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A device for the rapid transfer/transplantation of living cell sheets with the absence of cell damage

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ABSTRACT

In this study, we developed a device that could easily, rapidly, and completely transfer cell sheets from one material to another or transplant cell sheets onto the dorsal subcutaneous tissues of rats without leaving residual cells. Because the manipulation is as simple as pipetting, technical expertise is not required to transfer cell sheets very rapidly (the transfer time was 3.7 ± 1.6 s) using the device compared with that of a conventional method using a pipette (430 ± 180 s). After transfer by the device, C2C12 skeletal myoblast sheets showed active cell metabolism, cell viability, and very high production of vascular endothelial growth factor and stromal-derived factor-1α, indicating transfer without cell damage. Cardiac cell sheets after transfer showed spontaneous and synchronous beating, indicating intact cell–cell junctions and ion channel proteins on the cell surface. In addition, the device allowed us to transfer C2C12 cell sheets onto soft, rugged and curved surfaces such as human hands. Furthermore, cardiac cell sheets adhered rapidly and tightly onto the dorsal subcutaneous tissues of rats. This transfer/transplantation device may be a powerful tool in cell sheet-based tissue engineering and regenerative medicine.

1. Introduction

Cell-based therapy and regenerative medicine have been progressing rapidly and a number of clinical trials have already been performed [1–3]. However, injection of dissociated cells shows poor survival of transplanted cells and, thus, such a transplantation method might impede the expected therapeutic effects. To overcome these problems, tissue engineering has been developed as the next generation of cell therapy, and clinical trials have already been performed [4–8]. Our laboratory has developed a scaffold-free tissue engineering methodology, which is called "cell sheet engineering", using a temperature-responsive culture surface, and cell sheet-based tissue engineering has already been successfully applied for regeneration of various damaged tissues [9–15]. Cell sheet transplantation shows significantly more effective tissue regeneration and therapeutic effects than those observed by injection of dissociated cells [16–18]. In addition, clinical trials using autologous cell sheets have already been performed to replace several tissues including cornea epithelial, esophageal and myocardial tissues [19–22].

On the other hand, generally, single-layer cell sheets were quite fragile and easily crumpled when picking up the cell sheets from culture medium with forceps etc. Therefore, we have been trying to develop manipulators/methods, which can manipulate the cell sheet easily and simply. The cell sheet is transferred from a temperature-responsive culture surface to another surface or *in vivo* tissues by several techniques/methods using pipettes, support membranes, plunger-like devices, and other [14,15,17,19–35]. However, a unifying transfer/transplantation method of cell sheets has not yet been established and the degree of success by these transfer methods depends largely on the skill and experience of investigators/technicians. Therefore, development of a system for easy transfer/transplantation of cell sheets, in which technical expertise is not essential, is required for advancing cell sheet-based

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tissue engineering and regenerative medicine, and to ensure research results are more reproducible. In this study, we developed a device that easily, rapidly, and completely transferred/transplanted cell sheets without cell damages *in vitro* and *in vivo*.

2. Materials and methods

All animal experiments were performed according to the Guideline of Tokyo Women's Medical University on Animal Use, The Principles of Laboratory Animal Care formulated by the National Society for Medical Research, and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

2.1. Cell sheet transfer/transplantation device

A cell sheet transfer/transplantation device was developed in this study. The device was mainly composed of two parts; a scooping part and a handling part (Fig. 1A). The scooping part was further composed of an inner plate made of aluminum and an outer polytetrafluoroethylene-glass belt (AS ONE, Osaka, Japan) that covered the inner plate. The inner plate was connected to a movable pushing rod in the handling part. The outer movable belt was also fixed to the handling part via two stainless rods. When the pushing rod was pushed by hand, the inner plate and the outer belt were extended by pushing the rod in the direction of the tip of the device.

The mechanism of cell sheet scooping by the device was as follows. (1) After the device was sterilized with rubbing alcohol, both the inner plate and outer movable belt were retracted into the device. (2) The tip of the device was extended and tilted toward the near edge of a cell sheet. (3) The pushing rod was pushed by hand, and then the inner plate was slid in the direction of the tip, and the outer belt was moved out with the movement of the inner plate and rolled up at the tip simultaneously. After contacting the cell sheet, the device could scoop the cell sheet by the movement of the outer belt. (4) After moving the device with the cell sheet, the tip of the device was placed onto another surface and then pulled into the device to release the cell sheet onto the surface. The mechanism of cell sheet scooping by the device is schematically illustrated in Fig. 1B. The outer belt, which contacts cell sheets and dish surfaces directly, of the devise is coated polytetrafluoroethylene, which is a nonadherent, low friction, and low wearing. Therefore, it is expected that the manipulation of the devise does not affect the cell sheet and the surface when the cell sheet was scooped and released.

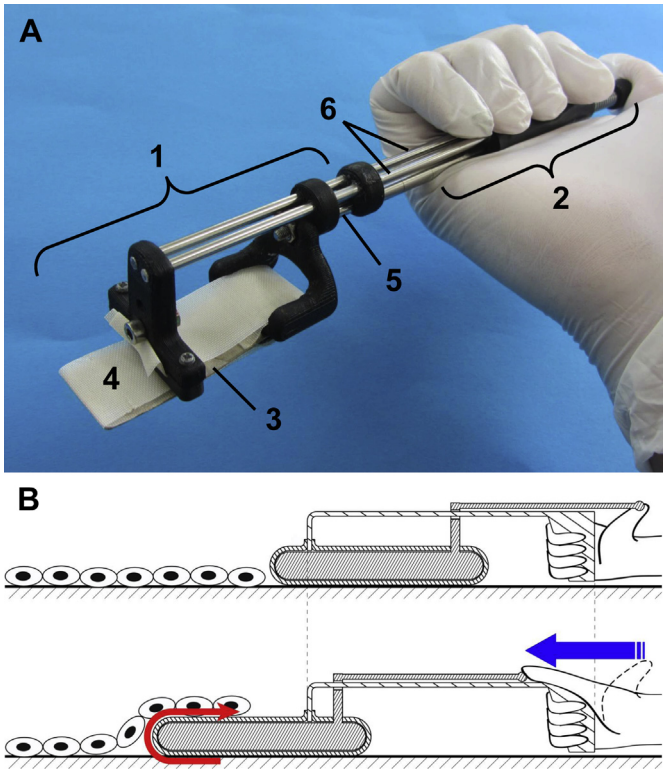

Fig. 1. Cell sheet transfer/transplantation device. An upper photograph (A) shows the device, which has several parts; a scooping part (1), a handling part (2), an inner plate (3), an outer movable belt (4), a pushing rod (5), and stainless rods (6). The mechanism of cell sheet scooping by the device is schematically illustrated in (B). The size of scooping part and cell sheet in (B) was largely exaggerated for easy understanding.

2.2. Preparation of C2C12 cell sheets and cardiac cell sheets

C2C12 mouse skeletal myoblasts (Dainippon Sumitomo Pharma, Osaka, Japan) were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Japan Bio Serum, Nagoya, Japan) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA). C2C12 cell sheets were fabricated as described previously [35]. Briefly, 6.0×10^5 C2C12 cells were plated onto a 35-mm temperature-responsive culture dish (Upcell; CellSeed, Tokyo, Japan) and cultured at 37 °C. After 3 days, the culture dish was transferred to a CO_2 incubator set at 20 °C for recovering a C2C12 cell sheet. A C2C12 cell sheet was photographed by a digital camera (GR Digital; Ricoh, Tokyo, Japan). For cardiac cell sheets, cardiac cells were isolated from the ventricles of 1-day-old Sprague–Dawley (SD) rats (CLEA, Tokyo, Japan), and prepared as described previously [11,15,17,24,25]. 2.4×10^6 rat cardiac cells were plated onto a 35-mm temperature-responsive culture dish and cultured at 37 °C. After 4 days of cultivation, a cardiac cell sheet was recovered by reducing the culture temperature (20 °C). The recovered cell sheets were used for next transfer/transplantation experiments.

2.3. Transfer of C2C12 cell sheets and cardiac cell sheets by the device

For confirming the mechanism of device visually, after a C2C12 cell sheet was stained with 0.001% neutral red solution, which was prepared by the dilution of 0.1% neutral red solution (Tokyo Chemical Industry, Tokyo, Japan) in the culture medium, for 15 min, the stained cell sheets were scooped and released by using the device. A C2C12 or cardiac cell sheet on a dish was transferred to another culture dish using the device or a conventional method using a pipette [15]. After transfer of a C2C12 cell sheet, the cell sheet was incubated at 37 °C for adherence to the culture dish. After the incubation, fresh culture medium was added to the cells, followed by incubation at 37 °C for 22 h. Then, the culture medium was collected and used for cell metabolic and damage analyses, and enzyme-linked immunosorbent assays (ELISAs). A C2C12 cell sheet was also transferred onto a human hand covered with a glove (JMS, Tokyo, Japan). The manipulations were recorded by a digital video camera (Handycam HDR-CX500V; Sony, Tokyo, Japan). Transfer times were measured by a stop-watch (Casio, Tokyo, Japan).

After the transfer, cardiac cell sheets were observed under a phase-contrast microscope (ET300; Nikon, Tokyo, Japan), and images were recorded by a digital video camera (DCR-TRV900; Sony) with CCD camera equipment (HV-D28S; Nikon).

2.4. Measurement of glucose consumption, lactate production, and released lactate dehydrogenase activity

The metabolic activities of transferred C2C12 cell sheets were monitored by measuring glucose consumption and lactate production in the culture medium. The release of lactate dehydrogenase (LDH) from cultured cells is used as a common index of cell injury and death. For measuring the values, culture medium samples were collected after cultivation of C2C12 cell sheets for 22 h. The concentrations of glucose and lactate, and LDH activities were determined by hexokinase UV method, lactic oxidase method, and LDH assay kit (Sicaliquid LDH J) (Kanto Chemical, Tokyo, Japan), respectively, as described previously [36,37].

2.5. ELISAs

A cytokine, vascular endothelial growth factor (VEGF), and a chemokine, stromal-derived factor-1α (SDF-1α), secreted from transferred C2C12 cell sheets for 22 h into the culture supernatant were quantitated by commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA).

2.6. Transplantation of a cardiac cell sheet by the device

Cardiac cell sheets were transplanted onto the dorsal subcutaneous tissue of SD rats as described previously [34]. The rats were anesthetized by inhalation of isoflurane (up to 3.5%). The dorsal skins were cut and opened, and then cardiac cell sheets were transplanted onto the dorsal subcutaneous tissues using the device. The tissue constructs were covered with silicone rubbers membrane (0.5 mm thick), and the skin incisions were closed. After 2 days, the transplanted portions were reopened and recorded by a digital camera (GR Digital) and a digital video camera (Handycam HDR-CX500V).