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Effects of Sprayable, Highly Adhesive Hydrophobized Gelatin Microparticles on Endoscopic Submucosal Dissection: A Swine Model

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Keywords
Endoscopic submucosal dissection · Adverse event · Anti-inflammation · ESD-induced wound · Perforation · Hydrophobized microparticles · Hydrophobically modified Alaska pollock gelatin

Abstract
Introduction: Sprayable wound dressings containing hydrophobized microparticles (hMPs) are characterized by strong adhesiveness. We examined the effect of hMPs derived from Alaska pollock gelatin on endoscopic submucosal dissection (ESD) ulcers. ***Methods:*** (1) In an in vivo model of miniature swine gastric ESD, gastric ulcers were created by ESD and then sprayed with hMPs or untreated followed by microscopic examination. (2) In an ex vivo ESD model of resected stomach, a pinhole-shaped perforation was created on the ESD ulcer of resected stomach; hMPs were then sprayed on the perforation; and air leakage and intragastric pressure were measured. (3) In an in vivo duodenal ESD model of miniature swine, duodenal artificial ESD ulcers with pinhole-shaped perforation were examined; ulcers were classified into hMPs-sprayed and nonsprayed groups, and inflammation in the intrinsic muscle layer and serosa were compared between the groups. ***Results:*** (1) Histological observation of submucosal tissues showed a decreased number of invading inflammatory cells in hMP-sprayed tissues compared with the control in miniature swine gastric ESD ($p < 0.05$). In addition, the rates of anti-alpha smooth muscle actin and type I collagen positivity were significantly lower in the hMPs group than in the control group ($p < 0.05$). (2) Intragastric pressure could not be measured in the nonsprayed group, whereas no air leakage was observed in the sprayed group when pressurized up to 26 mm Hg in the resected stomach model. (3) The sprayed group showed suppressed inflammation of the intrinsic muscular layer and serosa in both cases compared with the nonsprayed group in miniature swine duodenal ESD ($p < 0.05$). ***Conclusions:*** Sprayable, tissue-adhesive hMPs are a promising medical material for intraoperative and postoperative treatment of ESD-induced wound via anti-inflammation and strong adhesiveness.

Introduction

Endoscopic submucosal dissection (ESD) is a well-established technique used in endoscopic resection that enables the en bloc removal of gastrointestinal epithelial lesions [1]. It can be used to treat early gastrointestinal can-

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cer, irrespective of the lesion size or location. However, ESD is a difficult and complex procedure to perform and is associated with a higher risk of adverse events [2]. ESD for superficial duodenal epithelial tumors (SDETs) is technically difficult, with a reported intraoperative perforation rate of 21.4–35.7% [3]. Delayed perforation reportedly occurs in 6.3% of patients after ESD for SDETs [3, 4]. Although the precise mechanism of delayed perforations is unclear, bile and pancreatic juice containing digestive enzymes could play a pivotal role in the increased risk of delayed adverse events [3].

Several methods have been reported for closing post-duodenal ESD ulcers, such as the clipping method, over-the-scope clip (OTSC) closure method, polyglycolic acid (PGA) sheet shieling method, and laparoscopic and endoscopic cooperative surgery (LECS), to prevent delayed perforation [5, 6]. Furthermore, a method of autologous myoblast sheet transplantation to prevent perforation after duodenal ESD has also been reported [7].

We previously reported that hexanoyl (Hx:C6) group-modified alkaline-treated gelatin porous film (HAG) induced proper healing by decreasing inflammation for post-ESD gastric ulcers in miniature swine [8, 9]. We predicted that this sheet could be effective in preventing perforation of ESD-induced wound duodenal ulcers. However, we were unable to deliver it to ulcers in small spaces, such as the duodenum, due to its strong adhesiveness. Therefore, we developed a sprayable wound dressing comprising multifunctional hydrophobized microparticles (hMPs) derived from swine gelatin, which show strong tissue adhesion under wet environments and are effective in suppressing inflammation in rat skin ulcers and post-ESD gastric ulcers in miniature swine [10]. However, hMPs derived from swine gelatin did not produce clean particles [11]. Our sprayable wound dressing consisted of very fine hMPs derived from Alaska pollock gelatin that were able to close ex vivo perforation models using the duodenum, large intestine, and stomach under wet conditions [12]. However, the utility of hMPs derived from Alaska pollock for post-ESD ulcers in vivo and the evaluation of their closure potential using the whole stomach have not yet been investigated.

The present study examined the effect of hMPs derived from Alaska pollock on ESD ulcers via the healing process of miniature swine gastric ESD ulcers. Subsequently, the ability to close small perforations in ESD-induced ulcers in resected swine stomach and live miniature swine duodenum was also examined using the same material.

Materials and Methods

Experimental Animals

Eleven male miniature swine (age, 6 months; 19–21 kg; from the Kagoshima Miniature Swine Research Center, Kagoshima, Japan) were used in the present study. We used 3 swine for gastric ESD and 8 swine for duodenal ESD models.

The miniature swine were injected intramuscularly with 15 mg/kg ketamine (Daiichi Sankyo Propharma, Tokyo, Japan) and 2 mg/kg xylazine (Bayer Yakuhin, Osaka, Japan) for premedication and then treated with sevoflurane (Maruishi Pharmaceutical, Osaka, Japan) by inhalation. An endotracheal tube (100/199/065; Smith Medical Japan, Tokyo, Japan) was inserted, and anesthesia was maintained using sevoflurane. Animals were allowed free access to water on the day ESD was performed and solid food the following day. Feed dispensing was carried out by a professional animal experimenter at the Kagoshima University Animal Experimental Facility, and daily checks of symptoms were performed by the technician and experimenters. Autopsies were performed by several experimenters in accordance with the Ethical Guidelines of the Kagoshima University Animal Experimental Facility. No proton pump inhibitor was administered after ESD. Animal care, housing, and surgery were performed in accordance with the rules and regulations of the Committee for Animal Research of Kagoshima University, Japan.

hMPs

Hydrophobically modified Alaska pollock gelatin was prepared by reductive amination between gelatin and aldehyde as previously reported [13], and hMPs were prepared using a coacervation method in a water/ethanol mixed solvent as previously reported [11]. The optimized alkyl chain length (decyl groups, C10) and degree of substitution (43% to amino groups in ApGltn) of hydrophobic groups improved the mechanical strength of the hydrogel formed by hydration and fusion of the microparticles. Scanning electron microscope observation confirmed that the obtained hMPs possessed micrometer-sized particles (Fig. 1).

ESD Procedure

Artificial gastric ulcers were created by an expert endoscopist who performed ESD using an upper gastrointestinal endoscope (GIF-Q260J; Olympus, Tokyo, Japan) and a video scope system (EVIS LUCERA CV-260SL; Olympus). A glycerin solution containing a small amount of epinephrine and indigo carmine (Glycerol injection; Hikari Pharmaceutical, Tokyo, Japan) was injected into the submucosa of the planned ulcer site. The mucosa and submucosal layer were incised using an electric knife (Dual Knife, KD-650L; Olympus) and an electrosurgical generator (Pulse Cut Fast mode, 30W, ESG-100; Olympus) (Fig. 2a, b). We confirmed the diameters of the ulcer using measuring forceps (M2-K4; Olympus). All ESDs were performed entirely by M.K.

Delivery of hMPs

A total of 20 mg of hMPs were packed into small vials. A battery-powered Alto shooter endoscopic injector (Alto shooter Kaigen, Tokyo, Japan), which can be used for the application of powdered drugs for endoscopic hemostasis via endoscope, was used to spray the hMPs. A small vial of hMPs was attached directly to the Alto shooter, and the nozzle was taken out of the endoscope duct and sprayed toward the ulcer.

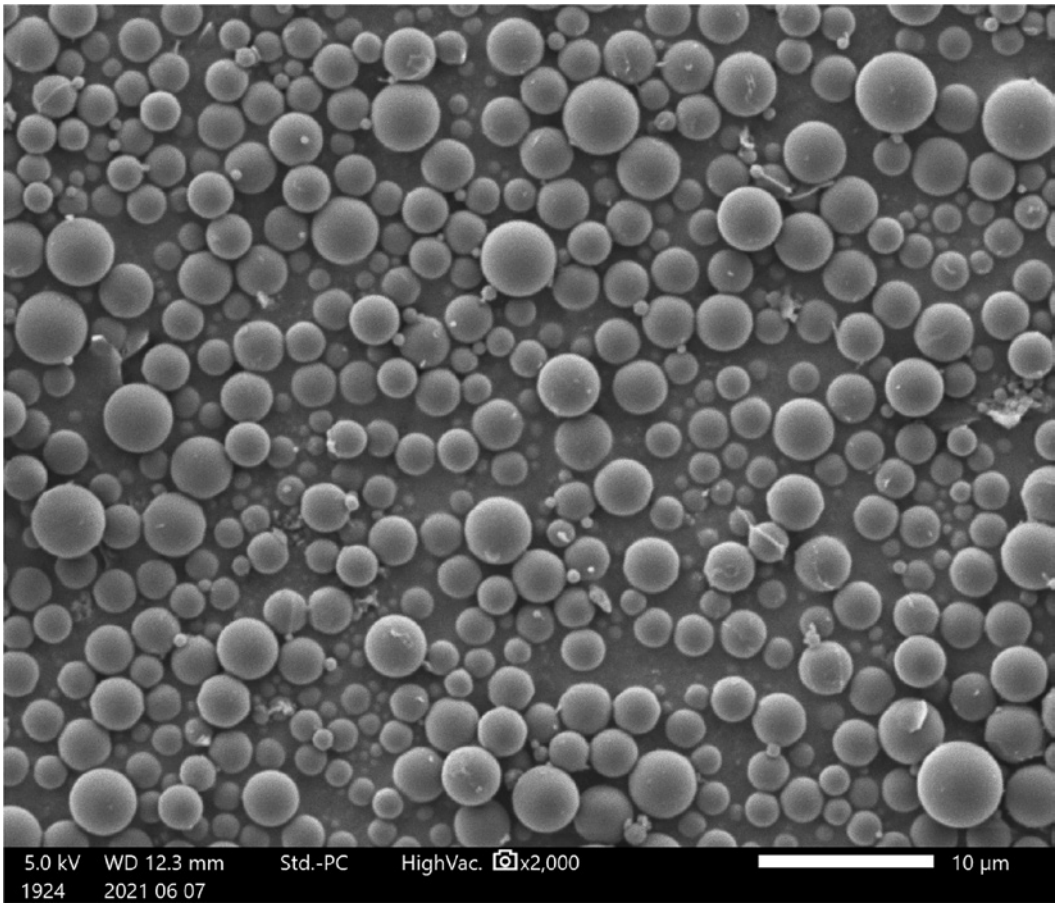

Fig. 1. Scanning electron microscopy image of hMPs. Hydrophobically modified Alaska pollock gelatin was prepared by reductive amination between gelatin and aldehyde. hMPs were prepared using a coacervation method in a water/ethanol mixed solvent. hMPs, hydrophobized microparticles.

Treatment of Ulcers after Gastric ESD

The wound healing process was evaluated using a swine gastric ESD model. As previously reported, 2 cm of gastric mucosa was resected to form 3-cm wounds using ESD [8] (Fig. 2b). Of the two ulcers prepared, one was treated by spraying with hMPs, and the other was left untreated (control ulcer site) (Fig. 2c, d). Three miniature swine were sacrificed on day 14 after ESD by intravenous administration of a lethal dose of sodium pentobarbital (Kyoritsu Seiyaku Co., Ltd., Tokyo, Japan). Miniature swine that underwent ESD were monitored daily and were euthanized under anesthesia if they exhibited distress and fever during experiments. All efforts were made to minimize animal suffering.

Evaluation of Perforation Closure Ability via ex vivo Model of Gastric ESD with Resected Swine Stomach

We evaluated whether hMPs could be used to close perforations using a resected swine stomach. Five resected stomachs were purchased from Tokyo Shibaura Zoki K.K and firmly fixed to the overtube (Top Overtube: Top corporation, Tokyo, Japan) at the esophageal side, whereas the duodenal side was fixed using scissors at the intestine (AE-06S YDM Tokyo corporation, Japan). The overtube was fitted with an adapter with an anti-degassing valve (Reek Cutter Top Corporation, Tokyo, Japan) to prevent air leaks and measure the pressure inside the stomach. The degassing valve and pressure gage (VBM Cuff Control Inflator: Smith Medical Japan, Tokyo, Japan) were connected by a tube, and the intragastric pressure was measured (Fig. 3a). ESD was performed as described previously (Fig. 3b) on the resected swine stomach, and a pinhole perforation (φ 2.7 mm) was created in the center of each ESD-induced ulcer (Fig. 3c, d). hMPs were sprayed on post-ESD ulcers with a pinhole perforation (Fig. 3e). The resected stomach that had undergone ESD with perforation was placed in water, and the pressure at the time of air leakage was measured before and after spraying hMPs. A total of four intragastric pressure measurements were performed on the same resected stomach: (1) before ESD, (2) after ESD, (3) ESD + perforation, and (4) ESD + perforation + spray (Fig. 3f, g).

Evaluation of Microperforation Closure Ability and Anti-Inflammation via in vivo Model of Duodenal ESD with Live Miniature Swine

In the in vivo model of miniature swine duodenal ESD, ESD was performed on the duodenal bulb to create an artificial ulcer around 10 mm in size. A minuscule pinhole perforation was then

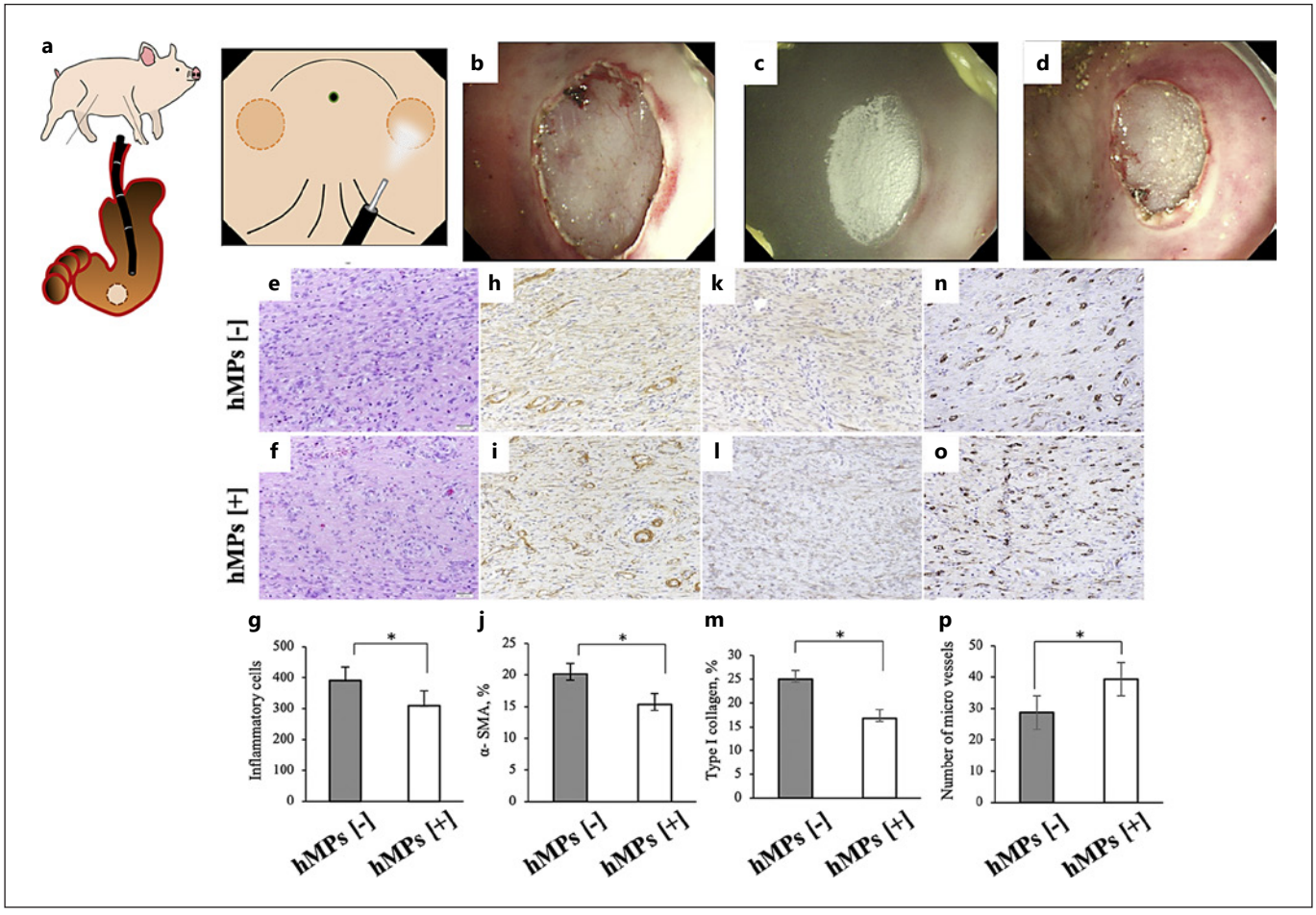

Fig. 2. hMPs decreased inflammatory cell infiltration and α-SMA-positive and type I collagen areas and increased the number of von Willebrand factor-positive cells in submucosal layers in a miniature swine gastric ESD model. **a** Two ulcers were prepared: one was sprayed with hMPs, and the other was untreated. **b** Artificial ulcer in the stomach antrum made by ESD. **c** hMPs were sprayed using a spray device onto an artificial gastric ulcer (image just after spraying). **d** hMPs were swelled in exudate (image approximately 5 min after spraying the same ulcer). **e**, **f**, **g** The number of inflammatory cells was significantly lower in hMPs-sprayed ulcers than in nonsprayed ulcers (control). **h**, **i**, **j** α-SMA-positive areas in submucosal layers were significantly lower in hMPs-sprayed ulcers than in nonsprayed ulcers (control). **k**, **l**, **m** There were significantly fewer type I collagen-positive areas in the submucosal layers in hMPs-sprayed ulcers compared with nonsprayed ulcers (control). **n**, **o**, **p** The number of cells positive for von Willebrand factor was higher in hMPs-sprayed ulcers than in nonsprayed ulcers (control). hMPs, hydrophobized microparticles; ESD, endoscopic submucosal dissection; α-SMA, α-smooth muscle actin.

created in the center of the ulcer using the tip of a dual knife (φ 0.65 mm). Eight miniature swine models of ESD-induced duodenal perforation were created and classified into sprayed ($n = 4$) and nonsprayed ($n = 4$) groups (Fig. 4a). Miniature swine that underwent ESD were monitored daily and euthanized under anesthesia if they showed distress and fever during experiments. All efforts were made to minimize animal suffering. Macroscopic and microscopic examinations were performed on post-ESD day 3. A scoring system was used to assess inflammatory spillover into the muscle layer and serosa as follows: (1) no inflammation spreading to the muscle layer; (2) inflammation limited to half of the muscle layer; (3) inflammation extending to all layers of the muscle layer; and (4) inflammation extending beyond the serosa.

Histological Analyses

Specimens were fixed in 10% neutral-buffered formalin (Kenei Pharmaceutical, Osaka, Japan) for 48 h, and each lesion was sliced at 4-mm intervals. The slices were then embedded in a paraffin block, cut into 2-μm-thick sections, and stained with hematoxylin and eosin (HE), Azan, or Masson trichrome staining. Inflammatory cells infiltrating into the submucosal layers in 12 random fields of view (×200 magnification) were counted on HE-stained tissues under a microscope. Damage to the muscularis propria was evaluated by Azan staining.

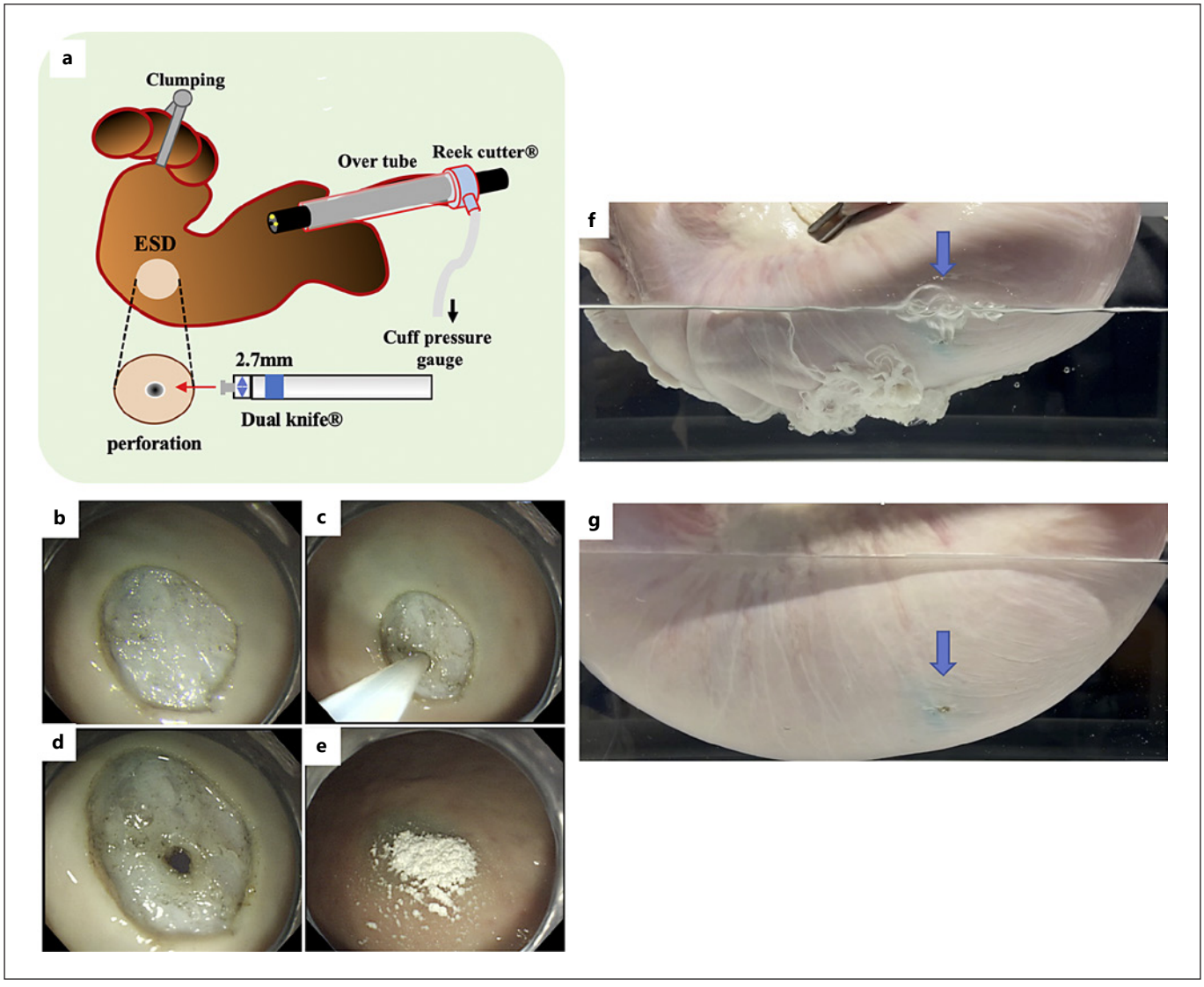

Fig. 3. hMPs closed microperforations in gastric ESD-induced ulcer (ex vivo). **a–e** A pinhole-shaped perforation was created on ESD ulcers. hMPs were sprayed onto ESD ulcers with a pinhole-shaped perforation. **f**, **g** Air leakage and intragastric pressure were measured. The pressure could not be measured in the nonsprayed group, whereas in the sprayed group, no air leak was observed when pressurized to 30 mm Hg in 4 of 5 cases. hMPs, hydrophobized microparticles; ESD, endoscopic submucosal dissection.

Immunohistochemistry

Immunohistochemical staining was performed using standard enzyme-labeled antibody methods [14]. The numbers of neutrophils and macrophages were determined using rabbit polyclonal antimyeloperoxidase antibody diluted 1:50 (AbCam, Cambridge, MA, USA) and goat polyclonal anti-Iba1 antibody diluted 1:4,000 (AbCam), respectively. Myofibroblasts and fibrosis were identified using mouse monoclonal anti-alpha smooth muscle actin (α-SMA) antibody (diluted 1:1,000; Progen Biotechnik, Heidelberg, Germany) and anti-collagen I alpha 1 antibody (diluted 1:400; Novus Biologicals, Littleton, CO, USA), respectively. Angiogenesis was evaluated using rabbit polyclonal antivon Willebrand factor antibody diluted 1:5,000 (AbCam). The samples were then incubated with anti-mouse or anti-rabbit secondary antibody (Nichirei, Tokyo, Japan). Specimens were fixed in 10% neutral-buffered formalin (Kenei Pharmaceutical Co., Ltd., Osaka, Japan) for 48 h, and each lesion was sliced at 4-mm intervals. The slices were then embedded in a paraffin block, cut to a thickness of 2 μm, and stained with HE, Azan, Masson trichrome, and immunostained. To evaluate fibrosis in the submucosal layer, we randomly selected 12 visual fields and quantified the areas stained with α-SMA and type I collagen using ImageJ, version 1.50b (National Institutes of Health, Bethesda, MD, USA). Additionally, blood vessels positive for von Willebrand factor were counted using ImageJ.

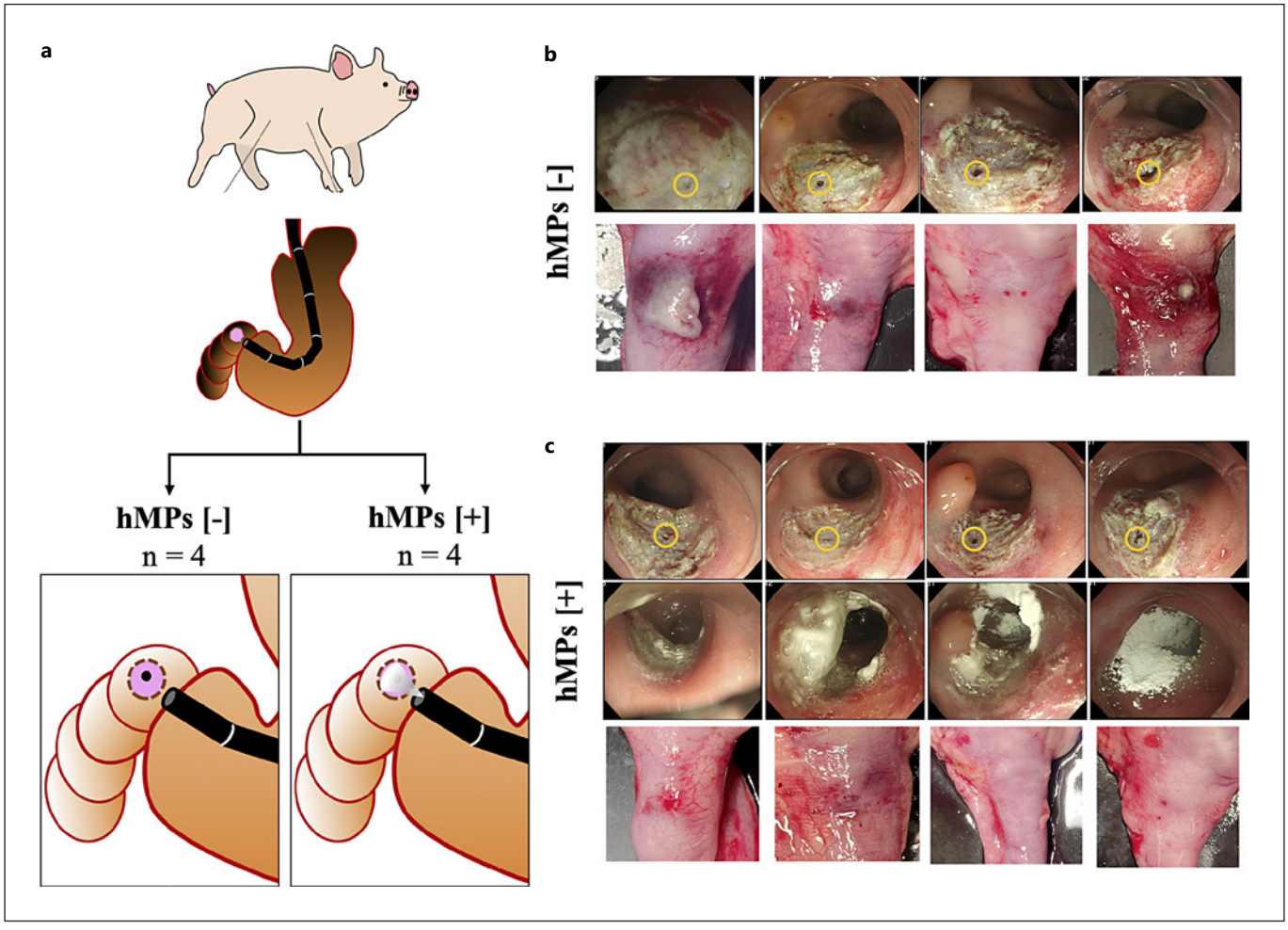

Fig. 4. hMPs closed microperforations during duodenal ESD in a miniature swine (in vivo). **a** Duodenal artificial ESD ulcers with pinhole-shaped perforations were created in our miniature swine model. **b**, **c** The appearance of the serosa of sprayed group was less red/purple than that of the nonsprayed group from the macroscopic findings on sacrifice. hMPs, hydrophobized microparticles; ESD, endoscopic submucosal dissection.

Table 1. Evaluation of perforation closure ability via an ex vivo model of gastric ESD with resected swine stomach

	Air leak (–)	Air leak (+)
Pressure before ESD (30 mm Hg)	5	0
Pressure after ESD (30 mm Hg)	5	0
ESD + perforation	0	5
ESD + perforation + spray	4	1

hMPs closed microperforations in gastric ESD-induced ulcer (ex vivo). No air leak was observed when pressurized to 30 mm Hg in 4 out of 5 cases. hMPs, hydrophobized microparticles; ESD, endoscopic submucosal dissection.

Statistical Analyses

The statistical significance of differences between the two groups was calculated using Student's *t* test or Mann-Whitney test depending on the results of Shapiro-Wilk and Levine's tests for normality and equality of variance, respectively. All p values <0.05 were considered significant. All statistical analyses were performed using the IBM SPSS Statistics Base 23 software program (IBM Corp., Armonk, NY, USA).

Results

hMPs Reduced Inflammation and Facilitated Angiogenesis in Submucosal Layers in Gastric ESD Model

ESD was performed safely in all swine without adverse events, such as intraoperative perforation. All miniature

swine were in good condition with no weight loss or fever after ESD. hMPs were sprayed using a spray device onto the artificial gastric ulcers swelled in exudate (Fig. 2b–d). The histological structures of the submucosal tissues were compared between the hMP-sprayed and nonsprayed groups. Histological observation of the submucosal tissues after 14 days showed that the number of invaded inflammatory cells was lower in the tissues sprayed with hMPs compared with the control (nontreated) tissue (345.72 ± 15.04 vs. 388.75 ± 22.10, respectively; $p < 0.05$) (Fig. 2e–g). In addition, the rates of α-SMA positivity were significantly lower in the hMPs group than the control group (14.94 ± 1.49 vs. 21.57 ± 2.42, respectively; $p < 0.05$) (Fig. 2h–j). Similarly, the rates of type I collagen positivity were significantly lower in the hMPs group than in the control group (16.04 ± 0.84 vs. 25.22 ± 2.23, respectively; $p < 0.05$) (Fig. 2k–m). The number of submucosal microvessels positive for von Willebrand factor was significantly higher in sprayed ulcers compared with the control (nonsprayed) ulcers (39.33 ± 1.89 vs. 28.67 ± 3.60, respectively; $p < 0.05$) (Fig. 2n–p).

hMPs Close Microperforations in Gastric ESD-Induced Ulcer

We performed a total of five repetitions of experiments to evaluate the ability of hMPs to close perforations in resected stomachs (Fig. 3a; online suppl. Video 1; see www.karger.com/doi/10.1159/000526650 for all online suppl. material). In all experiments, no air leakage was observed before and after ESD. After intentional pinhole perforation of the ESD-induced ulcer, pressure in the stomach could not be measured due to early air leakage in all cases (Fig. 3f; online suppl. Video 1). Four out of 5 cases treated with hMPs tolerated a pressure of 30 mm Hg without any air leakage (Fig. 3g; online suppl. Video 1). Even in the experiments where air leakage occurred, no air leakage was observed up to 26 mm Hg (Table 1; online suppl. Video 1).

hMPs Reduced Inflammation in the Muscle Layer and Serosa in Duodenal ESD Microperforation Model

In the in vivo model of miniature swine duodenal ESD, all swine models survived. Pre- and postoperative blood tests showed no change in inflammatory markers, such as white blood cell and C-reactive protein levels (data not shown). The appearance of the serosa of the sprayed group (hMPs [+]) was less red/purple compared with that of the nonsprayed group (hMPs [−]) in the macroscopic findings at sacrifice (Fig. 4b, c).

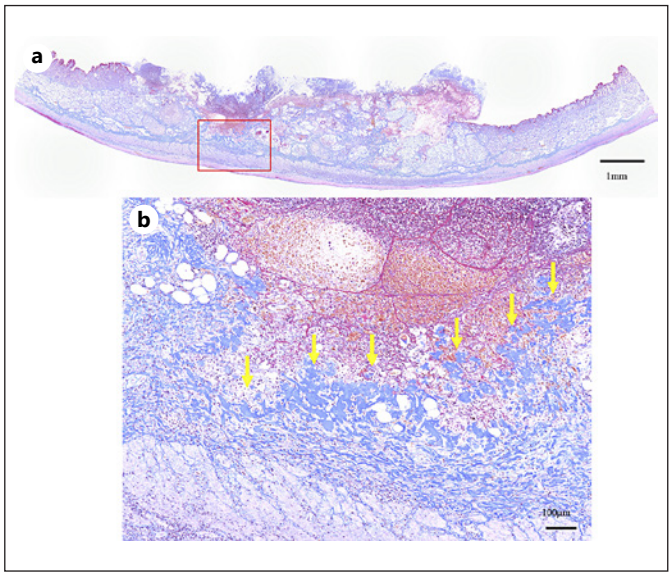

Fig. 5. Persistence of hMPs in a miniature swine in duodenal ESD-induced ulcer. **a** The presence of hMPs in post-ESD ulcers was confirmed using Azan staining at 3 days postoperatively. **b** The hMPs were present in submucosa above the intrinsic muscular layer (yellow arrow). hMPs, hydrophobized microparticles; ESD, endoscopic submucosal dissection.

The presence of hMPs in the post-ESD ulcers was confirmed by Azan staining up to 3 days postoperatively (Fig. 5). Histological examination revealed a significantly lower score for inflammatory spillover into the muscle layer in the sprayed group compared with the nonsprayed group (1.62 ± 0.30 vs. 2.92 ± 0.70, respectively; $p < 0.05$) (Fig. 6).

Discussion

The present study showed that hMPs can be used to efficiently suppress inflammation in submucosal tissues via physical and biochemical interactions after ESD. The inflammatory response initiated in the wounded tissue is associated with the early recruitment of neutrophils [15]. Neutrophils secrete chemokines and cytokines, which control the subsequent recruitment of monocytes that differentiate into macrophages. Many different cell types, including macrophages, fibroblasts, and contractile myofibroblasts, participate in wound repair [16]. In this study, hMPs reduced the number of infiltrating neutrophils and α-SMA-positive areas (Fig. 2). In addition, hMPs covered the microperforation of the ex vivo model of gastric ESD

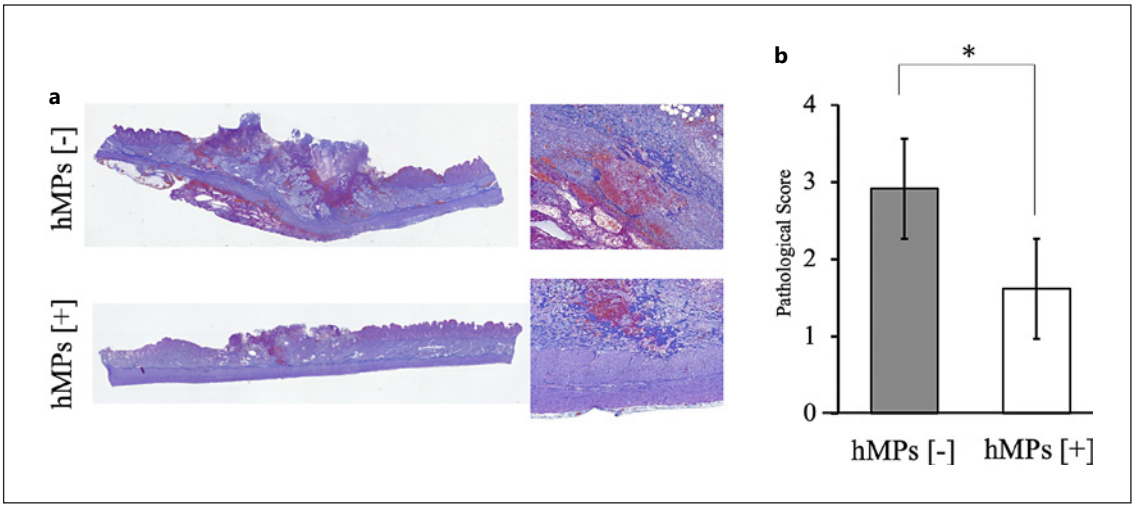

Fig. 6. hMPs decreased inflammation in the miniature swine duodenal ESD perforation model. **a** The hMPs-sprayed group showed suppressed inflammation of the intrinsic muscular layer and serosa in both cases compared with the nonsprayed group. **b** Pathological findings from scoring inflammatory spillover into the muscle layer revealed that the sprayed group significantly reduced inflammation. hMPs, hydrophobized microparticles; ESD, endoscopic submucosal dissection.

with high adhesion. Consequently, hMPs suppressed inflammation of the muscle layer and serosa owing to their anti-inflammatory and highly adhesive effects in the in vivo model of duodenal ESD.

In clinical practice, PGA sheets are reported to be effective in preventing delayed perforation after duodenal ESD [17]. However, PGA sheets have no inherent adhesive ability and require a fibrinogen solution as blood product and drop off easily. We previously reported that HAG induced proper healing by decreasing inflammation in post-ESD gastric ulcers in miniature swine [8, 9]. HAG has strong self-adhesiveness. However, it was not possible to deliver it to ulcers in a small space, such as the duodenum, due to its strong adhesiveness. Therefore, we developed a sprayable wound dressing comprising hMPs [10] made of swine gelatin, which showed strong tissue adhesion under wet environments and effective suppression of inflammation of rat skin ulcers and post-ESD gastric ulcers in miniature swine [10]. Ito et al. [12] developed sprayable wound dressing consisting of hMPs derived from Alaska pollock gelatin. In the present study, we found that the anti-inflammatory effects of hMPs of Alaska pollock gelatin, which gels quickly, in swine models were similar to those of swine gelatin [10] (Fig. 2). Swine gelatin and Alaska pollock gelatin differ in the amounts of proline and hydroxyproline among the constituent amino acids. Alaska pollock has a lower content of these constituents and is therefore more easily hydrated. Further, we have previously reported that swine gelatin did not produce clean particles. The reason for this was thought to be due to differences in the amount and molecular weight of proline [11]. As a result, we considered that the particles prepared with Alaska pollock hydrated and gelled more quickly when sprayed into gastric tissues. For the above reasons, we used gelatin derived from Alaska pollock as the material for our hMPs.

The hMPs inhibited inflammation, α-SMA-positive areas, and type I collagen areas in the submucosal layer of ESD-induced gastric ulcers. In addition, the hMPs sheet promoted angiogenesis in ESD-induced gastric ulcers. Therefore, hMPs induced post-ESD ulcer healing with less submucosal inflammation and muscularis propria injury and have the potential to decrease excess scarring via physical and biochemical interactions after ESD. Damage to the muscularis propria was reported to persist even after epithelialization in a canine artificial esophageal ulcer model [18]. The present results suggest that hMPs may be useful in preventing gastrointestinal stricture, such as after esophageal ESD.

We reported that hMPs are very fine particles that could close ex vivo perforation models in the duodenum, large intestine, and stomach under wet conditions [12]. However, previous reports have not used the entire duo-

denum, colon, or stomach. The present study evaluated the ability of hMPs to close perforations in ulcers created by ESD techniques using whole resected swine stomachs. The use of the whole resected stomach made the study more clinically relevant. After intentional pinhole perforation of ESD-induced ulcers, pressure in the stomach could not be measured due to early air leakage in all cases. However, no air leakage was observed up to 26 mm Hg in all cases treated with hMPs. Inoue et al. [19] reported an optimal maximum intragastric pressure of 18.7 mm Hg from opening of the cardia during gastric distension using the endoscopic pressure study integrated system. In the present study, spraying hMPs onto the gastric perforation resulted in no air leakage, even when applying an internal pressure of >18.7 mm Hg. These results suggest that hMPs alone may be able to close perforations with a diameter <2.7 mm. In clinical practice, gastrointestinal perforations caused by ESD or endoscopic mucosal resection are closed with clips or OTSC [3]. However, the use of clips in myofibrils or submucosa coagulated with a surgical knife can sometimes cause additional perforation [20]. An additional advantage of hMPs is that they can be used at any site. Some regions of the duodenum and stomach are also difficult to access, and hMPs may thus be useful in such areas. hMPs may be effective in areas where clip closure is difficult, as described above. Finally, the efficacy of a combined treatment of clips and hMPs for perforation closure should be investigated.

The findings of the present study demonstrate the usefulness of hMPs for duodenal ESD ulcer microperforation closure in miniature swine. Our results showed that there was no problem for survival even without any treatment after duodenal microperforation in a swine model, but there was a significant difference in the degree of localized inflammation in the duodenum. More specifically, spraying hMPs onto the duodenal microperforation site suppressed the spillover of inflammation into the muscular layer and the serosal side. ESD for SDETs is technically difficult, with a reported intraoperative perforation rate of 21.4–35.7% [3–21]. Nylund et al. [22] reported that the duodenum has a thinner wall than the gastric antrum using transabdominal ultrasonography. These findings suggest that electrical cautery with ESD caused duodenal perforation due to damages to the thin muscularis propria of the duodenum. Moreover, delayed perforation reportedly occurs in 6.3% of patients after ESD for SDETs [3, 21]. Bile and pancreatic juice containing digestive enzymes could play a pivotal role in the increased risk of delayed adverse events [3, 6, 23, 24]. Various measures are currently being taken to prevent intraoperative and postoperative duodenal perforation, such as the clipping method, OTSC closure method, PGA, and LECS [5, 6, 17, 25, 26]. It is difficult to close a large perforation or to suture the entire ulcer using clip closure alone. The OTSC method has been reported to be useful for intraoperative and postoperative perforations; however, there are limitations on the sites in which it can be used, and it must be performed by a skilled individual. PGA sheets have issues, such as lack of adhesive ability and difficulty delivering to the resection wound. Although LECS is a reliable method to prevent delayed perforation, it has some problems, such as limited suture sites and limited facilities. Furthermore, hMPs have the advantage that they can be used at any site. Delayed perforation reportedly occurs within 36 h after ESD for SDETs [4]. In the present study, the presence of hMPs in the post-ESD ulcers was confirmed using Azan staining up to 3 days postoperatively (Fig. 5). The persistence of hMPs in ESD ulcers for 3 days may be effective in preventing delayed perforation.

The present study has several limitations. First, the study included a small number of animals. Second, the effect of hMPs on post-ESD ulcers was not followed up in the long term. Further studies are required to examine the long-term antifibrotic and stenosis-preventive effects of hMPs on the submucosa. Third, duodenal ESD in this study was performed in the duodenal bulb, although the preferred site for SDET is the descending duodenum, which differs from actual clinical practice. However, the swine vater papilla is present in bulbs, which is exposed to bile and pancreatic juice. In addition, it was technically difficult to perform duodenal ESD in the descending part of the miniature swine.

In conclusion, hMPs efficiently suppressed inflammation in submucosal tissues via physical and biochemical interactions. In addition, hMPs covered small perforations with high self-adhesiveness. Sprayable, tissue-adhesive hMPs are a promising medical material for intraoperative and postoperative treatment of ESD via anti-inflammation and adhesiveness.

Acknowledgments

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Statement of Ethics

All experiments were approved by the Animal Care and Use Committee of Kagoshima University (Approval No. MD 15063). These animal experiments conform to the institutional standards of the Committee for Animal Research of Kagoshima University.

Conflict of Interest Statement

None of the authors have any conflicts of interest relevant to this study.

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Author Contributions

Masayuki Kabayama, Fumisato Sasaki, and Shuji Kanmura were responsible for the experimental design. Masayuki Kabayama, Fumisato Sasaki, Hiroki Yano, and Yusuke Fujino performed the animal experiments. Masayuki Kabayama and Hidehito Maeda performed the stomach and duodenal histochemical examinations. Fumisato Sasaki, Akihito Tanaka, Shiho Arima, Shiroh Tanoue, and Shinichi Hashimoto were responsible for statistical analyses. Hiromi Hirade, Akihiro Nishiguchi, and Tetsushi Taguchi created and provided hMPs. Akio Ido made substantial contributions to the conception and design of the study and the drafting of the manuscript. Masayuki Kabayama and Fumisato Sasaki wrote the manuscript. Masayuki Kabayama, Fumisato Sasaki, Hidehito Maeda, Akihito Tanaka, Shiho Arima, Shiroh Tanoue, Shinichi Hashimoto, Shuji Kanmura, Tetsushi Taguchi, and Akio Ido were involved in manuscript revisions.

Data Availability Statement

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

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