

Tissue shielding with polyglycolic acid sheets and fibrin glue on ulcers induced by endoscopic submucosal dissection in a porcine model

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Bibliography

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Background and study aims: The safety and efficacy of the application of polyglycolic acid (PGA) sheets with fibrin glue to ulcers induced by endoscopic submucosal dissection (ESD) have not been established in the treatment of lesions of the gastrointestinal tract, in which the influence of digestive fluid and peristalsis may affect treatment, and there may also be a risk of infection. The aims of this study were to evaluate the healing process of ESD-induced ulcers in animals treated with the application of a PGA sheet with fibrin glue and to verify experimentally the safety of this treatment procedure.

Materials and methods: Gastric ESD was performed in nine pigs under general anesthesia. Two ulcer sites were prepared in each pig; one ulcer was treated by applying a PGA sheet with fibrin glue (treated ulcer site), while the other ulcer

was left untreated (control ulcer site). Three pigs were euthanized at week 1, three at week 4, and three at week 8 after ESD, and the ulcer sites were macroscopically and histopathologically evaluated.

Results: Of the nine treated ulcer sites, seven ulcer sites, to which a PGA sheet had been applied without exposure to the mucosal fluid, showed no peeling of the sheet despite the influence of peristalsis and gastric acid. Histopathologic examination revealed abundant, newly formed blood vessels in the treated ulcers and good granulation. In the treated ulcers, no excessive inflammation, necrosis, or infection was observed.

Conclusions: Our animal study experimentally demonstrated that this treatment technique can be safely applied to ESD-induced ulcers.

Introduction

Endoscopic submucosal dissection (ESD) was developed as a treatment for early-stage gastric cancer with a low risk of lymph node metastasis, and it has been widely applied as a useful treatment technique with which larger lesions can be dissected en bloc [1]. However, in addition to the stomach, ESD is now applied in the esophagus, large intestine, and duodenum, and as the number of applications in difficult-to-treat cases has grown, so has the number of reports of serious procedural complications. Therefore, the reduction of these complications has become a major issue [2–5].

In recent years, a treatment technique in which polyglycolic acid (PGA) sheets (Neoveil 015G; Gunze Ltd., Kyoto, Japan) (● Fig. 1) are applied to ESD-induced ulcers with fibrin glue (Bolheal; Kaketsuken, Kumamoto-shi, Japan) (● Video 1) has exhibited great potential clinically for preventing procedural complications after ESD, such as postoperative bleeding, delayed perforation, and ste-

nosis [6–8]. The sheets consist of soft, elastic, nonwoven fabric made of PGA, and they are hydrolyzed in vivo, after which they undergo degradation and absorption within approximately 15 weeks. The sheets are hydrophilic, and their coating effect is known to be enhanced if they are administered together with fibrin glue [8]. This combination therapy of PGA sheets and fibrin glue has been widely used in multiple surgical fields, and studies have reported its safety and efficacy [9–12]. However, there are multiple issues concerning the use of this treatment in the gastrointestinal tract, including the delivery of the PGA sheet via endoscopy, whether the adhesion of the applied PGA sheet can be maintained in the presence of peristalsis and digestive fluid, and whether the adherent PGA sheet induces adverse events, such as excessive inflammation and infection.

The aims of this study were to evaluate the healing process of ESD-induced ulcers in animals treated with the application of a PGA sheet with fibrin glue and to verify the safety of this treatment procedure.

License terms



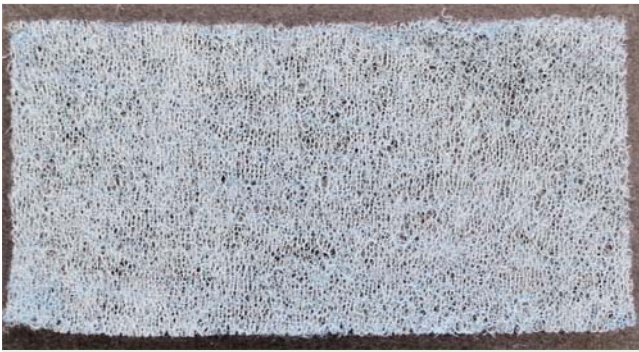


Fig. 1 The absorptive tissue reinforcement sheet, composed of polyglycolic acid, that was used in the study.

Materials and methods

Nine male pigs (strain, LWD; age, 2–3 months; weight, 20–30 kg) were used in this study. The animals were fasted from the day before ESD. One 10-mg tablet of rabeprazole sodium (Rabeprazole Na Tablet 10 mg [OHARA]; Daiichi-Sankyo Espha Co., Ltd., Tokyo, Japan) was orally administered on the day before ESD. On the day of ESD, 20 mg of omeprazole (Omeprazole Injection 20 mg [Nichi-iko]; Nichi-Iko Pharmaceutical Co., Ltd., Tokyo, Japan) was intravenously administered.

After endotracheal intubation and with the animal under general anesthesia, an upper gastrointestinal endoscope for veterinary use (VQ-8143B; Olympus, Tokyo, Japan) was inserted through the overtube into the stomach. Two ulcers were induced at the gastric fundus in each pig. A glycerin solution stained with indigo carmine (Glyceol Injection; Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) was injected into the submucosa of the planned ulcer site. The mucosa was incised circumferentially with an electric knife (IT knife nano, KD-612Q, Olympus, or Dual Knife, KD-650Q, Olympus). Submucosal dissection was performed subsequently at the incised site. Dissection was continued until resection of the mucosa with a snare (SD-17U-1, Olympus) became possible, at which point snaring was performed to shorten the procedure time and reduce the burden on the animal. The snaring ensured that all ulcers were uniform. In any animal with exposed blood vessels on the ulcer floor, coagulation was performed with a Coagrasper (FD-411QR, Olympus).

Delivery of polyglycolic acid sheet and affixation method

Of the two prepared ulcers, one was treated by applying a PGA sheet with fibrin glue (treated ulcer site), and the other was left untreated (control ulcer site). The PGA sheets were cut to a slightly size smaller than that of the ulcer floor to ensure that the PGA sheet did not extend to the mucosal epithelium. The PGA sheets were soaked in a fibrinogen solution before application and maintained in a storage sheet (Fig. 2). A hemostatic

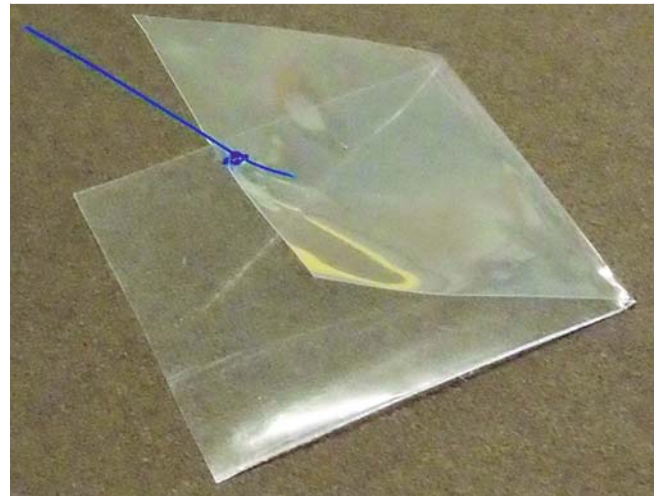


Fig. 2 An example of the storage sheet that was used. Using a storage sheet for the polyglycolic acid (PGA) sheet made it possible to protect the PGA sheet from mucus during delivery. To facilitate the delivery and recovery of the PGA sheet, a surgical suture (4–0 Prolene) was tied to one of its ends.

forceps was used to hold the storage sheet containing the PGA sheet, and the scope was inserted through the overtube into the stomach. The storage sheet was placed near the ulcer sites, and the PGA sheet was delivered to the ulcer floor with a forceps. An endoscopic catheter tube (Fine Jet; TOP Corporation, Tokyo, Japan) was used to place fibrinogen onto the PGA sheet. Then, thrombin solution was placed to cover the ulcer site and complete the adhesion procedure (Fig. 3 and Video 2).

The fibrin glue Bolheal is a plasma derivative containing a highly purified blood clotting component. It is composed of fibrinogen solution and thrombin solution, and it hardens when mixed (Video 1). Bolheal undergoes genetic screening and heat treatment and is filtered through a virus-removing membrane to eliminate viruses during production [13]. Its safety has been highly acclaimed.

Evaluation of ulcers induced with endoscopic submucosal dissection

Endoscopic observation with the animal under anesthesia was performed on the day after ESD. A 20-mg dose of omeprazole was administered intravenously. An endoscope was inserted through the mouth without an overtube. Each animal was examined for the presence or absence of postoperative bleeding and delayed perforation. For ulcers treated with PGA sheets, the presence or absence of residual sheets was determined.

Feeding was resumed at night on the day after ESD. From 2 days after ESD, one 10-mg tablet of rabeprazole sodium was administered orally daily until the day of euthanasia. Three pigs were euthanized at week 1, three at week 4, and three at week 8 after

Video 1

Bolheal for tissue adhesion is a plasma derivative with a highly purified blood clotting component. It is composed of fibrinogen solution and thrombin solution, and it hardens when mixed.

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Video 2

A hemostatic forceps was used to deliver the storage sheet containing a polyglycolic acid (PGA) sheet into the stomach. The PGA sheet was delivered to the ulcer floor with a forceps. The fibrin glue was placed onto the PGA sheet to complete the adhesion.

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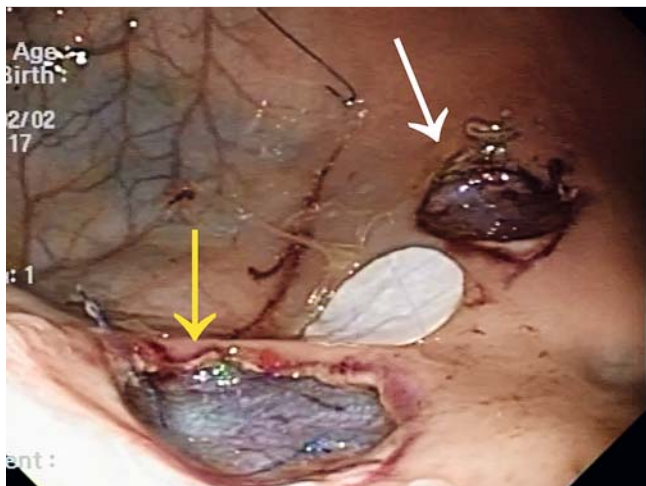


Fig. 3 A sheet of graph paper 15 mm in diameter was placed at the two planned ulcer sites. The circumference of the graph paper was marked to create two identical ulcers 20 mm in diameter. The polyglycolic acid sheet was trimmed to a size smaller than that of the ulcer and affixed to the ulcer floor with fibrin glue (yellow arrow). Ulcer prepared as a control (white arrow).



Fig. 4 Endoscopic finding in the treated ulcer site on the day after endoscopic dissection. The polyglycolic acid sheet remains firmly on the ulcer floor, shielding the ulcer site from exposure to gastric acid.

ESD, and the ulcer sites were observed macroscopically and histopathologically. For the purpose of verifying the safety of this treatment procedure, the ulcer floors were evaluated histopathologically for the presence or absence of excessive inflammation, necrosis, and infection.

This study protocol was prepared in accordance with “Guidelines for Proper Conduct of Animal Experiments” (Science Council of Japan) and was approved by the Institutional Animal Experiment Committee of Shizuoka Cancer Center Hospital, Shizuoka, Japan, and the Chemo-sero-therapeutic Research Institute (Kaketsuken), Kumamoto-shi, Japan. This animal experiment was performed at NAS Laboratory Co., Ltd., in Chiba, Japan. There was no conflict of interest to declare.

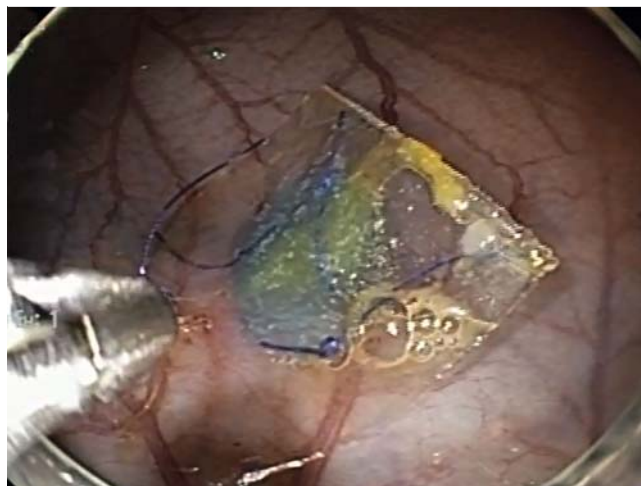


Fig. 5 Mucus was mixed in the storage sheet, delivered to the stomach with a pair of forceps.

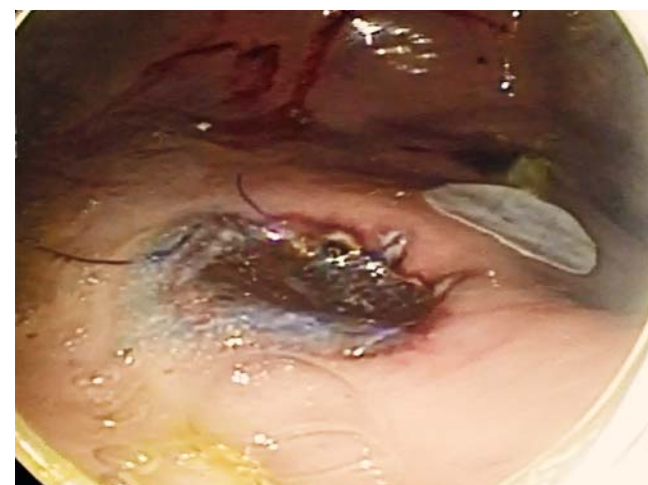


Fig. 6 The polyglycolic acid sheet extended to the mucosal epithelium around the ulcer. The graph paper used for marking was recovered with a forceps after the procedure.

Results

ESD-induced ulcers were successfully prepared in all nine pigs without complications, such as intraoperative perforation, and the PGA sheets were affixed to the ulcer floors with fibrin glue. In all animals, confirmatory endoscopy on the day after ESD revealed no complications, such as delayed perforation and post-operative bleeding, at either the control or the treated ulcer sites. During confirmatory endoscopy performed on the day after ESD, a residual PGA sheet was observed at seven of nine treated ulcer sites (Fig. 4). We reviewed the videos taken at application for the two instances in which the PGA sheets became dislodged. In one case, gastric mucus had infiltrated the storage film before the PGA sheet was placed onto the ulcer site (Fig. 5). In the other case, the PGA sheet extended to the mucosal epithelium when it was affixed (Fig. 6). Of the two animals in which the PGA sheets detached on the day after ESD, one was in the 4-week euthanasia group and the other was in the 8-week euthanasia group. In the three animals euthanized 1 week after ESD, both the treated and the control ulcer sites were reduced in size. At the treated ulcer sites, a white membranous film was observed (Fig. 7). The

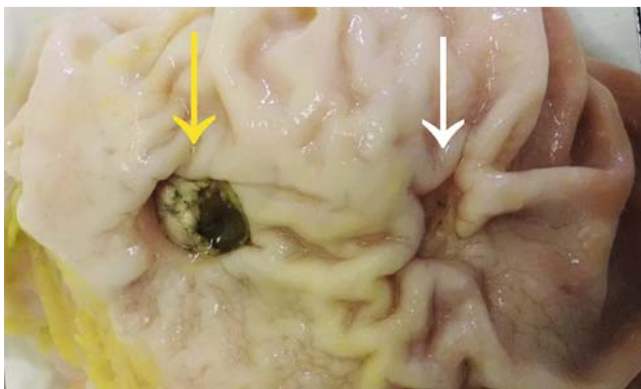


Fig. 7 Formalin-fixed specimen obtained 1 week after endoscopic sub-mucosal dissection. The residual polyglycolic acid sheet can be observed at the treated ulcer site (yellow arrow). The control ulcer site is not closed (white arrow).

treated ulcers tended to show reduced wound contraction in comparison with the control ulcers. Reduced wound contraction was also observed in the animal in which the PGA sheet detached early. On histopathologic examination, granulation tissue with inflammatory cell infiltration was observed in all the control ulcer floors, which is considered to be part of the usual process of ulcer healing (Fig. 8 a, b).

Histopathologic evaluation revealed that the sheet was detached in one of the three treated ulcers. At two treated ulcer sites, a thick layer consisting of a mixture of fibrin glue and necrotic substances covered the ulcer surfaces (Fig. 9 a, b). In moderately magnified images, a residual PGA sheet was observed in the mixture covering the ulcer. Newly formed blood vessels tended to be more abundant in the ulcer floor at the treated sites than in the ulcer floor at the control sites, with good granulation (Fig. 9 c). No excessive inflammation, necrosis, or infection was observed. All three animals showed similar pathologic findings.

In the three animals euthanized 4 weeks after ESD, all the treated and control ulcers were closed (Fig. 10). There was no residual PGA sheet at the treated ulcer sites, and no delayed ulcer healing or suspected infection was observed. Histopathologic examination revealed that the control ulcer sites were all closed, with newly formed blood vessels and fibrous growth, consistent with the usual process of ulcer healing. Like the ulcers at the control sites, the ulcers at the treated sites were all closed, and in moderately enlarged images of the sites, epithelial regeneration and newly formed blood vessels, as well as fibrous growth, were observed. As at the control ulcer sites, no excessive inflammation, necrosis, or infection was observed at the treated ulcer sites (Fig. 11 a, b).

In the three animals euthanized 8 weeks after ESD, all ulcers at both the control sites and the treated sites were closed. Macroscopic and histopathologic examinations 8 weeks after ESD revealed findings in the ulcers at both the control and the treated sites similar to those seen 4 weeks after ESD.

The time required to apply a PGA sheet to an ESD-induced ulcer and place fibrin glue averaged 472 ± 144 seconds (excluding data for the first attempt and examination of the application method) (Table 1).

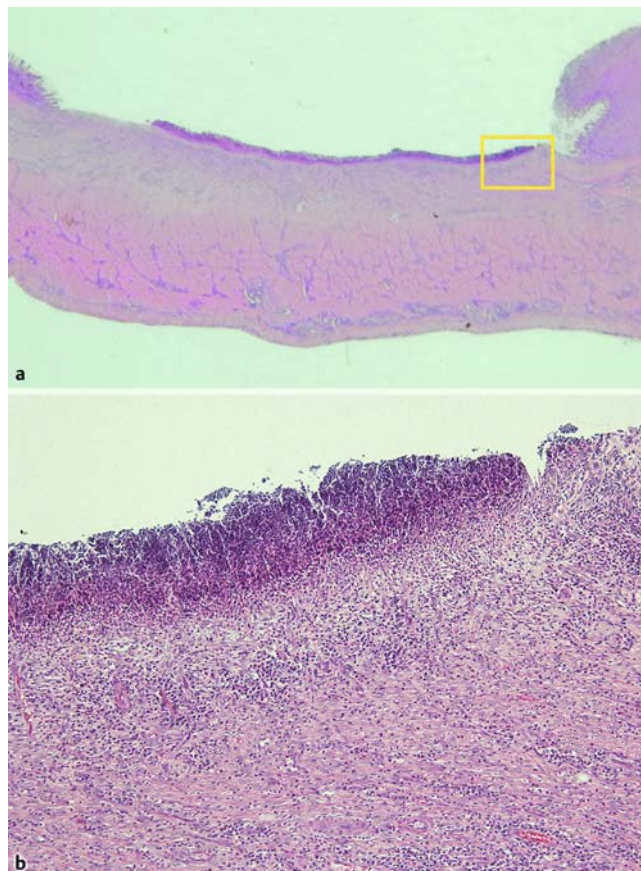
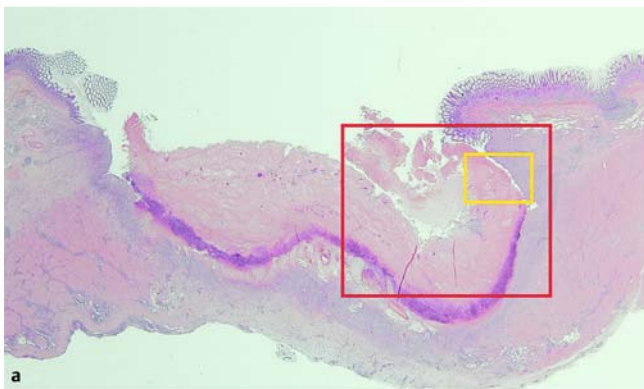


Fig. 8 a A magnified image of the control ulcer site 1 week after endoscopic dissection. The portion of the ulcer floor within the yellow box is shown enlarged in b. b At the control ulcer site, granulation tissue with inflammatory cell infiltration was observed, which is considered to indicate the usual process of ulcer healing. Some damage was observed on the surface, which may be attributable to gastric acid ($\times 10$).

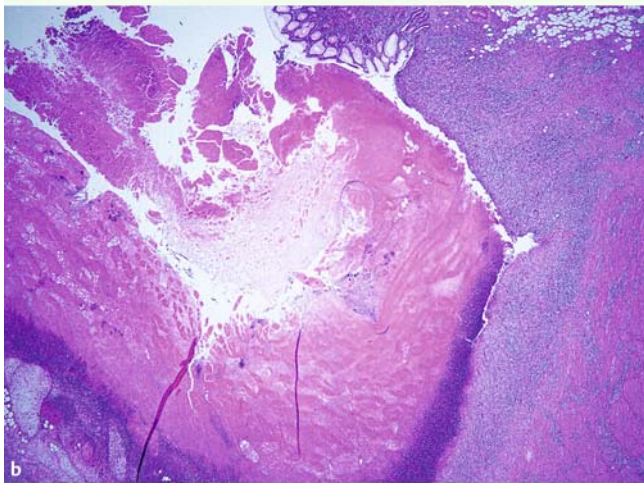
Discussion

No prior study has evaluated ESD-induced ulcers histopathologically following the application of PGA sheets with fibrin glue. We developed a method to affix PGA sheets efficaciously onto ESD-induced ulcers and demonstrated the safety of this procedure in pigs.

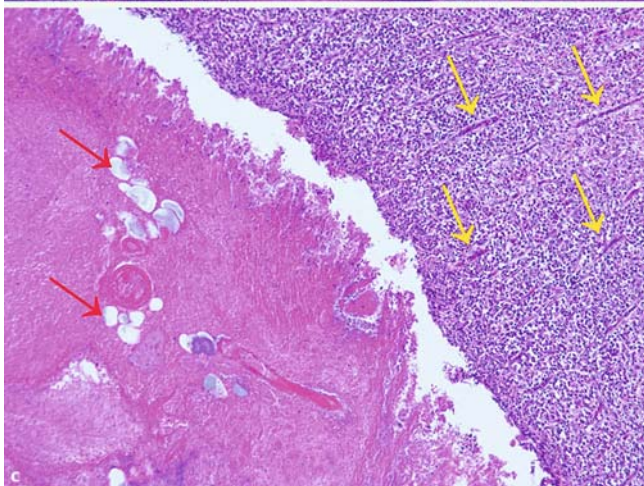
The method of applying PGA sheets to ESD-induced ulcers was first reported by Takimoto et al. To affix PGA sheets onto ESD-induced duodenal ulcers, the researchers applied small sheets in multiple portions via the channel [6]. However, this method requires a longer time to cover the ulcer floor; therefore, we affixed PGA sheets that were the same size as the ulcer floor in a single surgical session. In preliminary examinations, it was difficult to flatten large PGA sheets with forceps because larger sheets tend to cluster once wet. Therefore, we prepared a storage sheet by folding the film, into which the PGA sheet was tucked. This strategy prevented the PGA sheet from clustering, even when wet. Furthermore, our preliminary examinations revealed that when PGA sheets are exposed to mucus, their ability to adhere to the ulcer floor decreases. Therefore, the PGA sheets were soaked in fibrinogen and then tucked into storage sheets to prevent the PGA sheets from being soaked, even if mucus entered the storage sheets.



a



b



c

Fig. 9 **a** A magnified image of a treated ulcer site 1 week after endoscopic dissection. The portion of the ulcer within the red box is shown in Fig. 9b, and that within the yellow box is shown in **b**. **b** The ulcer was covered by a thick layer consisting of a mixture of fibrin glue and tissues treated with the electric knife ($\times 2$). **c** Under moderate magnification, the residual polyglycolic acid sheet was observed in the mixture covering the ulcer (red arrows). On the ulcer floor, new blood vessels were present in greater abundance than in the control sites, with good granulation (yellow arrows) ($\times 10$).

Confirmatory endoscopy on the day after ESD revealed that the PGA sheets detached at two treated sites. We reviewed the videos taken at application. It was presumed that in one animal, gastric mucus had infiltrated the storage sheet, which may have contributed to PGA sheet detachment (▶ Fig. 5). In the other animal, the PGA sheet extended to the mucosal epithelium (◀ Fig. 6). Prior basic examination confirmed that the adhesive properties of the

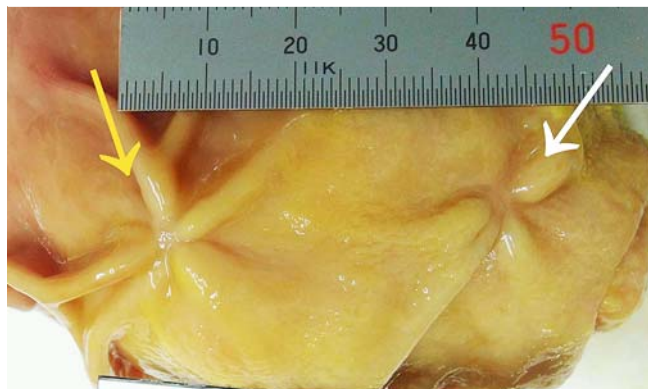
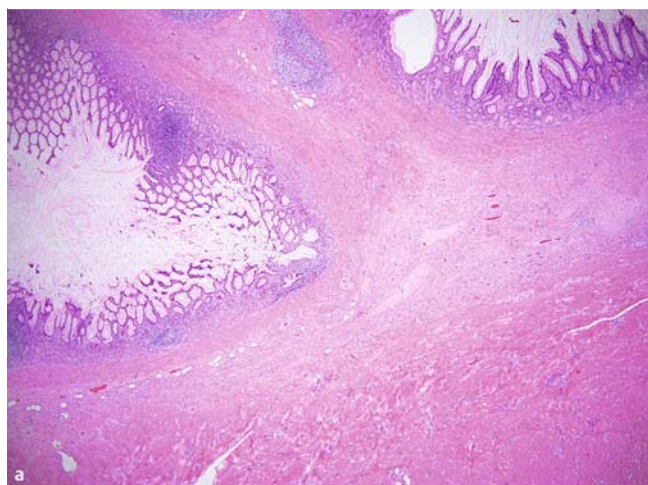
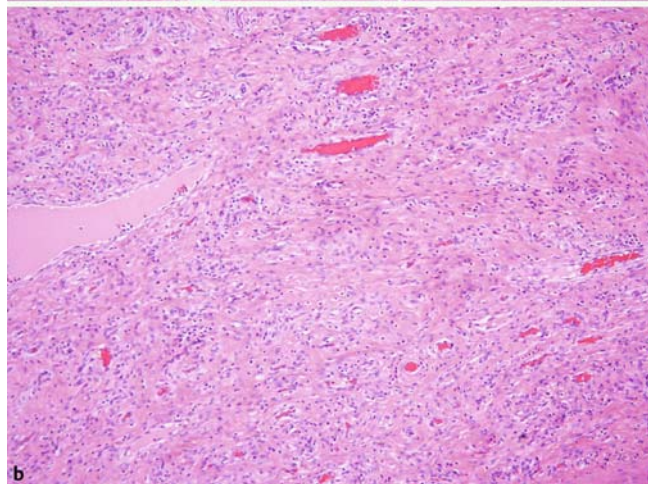


Fig. 10 Resected specimen obtained 4 weeks after endoscopic dissection. The treated ulcers (yellow arrow) were closed, and no polyglycolic acid sheet was observed. In addition, no delayed ulcer healing or suspected infection was noted. No difference was found between the control ulcer sites (white arrow) and the treated ulcer sites.



a



b

Fig. 11 Magnified images, $\times 2$ (**a**) and $\times 10$ (**b**), of the treated ulcer sites 4 weeks after endoscopic dissection. At the treated ulcer sites, epithelial regeneration and newly formed blood vessels, as well as fibrous growth, were observed. As at the control ulcer sites, the ulcers were healing well, with no excessive inflammation, necrosis, or infection.

PGA sheet were inadequate for adhesion to the mucosal epithelium, and the slightly extended edge of the sheet rolled up, leading to PGA sheet detachment. PGA sheet adhesion was maintained at the other treated ulcer sites, and it was considered pos-

Table 1 Polyglycolic acid sheet affixation time.

| Application attempt | Application time, s |
|---------------------|---------------------|
| 1 | 2595 |
| 2 | 258 |
| 3 | 426 |
| 4 | 330 |
| 5 | 259 |
| 6 | 642 |
| 7 | 427 |
| 8 | 477 |
| 9 | 598 |

sible for the PGA sheets to adhere effectively when the delivery and application methods in this study were properly performed. In the specimens resected 1 week after ESD, the ulcers were reduced in size at both the treated and the control sites. The treated ulcers tended to show reduced wound contraction in comparison with the control ulcers. This finding was also observed in the animal in which the PGA sheet detached early. Concerning oral surgery, studies in which the same method was used to cover raw surfaces after tumor dissection also reported reduced wound contraction. Reduced wound contraction is believed to prevent gastrointestinal stricture after semicircular and circular ESD, and we expect this finding to be explained by future studies.

Mixtures containing the PGA sheet and white membranous film were observed at the treated ulcer sites, potentially protecting the ulcer floor from exposure to gastric acid. A thick layer containing fibrin glue and necrotic substances covered the ulcer surface on histopathologic examination (► Fig. 9a, b). A mild inflammatory reaction was observed on the ulcer floor, which was considered to be an immune reaction to foreign proteins resulting from the use of human-derived fibrin glue in animals. No excessive inflammation or infection was observed histopathologically on the ulcer floors at the treated sites.

In the specimens resected between 4 and 8 weeks after ESD, all ulcers were closed at both the control and the treated sites. In the treated ulcers, no residual sheet was found, and an abundance of newly formed blood vessels in the ulcer floors and good granulation were observed. The amount and extent of fibrosis were similar in the control and the treated sites.

A limitation of this study was that not enough euthanasia points were set. It would have been better to perform an additional experiment and euthanasia 2 weeks after ESD in order to evaluate the healing process in the ESD-induced ulcers more precisely. Ideally, a preliminary experiment to measure the healing rate of gastric ulcers in swine would have been done before the study. However, because of financial limitations, it was not possible to breed and conduct experiments on more pigs.

The primary objective of this study was to demonstrate the safety of the procedure. When PGA sheets are used in the gastrointesti-

nal tract, they can become nonsterile. However, this study found that even under nonsterile conditions, PGA sheets do not become a source of infection. In addition, the study found that the hydrolytic degradation of the PGA sheet does not cause excessive inflammation and delay ulcer healing. We believe this treatment has utility in the gastrointestinal tract. Our examination of the application and safety of the technique can provide significant information that will further its clinical application.

Conclusion



Our animal study experimentally demonstrated that this treatment technique can be safely applied to ESD-induced ulcers.

Competing interests: None

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