Chapter 14
Cell Scooper: A Device for the Rapid Transfer of Living Cell Sheet

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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 opsurface. 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.

Keywords Mechanism · Device · Scooping · Transfer

14.1 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

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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 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 [44].

14.2 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).

14.2.1 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. 14.1a). The scooping part was further composed of an inner plate made of aluminum and

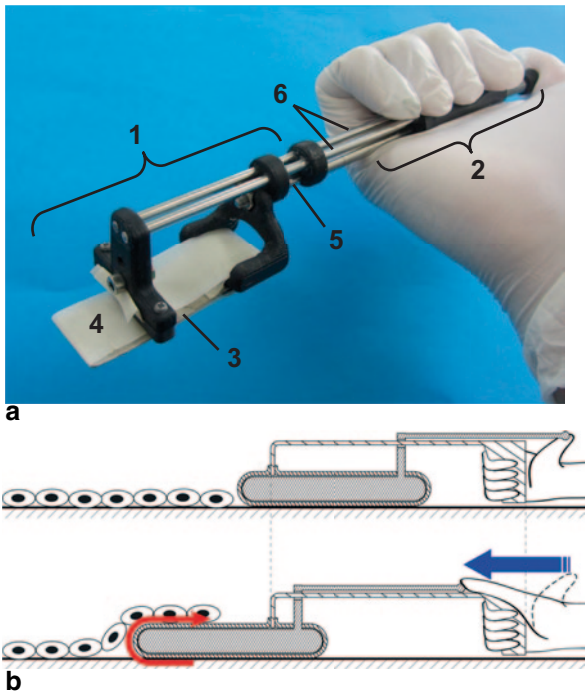

Fig. 14.1 Cell sheet transfer/transplantation device. An *upper* photograph **a** shows the device, which has several parts; a scooping part (*1*), a handling part (*2*), an inner plate (*3*), an outer movable belt (*4*), a pushing rod (*5*), and stainless rods (*6*). The mechanism of cell sheet scooping by the device is schematically illustrated in **b**. The size of scooping part and cell sheet in (*b*) was largely exaggerated for easy understanding

an outer polytetrafluoroethylene-glass cross (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 cross 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 cross 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 cross 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 cross 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 cross. (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. 14.1b. The outer cross, which contacts cell sheets and dish surfaces directly, of the device is coated polytetrafluoroethylene, which is a nonadherent, low friction, low wearing, and FDA approval material. 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.

14.2.2 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 × 105 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 CO2 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 × 106 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.

14.2.3 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, Toknyo, 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).

14.2.4 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].

14.2.5 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).

14.2.6 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).

14.2.7 Histological Analysis

Cell sheets on dishes were fixed with 4% paraformaldehyde. Specimens were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Prepared specimens were examined under a microscope (ELIPSE TE2000-U; Nikon).

14.2.8 Data Analysis

Data were expressed as the mean ± SD. The unpaired Student's t test was performed to compare two groups. A value of $p < 0.05$ was considered statistically significant.

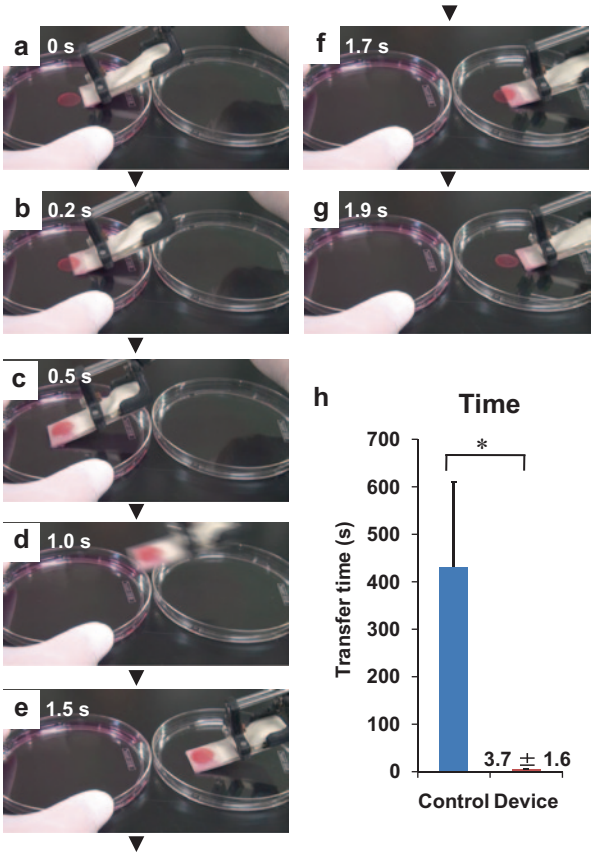

Fig. 14.2 Transfer a C2C12 cell sheet stained with neutral *red* from a dish to another culture dish using the transfer device. Scooping (**a**–**c**), transfer (**d**), and cell sheet-release (e–g). Numbers in the photographs indicate elapsed times. Comparison of transfer time by using the device (device) or a conventional method using a pipette (control) **h**. Each data point represents the mean ± SD ($n = 3$), $^*p < 0.05$

14.3 The Stiffness-Based Deformability

14.3.1 C2C12 Cell Sheet Transfer from Dishes to Other Surfaces Using the Device

We attempted to transfer C2C12 cell sheets from dishes to other surfaces using the device. As shown in Fig. 14.2a–g, a C2C12 cell sheet on a culture dish could be easily transferred to another culture dish without leaving residual cells by a simple manipulation, similar to a pipette manipulation. The device even allowed us to transfer a single-layer cell sheet without any breakages (Fig. 14.3a). In more than one hundred trials, no unsuccessful transfer occurred. Histological analysis showed that a C2C12 cell sheet could be transferred as a contiguous cell sheet with tight cell-cell junctions (Fig. 14.3b). In addition, even an inexperienced researcher successfully transferred C2C12 cell sheets very rapidly using the device (the transfer time was 3.7 ± 1.6 s, $n = 3$), whereas the inexperienced researcher required a longer time to

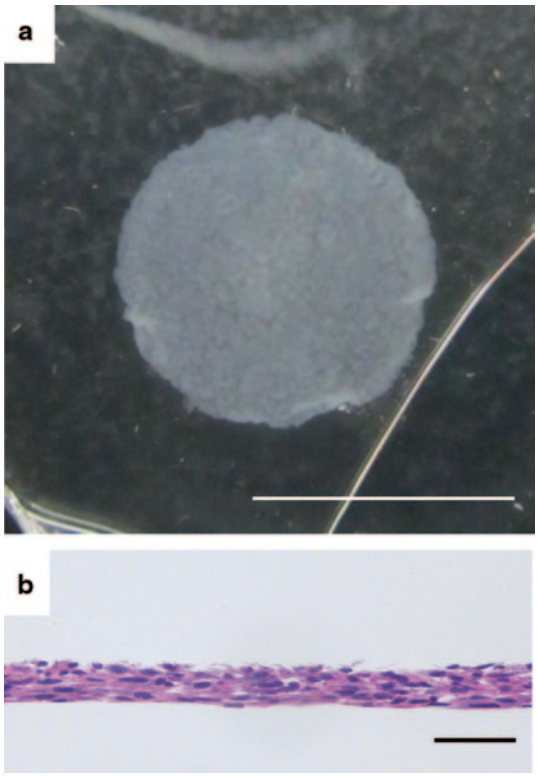

Fig. 14.3 Morphological and histological observations of a C2C12 cell sheet transferred using the device. An *upper* photograph shows a C2C12 cell sheet just after transfer **a** and a *lower* photograph shows *cross-sectional* observation of the cell sheet after 1 day of cultivation on a culture dish **b**. *White* and *black bars* represent 10 and 50 μm, respectively

transfer a cell sheet with a conventional method using a pipette (430 ± 180 s, $n = 3$) (Fig. 14.2h) [15]. Of course, the manipulation time of the cell sheet transfer was shortened by the skillful and experience of researchers/technicians. On the other hand, as shown in this study, the development of simple cell sheet manipulation devices as well as the skill and experience of the researchers/technicians is important to advance tissue engineering and regenerative medicine, and ensure research results are more reproducible.

Next, we examined whether the cell sheets were transferred by the device without cell damage. Cell sheets transferred by the device and those by the conventional method showed similar cell metabolisms, namely glucose consumption (Fig. 14.4a) and lactate production (Fig. 14.4b), which are indexes of the bioactivity of cells, and the release of LDH (Fig. 14.4c), a common index of cell injury and death, indicating that the cell sheets could be transferred using the device without any cell damage, similar to that using the conventional method. Furthermore, C2C12 cell sheets transferred using the device produced large amounts of VEGF and SDF-1α, similar to that of cell sheets transferred by the conventional method (Fig. 14.5). Autologous skeletal myoblast sheets are already used in various damaged myocardial animal models, resulting in inhibition of detrimental myocardial remodeling and improvement of myocardial functions [38]. It is generally believed

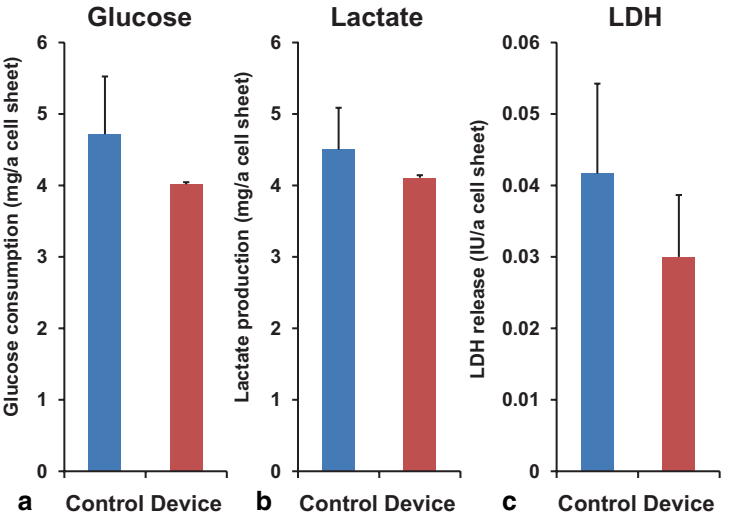

Fig. 14.4 Comparison of glucose consumption (**a**), lactate production (**b**), and lactate dehydrogenase (LDH) release (**c**) by C2C12 cell sheets transferred by using the device (device) or the conventional method (control). Total glucose consumption was calculated by subtracting the glucose concentration in the medium after cultivation for 22 h from that before incubation. The values of lactate production and LDH release were calculated by subtracting the backgrounds of lactate concentration and LDH activity in the fresh medium from those after cultivation for 22 h, respectively. Each data point represents the mean ± SD ($n = 3$)

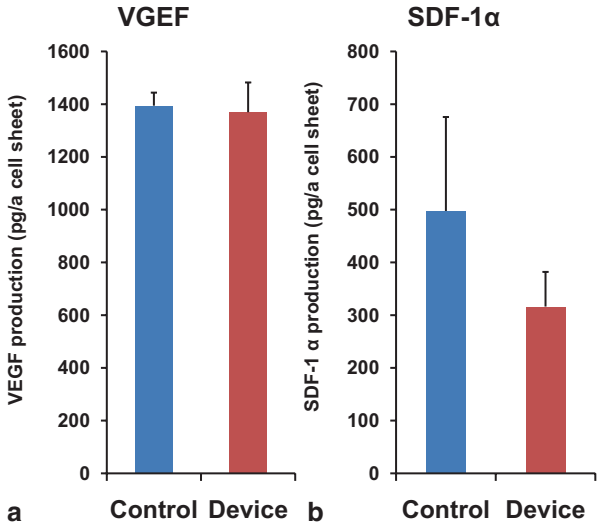

Fig. 14.5 Comparisons of VEGF (**a**) and SDF-1a (**b**) secretions from C2C12 cell sheets transferred using the device (device) and the conventional method (control). The concentrations of VEGF and SDF-1a in culture supernatants (conditioned for 22 h) were determined by ELISAs. The values of those secretions were calculated by subtracting the backgrounds of VEGF/SDF-1a concentration in the fresh medium from those after cultivation for 22 h. Values represent the mean ± SD ($n = 6$)

that the paracrine effects of bioactive factors, including VEGF and SDF-1α secreted from transplanted skeletal myoblast sheets, contribute toward the improvement of damaged myocardium after transplantation [16, 38]. VEGF induces strong angiogenesis and SDF-1α recruits several kinds of stem/progenitor cells, such as hematopoietic stem cells and endothelial progenitor cells, which express CXC chemokine receptor 4, the receptor of SDF-1α, and clinical trials for the repair of damaged myocardium using these bioactive factors have already been performed [39–40]. Our results also confirmed that the device allowed the transfer of bioactive cell sheets without cell damage. Moreover, a C2C12 cell sheet was compeletly transferred onto a human hand covered with a glove using the device, indicating that the device can transfer cell sheets onto soft, rugged and curved surfaces, as well as hardy and flat materials.

14.3.2 Cardiac Cell Sheet Transfer from Dishes to Other Surfaces Using the Device

Similar to C2C12 cell sheets, cardiac cell sheets on a dish could also be easily transferred to another culture dish using the device. Transferred cardiac cell sheet exhibited spontaneous and synchronous beating. In native cardiac tissue, electrical coupling between cardiomyocytes occurs via cell-cell contacts called gap junctions that mediate the exchange of small molecules and ions between neighboring cells. The electrical activities of cardiomyocytes occur via ion flows that mediate several ion channels on cardiomyocyte membranes, including sodium, calcium and potassium channels, which are critical for spontaneous and synchronous beating [41]. The spontaneous and synchronous beating of transferred cardiac cell sheet suggested that cardiomyocytes within the cell sheet had intact gap junctions and ion channels on the cell surface. Namely intact cell sheets without the damage of cell surface proteins and cell-cell junctions could be scooped and transferred by the devise.

Finally, transplantation of a cardiac cell sheet onto the dorsal subcutaneous tissue of rats was performed. As shown in Fig. 14.6, the cardiac cell sheet could also be easily transplanted onto the tissue. At 10 min after transplantation, the transplanted cardiac cell sheet could not be washed out by the dropping of a saline solution, indicating that the cell sheet adhered tightly and rapidly onto the tissue. The rapid and tight adhesion of transferred cell sheets onto the tissue suggested that the devise allowed us to be able to transplant cell sheets containing intact ECM matrix. Therefore, it is thought that intact cell sheets could be scooped and transplanted without the damage of ECMs by the devise. At 2 days after transplantation, the cardiac cell sheet became red, suggesting blood flow within the cell sheet (Fig. 14.7).

At present, clinical trials using autologous skeletal myoblast sheets are in progress [21]. In addition, pulsatile cardiac cell sheets have been fabricated with human induced pluripotent stem cells for the purpose of clinical usage [37, 42], and cardiac cell sheets have already been investigated in a porcine model [43]. The newly developed device allowed us to transfer skeletal myoblast sheets and cardiac cell

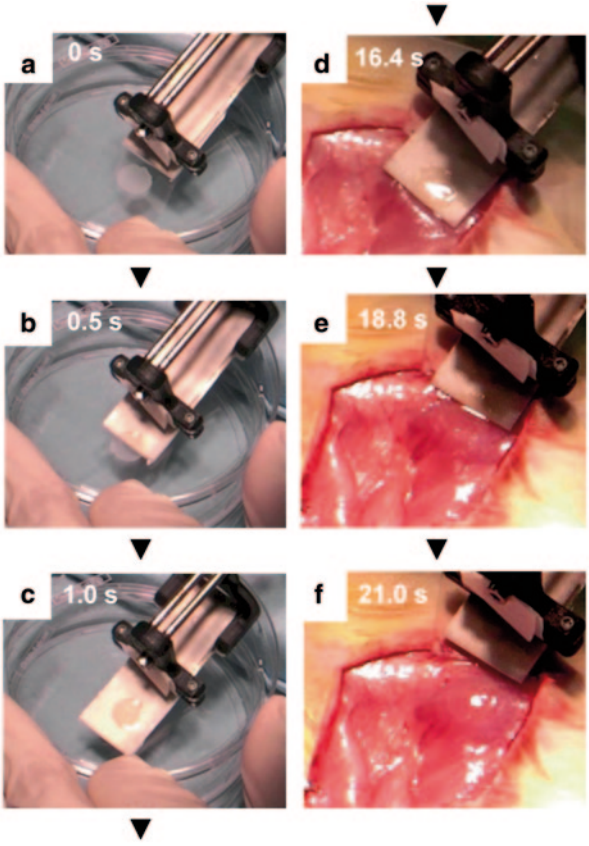

Fig. 14.6 Transplantation of a cardiac cell sheet onto the subcutaneous tissue of a rat using the device. Numbers in the photographs indicate elapsed times

sheets without cell damage. The skeletal myoblast sheets produced large amounts of paracrine factors nsevaluate the potential for clinical use, a large animal (porcine) experiment is ongoing.

In conclusion, we developed a device that can easily and rapidly transfer cell sheets between surfaces without leaving residual cells and undergoing cell damage. In addition, the device can be used for easy transplantation of cell sheets onto animal tissues. Because the device manipulation is simple, similar to a pipette manipulation, the device is not dependent on the skill and experience of the manipulators. Therefore, we are confident that this device can be used a powerful tool in the fields of cell sheet-based tissue engineering and regenerative medicine.

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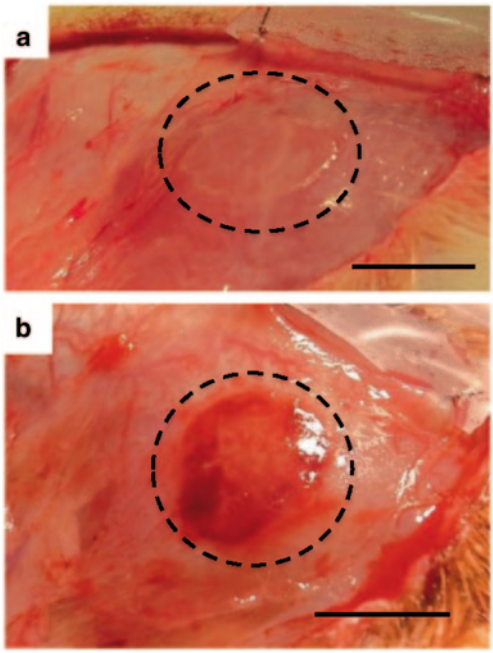

Fig. 14.7 Transplanted cardiac cell sheet onto the subcutaneous tissue of a rat using the device. Photographs (**a**) and (**b**) show a cardiac cell sheet (*dashed circles*) transplanted onto the subcutaneous tissue of a rat after 1 min and 2 d, respectively. *Black bars* represent 10 mm

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References

1. Menasché P (2008) Skeletal myoblasts and cardiac repair. J Mol Cell Cardiol 45:545–553
2. Atala A, Lanza R, Thomson JA, Nerem R (2011) Principles of regenerative medicine, 2nd edn. Academic Press, San Diego
3. Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, Beache GM, Wagner SG, Leri A, Hosoda T, Sanada F, Elmore JB, Goichberg P, Cappetta D, Solankhi NK, Fahsah I, Rokosh DG, Slaughter MS, Kajstura J, Anversa P (2011) Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. Lancet 378:1847–1857
4. Zimmermann WH, Melnychenko I, Wasmeier G, Didié M, Naito H, Nixdorff U, Hess A, Budinsky L, Brune K, Michaelis B, Dhein S, Schwoerer A, Ehmke H, Eschenhagen T (2006) Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. Nat Med 12:452–458
5. Chachques JC, Trainini JC, Lago N, Masoli OH, Barisani JL, Cortes-Morichetti M, Schussler O, Carpentier A (2007) Myocardial assistance by grafting a new bioartificial upgraded myocardium (MAGNUM clinical trial): one year follow-up. Cell Transplant 16:927–934
6. Shinoka T, Breuer C (2008) Tissue-engineered blood vessels in pediatric cardiac surgery. Yale J Biol Med 81:161–166

7. Lee K, Chan CK, Patil N, Goodman SB (2009) Cell therapy for bone regeneration-bench to bedside. J Biomed Mater Res Part B Appl Biomater 89:252–263
8. Iwasa J, Engebretsen L, Shima Y, Ochi M (2009) Clinical application of scaffolds for cartilage tissue engineering. Knee Surg Sports Traumatol Arthrosc 17:561–577
9. Yamada N, Okano T, Sakai H, Karikusa F, Sawasaki Y, Sakurai Y (1990) Thermo-responsive polymeric surface: control of attachment and detachment of cultured cells. Makromol Chem Rapid Commun 11:571–576
10. Okano T, Yamada H, Sakai H, Sakurai Y (1993) A novel recovery system for cultured cells using plasma-treated polystyrene dishes grafted with poly (N-isopropylacrylamide). J Biomed Mater Res 27:1243–1251
11. Shimizu T, Yamato M, Isoi Y, Akutsu T, Setomaru T, Abe K, Kikuchi A, Umezu M, Okano T (2002) Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. Circ Res 90:e40–e48
12. Matsuda N, Shimizu T, Yamato M, Okano T (2007) Tissue engineering based on cell sheet technology. Adv Mater 19:3089–3099
13. Yang J, Yamato M, Shimizu T, Sekine H, Ohashi K, Kanzaki M, Ohki T, Nishida K, Okano T (2007) Reconstruction of functional tissues with cell sheet engineering. Biomaterials 28:5033–5043
14. Obokata H, Yamato M, Tsuneda S, Okano T (2011) Reproducible subcutaneous transplantation of cell sheets into recipient mice. Nat Protoc 6:1053–1059
15. Haraguchi Y, Shimizu T, Sasagawa T, Sekine H, Sakaguchi K, Kikuchi T, Sekine W, Sekiya S, Yamato M, Umezu M, Okano T (2012) Fabrication of functional three-dimensional tissues by stacking cell sheets in vitro. Nat Protoc 7:850–858
16. Memon IA, Sawa Y, Fukushima N, Matsumiya G, Miyagawa S, Taketani S, Sakakida SK, Kondoh H, Aleshin AN, Shimizu T, Okano T, Matsuda H (2005) Repair of impaired myocardium by means of implantation of engineered autologous myoblast sheets. J Thorac Cardiovasc Surg 130:1333–1341
17. Sekine H, Shimizu T, Dobashi I, Matsuura K, Hagiwara N, Takahashi M, Kobayashi E, Yamato M, Okano T (2011) Cardiac cell sheet transplantation improves damaged heart function via uperior cell survival in comparison with dissociated cell injection. Tissue Eng Part A 17:2973–2980
18. Wei F, Qu C, Song T, Ding G, Fan Z, Liu D, Liu Y, Zhang C, Shi S, Wang S (2011) Vitamin C treatment promotes mesenchymal stem cell sheet formation and tissue regeneration by elevating telomerase activity. J Cell Physiol 227:3216–3224
19. Nishida K, Yamato M, Hayashida Y, Watanabe K, Yamamoto K, Adachi E, Nagai S, Kikuchi A, Maeda N, Watanabe H, Okano T, Tano Y (2004) Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. N Engl J Med 351:1187–1196
20. Ohki T, Yamato M, Ota M, Takagi R, Murakami D, Kondo M, Sasaki R, Namiki H, Okano T, Yamamoto M (2012) Prevention of esophageal stricture after endoscopic submucosal dissection using tissue-engineered cell sheets. Gastroenterology 143:582–588
21. Sawa Y, Miyagawa S, Sakaguchi T, Fujita T, Matsuyama A, Saito A, Shimizu T, Okano T (2012) Tissue engineered myoblast sheets improved cardiac function sufficiently to discontinue LVAS in a patient with DCM: report of a case. Surg Today 42:181–184
22. Burillon C, Huot L, Justin V, Nataf S, Chapuis F, Decullier E, Damour O (2012) Transplantation for the treatment of corneal limbal epithelial stem cell deficiency. Invest Ophthalmol Vis Sci 53:1325–1331
23. Nishida K, Yamato M, Hayashida Y, Watanabe K, Maeda N, Watanabe H, Yamamoto K, Nagai S, Kikuchi A, Tano Y, Okano T (2004) Functional bioengineered corneal epithelial sheet grafts from corneal stem cells expanded ex vivo on a temperature-responsive cell culture surface. Transplantation 77:379–385
24. Sekine H, Shimizu T, Kosaka S, Kobayashi E, Okano T (2006) Cardiomyocyte bridging between hearts and bioengineered myocardial tissues with mesenchymal transition of mesothelial cells. J Heart Lung Transplant 25:324–332

25. Haraguchi Y, Shimizu T, Yamato M, Kikuchi A, Okano T (2006) Electrical coupling of cardiomyocyte sheets occurs rapidly via functional gap junction formation. Biomaterials 27:4765–4774
26. Ohashi K, Yokoyama T, Yamato M, Kuge H, Kanehiro H, Tsutsumi M, Amanuma T, Iwata H, Yang J, Okano T, Nakajima Y (2007) Engineering functional two- and three-dimensional liver systems in vivo using hepatic tissue sheets. Nat Med 13:880–885
27. Tsuda Y, Shimizu T, Yamato M, Kikuchi A, Sasagawa T, Sekiya S, Kobayashi J, Chen G, Okano T (2007) Cellular control of tissue architectures using a three-dimensional tissue fabrication technique. Biomaterials 28:4939–4946
28. Kanzaki M, Yamato M, Yang J, Sekine H, Takagi R, Isaka T, Okano T, Onuki T (2008) Functional closure of visceral pleural defects by autologous tissue engineered cell sheets. Eur J Cardiothorac Surg 34:864–869
29. Kobayashi H, Shimizu T, Yamato M, Tono K, Masuda H, Asahara T, Kasanuki H, Okano T (2008) Fibroblast sheets co-cultured with endothelial progenitor cells improve cardiac function of infarcted hearts. J Artif Organs 11:141–147
30. Iwata T, Yamato M, Tsuchioka H, Takagi R, Mukobata S, Washio K, Okano T, Ishikawa I (2009) Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. Biomaterials 30:2716–2723
31. Maeda M, Yamato M, Kanzaki M, Iseki H, Okano T (2009) Thoracoscopic cell sheet transplantation with a novel device. J Tissue Eng Regen Med 3:255–259
32. Yaji N, Yamato M, Yang J, Okano T, Hori S (2009) Transplantation of tissue-engineered retinal pigment epithelial cell sheets in a rabbit model. Biomaterials 30:797–803
33. Asakawa N, Shimizu T, Tsuda Y, Sekiya S, Sasagawa T, Yamato M, Fukai F, Okano T (2010) Pre-vascularization of in vitro three-dimensional tissues created by cell sheet engineering. Biomaterials 31:3903–3909
34. Sasagawa T, Shimizu T, Sekiya S, Haraguchi Y, Yamato M, Sawa Y, Okano T (2010) Design of prevascularized three-dimensional cell-dense tissues using a cell sheet stacking manipulation technology. Biomaterials 31:1646–1654
35. Haraguchi Y, Sekine W, Shimizu T, Yamato M, Miyoshi S, Umezawa A, Okano T (2010) Development of a new assay system for evaluating the permeability of various substances through three-dimensional tissue. Tissue Eng Part C Methods 16:685–692
36. Sekine W, Haraguchi Y, Shimizu T, Umezawa A, Okano T (2011) Thickness limitation and cell viability of multi-layered cell sheets and overcoming the diffusion limit by a porous-membrane culture insert. J Biochip Tissue Chip S2:001
37. Haraguchi Y, Matsuura K, Shimizu T, Yamato M, Okano T (2013) Simple suspension culture system of human iPS cells maintaining their pluripotency for cardiac cell sheet engineering. J Tissue Eng Regen Med. doi:10.1002/term.1761
38. Haraguchi Y, Shimizu T, Yamato M, Okano T (2012) Cell therapy and tissue engineering for cardiovascular disease. stem cells. Trans Med 1:136–141
39. Penn MS, Pastore J, Miller T, Aras R (2011) SDF-1 in myocardial repair. Gene Ther 19:583–587
40. Lavu M, Gundewar S, Lefer DJ (2011) Gene therapy for ischemic heart disease. J Mol Cell Cardiol 50:742–750
41. Boron WF, Boulpaep EL (2003) Medical physiology. In: Radisic M, Michael VM (eds) The cardiovascular system (Chapter IV). Elsevier Science, Philadelphia, pp 421–590
42. Matsuura K, Wada M, Shimizu T, Haraguchi Y, Sato F, Sugiyama K, Konishi K, Shiba Y, Ichikawa H, Tachibana A, Ikeda U, Yamato M, Hagiwara N, Okano T (2012) Creation of human cardiac cell sheets using pluripotent stem cells. Biochem Biophys Res Commun 425:321–327
43. Kawamura M, Miyagawa S, Miki K, Saito A, Fukushima S, Higuchi T, Kawamura T, Kuratani T, Daimon T, Shimizu T, Okano T, Sawa Y (2012) Feasibility, safety, and therapeutic efficacy of human induced pluripotent stem cell-derived cardiomyocyte sheets in a porcine ischemic cardiomyopathy model. Circulation 126:S29–S37