A SIMPLE PDMS-BASED SUCTION DEVICE FOR STABILIZING IN VIVO REAL-TIME FLUORESCENCE IMAGING OF TRANSPLANTED CELLS IN LIVE ANIMALS
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ABSTRACT
We report a novel technique of in vivo real-time fluorescent imaging of transplanted cells for small laboratory animals. A PDMS-based device for stabilizing in vivo imaging has been developed. The suction device utilizes small negative pressure to fix the moving tissue on the cover glass for the imaging with an inverted microscope (Fig.1). The suction device realized to monitor the circulation of intravenously transplanted cells in the liver in live mice. Since the fabrication and usage are quite simple, this device provides opportunities to perform the imaging for many researchers and will be a powerful tool for cell therapy development.

KEYWORDS: Suction, In vivo imaging, Cell, Transplantation

INTRODUCTION
Although cell therapy has a great potential for the treatment of various diseases, the fundamental issues such as a fate of the transplanted cells in vivo remained poorly understood [1]. To investigate the cell fate, in vivo optical imaging method using fluorescence is a promising method [2]. However, the optical method is difficult because it requires extensive knowledge and skill of the technique. Especially, imaging of the tissues (e.g., liver, lung, and heart) was difficult because they moved automatically by physiological phenomena.

Previously, we developed a pneumatic suction device which utilizes negative pressure to guarantee relative positioning between the medical MEMS devices (a temperature sensor and micropump) and the moving object in the body [3]. In the present paper, we aimed to apply the micro suction device to perform in vivo real-time imaging of the moving tissue with a commercially available inverted confocal laser scanning microscope.

Figure 1: Schematic drawing of the developed suction device.

Figure 2: Image of the developed suction device. (a) Whole image of the suction device. The device was 2 cm in width, 5 cm in length and 1 mm in thickness. Arrows: a microchannel; asterisk: a tissue suction hole; pounds: posts. Scale bar, 5 mm. (b) Magnified view of the tissue suction hole. The average width of a rectangular hole was 550 μm (n=12). Arrows: microholes; asterisk: a tissue suction hole. Scale bar, 1 mm.
RESULTS AND DISCUSSION

The fabricated suction device was shown in Fig.2. Three posts were designed to prevent the top of the device collapsed (Fig.2a). Three micro holes were designed in the suction hole and used to apply negative pressure from 3 different directions (Fig.2b). The fabrication process of the device is so easy that a molding process was mainly used (Fig.3). A tape with a thickness of 150 μm cut into required shape was used as a mold for PDMS.

![Fabrication process of the suction device.](image)

The imaging of a living mouse liver was demonstrated (Fig.4). The liver of an anesthetized mouse was put directly on the tissue suction hole of the device placed on the stage of the microscope. Then, the device was activated with a small negative pressure to touch the liver surface with the cover glass. The continual observation of the liver was succeeded with the device (Fig.4a), whereas it was failed without the device (Fig.4b). Any severe damage wasn’t observed at the liver surface after the suction. Since the observation was failed when the negative pressure was off, applying the pressure was necessary during the observation. Changing objective lenses in the microscope did not hamper the real-time observation of the liver surface with the suction device. The fluorescent labeled mesenchymal stem cells were transplanted into a mouse intravenously and then, the liver was observed with the device. The circulating transplanted cells could be observed in a real time manner at the liver (Fig.5).

![In vivo real-time imaging of liver in mice with or without using the device.](image)

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CONCLUSION
In this study, we developed a suction device for stabilizing in vivo real-time fluorescence imaging of tissue in live animal. We show that the device was successfully used for the observation of transplanted stem cells in liver in a minimally invasive manner. Since the production and usage of the device are quite simple, it will be a powerful tool for development of cell therapy.

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REFERENCES

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