



Review

Esophageal regenerative therapy using cell sheet technology

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ABSTRACT

We have been conducting research on esophageal regenerative therapy using cell sheet technology. In particular, in the endoscopic field, we have pushed forward clinical research on endoscopic transplantation of cultured autologous oral mucosal epithelial cell sheets to esophageal ulcer after endoscopic submucosal dissection (ESD). We started research in this direction using animal models in 2004 and performed clinical research in 2012 in collaboration with Nagasaki University and Karolinska Institute. Although in full-circumferential cases it was difficult to prevent esophageal stricture after ESD, there were no complications and stricture could be suppressed. The cell sheet technology is still in its infancy. However, we are convinced that it has a high potential for application in various areas of gastrointestinal science. In this review, we focus on the pre-clinical and clinical trial results obtained and on the theoretical aspects of (1) stricture prevention, (2) esophageal tissue engineering research, and (3) endoscopic transplantation, and review the esophageal regenerative therapy by cell sheet technology.

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Abbreviations: ESD, endoscopic submucosal dissection; EMR, endoscopic mucosal dissection; EBD, endoscopic balloon dilation; OMECS, oral mucosal epithelial cell sheet; PIPAAm, poly(N-isopropylacrylamide); ECM, extracellular matrix; CPC, cell-processing center; GMP, good manufacturing practice; PVDF, polyvinylidene difluoride; TAC, triamcinolone; SEMS, self-expandable metallic stent; CMC, carboxymethyl cellulose; PGA, polyglycolic acid.

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1. Introduction

Esophageal stricture is the major complication occurring after substantial endoscopic submucosal dissection (ESD) of superficial esophageal neoplasms [1]. In the endoscopic mucosal resection (EMR) era before ESD was developed, Katada et al. [2] reported that mucosal removal of more than 3/4 of the luminal circumference was associated with a high rate of stricture in patients with esophageal squamous cell carcinoma, and that mucosal defects longer than 30 mm were associated with greater stricture severity. Ono et al. [3] reported that 90% of the patients, in whom the lesion extended more than 3/4 of the esophageal circumference, needed periodical balloon dilation approximately every 1–2 weeks. To prevent post-ESD esophageal stricture, subsequent endoscopic balloon dilation (EBD) is often required [4], but there is a risk of perforation after EBD [5]. The problems associated with an esophageal stricture can severely affect the quality of life for patients. Yamaguchi et al. [6] reported the need for an average of 15.6 sessions of endoscopic balloon dilation in cases of 2/3-circumferential esophageal ESD, and an average of 32.7 sessions in full-circumferential esophageal ESD.

To prevent esophageal stricture, we started research on the endoscopic transplantation of tissue-engineered autologous oral mucosal epithelial cell sheets (OMECS) in 2004 and developed a therapy for clinical use [7]. In this review, we focus on the pre-clinical and clinical trial results obtained and on the theoretical aspects of (1) stricture prevention, (2) esophageal tissue engineering research, and (3) endoscopic transplantation, and review the esophageal regenerative therapy by cell sheet technology.

2. Cell sheet technology by temperature-responsive cell culture dishes

Okano et al. [8] developed novel cell culture dishes grafted with a temperature-responsive polymer, poly (N-isopropylacrylamide) (PIPAAm), that is able to change its surface adhesion properties in response to temperature. The surface of these temperature-responsive cell culture dishes is hydrophobic at temperatures greater than 37 °C, allowing cells to attach to the surface of the culture dish and proliferate (Fig. 1a). When the temperature is reduced to below 32 °C, the polymer changes its physical surface structure and becomes hydrophilic. These physical changes cause the spontaneous detachment of cells as cell sheet formation progresses.

Temperature-responsive polymers preserve the extracellular matrix (ECM), as well as cell morphology and functionality without the use of protein enzymes (Fig. 1b) [9]. The detached cell sheets can then be harvested as a contiguous cell sheet, allowing quick integration of the sheets when transplanted. Thus, cell sheet technology is supported by scientific evidence on the mechanism of temperature-responsive cell culture dishes [10,11].

3. Development of regenerative therapy by endoscopic transplantation of tissue-engineered OMECS

3.1. Investigation of regenerative therapy using tissue-engineered OMECS in an animal model

At the time of development, gastric ESD was extensively performed, but active treatment of large post-ESD ulcerations was not performed. Additionally, in the surgical field, dressing or skin grafting techniques are usually used to treat wounds on the surface of the body, but since the digestive tract, which consists of the same epithelial system, has been overlooked in terms of treatment options, we addressed this issue and focused on the development of a suitable therapy. Nishida et al. [12] reported a corneal reconstruction procedure using autologous OMECS for the treatment of bilateral ocular traumas or diseases such as Stevens-Johnson syndrome and Pemphigoid, and demonstrated that OMECS can be transplanted without suturing. These results prompted us to apply this therapy in the endoscopy field, and we focused on oral mucosal epithelial cells as the cell source instead of esophageal epithelial cells. Histologically, both the oral mucosal and esophageal epithelium represent the squamous cell type; thus, they are essentially identical. Moreover, oral mucosal cells are easy to harvest, the harvesting methods are minimally invasive, and the methods of removal do not cause perforation of the oral wall. When it comes to harvest the esophageal mucosa for esophageal tissue engineering, there is always a risk of perforation because the esophageal mucosa is actually removed by endoscopy.

We previously investigated the use of epithelial cell sheets harvested from oral mucosal tissue in a canine model [13]. We concluded that cell sheets could be endoscopically transplanted to the ulcerative site immediately after esophageal ESD. Culture of

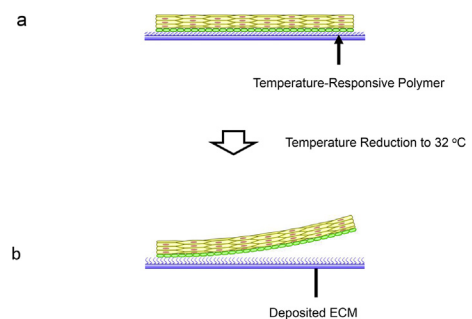


Fig. 1. Technology of temperature-responsive cell culture dishes. The surface of temperature-responsive dishes is hydrophobic at 37 °C, which allows cell adhesion, spreading, and proliferation. The same surface becomes hydrophilic once the temperature is reduced to <32 °C, and all the cells cultured on it are detached without the need for proteolytic enzymes such as trypsin. Cells cultured until confluence are harvested as a single contiguous cell sheet. (a) Culture condition on temperature-responsive dishes at 37 °C. (b) Harvest of the cell sheet with attached protein (after decreasing the temperature). Adapted with permission from Elsevier [7].

epithelial cells can be performed by Green's methods using 3T3 cells [14–16]. This standard fibroblast cell line was established in mice, but for this reason, the possibility of the implantation of xenogeneic 3T3 cells into humans has been a concern. Therefore, in order to facilitate clinical research, a method that does not include 3T3 cells is required, which led to research on new culture methods [17–19].

3.2. Preparation for clinical study of endoscopic transplantation of OMECS

The novel culture method using the insert culture dishes to prepare cell sheets for clinical research is different from the conventional method. The insert surfaces of these dishes were grafted with a temperature-responsive polymer (PIPAAm), and because only human cells without 3T3 cells were used in this process, the patient compliance and acceptance were high with this therapy.

We ensured that our cell-processing center (CPC) complied with the good manufacturing practice (GMP) standards. After all procedures had been completed, a “cold run” was performed, i.e., an experiment in which the same protocol is applied in animals before actual human cell sheet transplantation to humans. Specifically, human cells were harvested from volunteer donors and cultured for 16 days at the CPC in our institute, and the esophageal ESD procedure was performed in an animal endoscopy room equipped with two incubators that can be maintained at 20 °C and 37 °C. Simultaneously, the cell sheets were transplanted to a canine esophageal ulceration site to evaluate whether all procedures could be performed as planned without problems in the animals [20]. Before cell sheet transplantation, an EMR-tube was inserted in the esophagus to keep the cervical esophageal cavity straight. Because the canine neck is straight, and the human esophagus entrance is curved, in the first trial in humans, we were concerned that the endoscopic tube may move the cell sheets. After confirming that all procedures can be performed as planned, we are ready to begin a clinical research in Tokyo Women's Medical University, described in the following sections.

3.3. Regenerative therapy of tissue-engineered autologous OMECS

A specimen of the patient's own oral mucosal tissue was surgically collected from the interior buccal mucosa (Fig. 2a) [21]. The specimen size was approximately 6 mm in diameter for one cell sheet, and the area of the tissue was determined according to the planned number of transplantations of cell sheets.

Oral mucosal epithelial cells were collected after the treatment with dispase I (1000 PU per milliliter, Godo Shusei), at 37 °C for 2 h, and the collected epithelia were mixed in trypsin and ethylenediaminetetraacetic acid (EDTA) for 20 min. Using commercial cell culture inserts (Falcon, Becton Dickinson), the surface of the culture dish of the inserts were grafted with temperature-responsive polymer, and new temperature-responsive cell culture inserts (UpCell Insert, CellSeed) were prepared. Then, the collected epithelial cells were seeded onto the temperature-responsive culture inserts and cultured with autologous serum for 16 days at 37 °C (Fig. 2b).

After culture in vitro for 16 days, OMECS (23.4 mm in diameter) were harvested by reducing the temperature to 20 °C while esophageal ESD was being performed (Fig. 2c). The autologous oral mucosal epithelial cell sheets were transplanted onto the esophageal ulcerative site immediately after ESD (Fig. 2d).

3.4. Endoscopic transplantation methods

Endoscopic transplantation of cell sheets has been performed by one of the two methods described below. However, this technology is not yet completely translatable to the ulcer area and needs further development.

3.4.1. Membrane method

Since the cell sheet is very thin, endoscopic transplantation is not possible with the cell sheet alone; thus, it requires a polyvinylidene difluoride (PVDF) thin-sheet support membrane (Immobilon-P, DURAPORE®, Millipore) [22]. Cell sheets were individually transplanted to the esophageal ulcerative site using endoscopic forceps immediately after ESD (Fig. 3). The PVDF support membrane with an attached autologous OMECS sheet was held using endoscopic forceps, and the endoscope was moved to the ulcerative site through the EMR-tube (Create Medic) for cell sheet transfer. The cell sheets were then carefully turned and placed directly onto the center of ulcerative site. Gentle pressure was applied to the overlying PVDF support membrane with a cell sheet using the endoscopic forceps for 10 min. The cell sheets were then securely transplanted on the ulcer wound beds. The support membrane was not removed so that the cell sheet would not be lost by this procedure, and since the support membrane is thin and small, it can be naturally excreted through the gastrointestinal tract. This protocol was repeated as necessary to ensure that the ulcerative site was sufficiently covered by the transplanted cell sheets. The membrane method allows for precise placement of the cell sheets on the target sites; however, it requires advanced professional and technical skills, and any contact with the EMR-tube will damage the cell sheets permanently.

3.4.2. Balloon device method

In response to the difficulties imposed by endoscopic transplantation procedures, a new endoscopic device was developed and produced using a 3D printer [23]. To prevent losing or damaging the cell sheet at the inner wall of the EMR-tube during transfer, the balloon placed on the cell sheet is deflated, keeping the cell sheet within the protective wall of the device. Once positioned on the ulcer site, air pressure is applied to expand the balloon surface directly attached to the cell sheet, thereby providing a rapid, easy, and relatively accurate means of cell sheet transplantation. However, the influence of cell sheet and cell damage caused by balloon expansion remain unclear.

The combination of both methods (membrane method and balloon device method) is also possible and can be advantageous for covering the general area of the site with the transplant cell sheets using the balloon device method first, and for placing the cell sheets at specific locations using the membrane method afterward. Nonetheless, it is important to understand the advantages and disadvantages of each transplant method.

4. Clinical research in Tokyo Women's Medical University

According to the guideline for esophageal cancer treatment at that time, the absolute indication for endoscopic resection was limited to 2/3 circumference. Circumference exceeding this limit, in our institute, was out of indication for endoscopic treatment and, as a result, many of these patients underwent surgery. Since this first human clinical research that investigated endoscopic transplantation of cell sheets, a protocol that emphasizes safety was established.

We started a clinical trial (UMIN000000473) in Tokyo Women's Medical University in 2006. In this work, we produced tissue-engineered autologous oral mucosal epithelial cell sheets and

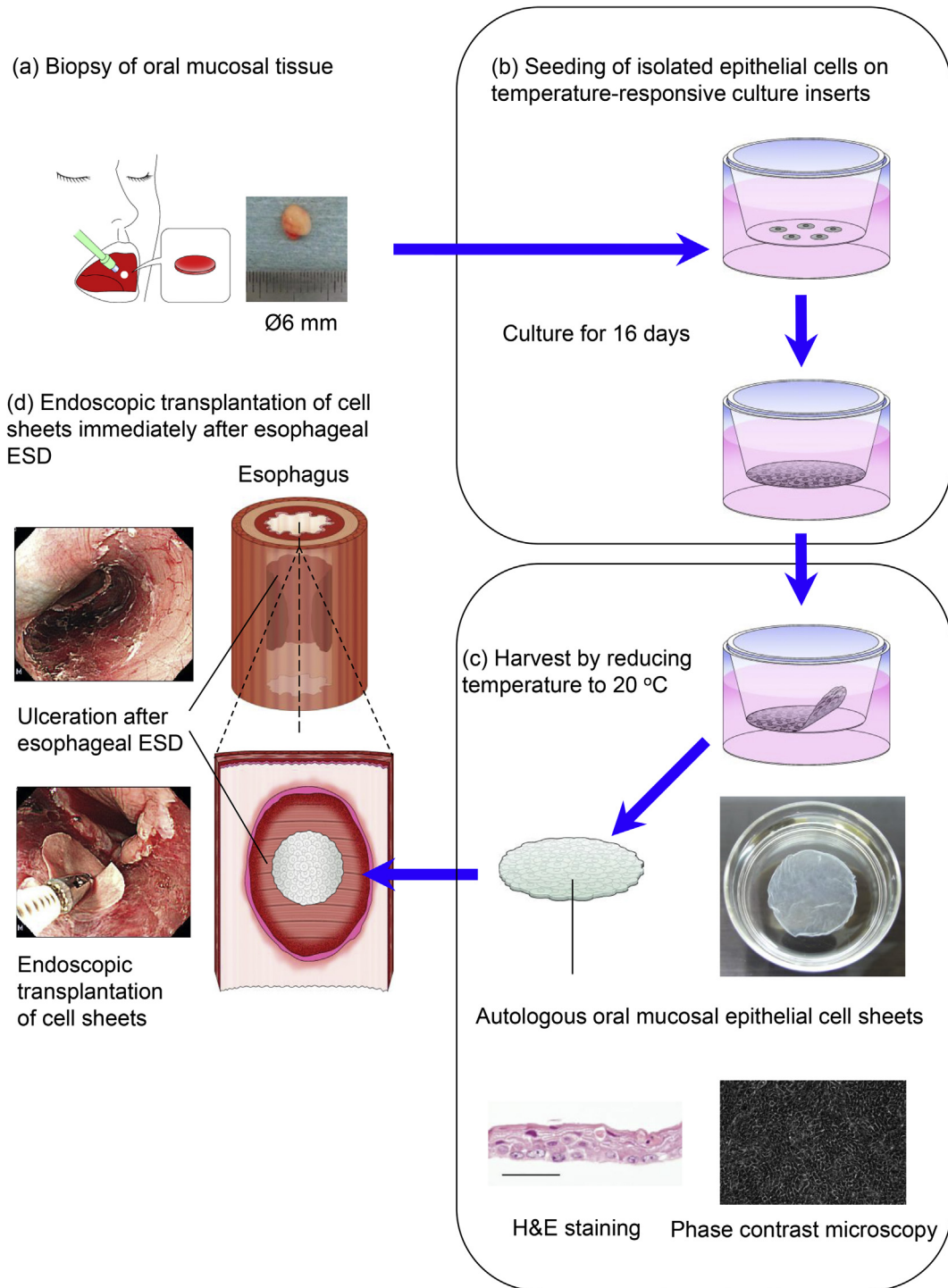


Fig. 2. Treatment of the artificial ulceration after esophageal ESD by transplantation of autologous oral mucosal epithelial cell sheets fabricated on temperature-responsive culture inserts. (a) Biopsies were taken from the patient's own oral buccal mucosal tissue. Oral epithelial cells were isolated from the tissue by proteolytic enzyme treatment. (b) The epithelial cells were seeded onto temperature-responsive culture inserts without a 3T3 feeder layer, and cultured with autologous serum for 16 days at 37 °C. (c) Oral mucosal epithelial cell sheets (23 mm in diameter) were harvested by reducing the culture temperature to 20 °C. Scale bar = 50 micron. (d) Autologous oral mucosal epithelial cell sheets on a support membrane were transplanted with endoscopic forceps onto the bed of the esophageal ulceration immediately after ESD. Adapted with permission from Elsevier [7].

investigated the safety and efficacy of endoscopic transplantation of these cell sheets for preventing stricture after esophageal ESD [7,24]. Eight out of nine patients did not experience dysphagia or stricture after the regenerative therapy (Table 1). The results of the study showed that the transplantation of cell sheets could safely and effectively prevent post-esophageal ESD stricture. One case of

EMR was reported in *Techniques in Gastrointestinal Endoscopy* [25]. This was the first in human case in April 2008.

All patients could be successfully transplanted with cell sheets by the membrane method. The patients underwent endoscopic examination once a week until epithelialization was complete, showing ulceration healing after esophageal ESD. Complete

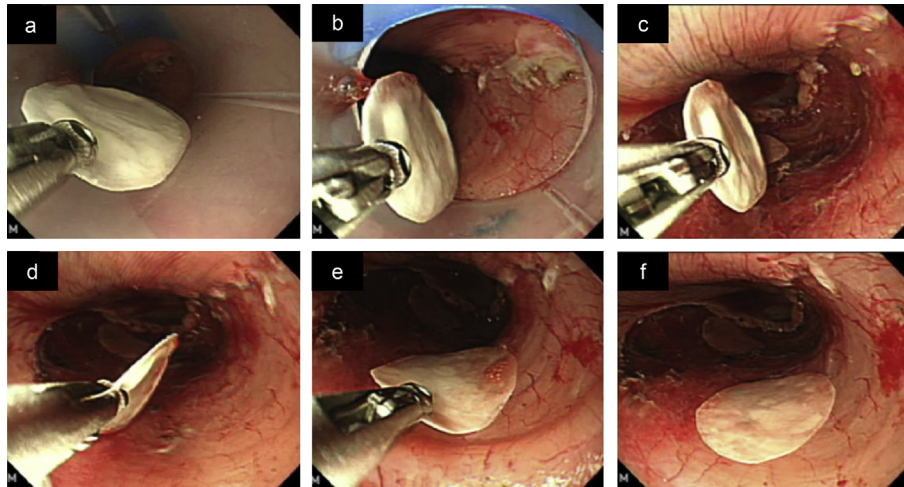


Fig. 3. Endoscopic transplantation of cell sheets. (a) The cell sheet on a support membrane passed through the EMR tube. The membrane was passed down to the esophagus with the side attaching the cell sheet facing the center of the esophagus and the side without the cell sheet facing the esophageal wall. (b) An entrance balloon was inflated to keep cavity dilated. (c) Operation of the endoscope continued as the entrance balloon maintained a wide working space by preventing air leakage. (d) When the cell sheet reached the ulceration site, the membrane was rotated by 180° using a pair of endoscopic forceps so that the side of the membrane containing the cell sheet faced the ulceration site. (e) The cell sheet was placed directly onto the esophageal ulceration site. (f) The cell sheet was held in place for approximately 10 min. Adapted with permission from Elsevier [7].

epithelialization occurred within a median time of as early as 3 weeks in this study [7]. Only one case (case 5) with a full circumferential ulceration that expanded to the esophagogastric junction (esophagus physiological constriction) suffered from post-ESD stricture. The cell sheets could not be transplanted in this area. Despite a widespread area, epithelialization of the large ulcerative area was achieved within 2 weeks, but the non-transplanted area showed the development of refractory strictures.

5. Clinical research in Nagasaki University

We also collaborated with Nagasaki University and started a clinical research trial (UMIN00010251) in 2013. The purpose of this research, and also the main challenge, was to determine whether the cell sheets cultured at Tokyo Women's Medical University could remain viable after being transported to Nagasaki University for subsequent transplantation, and to determine whether the cell sheets would be affected by external factors such as variable pressure and humidity.

In detail, oral mucosal tissues were collected from patients at Nagasaki University and transported to Tokyo Women's Medical University by air. These tissues were cultured in our laboratory and then transported back to Nagasaki University Hospital for the transplantation of the cell sheet to the esophageal lumen of patients (Fig. 4). Since temperature-responsive cell culture dishes were used, technology to stabilize the environment, such as temperature and pressure, was necessary. It was proved that cell sheets can be transferred from the main hospital and treatment is possible at any other facility if the required conditions are met.

Yamaguchi et al. [26] reported the efficacy results of transplantation of cell sheets that were prepared in one location and transported by air to another institute in cases of over 3/4 circumference. Of the 10 cases investigated, only four (case 12, 14, 18, 19) had stricture, and in particular, the two full-circumferential ESD cases (case 18, 19) had stricture (Table 1).

In this clinical study, basic research aspects were also investigated. Takagi et al. [27] revealed that the supplementation of transportation medium with antimicrobials is useful for preventing contamination with *Candida albicans* derived from the oral mucosa without hampering cell proliferation. Kasai et al. [28] reported that

brush biopsy contributes to quality control of the fabrication of autologous OMECS.

6. Clinical research in Karolinska Institute

In the Western world, the rate of adenocarcinoma of the esophagus (Barrett's cancer) has increased [29–31]. In Barrett's esophageal neoplasms, a stricture occurs after full-circumferential removal by ESD. Barrett's esophageal neoplasm is a wide circumferential occupation cancer, therefore extensive endoscopic resection is needed [32]. Chung et al. [33] reported complete Barrett's excision by stepwise endoscopic resection in short-segment Barrett's high-grade dysplasia, and that esophageal dilation was required in 33% of the EBD cases. Reported stricture rates after circumferential ESD were 90% [34,35]. Additionally, radiofrequency ablation (RFA) has few comparative complications, but esophageal stricture rates after RFA range from 4 to 5% [36,37]. Our approach for Barrett's esophageal neoplasm treatment is the combination of circumferential Barrett's epithelium resection and cell sheet transplantation to reduce complications.

Tokyo Women's Medical University and Karolinska Institute signed a collaboration agreement between research and education in 2010. After one year of preparation and education on the cell culture method and transplantation technique of cell sheets, we started clinical research with Karolinska Institute in 2012. Barrett's neoplasms were treated by ESD and RFA and subsequent transplantation of cell sheets, and we showed that five (case 21–25) patients were treated [38] (Table 1). Esophageal ESD was extensive with resections being circumferential in three patients and 9–10 cm in length in two patients. Three (case 22, 23, 25) of the five patients developed strictures requiring two to five dilatation sessions after transplantation. All cases with stricture were full-circumferential ESD cases. Cell sheet therapy decreased both the risk for and extent of stricture. This was the first clinical research trial in Europe using cell sheet technology for esophageal therapy.

7. Evaluation of regenerative therapy by endoscopic transplantation of tissue-engineered OMECS

Endoscopic transplantation of tissue-engineered OMECS encompasses the fundamental aspects of (1) stricture prevention, (2)

Table 1
Result of cell sheet therapy for esophageal ESD. Results of clinical research by using tissue-engineered oral mucosal epithelial cell sheets [7,25,26,38].

Cases	Institute	Age/Sex	EMR/ESD	Circumference of ulceration (%)	Length (cm)	Transplanted cell sheets (n)	Specimen size (mm)	Approximate proportion of cell sheet coverage (%)	Stricture	EBD (n)	Duration of wound healing (weeks)
1	TWMU	52/M	EMR	50	3	1	22 × 17	25.7	(-)	0	3
2	TWMU	70/M	ESD	67	6	2	43 × 36	12.4	(-)	0	4
3	TWMU	73/M	ESD	67	2	2	21 × 11	83.1	(-)	0	3
4	TWMU	73/M	ESD	67	3	3	43 × 15	44.7	(-)	0	3
5	TWMU	65/M	ESD	100	11	7	55 × 70	17.5	(+)	21	23
6	TWMU	64/M	ESD	50	4	2	24 × 23	34.8	(-)	0	3
7	TWMU	55/M	ESD	75	7	7	45 × 40	37.3	(-)	0	3
8	TWMU	80/M	ESD	67	6	8	43 × 28	63.8	(-)	0	4
9	TWMU	70/M	ESD	75	6	4	45 × 30	28.4	(-)	0	5
10	TWMU	68/M	ESD	75	7	6	30 × 42	45.7	(-)	0	3
11	NU	55/M	ESD	88	Undescribed	6	80 × 55	13.1	(-)	0	4
12	NU	68/M	ESD	90	Undescribed	7	75 × 69	12.9	(+)	1	5.1
13	NU	73/M	ESD	83	Undescribed	5	45 × 30	35.6	(-)	0	5.7
14	NU	58/M	ESD	88	Undescribed	8	55 × 46	30.4	(+)	2	4.1
15	NU	67/M	ESD	83	Undescribed	8	50 × 33	46.5	(-)	0	4.1
16	NU	56/M	ESD	83	Undescribed	6	55 × 40	26.2	(-)	0	4.1
17	NU	63/M	ESD	90	Undescribed	8	73 × 55	19.1	(-)	0	7
18	NU	72/M	ESD	100	Undescribed	13	95 × 84	15.6	(+)	7	24.6
19	NU	62/F	ESD	100	Undescribed	5	53 × 50	18.1	(+)	1	70
20	NU	74/M	ESD	87	Undescribed	6	46 × 45	27.8	(-)	0	10
21	KI	70/M	ESD	75	5	2	Undescribed	Undescribed	(-)	0	2
22	KI	68/M	ESD	100	5	6	Undescribed	Undescribed	(+)	4	3
23	KI	55/M	ESD	100	10	5	Undescribed	Undescribed	(+)	5	3
24	KI	69/M	ESD	75	9	5	Undescribed	Undescribed	(-)	0	3
25	KI	69/M	ESD	100	4	4	Undescribed	Undescribed	(+)	2	2

Note: TWMU: Tokyo Women's Medical University; NU: Nagasaki University; KI: Karolinska Institute.

esophageal tissue engineering research and (3) endoscopic transplantation. We discuss these three aspects below.

7.1. From the perspective of stricture prevention

Lewis et al. [39] reported the factors associated with esophageal stricture after EMR monotherapy for neoplastic Barrett's esophagus. Resection of >50% of the circumference was strongly associated with stricture formation, and patients with a history of ≥25 pack-years tobacco trended toward esophageal stricture formation following EMR. Esophageal stricture often occurs when patients suffer from post-ESD ulceration of over 3/4 of the circumference of the esophagus, and stricture rate can range from 36 to 90% [3,40–42]. In another study, strictures occurred in all patients after circumferential esophageal ESD [43]. Miwata et al. [44] reported that refractory post-ESD stricture occurs after full circumferential esophageal ESD with muscle layer damage and ≥5 cm of longitudinal mucosal defect length.

Yu et al. [45] classified strategies to prevent esophageal stricture after ESD into five categories: (1) Pharmacological prophylaxis, (2) Mechanical strategies, (3) Tissue engineering strategies, (4) Autologous transplantation, and (5) Other novel strategies. Cell sheets transplantation and extracellular matrix approaches are included in the Tissue engineering strategies.

The most common reports refer to the use of steroids to prevent esophageal stricture [46]. Hashimoto et al. [47] reported local injection of a steroid (triamcinolone: TAC) in the wound by endoscopy, and subsequent reports have been made [46,48–50]. However, Hanaoka et al. [48] reported that a tumor circumferential greater than 75% is an independent risk factor for refractory stricture despite steroid injections. These authors emphasized that the development of more extensive interventions is warranted to prevent refractory stricture. Locoregional TAC may cause deep mural damage when it is injected into the *muscularis propria*. Thus, care should be taken not to inject TAC into the muscle layer when it is used to prevent post-ESD stricture [51].

Yamaguchi et al. [6,26] reported the usefulness of systemic steroids. Oral administration is a simple method and does not require the use of an endoscope. Pih et al. [52] also reported the stricture rates after ESD for superficial esophageal neoplasms with mucosal defects in >75% of the esophageal circumference in 53 patients. The stricture rate was 50.0% in the ESD-alone group, 20.0% in the oral steroid group, and 33.3% in the steroid injection group. Oral steroid prophylaxis appeared to be a safe and effective treatment in preventing post-ESD stricture. However, the side effects of systemic steroids are more severe and include delayed wound healing, immune suppression, optical damage, psychiatric disturbances, diabetes, peptic ulcerations, osteoporosis, and a higher susceptibility to tuberculosis infection. Iizuka et al. [53] reported the occurrence of pneumonia and oral herpes infection, which are adverse events potentially associated with steroid administration, in the original group that was given a 30-mg/day dose of prednisolone and tapered over 8 weeks. *Candida* esophagitis, arthritis, and steroid-related myopathy were observed in the other group (30 mg of prednisolone was administered orally for 3 weeks and then the dose was reduced via 5-mg decrements every 3 weeks).

Yang et al. [54] reported that long-term oral steroid therapy appears to be the optimal prevention method for postoperative stricture. On the other hand, Wang et al. [55] reported that a locally injected steroid was superior to oral steroid in EBD reduction. Abe et al. [56] reviewed the oral and locally injected/administered steroids used as first-line options for the prevention of esophageal strictures. The clinical trial JCOG1217, a randomized controlled trial of oral steroid administration versus local steroid injection therapy, is currently in progress [57] and the results are awaited. Barrett et al. [58] reviewed that, although oral or locally injected steroids are promising options, no currently available technique is sufficiently effective and devoid of significant safety concerns to recommend its routine use for the prevention of strictures after extensive endoscopic resection.

Shibagaki et al. [59] revealed that TAC-filling was effective to prevent esophageal post-ESD stricture. A similar method reported by Mori et al. [60] involves the combination of steroid gel

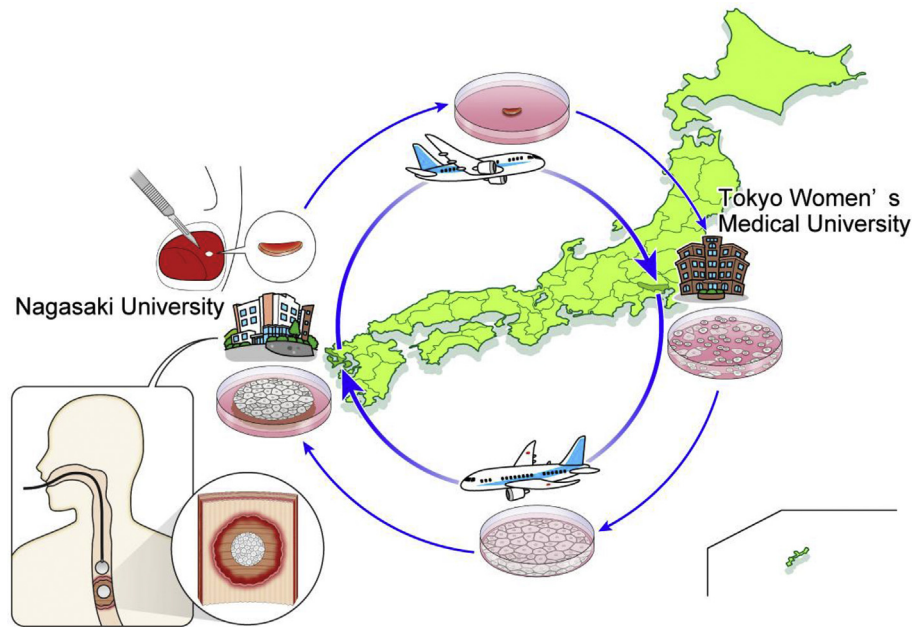


Fig. 4. Clinical research workflow of cell sheets delivered by air. Oral mucosal tissues were removed from patients at Nagasaki University and transported to Tokyo Women's Medical University by air. These tissues were cultured in our laboratory and then transported back to Nagasaki University Hospital for cell sheet transplantation.

applications and balloon dilatations for esophageal stricture prevention. Mechanical strategies for EBD [4], self-expandable metallic stent (SEMS) implantation [41], and biodegradable stent implantation [61] have a potential risk of perforation. A pseudoaneurysm of aberrant right subclavian artery caused by esophageal stent placement because of esophageal stricture after ESD was also reported [62]. Oliveira et al. [63] reviewed the use of preventive therapy after extensive ESD of the esophagus. The meta-analysis reveals that this preventive therapy reduces the risk of stricture and the number of endoscopic dilatations for resolution of stricture without increasing the number of complications.

Cell sheet transplantation is still in the preclinical stage and involves high costs in the present circumstances. However, this method is safe and has great potential for development. Full circumferential mucosal defects are difficult to control without EBD because the transplant cell sheets cannot fully cover the lesion. The desired effect can be expected once the transplantation method is improved.

7.2. From the perspective of esophageal tissue engineering research

Research on esophageal tissue engineering has been conducted for a long time [64–67]. Recently, Arakelian et al. [68] reviewed the two main applications used in tissue engineering for esophageal repair with cell sheet technology without involving the use of scaffolds and the production of biomaterials for full-thickness circumferential esophageal replacement. When full-thickness circumferential esophageal replacement is necessary, a combination of scaffold and cells is the most effective method for inducing regeneration of all layers of the esophagus and recovering its functionality [69].

Badylak et al. [70] reported esophageal preservation in five patients. After endoscopic long segment and *en bloc* circumferential resection of the esophageal mucosa and submucosa was performed, a biologic scaffold (an ECM from decellularized tissues) was transplanted and a stent was placed. However, some of the five cases had some complications (compression, muscular tear, perforation, migration).

In a study using minipigs, Poghosyan et al. [71] showed that the circumferential replacement of the cervical esophagus by a tube-

shaped tissue-engineered substitute under the temporary cover of an esophageal endoprosthesis allowed esophageal nutritional autonomy and tissue remodeling. The tissue-engineered substitute was made of a tubulized acellular matrix (small intestinal submucosa) cellularized with autologous skeletal myoblasts and covered by a human amniotic membrane seeded with autologous oral epithelial cells. Nakase et al. [72] showed that a substitute biomaterial composed of oral keratinocytes and fibroblasts cultured on a human amniotic membrane and sheeted on polyglycolic acid filled with smooth muscle tissue successfully replaced the thoracic esophagus without stent calibration.

Moreover, the first case of successful full-thickness circumferential replacement of the esophagus by tissue engineering approaches in humans was reported in 2016. Dua et al. [73] reported the repair of the esophagus of a 24-year-old man perforated by a metal plate placed in his cervical spine after a severe accident using AlloDerm [74], which is a commercially available dermal ECM, sprayed with autologous platelet-rich plasma to promote stem cell recruitment. The matrix was applied around a non-biological stent, which was then introduced into the defect area to prevent strictures. Three years after surgery, the stent was removed, and the esophagus of this patient had sufficient nutritional autonomy.

Although our studies focused on oral mucosal epithelial cells, research on adipose tissue-derived stromal cells (ADSCs) as a cell source is also progressing [75–77], and this approach is an interesting alternative because large amounts of ADSCs can be easily obtained. Moreover, OMECS transplantation research is expanding to other fields. Kuramoto et al. [78] demonstrated that OMECS transplantation was highly effective in preventing intrauterine adhesions caused by endometrial damage, highlighting a new therapeutic technique to prevent re-adhesion after the treatment of intrauterine adhesions.

7.3. From the perspective of endoscopic transplantation

Pasricha et al. [79] presented an overview of the developments in endoscopy and showed the possibilities for endoscopic intervention in the submucosal space as third space outside the lumen

or the peritoneal cavity, and for repair and prevention of injury of the gut wall by tissue regeneration. Additionally, Legget et al. [80] reviewed the basic principles of tissue engineering with emphasis on the potential role on the gastrointestinal field. Based on these reports and on the progress made in the field, we believe the availability of engineered tissues for endoscopic application will increase with advances in cell culture techniques. For example, Badylak et al. [70] performed endoscopic transplantation using a material called biologic scaffold (an ECM from decellularized tissues), and we performed endoscopic transplantation on a clump called oral mucosal epithelial cell sheets [7].

Hochberger et al. [81] reported a case of successful transplantation of mucosa from the stomach to the esophagus to prevent stricture after circumferential ESD of high-grade intraepithelial neoplasia, suspicion of an early squamous cell cancer in the cervical esophagus. The gastric transplant was attached to the ulcerative site by means of endoscopic clips and a non-covered self-expanding metal stent. After five months, biopsy proved the presence of *Helicobacter pylori*-negative antral mucosa at the transplant site. Liao et al. [82] reported esophageal mucosa autologous transplantation performed in 9 patients. In addition, Chai et al. [83] performed transplantation of an autologous skin graft with stent to post-ESD mucosal defect in 8 patients, and reported that complications, such as perforation, bleeding, wound infection, and stent migration, did not occur. Despite full-circumferential resection, EBD was performed in three of the eight patients. This is a very interesting method and we expect further reports in the future.

Other studies using animal models, amniotic membrane grafts [84], high-density collagen patch [85], autologous flap [86], and carboxymethyl cellulose (CMC) sheets [87], were also published previously. Lua et al. [88] reported the use of CMC sheets in seven patients in a randomized controlled study, in which the incidence rate of post-ESD stricture was 57% (4/7 patients). Moreover, Iizuka et al. [89] described the results of polyglycolic acid (PGA) sheets and demonstrated that these sheets decreased the incidence of esophageal stricture. Likewise, other authors used PGA sheets with fibrin glue [90] or the combination of PGA sheets with fibrin glue and a locoregional steroid injection [91] and proved their efficacy in preventing esophageal strictures. However, transplantation of materials in vivo can always be associated with the risk of infection and inflammation.

8. Future perspectives

Using our methods, tissue-engineered oral mucosal cell sheets can be created before endoscopic transplantation that, when performed immediately after ESD, is conducive to successful engraftment. The submucosal layer is rich in blood vessels for blood supply, and transplantation of the cell sheets is physically beneficial on the dissected surface where fibrin meshes cannot adhere.

In an animal model, Kobayashi et al. [92] reported that even intractable anastomotic stricture occurs at the stage of repair of mucosal laceration after EBD. In this study, transplanting epidermal cell sheets to the laceration site after EBD could reduce the extent of stricture. In our clinical researches, oral mucosal cell sheets could only be made before esophageal ESD, but alternative treatment is possible in cases where the stricture has already occurred. By using epidermal cells instead of oral mucosa, it is possible to collect a larger amount compared to oral mucosa. Thus, our next step will be to replicate these results in clinical research.

9. Conclusion

The tissue-engineered autologous cell sheet technology has been proven to be a highly safe regenerative therapy. Although this

therapeutic approach is still in an early phase of development, we believe that future research will allow it to grow into a therapy that will benefit patients, and we are convinced that this technology has a high potential for application in various areas of the gastrointestinal field.

Declaration of competing interest

All authors declare no conflicts of interest.

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