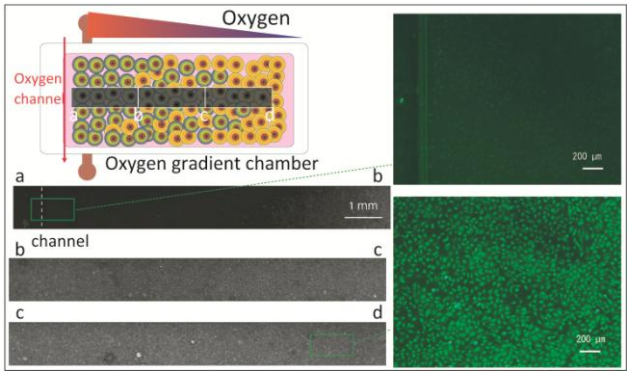


Figure 5 Immunofluorescence of pimonidasole in HeLa cells which were cultured in the oxygen gradient chamber. Fluorescent intensity increased with increasing distance from the oxygen channel.

Figure 4 Oxygen tension in the oxygen gradient chamber. Theoretical oxygen tension in the chamber was stimulated by software, FlexPDE. (A) Oxygen tension in the two dimensional model of sectional surface (B) The actual result of oxygen tension measurement obtained from the phosphorescence decay curve of porphyrin derivertive (Pd-TCPP).

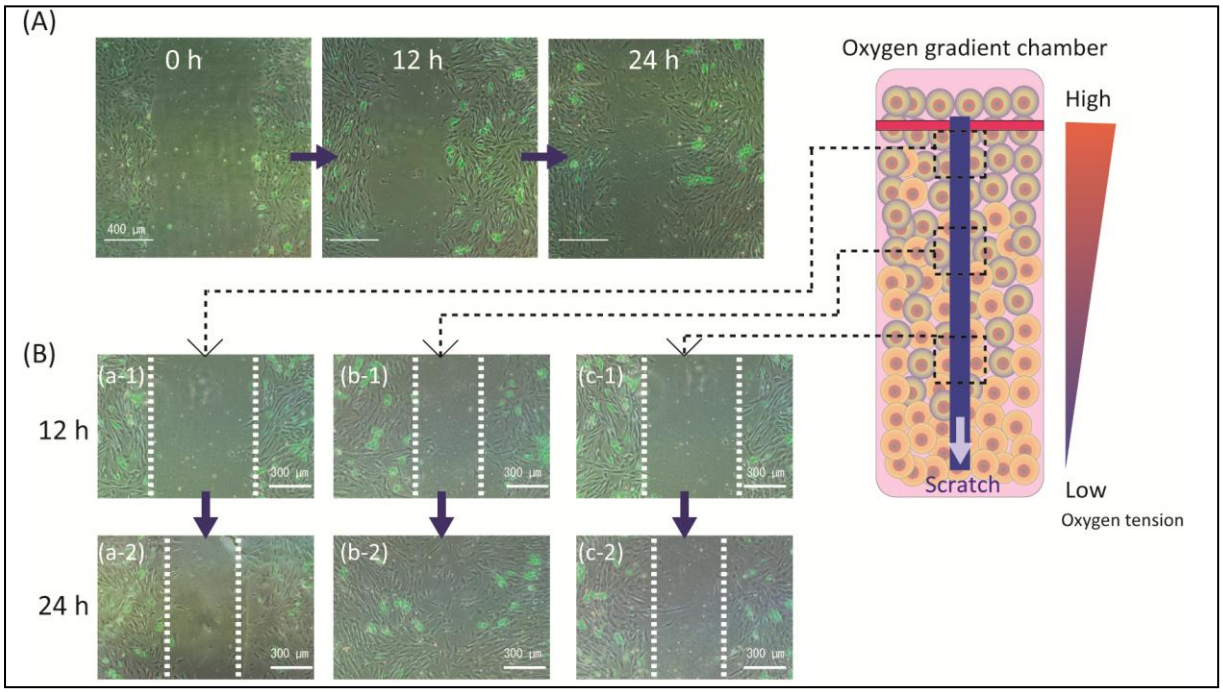


Figure 6 Cell-scratching on the surface of oxygen gradient chamber. Cell-scratching on the surface was accomplished by the micro automated scratcher. (A) The created scratching was covered with cell migration. (B) Images of the scratch closing. (a-1, and -2) At 12 and 24 h after scratching, normal oxygen tension (100 ~150 mmHg)(normaxia) gave a normal cell closing status, after scratching. Middle oxygen tension (4 ~ 10 mmHg) (hypoxia) promoted the closing of the scratched area, comparing that in normaxia (b-1, and -2). However, significantly low oxygen tension (0 ~4 mmHg) (anoxia) inhibited the closing(c-1, and -2). Apparently, middle oxygen tension was able to enhance the closing rate.