Article

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Prevention of Esophageal Stricture after Endoscopic Submucosal Dissection with an Autologous Esophageal Epithelial Cell Suspension: An Animal Study

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Background: Severe esophageal stricture decreases patient's quality of life after circumferential endoscopic submucosal dissection (ESD). We aimed to evaluate the efficacy of autologous esophageal epithelial cell suspensions in preventing esophageal stricture after circumferential ESD.

Methods: Twelve male mini-pigs underwent circumferential ESD and were randomized into four groups: G1 (control), G2 (esophageal stent), G3 (autologous esophageal epithelial cell suspension), and G4 (autologous esophageal epithelial cell suspension combined with esophageal stent). Post-ESD status was observed in each group, and endoscopy was performed weekly. Esophageal stents were removed 3 weeks after ESD. The esophageal stricture rates and histologic characteristics were assessed 4 weeks after ESD.

Results: G1 showed the greatest weight loss ($p < 0.05$). Dysphagia scores were not significantly different among the groups. The esophageal mucosal stricture rates were 77.7 $\pm$ 2.9%, 74.2 $\pm$ 1.9%, 69.2 $\pm$ 3.8% and 65.9 $\pm$ 1.9% in G1-4, respectively; with the highest in G1 (G1 vs. G3, $p = 0.005$; G1 vs. G4, $p = 0.001$). The regenerated epithelium lengths were 4.408 $\pm$ 1.980 mm, 8.319 $\pm$ 0.857 mm, 11.801 $\pm$ 2.455 mm and 12.353 $\pm$ 1.111 mm in G1-4, respectively. The lowest degree of re-epithelialization was observed in G1, followed by G2, with the highest degrees in G3 and G4 (G1 vs. G3, $p = 0.001$; G1 vs. G4, $p = 0.000$). The maximum wound fibrosis thicknesses were 2.546 $\pm$ 0.389 mm, 2.136 $\pm$ 0.231 mm, 1.126 $\pm$ 0.211 mm and 1.131 $\pm$ 0.438 mm in G1-4, respectively, with higher degrees in G1 and G2 than in G3 and G4 (G1 vs. G3, $p = 0.001$; G1 vs. G4, $p = 0.001$).

Conclusions: Autologous esophageal epithelial cell suspensions can promote re-epithelialization and reduce fibrosis, thus decreasing esophageal stricture severity after ESD.

Keywords: endoscopic submucosal dissection (ESD); esophageal stricture; cell suspension; esophageal stents; esophageal fibrosis

Introduction

Endoscopic submucosal dissection (ESD) is a widely used technique for the treatment of high-grade intraepithelial neoplasia and early carcinoma of the gastrointestinal mucosa because of its less invasive nature and its high success rate in achieving en bloc resection [1,2]. However, postoperative strictures still frequently occur after esophageal ESD, especially after extensive resection, involving more than 3/4 of the esophageal circumference. The postoperative stricture is as high as 100% in patients who undergo whole circumferential ESD [3,4]. Such strictures can lead to severe dysphagia and a significant decrease in patient quality of life, thus becoming a major problem that needs to be addressed postoperatively. For these reasons, the primary methods currently used to prevent post-ESD esophageal strictures include prophylactic endoscopic balloon dilation, covered self-expanding metal stent placement, administration of systemic steroids, and local steroid injections [5–8]. These measures reduce the incidence of esophageal strictures after ESD to a certain extent, but the results are unsatisfactory when the wounds are relatively large, and some complications such as bleeding, perforation and infection still exist [9]. In recent years, there have been continued investigations on new mechanical dilatation methods, such as the esophageal insertion of self-help inflatable balloons [10] and biodegradable stents [11].

Local administration of medication is also a hotspot of research; examples of such injections include botulinum toxin type A (BTX-A), mitomycin C (MMC), thymosin β4 (Tβ4), and small interfering ribonucleic acid (siRNA) [12–14]. Also, it has been found that the combined application of trauma-protective materials such as polyglycolic acid (PGA) diaphragms has a beneficial effect in the prevention of post-ESD esophageal strictures [15,16]. Nevertheless, the results of these studies have not yet been replicated in clinical settings due to their limited efficacy, lack of validation in large study population samples, and difficulties in obtaining raw materials.

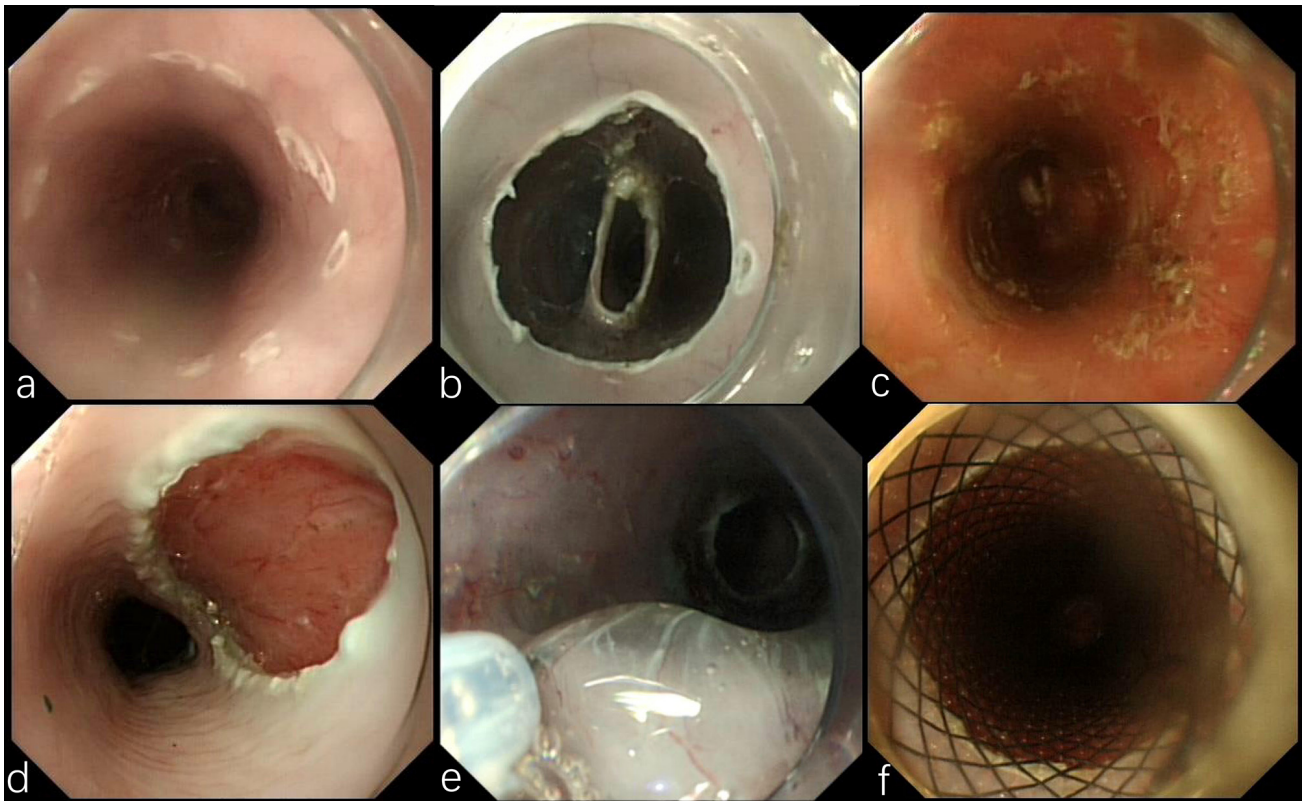

Fig. 1. Esophageal circumferential endoscopic submucosal dissection (ESD) and intervention. (a) Circumferential marking. (b) Establishment of bilateral submucosal tunnels. (c) Separation of submucosal tissue between the two tunnels to complete circumferential ESD. (d) Electrical trap pre-circumscription of a normal esophageal mucosa, approximately 1 cm in diameter, at 30–33 cm depth of the esophagus. (e) Injection of esophageal cell suspension. (f) Placement of esophageal stent.

Regenerative medicine is an emerging and crucial field that addresses the pressing need for preventing esophageal strictures. These studies have focused on extracellular matrix scaffolds, autologous cell suspensions, cell membrane sheets, autologous tissue transplants, etc. [17–19]. Autologous epidermal active cell transplantation regeneration technology is a method of transplanting a cell suspension to the wound surface to regenerate the epithelium uniformly and rapidly and avoid scar formation; this approach has been applied in the treatment of burns and skin diseases and has exhibited excellent efficacy [20,21]. Early studies [22,23] showed that cell suspensions with recognized effectiveness for the prevention of esophageal strictures included skin keratin-forming cells, oral keratin-forming cells, adipose-derived stromal cells, and mesenchymal stem cells. However, the use of autologous esophageal cell suspensions has not been reported. Therefore, we aimed to construct animal models of circumferential esophageal ESD resection in order to explore the effect of autologous esophageal epithelial cell suspensions in the prevention of post-ESD strictures.

Materials and Methods

Animals

A total of 12 healthy male mini-pigs, with an average weight of 25 kg, were provided and housed by the Animal Experiment Center of Changhai Hospital for this experiment. The animals were randomly assigned to the control group (G1), esophageal stent placement group (G2), esophageal epithelial cell suspension injection group (G3), or esophageal epithelial cell suspension injection combined with esophageal stent placement group (G4). Each group contains three animals. We observed their general health conditions and recorded their body weight and feeding habits weekly, before and after surgery. All experimental protocols were approved by the Ethics Committee of the Changhai Hospital, Shanghai, China (Approval number: CHEC2020-241), and followed the guidelines of the International Committee of Animal Care (US National Institutes of Health and European Commission).

Study Design

The pigs were subjected to circumferential ESD and randomized into G1, G2, G3 and G4 in a 1:1 ratio. Endoscopy was performed to check for esophageal strictures

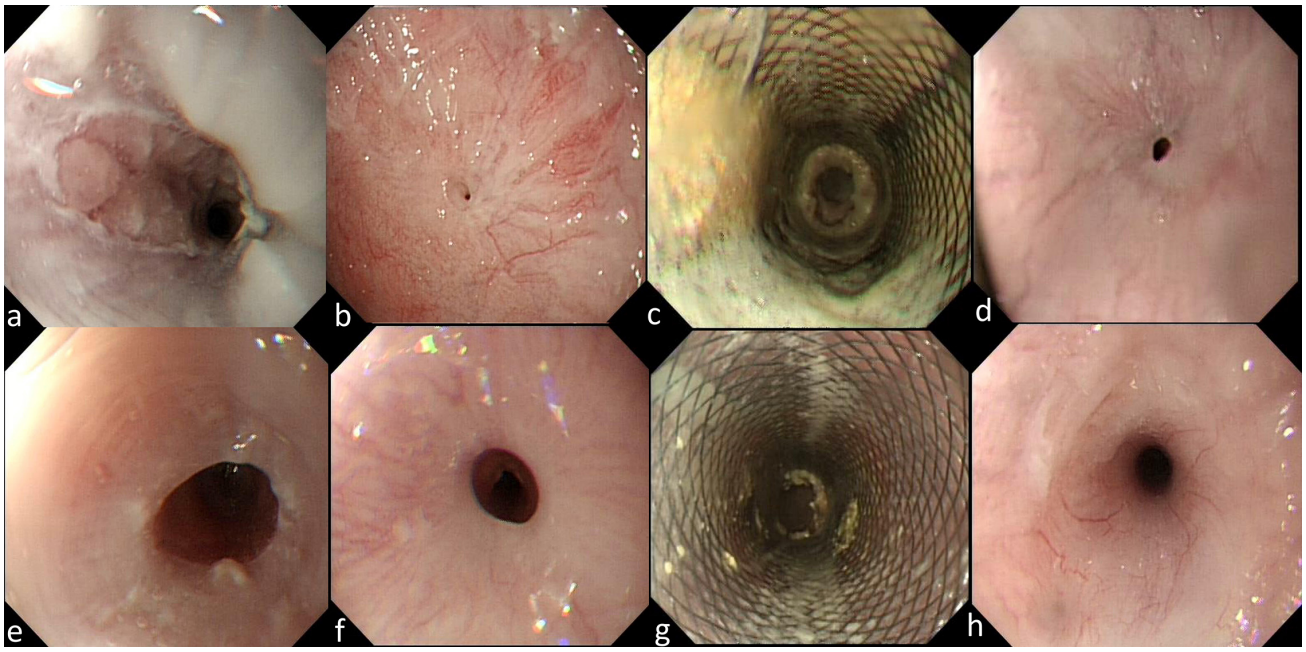

Fig. 2. Endoscopic image of each group two and four weeks after ESD. (a,b) Endoscopic image of the group G1. (c,d) Endoscopic image of the group G2. (e,f) Endoscopic image of the group G3. (g,h) Endoscopic image of the group G4.

and healing status. The esophageal stents were removed 3 weeks post-ESD and the animals were euthanized at the fourth week by isoflurane (CAS 26675-46-7, Shanghai Jinjinle Industry Shanghai, China) excess, according to the protocol regulations. The entire esophagus of the animals was harvested and macroscopic and histological examination was conducted independently by two pathologists. A third pathologist reviewed the results in case of disagreements.

ESD Procedures

Endotracheal intubation was performed for general anesthesia, which was introduced with 3–4% of isoflurane and maintained with 0.5–2.0% of the same drug. The equipment used for ESD included a carbon dioxide (CO_2) insufflator (UCR, Olympus, Tokyo, Japan), a forward-viewing endoscope (GIFQ260J, Olympus) with a transparent distal cap attachment (D-201–11, 804, Olympus) and an injection needle (NM-200L-0423, Olympus). This study adopted the "double-tunnel method", and a submucosal dissection was performed using either a dual knife (KD-650L, Olympus), an insulation-tipped-2 (IT-2) knife (KD-611L, Olympus) or a hybrid knife (ERBE, Tübingen, Germany). During the ESD procedure, 0.9% saline was premixed with indigo carmine, and 1:10,000 epinephrine was injected into the submucosal layer to lift the surrounding mucosa. Minor bleeding points were addressed by electrocoagulation while large bleeding points were managed with hemostatic clips until the esophageal mucosa was completely removed at a depth of 35–40 cm (Fig. 1a–c).

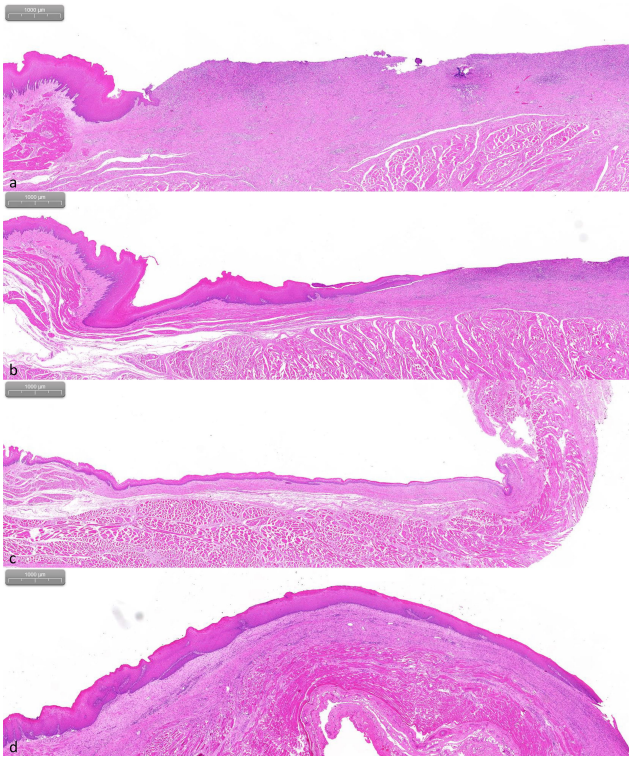

Fig. 3. The length and site of re-epithelization at four weeks after surgery (hematoxylin-eosin (HE) staining, 40×). Images show greater epithelial tissue formation in G3 and G4. (a) The length of re-epithelization of G1. (b) The length of re-epithelization of G2. (c) The length of re-epithelization of G3. (d) The length of re-epithelization of G4.

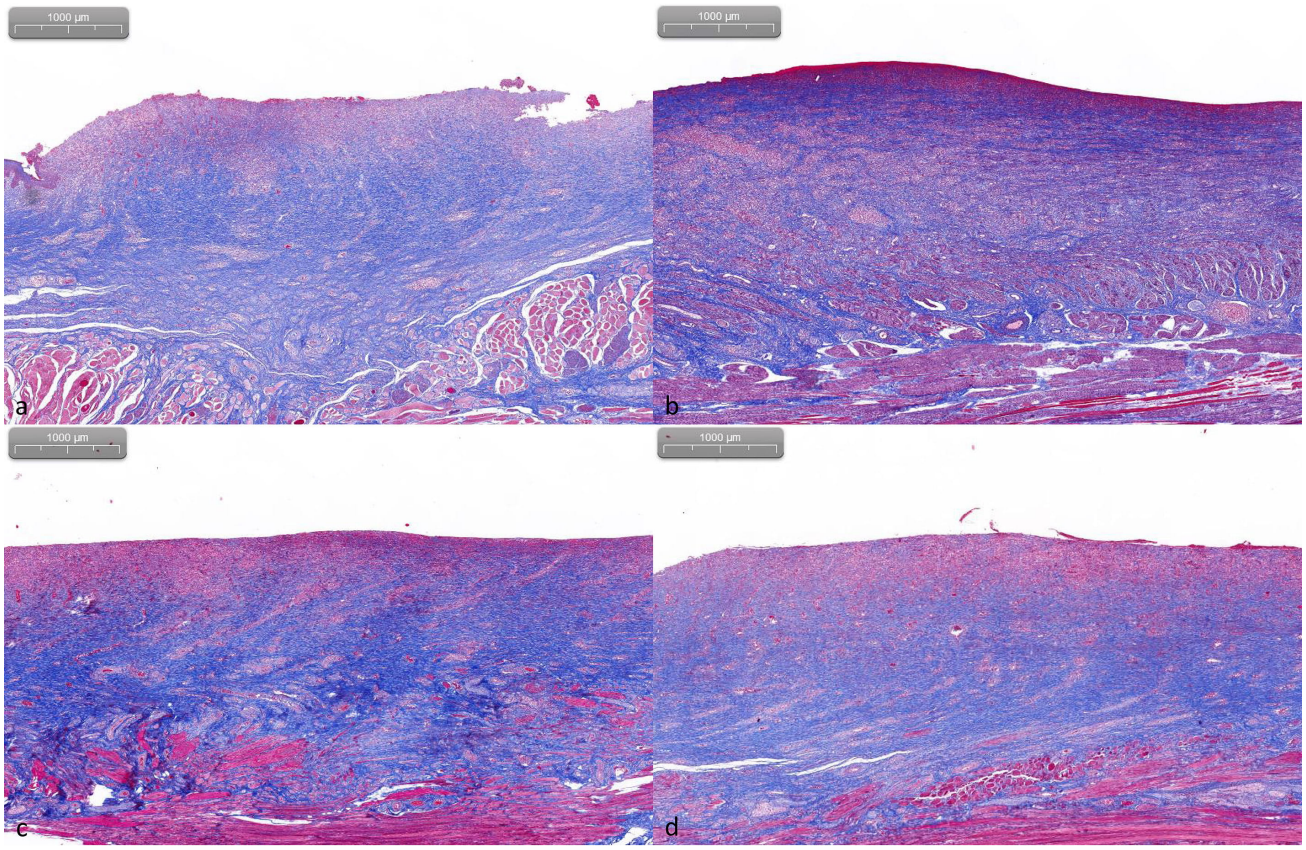

Fig. 4. Maximum thickness of submucosal fibrosis in esophageal wounds four weeks after surgery (Masson's trichrome staining, 40×). Images show less fibrosis in the transplanted animals from G3 and G4 groups. (a) Maximum thickness of submucosal fibrosis of G1. (b) Maximum thickness of submucosal fibrosis of G2. (c) Maximum thickness of submucosal fibrosis of G3. (d) Maximum thickness of submucosal fibrosis of G4.

Preparation of Autologous Esophageal Epithelial Cell Suspension

In groups G3 and G4, a normal mucosa of approximately 1 cm in diameter was circumscribed at 30–33 cm esophagus depth using an electrical trap before the ESD procedure (Fig. 1d). Tiny blood vessels and extra tissues were removed from the esophageal mucosa. Then, the tissue was immersed in 0.05% chlorhexidine acetate solution for 3 minutes and rinsed during 20 min with a 4 °C PBS solution (Gibco BRL, Grand Island, NY, USA) containing double antibiotics, 100 IU/mL penicillin and 100 µg/mL streptomycin. Digestion of the esophageal mucosal tissue was performed for 2 hours with dispase-II and trypsin enzymes (Gibco BRL), followed by the addition of fetal bovine serum (FBS, Gibco BRL) to terminate the digestion and a filtering step. Finally, the suspensions were centrifuged, the supernatant was removed and the cells obtained were washed with PBS. Repeat the centrifugation and washing steps three times. Five ml of epithelial cell medium 2 was added to obtain the final cell suspension.

Endoscopic Cell Suspension Injection and Esophageal Stent Placement Procedure

After esophageal circumferential ESD was performed and hemostasis was achieved, a covered metal stent was placed to G2 animals (Fig. 1f) while the ones of G3 and G4 were immediately injected with cell suspensions on the surgical site. Four points were chosen in each of the distal (40.0 cm), proximal (35.0 cm) and medial (37.5 cm) sides of the surgical site where 0.4 mL of esophageal epithelial cell suspension was injected into the residual submucosa using an injection needle (NM-200L-0423, Olympus) (Fig. 1e). After the injection was completed, the same esophageal stent as in G2 was placed in G4, the wound was checked for bleeding and perforation, and the operation was finished.

Postoperative Management and Follow-Up

All the animals were fasted on the first day after ESD and gradually transitioned to a normal pelleted diet. Proton pump inhibitors, antibiotics, and hemostatic agents were routinely used to promote rehabilitation. Endoscopy was performed weekly to assess esophageal patency and monitor the timing of stricture formation. The dysphagia score

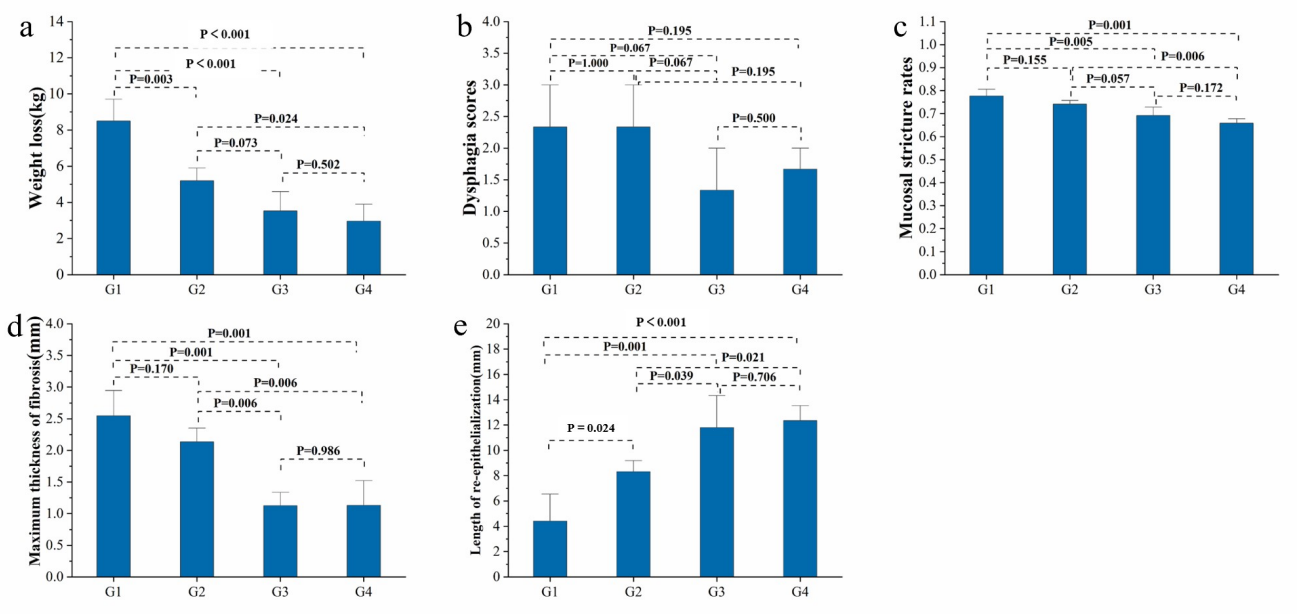

Fig. 5. Multiple comparisons between groups. (a) A comparison of weight loss. (b) A comparison of dysphagia scores. (c) A comparison of mucosal stricture rates. (d) A comparison of mucosal maximum thickness of fibrosis. (e) A comparison of length of re-epithelialization. Groups: G1, control; G2, esophageal stent; G3, autologous esophageal epithelial cell suspension; G4, autologous esophageal epithelial cell suspension combined with esophageal stent.

was evaluated as follows: 0 = no dysphagia, 1 = dysphagia to semi-liquids, 2 = dysphagia to liquids, and 3 = complete dysphagia. The stricture was defined as a <9.8 mm opening that did not allow the passage of a standard endoscope (GIF H260, Olympus Medical Systems, Tokyo, Japan) through the stenotic area.

Endpoints

The esophageal stents were removed at 3 weeks postoperatively, the pigs were euthanized at 4 weeks postoperatively. In the final step, histopathological examination was carried out to measure the stricture rate [24] using the formula [1 – Lmax/50% (Lno + Lna)] × 100, where Lmax represents the length of the short axis at the site of the narrowest regenerative mucosa, and Lno and Lna are the lengths of the short axis at the normal mucosa on the cranial and caudal sides, respectively. A histopathological examination was performed to address two specific aspects: Wound healing, re-epithelialization was observed through hematoxylin-eosin (HE) staining; and wound fibrosis, the maximum vertical thickness of the fibrous layer of the esophageal wound was estimated through Masson staining. The primary endpoints we observed were stricture rate, wound healing, and fibrosis after ESD, and the secondary endpoints were body weight and dysphagia score.

Statistical Analysis

Statistical analysis was performed with SPSS statistical software (26.0 IBM Corp., Armonk, NY, USA). The data of each group were expressed as percentages or as mean ± standard deviation (mean ± SD). Fisher's exact test was used to analyze categorical data while the one-way analysis of variance (ANOVA) test and *T* test were used to analyze continuous data. We applied a Bonferroni correction as a post-hoc test method to account for multiple comparisons. p values < 0.05 were considered statistically significant.

Results

All 12 pigs in this study safely underwent circumferential ESD. The intervention was performed based on the assigned grouping. The body weight, feeding habits, endoscopic manifestations, gross specimen stricture, wound healing and fibrosis were compared between the groups to evaluate the effectiveness of esophageal stents and autologous esophageal epithelial cell suspensions in preventing stricture after circumferential ESD. Four weeks after ESD, the weight loss of G1 group was 8.5 ± 1.2 kg, which was greater than that in the other three groups [5.2 ± 0.7 kg (G2), 3.5 ± 1.2 kg (G3) and 3.0 ± 0.8 kg (G4), $p < 0.05$]. On the 28th day after ESD, the dysphagia scores of G1 (2.3 ± 0.6), G2 (2.3 ± 0.6), G3 (1.3 ± 0.6), and G4 (1.6 ± 0.6) showed no statistical difference. On the endoscopic examinations, at 2 weeks after ESD, three animals in group G1 and two in group G3 showed esophageal strictures; the gastroscope could pass normally through the esophagi in groups G2 and G4 (Fig. 2a,c,e,g). The esophageal stent in groups G2 and G4 was removed at three weeks postoperatively. At four weeks esophageal strictures that made

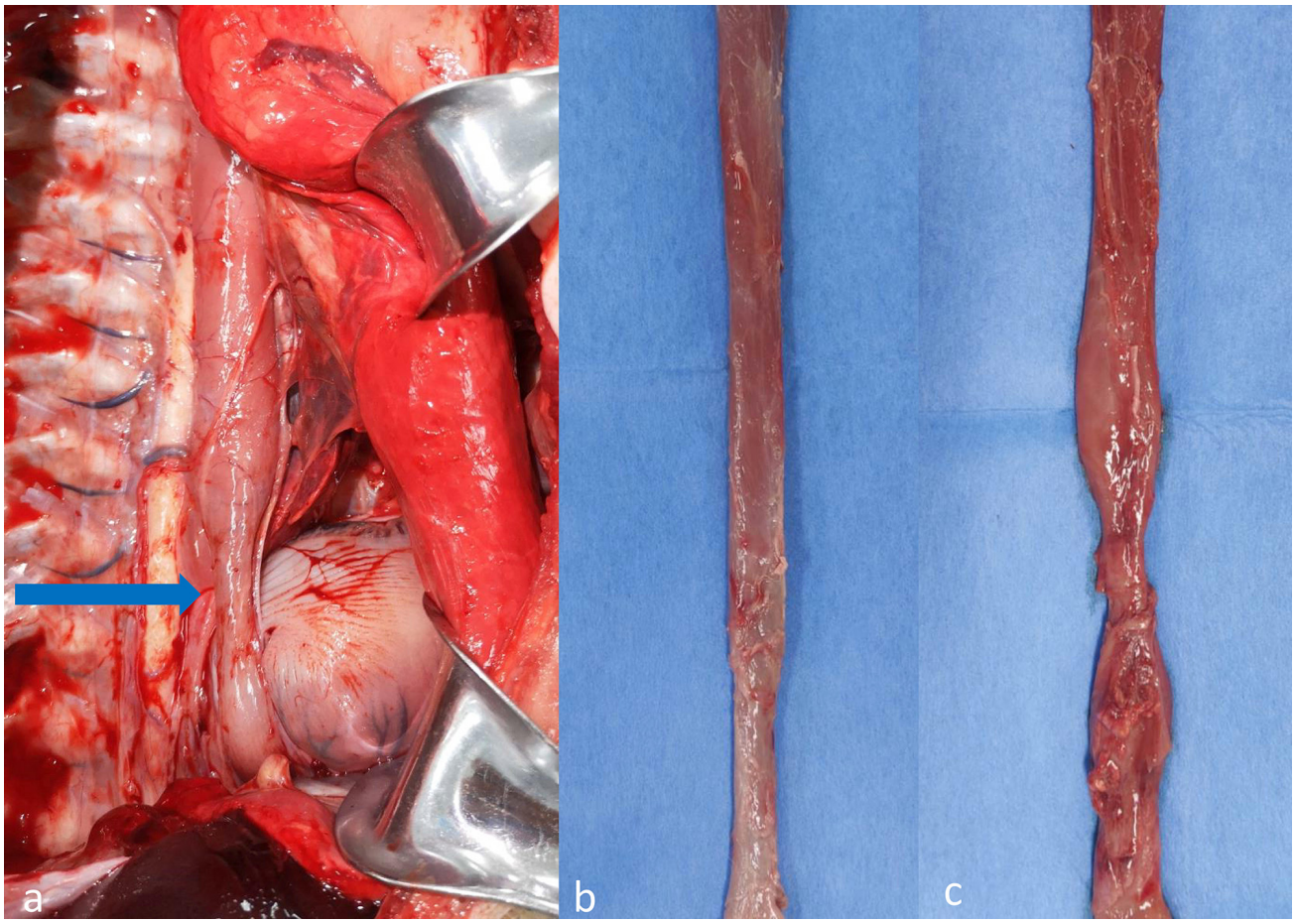

Fig. 6. Gross esophageal specimen. (a) Animal anatomy, blue arrow shows stricture esophagus. (b) Non-stent group, the esophageal adventitia is smooth. (c) Stent group, the esophageal adventitia is irregular, there is connective tissue adhesion.

it difficult for a gastroscope to pass were observed in all pigs of all groups (Fig. 2b, d, f, h). The degree of stricture in the animals of group G2 was comparable to those in G1. The supra-traumatic incision was pinhole-like shape. In groups G3 and G4, closed biopsy forceps could be passed through the incision, and the degree of esophageal stricture was lower than that in the other two groups. We found that the esophageal mucosal stricture rates of the four groups were 77.7 ± 2.9% (G1), 74.2 ± 1.9% (G2), 69.2 ± 3.8% (G3) and 65.9 ± 1.9% (G4). This rate in G1 was the highest (G1 vs. G3, $p = 0.005$; G1 vs. G4, $p = 0.001$).

The total lengths of re-epithelialization in each group were 4.408 ± 1.980 mm (G1), 8.319 ± 0.857 mm (G2), 11.801 ± 2.455 mm (G3) and 12.353 ± 1.111 mm (G4). There was no significant difference between G3 and G4 ($p = 0.706$) (Fig. 3a–d). Pairwise comparisons among the groups showed that the G1 had the lowest degree of re-epithelialization, followed by G2, and wound healing was the best in G3 and G4. The maximum fibrosis thickness in each group were 2.546 ± 0.389 mm (G1), 2.136 ± 0.231 mm (G2), 1.126 ± 0.211 mm (G3), and 1.131 ± 0.438 mm (G4) (Fig. 4a–d). There were no significant differences between G1 and G2 or between G3 and G4 (G1 vs. G2, $p = 0.170$; G3 vs. G4, $p = 0.986$). In pairwise comparisons among the other groups, $p < 0.05$, the differences between groups were statistically significant. The degree of wound fibrosis in G1 and G2 was significantly higher. The various comparisons between groups are summarized in Fig. 5a–e.

We found that the outer membrane of the surgical segment from the esophagus was adhered to the thoracic tissue in the animals from the groups with esophageal stent placement (G2 and G4), and a large amount of connective tissue hyperplasia was visible, while the outer membrane of the surgical segment of the esophagus was smooth in G1 and G3 (Fig. 6a–c). The stricture was visible on the oral and anal sides of the specimen in group G4, unlike in the other groups, where the most severe stenosis occurred only in the middle esophagus (Fig. 7a–d).

Discussions

Early explorations of cell suspensions were limited to animal studies on endoscopic mucosal resection (EMR), in which efficacy was seen. However, oral cell suspension is less effective for whole circumferential ESD [22,23,25]. In recent years, both domestic and international researchers

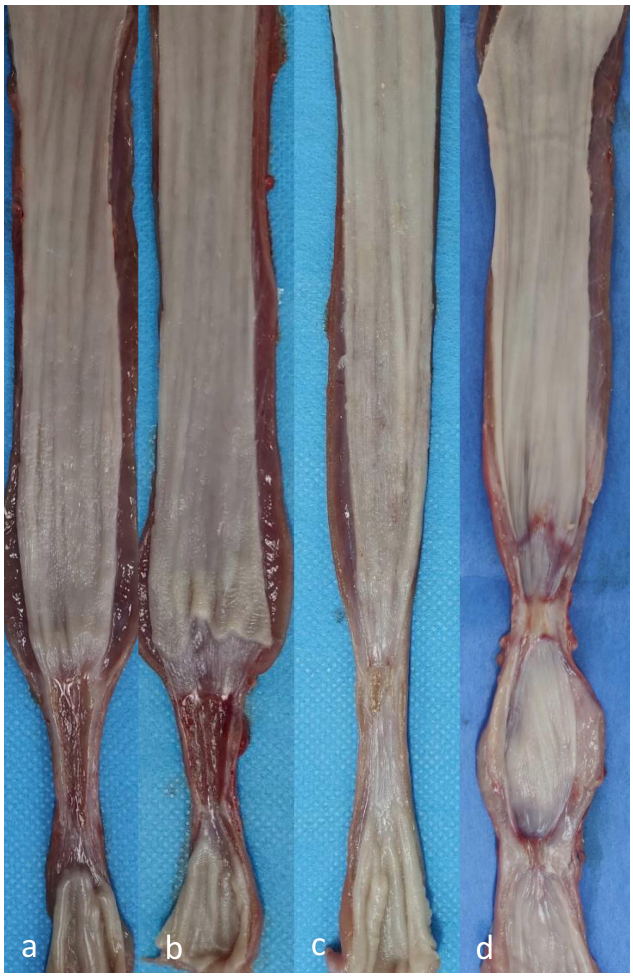

Fig. 7. Image of gross specimen of esophageal wound stricture. (a–d) Image of gross specimen of esophageal wound stricture four weeks post-surgery of animals from G. The stricture was visible on the oral and anal sides of the specimen.

have attempted to prevent esophageal stricture by autologous mucosal transplantation to alleviate esophageal strictures [19,26,27]. In 2018, autogenous esophageal mucosa transplantation was conducted on nine patients with esophageal cancer who had undergone peri-circumferential ESD. Subsequently, postoperative strictures developed in eight of these patients, with an average of 2.7 balloon expansions performed under endoscopy; which represents half the number reported in other studies [28]. The effect of cell suspensions and esophageal mucosal tissue transplantation reported on the prevention of esophageal stricture provided the idea for this study. To our understanding, there is no previous documentation on the prevention of esophageal stricture with cell suspensions derived from the esophagus

In this study, endoscopic examinations showed that after stent removal at the 3rd week, different degrees of esophageal stricture were observed in animals from both G2 and G4 groups, further confirming the effectiveness of esophageal stents in alleviating stricture. General health characteristics and histological findings of the specimens were observed and recorded. The stricture rate was higher in animals from G1 and G2; the ones from G3 and G4 had a better degree of wound re-epithelialization and lower degree of wound fibrosis (p values < 0.05). Therefore, the approaches adopted in the G3 and G4 turn out to be more effective in the alleviation of esophageal stenosis, promoting wound re-epithelialization and diminishing wound fibrosis.

In previous animal studies, cell suspensions played a certain role in the prevention of esophageal strictures by secreting humoral factors, inducing cell differentiation, inhibiting the inflammatory response and promoting angiogenesis [29]. The limitations of such studies consist in the need to culture these cells *in vitro* for an approximate period of two weeks after they were obtained. Due to the strict conditions for cell culture and transportation, these cells could not effectively prevent esophageal stenosis after whole circumferential ESD was performed. In our study, the autologous esophageal epithelium was used to prepare cell suspensions that were directly transplanted into the wound with the advantages of stable cell number, short preparation time and viable cells used. The time required to obtain the number of cells employed (1.5×10^6 to 5.0×10^6) was no longer than 2.5 hours. We confirmed the activity of the esophageal epithelial cells by *in vitro* culture. In addition, the use of autologous esophageal epithelium cell eliminated the risk of rejection reactions, as observed in this study. We hypothesize that the cells and extracellular matrix in the esophageal epithelial cell suspension constitute a suitable microenvironment for the growth of esophageal epithelial cells, inhibit the local inflammatory response, reduce wound fibrosis, thus playing a role in alleviating esophageal stricture.

Compared to animals that did not receive esophageal stents, those that underwent esophageal stent placement had extensive adhesions in the outer membrane of the esophagus and thoracic tissue. This kind of adhesion has not been reported before, according to our sources. Its effect on the esophageal mechanical movement and thoracic surgery should be further explored. The rapid occurrence of severe esophageal stricture after stent removal three weeks post-surgery indicates that early removal of the stent may not be an appropriate option. In addition, a stricture ring was visible on both the oral and anal sides of the specimens in the G4 group. Besides, the re-epithelialization process is considered to have occurred in the middle part of the esophagus from the wound surface. This may be due to the combined effect of the friction between the esophageal stent and the re-epithelialization promoted by the cell suspension.

Nonetheless, this study also has certain limitations. First, the sample size is small, and some results only show trends but no significant differences. Second, since it constitutes an autologous epidermal activated cell transplantation regeneration technique, the source of the cell suspension was selected as an area of esophageal epithelium measuring 1 cm diameter, and there was no comparative study

in the donor's area. This area should be adjusted subsequently in a comparative study in order to determine the appropriate ratio between the area of esophageal wound and the one of the donor. Finally, we did not investigate the presence of inflammatory factors in the esophagus and serum, which is important because the specific mechanism of action of the cell suspension in preventing esophageal strictures is unknown. Subsequent studies on the combination of cell suspensions with PGA diaphragms and Extracellular matrix (ECM) scaffolds can also be carried out to help achieve a breakthrough in the prevention of postoperative esophageal strictures after circumferential ESD.

Conclusions

Autologous esophageal epithelial cell suspensions can be elaborated in a simple way. They promote tissue healing and reduce wound fibrosis, thus decreasing the degree of esophageal stricture. Prophylactic esophageal stent placement may delay the onset of esophageal stricture. Premature stent removal may not provide sustained benefits to the patient. The appropriate timing of stent removal needs to be explored. This study provides a new strategy for the clinical prevention of post-ESD esophageal strictures and represents a useful exploration of this technique.

Abbreviations

ESD, endoscopic submucosal dissection; BTX-A, botulinum toxin type A; MMC, mitomycin C; Tβ4, thymosin β4; siRNA, small interfering ribonucleic acid; PGA, polyglycolic acid; EMR, endoscopic mucosal resection; FBS, fetal bovine serum; SD, standard deviation.

Availability of Data and Materials

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Author Contributions

SCS, JC and TTP designed the study, and performed the study. SCS and TTP collected the data and drafted the manuscript. YFW and JC performed the study, analyzed data, and revised the article critically for important intellectual content. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All experimental protocols were approved by the Ethics Committee of the Changhai Hospital, Shanghai, China (Approval number: CHEC2020-241), and followed the guidelines of the International Committee of Animal Care (US National Institutes of Health and European Commission).

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Han C, Sun Y. Efficacy and safety of endoscopic submucosal dissection versus endoscopic mucosal resection for superficial esophageal carcinoma: a systematic review and meta-analysis. Diseases of the Esophagus: Official Journal of the International Society for Diseases of the Esophagus. 2021; 34: doaa081.

[2] Oza K, Peesay T, Greenspun B, Carroll JE, Shafa S, Zeck JC, *et al*. Long-term outcomes of endoscopic mucosal resection for early-stage esophageal adenocarcinoma. Surgical Endoscopy. 2022; 36: 5136–5143.

[3] Shi Q, Ju H, Yao LQ, Zhou PH, Xu MD, Chen T, *et al*. Risk factors for postoperative stricture after endoscopic submucosal dissection for superficial esophageal carcinoma. Endoscopy. 2014; 46: 640–644.

[4] Miwata T, Oka S, Tanaka S, Kagemoto K, Sanomura Y, Urabe Y, *et al*. Risk factors for esophageal stenosis after entire circumferential endoscopic submucosal dissection for superficial esophageal squamous cell carcinoma. Surgical Endoscopy. 2016; 30: 4049–4056.

[5] Zhang BZ, Zhang Y, Wang YD, Liao Y, Zhang JJ, Wu YF, *et al*. Stent placement to prevent strictures after esophageal endoscopic submucosal dissection: a systematic review and meta-analysis. Diseases of the Esophagus: Official Journal of the International Society for Diseases of the Esophagus. 2021; 34: doab015.

[6] Abe S, Iyer PG, Oda I, Kanai N, Saito Y. Approaches for stricture prevention after esophageal endoscopic resection. Gastrointestinal Endoscopy. 2017; 86: 779–791.

[7] Pih GY, Kim DH, Na HK, Ahn JY, Lee JH, Jung KW, *et al*. Comparison of the Efficacy and Safety of Endoscopic Incisional Therapy and Balloon Dilatation for Esophageal Anastomotic Stricture. Journal of Gastrointestinal Surgery: Official Journal of the Society for Surgery of the Alimentary Tract. 2021; 25: 1690–1695.

[8] Xiang J, Linghu E, Li L, Zou J, Wang X, Chai N. Utility of radial incision and cutting with steroid injection for refractory stricture after endoscopic submucosal dissection for large superficial esophageal squamous cell carcinoma. Surgical Endoscopy. 2021; 35: 6930–6937.

[9] Kadota T, Yoda Y, Hori K, Shinmura K, Oono Y, Ikematsu H, *et al*. Prophylactic steroid administration against strictures is not

enough for mucosal defects involving the entire circumference of the esophageal lumen after esophageal endoscopic submucosal dissection (ESD). Esophagus: Official Journal of the Japan Esophageal Society. 2020; 17: 440–447.

[10] Li L, Wang Z, Wang N, Zhang B, Zou J, Xiang J, *et al*. Self-help inflatable balloon versus autologous skin-grafting surgery for preventing esophageal stricture after complete circular endoscopic submucosal dissection: a propensity score matching analysis. Surgical Endoscopy. 2023; 37: 3710–3719.

[11] Yang K, Cao J, Yuan TW, Zhu YQ, Zhou B, Cheng YS. Silicone-covered biodegradable magnesium stent for treating benign esophageal stricture in a rabbit model. World Journal of Gastroenterology. 2019; 25: 3207–3217.

[12] Zhou X, Chen H, Chen M, Ding C, Zhang G, Si X. Comparison of endoscopic injection of botulinum toxin and steroids immediately after endoscopic submucosal dissection to prevent esophageal stricture: a prospective cohort study. Journal of Cancer. 2021; 12: 5789–5796.

[13] Wang H, Shuai Q, Tang J, Long D, Xu C, Liu F, *et al*. Local Thymosin β4 Gel Injection Prevents Esophageal Stricture after Circumferential Endoscopic Submucosal Dissection in a Porcine Model. Digestive Diseases (Basel, Switzerland). 2019; 37: 87–92.

[14] Sato H, Sagara S, Nakajima N, Akimoto T, Suzuki K, Yoneyama H, *et al*. Prevention of esophageal stricture after endoscopic submucosal dissection using RNA-based silencing of carbohydrate sulfotransferase 15 in a porcine model. Endoscopy. 2017; 49: 491–497.

[15] Bao Y, Li Z, Li Y, Chen T, Cheng Y, Xu M. Recent Advances of Biomedical Materials for Prevention of Post-ESD Esophageal Stricture. Frontiers in Bioengineering and Biotechnology. 2021; 9: 792929.

[16] Nishimura Y, Ono M, Okubo N, Sone T, Higashino M, Matsumoto S, *et al*. Application of polyglycolic acid sheets and basic fibroblast growth factor to prevent esophageal stricture after endoscopic submucosal dissection in pigs. Journal of Gastroenterology. 2023. (online ahead of print)

[17] Zhou XB, Li SW, He SQ, Xu SJ, Cai Y, Xu SW, *et al*. Transplantation of acellularized dermis matrix (ADM) plus fully covered metal stent to prevent stricture after circumferential endoscopic submucosal dissection of early esophageal cancer (with video). Regenerative Therapy. 2021; 18: 441–446.

[18] Zhou XB, Xu SW, Ye LP, Mao XL, Chen YH, Wu JF, *et al*. Progress of esophageal stricture prevention after endoscopic submucosal dissection by regenerative medicine and tissue engineering. Regenerative Therapy. 2021; 17: 51–60.

[19] Liu Y, Li Z, Dou L, Zhang Y, He S, Zhu J, *et al*. Autologous esophageal mucosa with polyglycolic acid transplantation and temporary stent implantation can prevent stenosis after circumferential endoscopic submucosal dissection. Annals of Translational Medicine. 2021; 9: 546.

[20] Ren J, Liu J, Yu N, Zhang W, Qian H, Liu Z, *et al*. The use of noncultured regenerative epithelial suspension for improving skin color and scars: A report of 8 cases and review of the literature. Journal of Cosmetic Dermatology. 2019; 18: 1487–1494.

[21] Chen Q, Yu N, Liu Z, Zhang W, Long F, Zeng A, *et al*. The Clinical Efficacy of ReCell® Autologous Cell Regeneration Techniques Combined with Dermabrasion Treatment in Acne Scars. Aesthetic Plastic Surgery. 2020; 44: 535–542.

[22] Zuercher BF, George M, Escher A, Piotet E, Ikonomidis C, Andrejevic SB, *et al*. Stricture prevention after extended circumferential endoscopic mucosal resection by injecting autologous keratinocytes in the sheep esophagus. Surgical Endoscopy. 2013; 27: 1022–1028.

[23] Juhásová J, Klíma J, Martínek J, Walterová B, Dolezel R, Vacková Z, *et al*. Two types of autologous cells in stricture development prevention after complete circular endoscopic dissection in minipig. Rozhledy V Chirurgii: Mesicnik Ceskoslovenske Chirurgicke Spolecnosti. 2019; 98: 497–508.

[24] Tang J, Ye S, Ji X, Liu F, Li Z. Deployment of carboxymethyl cellulose sheets to prevent esophageal stricture after full circumferential endoscopic submucosal dissection: A porcine model. Digestive Endoscopy: Official Journal of the Japan Gastroenterological Endoscopy Society. 2018; 30: 608–615.

[25] Honda M, Hori Y, Nakada A, Uji M, Nishizawa Y, Yamamoto K, *et al*. Use of adipose tissue-derived stromal cells for prevention of esophageal stricture after circumferential EMR in a canine model. Gastrointestinal Endoscopy. 2011; 73: 777–784.

[26] Hochberger J, Koehler P, Wedi E, Gluer S, Rothstein RI, Niemann H, *et al*. Transplantation of mucosa from stomach to esophagus to prevent stricture after circumferential endoscopic submucosal dissection of early squamous cell. Gastroenterology. 2014; 146: 906–909.

[27] Zou J, Chai N, Linghu E, Li H, Chai M, Shi Y, *et al*. Autologous skin-grafting surgery to prevent esophageal stenosis after complete circular endoscopic submucosal tunnel dissection: a case-matched controlled study. Surgical Endoscopy. 2021; 35: 5962–5970.

[28] Liao Z, Liao G, Yang X, Peng X, Zhang X, Xie X, *et al*. Transplantation of autologous esophageal mucosa to prevent stricture after circumferential endoscopic submucosal dissection of early esophageal cancer (with video). Gastrointestinal Endoscopy. 2018; 88: 543–546.

[29] Gao Y, Jin SZ. Strategies for treating oesophageal diseases with stem cells. World Journal of Stem Cells. 2020; 12: 488–499.