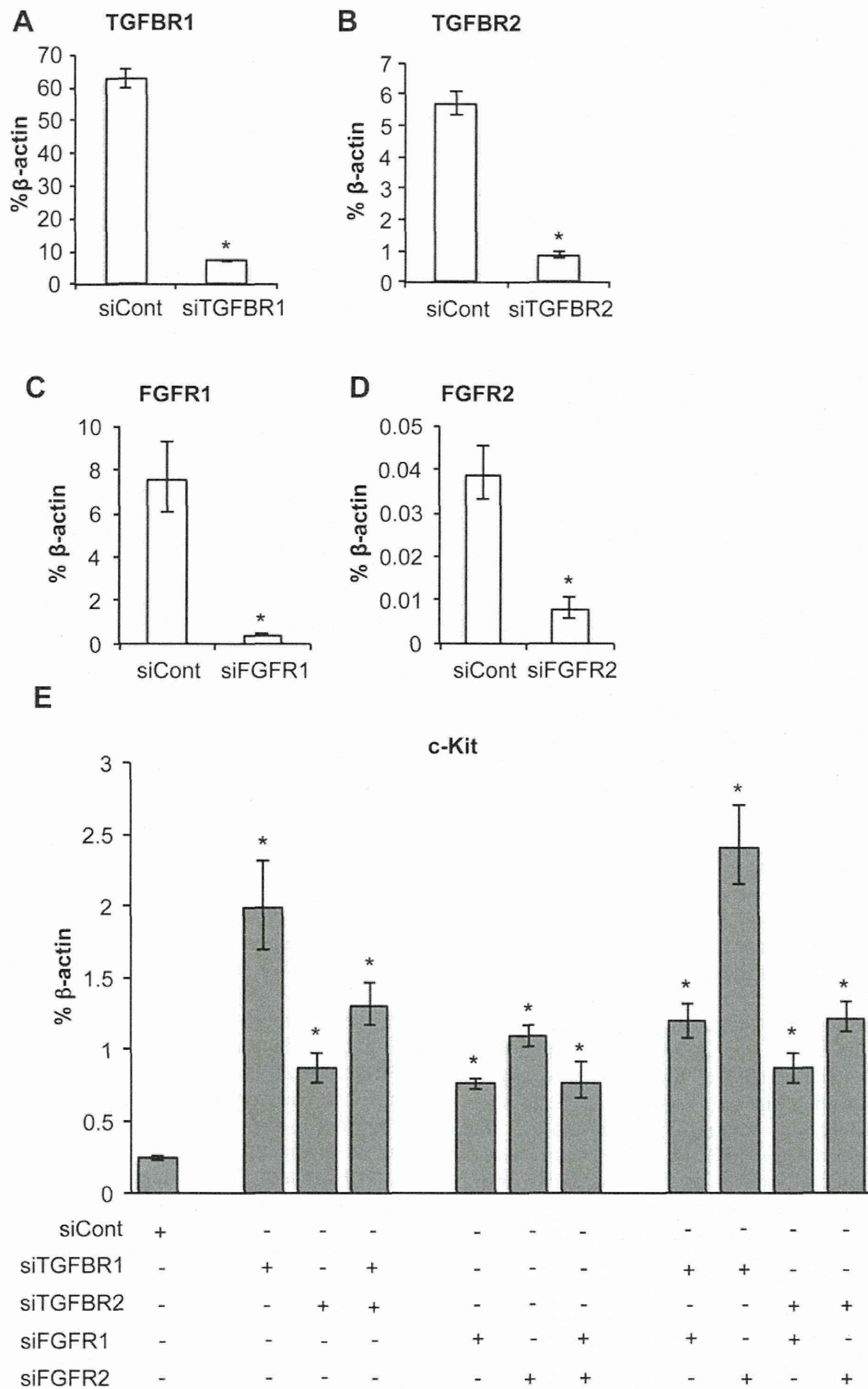

Fig. 7. The efficacy of siRNA in inhibiting mRNA expression of TGFBR1 (A), TGFBR2 (B), FGFR1 (C), and FGFR2 (D) in hMSCs. Knockdown effect of TGFBR1/2 and/or FGFR1/2 on mRNA expression of c-Kit (E). $^*P < 0.05$.

with previous reports in which FACS analysis was used to detect human lung stem cells, human cardiac stem cells, and hMSCs derived from bone marrow, periosteum, synovium, adipose tissue, and skeletal muscle [20–22]. In some studies, c-Kit was regarded as a negative marker of hMSCs [21,35]. This study, however, clearly showed the higher capacity of c-Kit$^+$ cells in forming colonies compared to the main population.

In terms of differentiation potential, c-Kit$^+$ cells showed a lower potential to differentiate into osteoblasts or adipocytes than that of the main population. This observation, together with the results from CFA, suggested that c-Kit/SCF signaling might play an important role in the maintenance of undifferentiated hMSCs. To clarify this, siRNA-mediated gene silencing was used to knockdown endogenous c-Kit and SCF genes, and the effects on ALP activity and

expressions of osteoblast and adipocyte lineage specific genes were determined. The knockdown of c-Kit/SCF signaling significantly enhanced the expression of specific osteoblast markers, including ALP enzyme, Runx2, OCN, and OPN. In the ALP assay, siRNA-mediated knockdown of the c-Kit gene remarkably increased the ALP activity in cells cultured in both 0.5% and 10% FBS conditions, when cultured with OIM. OIM is important in upregulating expression of Cbfa1/Runx2, ALP, osteonectin, OPN, OCN, bone sialoprotein, and type I collagen, to induce the osteogenic differentiation of human PDL cells [3,36]. However, in 10% FBS culture condition without supplementary OIM, the significant enhancement of ALP activity was observed when c-Kit gene was knocked down. Furthermore, this study found that OCN and OPN mRNA was upregulated significantly when c-Kit and/or SCF genes were knocked down, regardless of OIM. Unlike OCN or OPN, complete inhibition of c-Kit/SCF signaling by dual knockdown of c-Kit and SCF genes was required for the significant upregulation of Runx2 by 1.8-fold when the cells were cultured without OIM, suggesting that c-Kit/SCF signaling more strictly controlled the expression of Runx2 gene. Previous evidence has shown a 1.5-fold increase in Runx2 mRNA expression in hPDLCs after a 5-day induction with OIM [36]. However, the 4- to 10-fold upregulation of the Runx2 gene was observed when c-Kit and/or SCF genes were simultaneously knocked down along followed by a 3-day osteogenic induction. Interestingly, in the presence of OIM, c-Kit and SCF, but not Runx2, synergistically regulated the expression of OCN and OPN mRNA. This result suggested the additional role of c-Kit/SCF signaling in the control of the expression of osteogenic-related genes. Runx2 is a core transcription factor that is required for the commitment of hMSCs to osteoblastic progenitors fate [37]. OCN and OPN express transiently, albeit at a low level, during osteoblast maturation and are highly expressed in mature osteoblasts [37]. c-Kit/SCF signaling was surprisingly found to be indispensable for the control of bone-related gene expression in hMSCs throughout a lineage transition from hMSCs to mature functional osteoblasts. Results from both ALP activity and gene expression assay indicated the essential role of c-Kit gene in the osteogenic differentiation of hMSCs. However, in the ALP assay, the knockdown of the SCF gene had no influence on the ALP activity and decreased the effect of c-Kit knockdown in the dual knockdown experiments. This phenomenon differed from the results observed at the mRNA level as measured by real-time PCR, suggesting that the ALP activity of hMSCs was not solely dependent on c-Kit/SCF signal, but also required other possible coordinating signals. In terms of adipogenic differentiation, the results showed that the expression of PPARγ, a key transcriptional factor for adipogenic differentiation [38], and LPL, an early marker gene in adipocyte [39], were significantly upregulated when both c-Kit and SCF genes were concurrently knocked down. These findings suggested that c-Kit/SCF signaling suppressed the differentiation potential of hMSCs.

Because hMSCs have the characteristic of phenotypic interconversion, which appears at the progenitor level [40], the differentiation potential of hMSCs can be affected by changes in the microenvironment [41,42]. However, the results demonstrated that decreased mRNA expression of c-Kit and SCF suppressed the lineage transdifferentiation independent of OIM or AIM during the culture of hMSCs. In addition, recently, the whole genome transcriptional profile of murine c-Kit^{+} cardiac progenitor cells has been uncovered. c-Kit^{+} cardiac progenitor cells appear to be undifferentiated as indicated by high expression of genes encoding mesodermal-specific and stem cell-related genes and the down-regulation of cardiomyocyte-specific genes [43]. Altogether, these findings emphasized the role of c-Kit/SCF in the maintenance of undifferentiated hMSCs by restricting the lineage transition of stem cells.

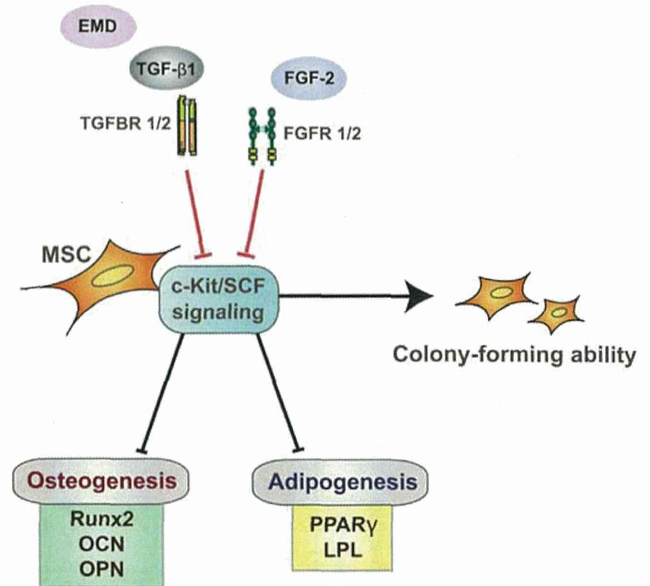

Fig. 8. Schematic illustration of the proposed relationship between growth factors and c-Kit/SCF signaling in controlling the differentiation of hMSCs. c-Kit/SCF signaling enhanced the colony-forming ability of hMSCs. Stimulation with TGF-β1, FGF-2, and EMD suppressed gene expression of c-Kit. The downregulation of c-Kit/SCF genes upregulated lineage-specific genes; e.g., Runx2, OCN, OPN, PPARγ, and LPL, that allowed hMSCs to differentiate into osteoblast or adipocyte.

The applications of 1 ng/mL rhTGF-β1 or 10 ng/mL rhFGF-2 have been reported as optimal dosages for enhancing the mitogenic activity of hPDLCs, respectively [26,27]. A significant increase in hyaluronan and proteoglycan synthesis in hPDLCs has been demonstrated when cells were cultured with 50–150 μg/mL EMD, suggesting the role of EMD in enhancing the lining of extracellular matrix and facilitating cell–cell interaction [25]. Interestingly, the present study showed that stimulation with 1 ng/mL rhTGF-β1 and 10 ng/mL rhFGF-2 remarkably suppressed the gene expression of c-Kit in hMSCs. A similar phenomenon was also observed by stimulating cells with 25–100 μg/mL EMD in the presence of either 10% FBS or 0.5% FBS (data not shown). Knockdown of TGFBR1/2 and FGFR1/2, specific receptors for TGF-β1, EMD, and FGF-2, with siRNA resulted in the significant upregulation of c-Kit gene expression. Furthermore, dual knockdown of TGFBR1 and FGFR2 receptors resulted in the maximal upregulation of c-Kit mRNA, indicating that TGFBR1 and FGFR2 functioned as predominant receptors in regulating the mRNA expression of c-Kit. The results suggested the specific relationship between the function of growth factors and c-Kit/SCF signaling in controlling stem cell activity.

5. Conclusions

This study demonstrated an important role of c-Kit in maintaining the undifferentiated stage of hMSCs by (1) enhancing the colony-forming ability and (2) inhibiting the expression of lineage-specific genes. The gene expression of c-Kit was specifically modulated by surrounding growth factors such as TGF-β1, FGF-2, or EMD (Fig. 8). The modulation of c-Kit/SCF signaling might be considered as a future regenerative approach in directing the differentiation cues of hMSCs.

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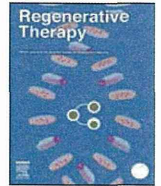

Original article

How to prevent contamination with *Candida albicans* during the fabrication of transplantable oral mucosal epithelial cell sheets

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ABSTRACT

We have utilized patients' own oral mucosa as a cell source for the fabrication of transplantable epithelial cell sheets to treat limbal stem cell deficiency and mucosal defects after endoscopic submucosal dissection of esophageal cancer. Because there are abundant microbiotas in the human oral cavity, the oral mucosa was sterilized and 40 μg/mL gentamicin and 0.27 μg/mL amphotericin B were added to the culture medium in our protocol. Although an oral surgeon carefully checked each patient's oral cavity and although candidiasis was not observed before taking the biopsy, contamination with *Candida albicans* (*C. albicans*) was detected in the conditioned medium during cell sheet fabrication. After adding 1 μg/mL amphotericin B to the transportation medium during transport from Nagasaki University Hospital to Tokyo Women's Medical University, which are 1200 km apart, no proliferation of *C. albicans* was observed. These results indicated that the supplementation of transportation medium with antimycotics would be useful for preventing contamination with *C. albicans* derived from the oral mucosa without hampering cell proliferation.

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Abbreviations: *C. albicans*, *Candida albicans*; DMEM, Dulbecco's modified Eagle's medium.

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Cultured oral mucosal epithelial cells have been utilized for sympatric and ectopic transplantation to reconstruct stratified epithelia such as the oral mucosa, skin, and cornea [1–3]. After optimizing culture medium containing autologous serum for fabricating autologous oral mucosal epithelial cell sheets, we have treated an esophageal ulcer resulting from endoscopic mucosal dissection of a mucosal tumor by performing endoscopic transplantation of autologous oral mucosal epithelial cell sheets fabricated on temperature-responsive cell culture surfaces to promote wound healing and prevent stenosis [4–6].

Because the human oral cavity contains abundant microbiota, biopsies of oral mucosa are treated with povidone-iodine. Furthermore, biopsies are stored in Dulbecco's modified Eagle's medium (DMEM) supplemented with 86 μg/mL ampicillin-sulbactam (Unasyn-S; Pfizer, NY, USA) and 100 μg/mL streptomycin (Meiji Seika Pharma, Tokyo, Japan) during transport from the oral surgery department to the cell culture facility. Moreover, the tissue is treated with povidone-iodine in the cell culture facility and is treated with dispase in DMEM including the same concentrations of ampicillin-sulbactam and streptomycin for epithelium separation. In addition, we add 40 μg/mL gentamicin (Gentacin; Schering-Plough, NJ, USA) and 0.27 μg/mL amphotericin B (Fungizone; Bristol-Myers Squibb, NY, USA) to the culture medium to maintain a sterile environment. Therefore, we have not experienced bacterial or fungal contamination in 8 biopsies from healthy volunteer donors in a preclinical study or in 10 biopsies from patients suffering from esophageal cancer treated at Tokyo Women's Medical University [6,7]. We have performed another clinical research study to examine the safety of long-distance transport of fabricated cell sheets between Tokyo Women's Medical University and Nagasaki University Hospital, which are approximately 1200 km apart, with transport taking 5–7 h by air and train. The protocol for oral mucosal epithelial cell sheet transplantation into patients was approved by the Ethical Committees and Internal Review Boards of Nagasaki University and Tokyo Women's Medical University. Approval of this clinical study by the Health, Labour and Welfare Ministry was gained on March 29th, 2013. Unfortunately, we experienced contamination with a yeast-like fungus in the culture supernatant of a patient's oral mucosal epithelial cells, so we abandoned the fabricated cell sheets for transplantation. We then performed sterilization tests to identify the source of the contamination and the strain of the fungus. Supernatants from each sample were cultured in soybean-casein digest broth (Wako Pure Chemical Industries, Osaka, Japan) and alternative thioglycollate medium (Wako Pure Chemical Industries). The strain of the cultured fungus was identified using CHROMagar Candida (Becton, Dickinson and Company, NJ, USA) and API 20C AUX (bioMérieux, Lyon, France). The obtained results revealed that the patient's oral mucosa was the source of *C. albicans* (*C. albicans*), as described below (Table 1). The oral mucosal tissue appeared macroscopically healthy (Fig. 1A), and there was no *Candida* antigen or infection with *C. albicans* in the patient's serum, which was added to the culture medium (Table 1). In addition, the cultured oral mucosal epithelial cells exhibited normal cell morphology (Fig. 1B,C). However, contaminating *C. albicans* and hyphal formation were detected during epithelial cell culture (Fig. 1D,E). It should be noted that hyphal formation by *C. albicans* was inhibited under anaerobic conditions [8].

We then tested the susceptibility of the *C. albicans* strain obtained from the conditioned medium and the oral surface of the patient to antimycotic agents using a commercially prepared colorimetric microdilution panel (ASTY; Kyokuto Pharmaceutical Industrial, Tokyo, Japan) [9]. The proliferation of the strain was completely inhibited by 0.5 μg/mL amphotericin B. In comparison, in previous susceptibility testing, the proliferation of nearly all *Candida* species was inhibited by 1.0 μg/mL amphotericin B [10], and a higher concentration of amphotericin B often hampers mammalian cell proliferation [11]. Therefore, we changed our protocol for the transport of oral mucosal biopsies from Nagasaki University Hospital to Tokyo Women's Medical University. The DMEM used for the transportation was supplemented with 1.0 μg/mL amphotericin B, and the concentration of amphotericin B in the culture medium was kept at 0.27 μg/mL, with no modification.

It took approximately 6 h to transport the biopsy by air and train, and then the transported biopsy was subjected to harvesting of the oral mucosal epithelial cells using dispase treatment for 2 h at 37 °C in DMEM supplemented with the same concentration of amphotericin B. As a result, no contamination with *C. albicans* was observed in the supernatant of the culture medium used for the fabrication of transplantable epithelial cell sheets from the same

Table 1
The results of quality control tests.

Sample	Items		Result
Cell culture supernatant (1st trial)[a]	Sterilization test	Bacteria	Negative
		Fungi	*Candida albicans*
	Mycoplasmal culture		Negative
	Mycoplasma test (PCR)[b]		Negative
	Endotoxin		0.062 EU/mL
Reagents for cultivation	Sterilization test	Bacteria	Negative
		Fungi	Negative
Serum (patient)	Sterilization test	Bacteria	Negative
		Fungi	Negative
		Candida antigen	Negative
Oral surface (patient)	Sterilization test	Fungi	*Candida albicans*
Oral surface (operator 1)	Sterilization test	Fungi	Negative
Oral surface (operator 2)	Sterilization test	Fungi	Negative
Cell culture supernatant (2nd trial)[a]	Sterilization test	Bacteria	Negative
		Fungi	Negative
		Mycoplasmal culture	Negative
		Mycoplasma test (PCR)[b]	Negative
		Endotoxin	0.136 EU/mL
Oral surface (patient)	Sterilization test	Fungi	*Candida albicans*

[a] Cell culture supernatants were routinely used for quality control tests.
[b] PCR for detecting *Mycoplasma pneumoniae* was performed in accordance with method shown by Jensen JS et al. [12].

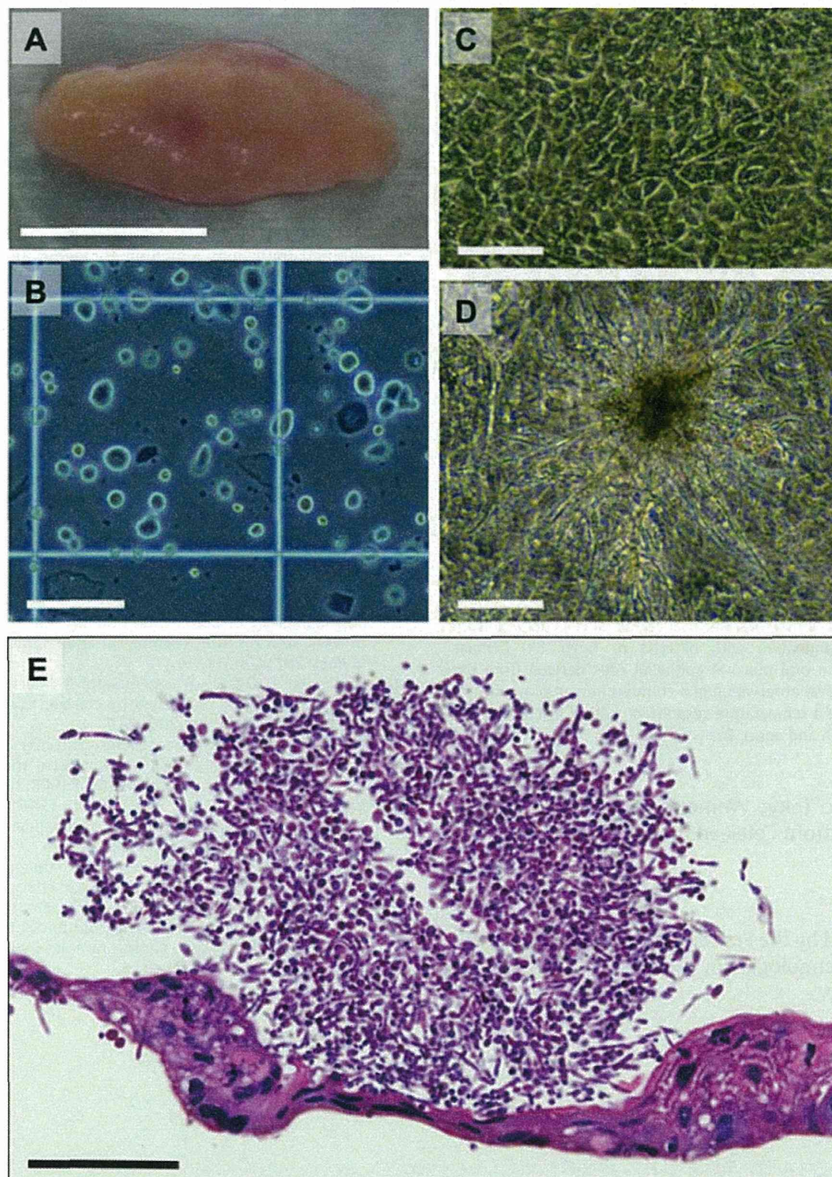

Fig. 1. *Candida albicans* (*C. albicans*) proliferating in the cell culture supernatant of human oral mucosal epithelial cells in this clinical study. (A) Human oral mucosal tissue of the patient. Bar = approximately 1 cm. (B) Oral mucosal epithelial cells derived from the patient after cell preparation. Bar = 100 μm. (C) Cellular morphology of the cultured human oral mucosal epithelial cells. Bar = 100 μm. (D) *C. albicans* observed on the cultured epithelial cells in a culture vessel. Bar = 100 μm. (E) Histological observation of the *C. albicans* adhering to a cultured epithelial cell sheet harvested from a temperature-responsive culture insert. The cell sheet and *C. albicans* were stained with hematoxylin and eosin. Bar = 50 μm.

patient, and the cultured epithelial cells were successfully harvested as cell sheets (Table 1, Fig. 2). To maintain a sterile environment, the temperature-responsive cell culture inserts to which the cultured epithelial cell sheets adhered were placed in transportable containers while in the safety cabinet of a clean room specialized for fabricating transplantable cell sheets for a clinical setting. The containers were then transported to Nagasaki University Hospital in the transportation box, which was mounted on a hot plate to keep the temperature at 37 °C. After transport, the epithelial cell sheets were finally transplanted onto the esophageal ulcer of the patient after endoscopic dissection to remove esophageal cancer.

Here, we have reported our experience of contamination with *C. albicans* during the fabrication of transplantable oral mucosal epithelial cell sheets derived from a patient who was not suffering from candidiasis. By adding 1 μg/mL amphotericin B to the transportation medium, fungal proliferation was completely inhibited and esophageal mucosal regeneration was successfully observed. Therefore, the method described in this report should be useful for preventing contamination with *C. albicans* without increasing the concentration of amphotericin B in the culture medium.

Disclosure statement

Teruo Okano is a founder and director of the board of CellSeed Inc., licensing technologies and patents from Tokyo Women's Medical University. Teruo Okano and Masayuki Yamato are

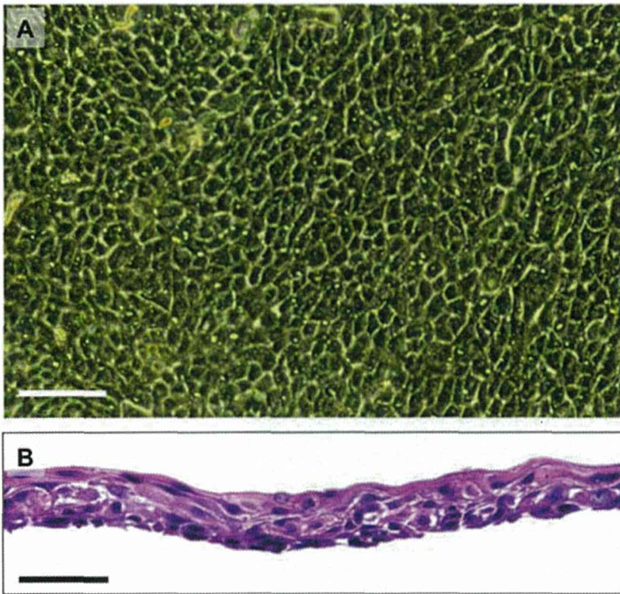

Fig. 2. Second trial of cultivation of human oral mucosal epithelial cells derived from the same patient, without contamination with bacteria or fungi. (A) Cellular morphology of the cultured human oral mucosal epithelial cells derived from the patient. Bar = 100 μm. (B) Histological observation of a cultured human oral mucosal epithelial cell sheet harvested from a temperature-responsive culture insert. The cell sheet was stained with hematoxylin and eosin. Bar = 50 μm.

shareholders of CellSeed Inc. Tokyo Women's Medical University is receiving research funding from CellSeed Inc.

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NEW METHODS

Endoscopic cell sheet transplantation device developed by using a 3-dimensional printer and its feasibility evaluation in a porcine model

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Background: To prevent severe esophageal stenosis after aggressive endoscopic submucosal dissection (ESD), our group previously reported an efficient treatment using cell sheets that had been fabricated from patient cells. However, this transplantation procedure had not been easy for every endoscopist and needed to be improved to derive the full effect of epithelial cell sheets.

Objective: To develop an endoscopic device that enables easy and effective cell sheet transplantation and to evaluate its performance and clinical feasibility.

Design: Animal study.

Setting: Animal experimentation laboratory.

Intervention: Three pigs underwent circumferential esophageal ESD while under general anesthesia. A total of 12 cell sheets were endoscopically transplanted to the ESD site; 6 cell sheets were transplanted by using an endoscopic device that we developed, and 6 cell sheets were transplanted by using the conventional method.

Main Outcome Measurements: Procedure time, transplanted area on the ESD site, transplantation success rate, and monitoring of adverse events or incidents.

Results: The device allowed successful transplantation of all cell sheets with a shorter procedure time than with the conventional method (4.8 ± 0.8 minutes vs 13.3 ± 5.7 minutes, respectively) ($P = .005$) and onto a larger area (111.3 ± 56.3 mm^2 vs 41.8 ± 4.2 mm^2, respectively) ($P = .023$) with a higher success rate (100% vs 83%, respectively). No adverse incidents were monitored in each method.

Limitations: Animal study, small sample.

Conclusion: A newly designed endoscopic cell sheet transplantation device would be useful.

BACKGROUND

Esophageal stenosis is one of the major adverse events after aggressive endoscopic submucosal dissection (ESD) for early-stage esophageal cancer.[1-4] For treating stenosis, endoscopic balloon dilation has been widely used, although repeated stenosis is still an issue.[2] Clinical research involving steroid therapies[5-7] and stent treatments[2,8,9] have been studied to overcome.

Our group previously reported a regenerative approach in which autologous epithelial cell sheets were transplanted onto an ESD site to promote structural and functional

Abbreviation: ESD, endoscopic submucosal dissection.

DISCLOSURE: *Dr Okano is a founder of CellSeed Inc, is a member of the Board of Directors of CellSeed Inc and has licensed technologies and patents from Tokyo Women's Medical University related to this study and is a shareholder in CellSeed Inc. Dr Yamato is a consultant and shareholder in CellSeed Inc. All other authors disclosed no financial relationships relevant to this article. This study was supported by Grant-in-Aid for Scientific Research (14483609 to Dr Kanai), Cell Sheet Tissue Engineering Center (CSTEC), and a Health Labor Scientific Research Grants (26220101 to Dr Okano and 26270801 to Dr Yamato).*

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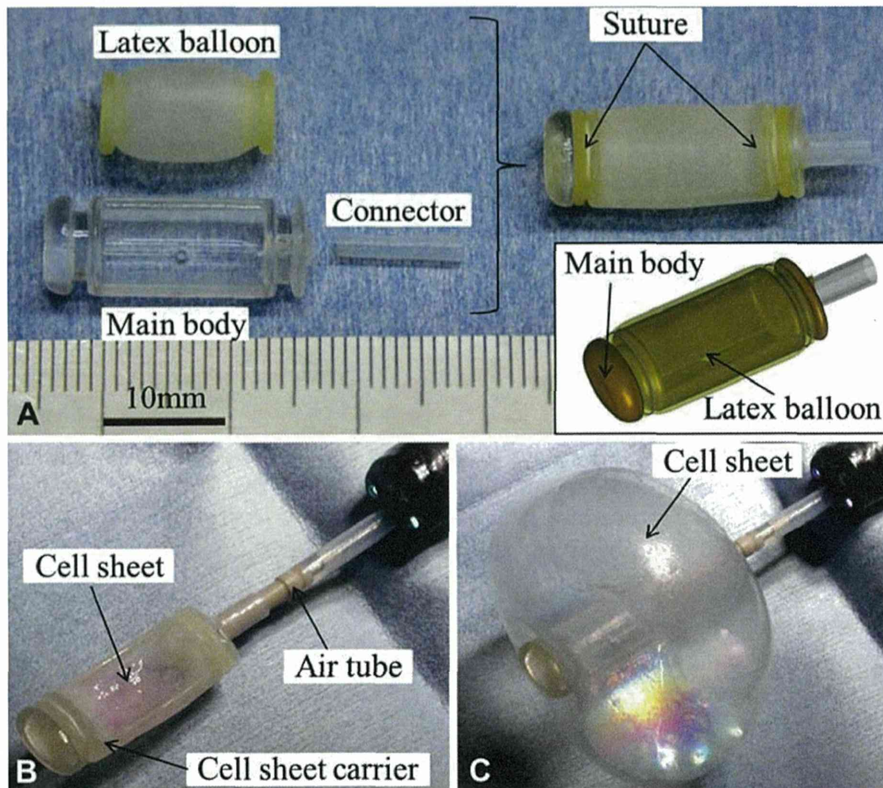

Figure 1. The endoscopic cell sheet transplantation device composed of a cell sheet carrier and an air tube. **A,** The structure of a cell sheet carrier. A latex balloon for US endoscope (MH-525; Olympus) was covered on the main body and secured at both sides with surgical sutures, and a connector (polytetrafluoroethylene tube) was glued at the proximal end of the main body. **B,** Delivery mode. After a cell sheet carrier was connected to an air tube that was passed through a biopsy channel (>2.8 mm), a cell sheet was loaded on a balloon and stored inside of the device by balloon deflation for easy delivery. **C,** Transplantation mode. The cell sheet could be expanded and adhered to an ulcer site for transplantation by balloon inflation.

re-epithelialization.[10,11] Cell sheets are fabricated from a patient's cells in our original designed temperature-responsive culture dishes,[12] and the thickness is usually less than 0.1 mm. A cell sheet can be grafted by just placing it on an ESD site without suturing or glue due to the existence of extracellular matrix underneath the cell sheet.[13] Several clinical studies have had good results.[14] To date, the cell sheets have been transplanted as previously reported.[10,11,14] Briefly, after a cell sheet was harvested on a round plastic membrane (~8 mm in diameter), it was delivered and placed on an ESD site by using conventional endoscopic forceps. Because the current transplantation procedure has not been easy for all endoscopists, we developed a novel device that allows easy delivery and transplantation of cell sheets. In this study, the clinical feasibility and the superiority of the device were evaluated in an in vivo porcine model.

METHODS AND MATERIALS

All animal experiments were performed according to the Guidelines of Tokyo Women's Medical University on Animal Use.

Prototyping for a novel endoscopic device

The device was composed of a cell sheet carrier (Fig. 1A) and an air tube. A cell sheet carrier was made of a main body fabricated by a 3-dimensional printer with a biocompatible plastic material (Objet 350 and MED610; Stratasys, Eden Prairie, Minn), a latex balloon for an US endoscope, a polytetrafluoroethylene tube, and surgical sutures. A balloon was covered on a main body and tied at both ends with a suture. A polytetrafluoroethylene tube was glued at the proximal end for a connector to an air tube. The cell sheet carrier was connected to an air tube which was passed through an endoscope's biopsy channel (>2.8 mm) ~~and could be supplied/suctioned air inside the balloon.~~ The air tube could transfer linear rotation torque to the cell sheet carrier.

A harvested cell sheet was loaded and kept in place on the balloon with a little water by water surface tension. The cell sheet was then stored inside the device by air suction because the balloon with the expandable cell sheet was adhered to the inner wall of the device (Fig. 1B) and easily delivered through an overtube into an esophagus. Conversely, the cell sheet could be attached to an ESD site for transplantation by balloon inflation (Fig. 1C).

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TABLE 1. Study results

Transplantation order	Animal	Transplantation method	Name of cell sheet	Procedure time, min:s	Transplanted area, mm^2	Adverse events
1	1	Membrane	M-1	15:03	38.0	None
2		Membrane	M-2	9:01	40.7	None
3		Membrane	M-3	23:43	46.0	None
4	2	Device	D-1	4:45	163.6	None
5		Device	D-2	6:16	32.6	None
6		Device	D-3	4:33	175.5	None
7		Membrane	M-4	10:10	38.0	None
8		Membrane	M-5	13:30	— (detached)	None
9		Membrane	M-6	8:32	46.4	None
10	3	Device	D-4	4:29	131.4	None
11		Device	D-5	3:47	100.6	None
12		Device	D-6	4:52	63.8	None

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Fabrication of human skin cell sheets[15]

243 Normal human epidermal keratinocytes (Lonza, Basel, Switzerland) were cultivated on temperature-responsive cell culture inserts (CellSeed, Tokyo, Japan) for 14 days at 37°C. They were harvested as a cell sheet (~18 mm in diameter) by reducing the temperature to 20°C for 30 minutes.

Animal experiments: transplantation procedure time

Three miniature pigs (6 months old, 16-20 kg, female, from the Nippon Institute for Biological Science) got artificial ulcerations (270-360° in range, 5 cm in length) in the lower esophagus by applying the hook-knife method of ESD, by using a GI endoscope (GIF-XQ260; Olympus, Tokyo, Japan) and a hook-knife (KD-620 LR; Olympus). An overtube (EMR tube; Create Medic, Tokyo, Japan) was then inserted into the esophagus. Twelve cell sheets were individually transplanted to the ESD sites by using the device or a conventional method as shown in Table 1. Procedure times (endoscope insertion time) were monitored.

Macroscopic analysis: transplantation success rate and area

All animals were killed 6 hours after transplantation. The esophagi were extracted and cut open. The macroscopic views were photographed, and the transplanted cell sheets were counted to calculate the transplantation success rate (%) (number of cell sheets adhered to the esophagus/number of cell sheets attempted to be transplanted × 100). Then each area covered by a cell sheet was calculated by using image analysis software (Image J; U.S. National Institutes of Health, Bethesda, Md), and the statistical analysis was performed by using a 2-tailed unpaired Student *t* test.

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TABLE 2. Study results: statistical analysis

Outcome	Device method (n = 6)	Membrane method (n = 6)	*P* value
Transplantation success rate, no. (%)	6 (100)	5 (83)	.341
Procedure time, mean ± SD, min	4.8 ± 0.8	13.3 ± 5.7	.005
Transplanted area, mean ± SD, mm^2	113.0 ± 56.3	41.8 ± 4.2	.023

Histological analysis

To confirm the basal layer of the transplanted cell sheets adhered to host tissues, immunohistochemistry was performed with antibodies anti-cytokeratin 14 (MAB9766; Abnova Corporation, Taipei, Taiwan) and anti-p63 and anti-integrin β4 (SC-8431, SC-9090; Santa Cruz Biotechnology, Dallas, Tex).

RESULTS

The results of this study are summarized in Tables 1 and 2.

Cell sheet transplantation

The cell sheet transplantation device method is shown in Figures 2A and 2B. After a cell sheet was stored inside the device by vacuuming approximately 1.5 mL of air, the device was then passed through an overtube to the ESD site. The balloon was inflated slowly by supplying approximately 15 mL air, and the cell sheet was kept attached for approximately 30 seconds. After the balloon

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