Submucosal endoscopy with mucosal flap safety valve

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Background: There is no reliable endoscopic method to selectively resect deeper layers of the gut wall or to access the peritoneal cavity and prevent peritoneal soiling.

Objectives: To determine the technical feasibility and safety of submucosal endoscopy with mucosal flap (SEMF) in accessing the peritoneal cavity through a large full-thickness gastric-muscle–wall resection.

Design: Ex vivo feasibility exploration and survival animal study.

Settings: Ex vivo samples were obtained from fresh harvested organs. In vivo procedures were conducted with the pigs under standard general anesthesia.

Interventions: High-pressure carbon dioxide (CO_2) injection and balloon dissection created a large submucosal working space for insertion of a cap-fitted endoscope. By using the EMR cap, a full-thickness resection of the muscularis propria was performed. This full-thickness defect was sealed with the overlying mucosal flap and the use of hemoclips or tissue anchors.

Results: By using the SEMF technique in the ex vivo experiment, the gastric wall was successfully traversed in each stomach after submucosal dissection and full-thickness resection of the musclaris. Similarly, by using the SEMF technique in the in vivo procedures, the peritoneal cavity was successfully accessed and the defect was completely sealed by using the mucosal flap. All animals survived 1 week after the procedure. Ulceration was noted in 3 pigs, and a small bowel injury was noted in 1 pig. Leak testing was negative in all stomachs.

Conclusions: By using the SEMF technique, submucosal space endoscopy and deep-layer gastric-wall resection were successfully performed. Furthermore, the mucosa overlying the dissected submucosal space served as a safe flap valve, preventing peritoneal leakage.

Our developmental endoscopy unit has explored, over several years, our concept of using the submucosa as a working space for endoscopic interventions, such as resection of Barrett's esophagus, laterally spreading mucosal polyps, noninvasive early cancers, and submucosal tumors, as well as acquisition of muscular tissue for etiologic analysis of motility disorders.[1-6] The submucosal space can also be a protective tunnel preventing peritoneal soiling by using the free overlying mucosa, as a sealant flap, and permitting a safer offset entry into the peritoneal cavity for natural orifice translumenal endoscopic surgery (NOTES).[7-15]

In this study, the muscular layer was resected from within a submucosal working space created with high-pressure carbon dioxide (CO_2) injection and balloon dissection. The isolated overlying mucosa was used as a biologic safety valve to control contamination from a perforating muscular layer resection. We explored the technical feasibility of this technique, initially ex vivo and then by an in vivo survival study. A secondary aim was to determine the technical feasibility and safety of submucosal endoscopy with mucosal flap (SEMF) in accessing the peritoneal cavity through a large full-thickness gastric-muscle–wall resection.

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MATERIALS AND METHODS

Organ and pig preparation

Three esophagogastric organ specimens were harvested, within 3 hours, from euthanized pigs.

Four 41.2 ± 2.71 kg (mean [standard deviation]) domestic pigs, under 1.5% to 2% isoflurane general anesthesia, were studied after a 48-hour fast.

Procedures

A dual-channel endoscope (GIF 2T100B; Olympus America Corp, Melville, NY), with a 19-mm, large EMR cap was used. First, the mucosa of the gastric body was lavaged with sterilized water and 20 mL of 10% povidone-iodine via the endoscope channel (omitted in ex vivo procedures). CO_2 injection used a commercially available CO_2 cylinder (CO_2 Duster; American Recorder Technology Inc, Simi Valley, Calif) used to clean electronic equipment. The published pressure of the cylinder is 3620 to 7240 mm Hg, with an average of 5172 mm Hg (7 Bar). High-pressure millisecond bursts were injected into the submucosal layer through a standard 23-gauge injection needle (Injector Force; Olympus America) to create a submucosal gas cushion (greater than 10 cm in diameter) (Fig. 1A). A total of 6 mL of 5% hydroxypropyl methylcellulose (Gonak; Akorn Inc, Buffalo Grove, Ill) was then injected to prevent gas escape and to maintain the cushion. A mucosal incision (10 mm) was made at one margin of the cushion with a bipolar needle knife (B-Knife; Zeon Medical Inc, Tokyo, Japan). A 15-mm biliary retrieval balloon (Multi-3; Olympus America) was then repeatedly inserted into the insufflated submucosal layer from the mucosal incision and was distended to dissect strands of connective tissue (Fig. 1B) to create a space to easily insert the endoscope with the attached EMR cap (Fig. 1C). In vivo, the length of the submucosal space was measured before the resection by using a handmade measuring guidewire. Opposite the mucosal entry point and within the submucosal space, the muscular layer was resected by cap EMR (Fig. 1D and E). In the exploratory phase ex vivo procedure, the length of the submucosal space between the mucosal incision and the leading edge of the muscular defect was measured directly by a ruler. The mucosal incision was closed by mucosal apposition with metal clips (HX-6UR-1; Olympus America). If clipping failed, then fixation of the mucosal flap onto the muscular layer was performed by using tissue anchors (Olympus Optical Co, Ltd, Tokyo, Japan) (Fig. 2) or medical acrylate glue (Indermil; Tyco Healthcare, Norwalk, Conn) through an ERCP catheter.

Postprocedure follow-up

In the in vivo study, oral intake was withheld during the immediate postanesthesia recovery period (6 hours). The animals were on a liquid diet for 48 hours, a soft diet on day 3, and then followed by a diet as tolerated. Antibiotics were given for 5 days (cefazolin 20 mg/kg, twice a day; and enrofloxacin 10 mg/kg, twice a day) and esomeprazole magnesium 40 mg, twice a day for 7 days. At 1 week, follow-up endoscopy was performed, and the animals were euthanized. A necropsy was performed to evaluate the interventional site. A leak test was performed by using harvested stomachs for each ex vivo specimen and each in vivo animal. The leak test was performed by clamping the gastric outlet and filling the stomach via the esophageal remnant with 1500 to 3000 mL water and observing the mucosectomy site for leakage, grossly and after dry wiping the site with gauze.

> **Capsule Summary**
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> **What is already known on this topic**
>
> - No reliable endoscopic method allows resection of deep layers of the gut wall or peritoneal cavity access without the risk of peritoneal soiling.
>
> **What this study adds to our knowledge**
>
> - In a preclinical study that used a porcine model, the muscular layer was resected from within a submucosal working space that was created with high-pressure CO_2 injection and balloon dissection, thus using the overlying mucosa to control contamination from a perforating muscular layer resection.

RESULTS

Ex vivo study

The distances between the mucosal incision and the leading edge of the muscular defect were 20, 20, and 30 mm (average, 23.3 mm). All mucosal incisions were successfully closed with clips. The gastric wall was successfully traversed in each stomach after full-thickness resection of the gastric wall. There were no water leaks.

In vivo study

The in vivo study results are summarized in Table 1. The anterior wall was selected in 3 pigs and the posterior wall in 1. In each animal, 65- to 80-mm submucosal spaces were formed. In 1 pig, the free space was created with CO_2 injection only (pig no. 3). The peritoneal cavity was successfully accessed in each animal after full-thickness resection of the muscularis. The average size of the resected muscular layer was 19 mm (Fig. 3). The endoscope was advanced with ease through the gastric-wall defect into the peritoneal cavity. Upon endoscope withdrawal into the gastric lumen, gastric distension was maintained without obvious secondary pneumoperitoneum. Attempts at mucosal-clip apposition succeeded in 1 pig only (Fig. 4). The free mucosal edge had become too edematous to grasp with standard clips. By using T-tag tissue anchors, the free mucosal edge at the mucosal entry site was fixed to the deeper gastric wall layers in 2 pigs; acrylic glue was used in 1 pig. During the survival period, 3 pigs resumed a normal diet, whereas 1 pig was eating poorly. Repeat endoscopy at 1 week showed ulcerations at the closure sites in all animals except for the 1 pig with successful clip mucosal apposition. Necropsy

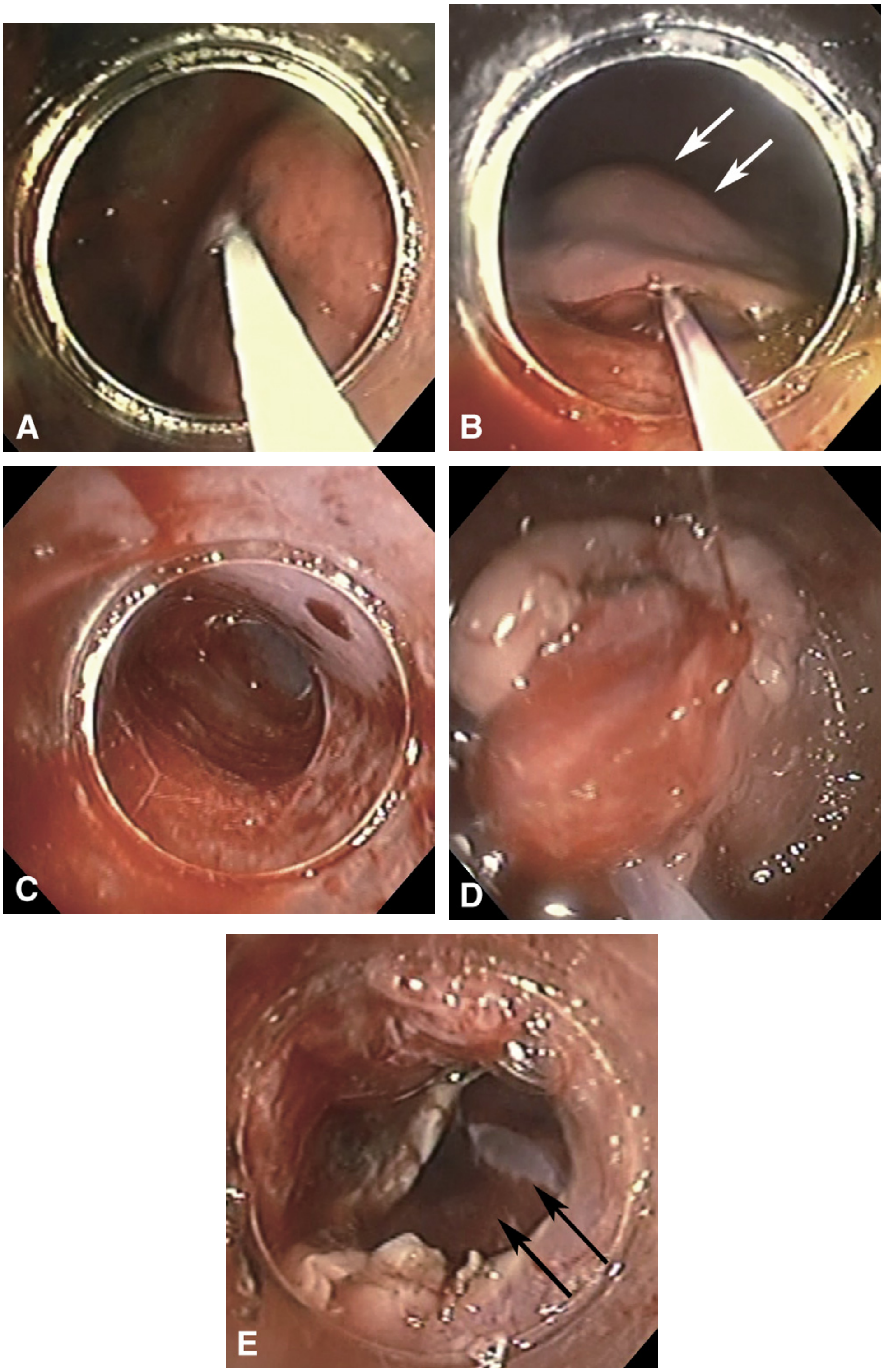

Figure 1. Procedural steps. **A,** Submucosal gas separation with CO_2 injection. **B,** A balloon distended in the submucosal space to dissect connective tissue (*arrows*). **C,** Submucosal working space. **D,** Muscular layer resected with the cap EMR technique. **E,** Perforating muscular defect; the liver is visualized through a muscular defect (*arrows*).

revealed an open serosal defect (greater than 2 cm) in 3 pigs, with mucosa sealing the defect completely (Fig. 5). A single tissue anchor used to fix the mucosal flap at the anterior wall had penetrated the liver in 1 animal. The animal with a posterior-wall approach was noted to have a localized transmural small-bowel injury. Water-leak testing on the resected stomachs showed no water seepage.

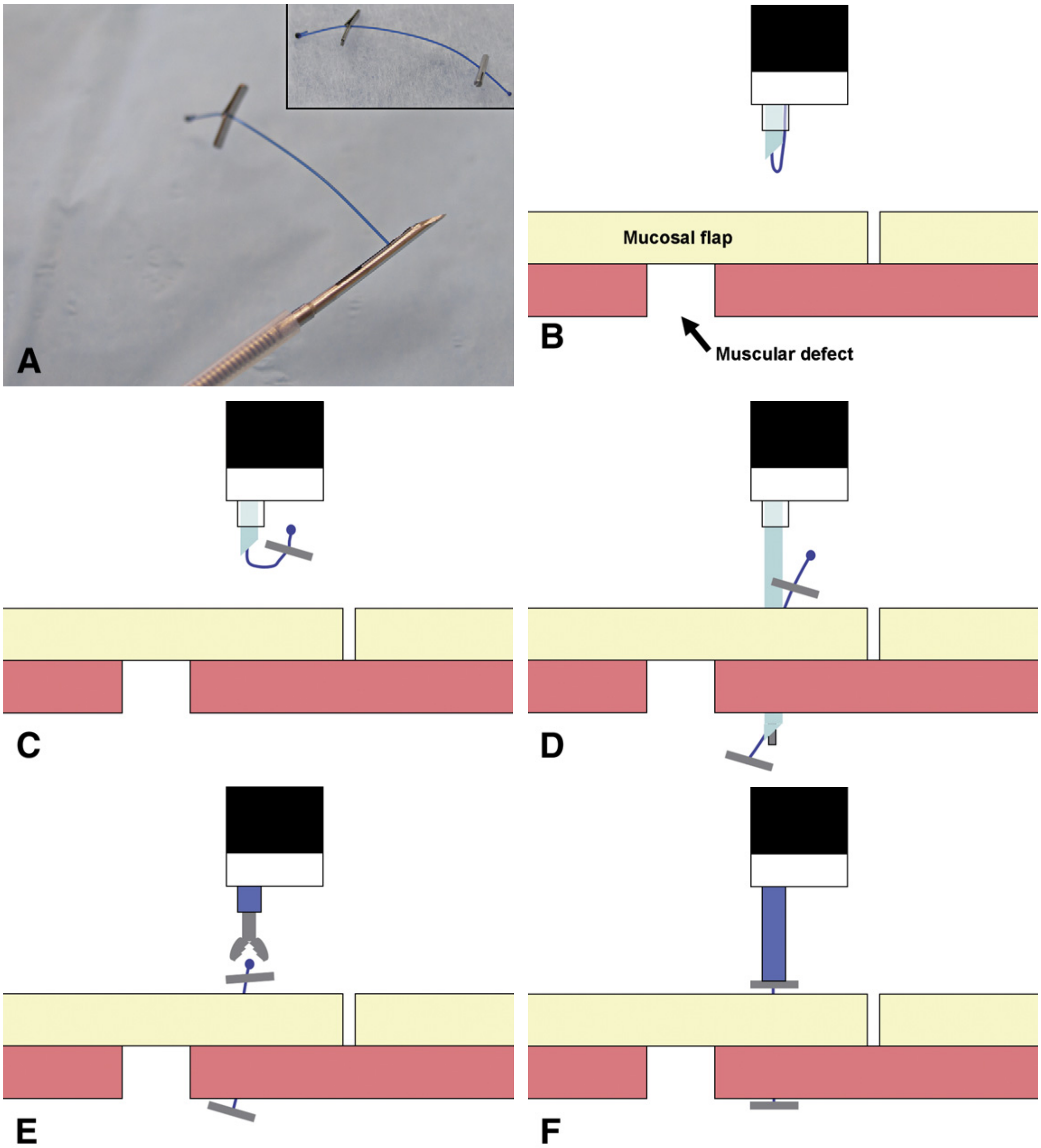

Figure 2. Images and schematic presentation of the prototype tissue-anchoring device. **A,** Images of tissue anchor with a 18-gauge needle delivery system; a tissue anchor consists of a thread and 2 tags attached at both ends of the thread (imposed image). **B,** Needle-delivery system passed through an accessory channel of the scope. **C,** Proximal T tag was released from the outer sheath. **D,** The mucosal and the muscular layer are penetrated with the needle, and a distal T tag is deployed from the needle. **E,** A proximal tip of the thread is grasped with a grasping forceps. **F,** A tissue anchor is cinched by pushing the proximal T tag with a pushing catheter over the forceps.

DISCUSSION

This effort explored our methodologic concept of using a submucosal space created by a combination of gas insufflation and balloon dissection to serve as a protective tunnel, allowing access to the peritoneal cavity and preventing peritoneal soiling by using the free overlying mucosa as a sealant flap. The SEMF methodology would permit safe endoscopic access to the peritoneal cavity during procedures such as NOTES. This approach could also be applied to acquisition of muscular tissue for motility disorders, as we have demonstrated.[16,17] The balloon dissection, which separated and opened the submucosal layer into a free working space, is similar to urologic laparoscopic procedures within the retroperitoneum.[18,19] CO_2 insufflation creates a widespread but controlled instantaneous submucosal gas cushion when compared with injected fluid. Balloon dissection was simple to perform and is theoretically safer, avoiding perforation and bleeding by eliminating tedious and error-prone electrosurgical knife cutting during electrosurgical endoscopic submucosal dissection.[20-22]

TABLE 1. Results of in vivo studies

Pig	1	2	3	4
Location	Anterior	Posterior	Anterior	Anterior
Sample size, mm	18	25	10	23
Closure method	Glue	Clips	Tissue anchors (2)	Tissue anchors (2)
Depth of the submucosal tunnel, mm	65	70	80	80
Food intake during follow-up period	Good	Poor	Good	Good
Serosal defect size after 1 wk, mm	20	20	0 (scar)	22
Weight gain 1 wk after the procedure, kg	2.2	−5.4	1.4	4
Endoscopic finding 1 wk after the procedure	Ulcer, 10 × 0.5 mm	Ulcer scar	Ulcer, 25 × 55 mm	Ulcer, 15 × 7 mm
Complication	None	Small-bowel injury	None	A tissue anchor penetrated the liver

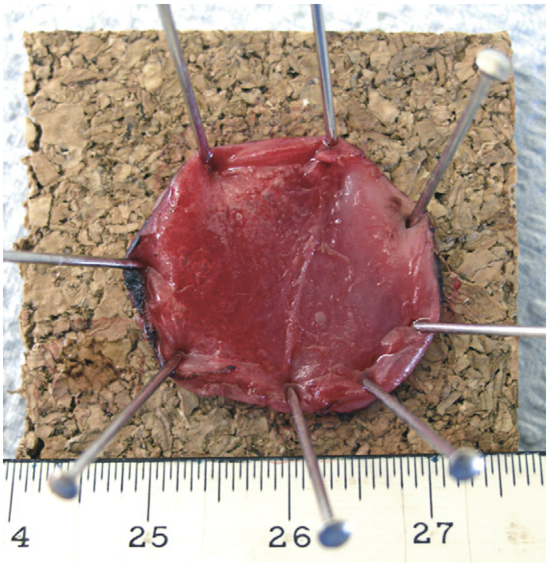

Figure 3. A muscular layer sample (pig no. 4 in Table 1).

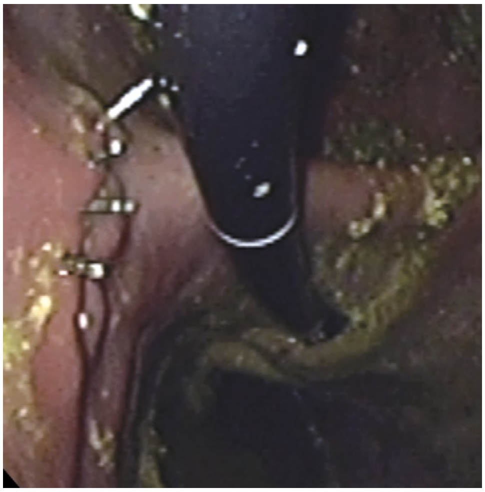

Figure 4. A manipulated site after the clip mucosal apposition (pig no. 2 in Table 1). A tissue anchor is penetrating the mucosal flap.

The results demonstrated that access into the peritoneal cavity by using this unique approach was feasible and safe when using an anterior gastric approach. The posterior gastric wall, although tested in only 1 animal, appeared to carry a higher risk for complications. This animal had a significant nonperforating small-bowel necrotic injury that resulted in a poor appetite and post-procedure weight loss. A posterior gastrotomy site allowed the endoscope and the other devices prompt contact with major structures (vessels), organs (pancreas), and the small bowel, with risk of inadvertent injury. In the case of the single pig for which a posterior approach was used, we speculated that the suction applied to perform the full-thickness resection of the muscularis included small bowel, and there was ‘‘bystander’’ thermal injury during the snare excision of the muscle sample. Use of this SEMF technique in NOTES procedures does not require the performance of a cautery-based full-thickness resection of the muscularis. The value of the technique is the creation of the submucosal space and the offset transmural exit site distant from the mucosal entry site. For NOTES procedures, a puncture followed by balloon dilation of the exit site would be safer.

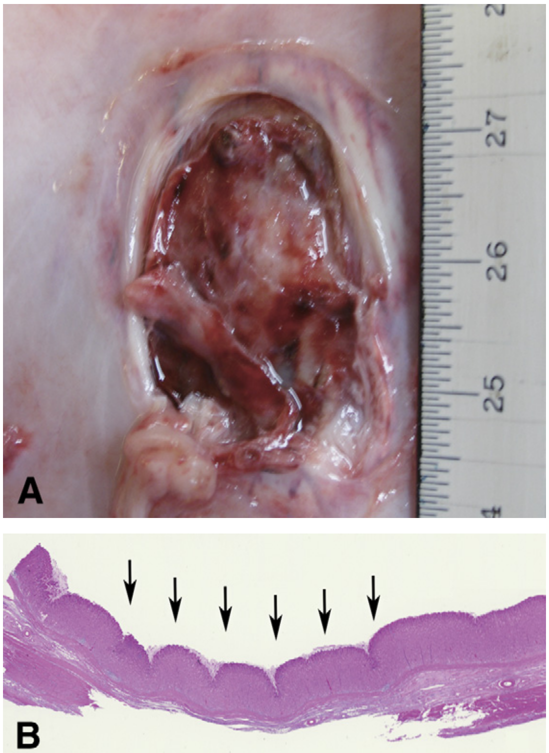

Figure 5. A, Serosal defect at 1 week after the procedure (pig no. 2 in Table 1). **B,** Low-power histologic overview of a muscular defect sealed by normal mucosa at 1 week after the procedure (pig no. 2 in Table 1) (H&E, orig. mag. ×3).

The observed ulceration represented partial necrosis of the overlying free mucosa. We suspect that these ulcerations may be because of a combination of factors, including suboptimal mucosa-to-mucosa apposition for tissue closure or trauma from endoscope manipulation, within a suboptimal submucosal working space or inadequate mucosal entry-point incisions. Additional long-term studies are required to clarify the cause for this ulceration, which may not be an unexpected finding when using this technique.

Some of the challenges for this methodology include the following: (1) identifying a reliable method to fix the incised mucosal flap onto the underlying muscular layer; this might be accomplished by means of a more robust clip or a clip-like device, which provides secure mucosa-to-mucosa apposition at the entry incision, or the use of a biologic sealant to adhere the mucosal flap onto the muscular layer, effectively sealing the submucosal space; (2) determining the appropriate size of the mucosal entry incision and submucosal working space for a specific intended procedure.

The inadvertent capture of the liver surface by the tissue anchor used in this study is not acceptable, despite the animal doing well clinically. Although tissue anchors have appeal, further refinements in their deployment are needed.

In this preliminary preclinical study, we successfully applied a unique endoscopic approach for access and manipulation within the submucosal space and the peritoneal cavity, while minimizing peritoneal leakage by using a novel mucosal-flap technique.

DISCLOSURE

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EDITORIAL

Natural orifice transluminal endoscopic surgery: a step toward clinical implementation?

Recently, preclinical investigators[1-6] reported enthusiasm for transgastric endoscopic surgery. In this issue of *Gastrointestinal Endoscopy*, an experimental study by Sumiyama et al[7] describes procedures for gaining access to the peritoneal cavity and for closing the resulting gastric-wall defect. A working group,[8] consisting of endoscopists from the American Society for Gastrointestinal Endoscopy (ASGE) and laparoscopic surgeons from the Society of American Gastrointestinal Endoscopic Surgery (SAGES) designated transgastric endoscopic surgery as ''natural orifice transluminal endoscopic surgery'' (NOTES).

In the history of the development of medical therapies, successive new interventional procedures have been developed that aim at decreasing invasiveness. For example, pancreatic-cyst–drainage guided EUS has been reported to be safe and feasible.[9,10] Endoscopic debridement of necrotic pancreatic tissue[11] and aggressive endoscopic therapy for pancreatic abscess[12] have been reported. These investigators performed daily endoscopic necrosectomy and lavage with saline solution after dilation of the cystogastrostoma or the cystoduodenostoma, and the pancreatic fistula was then sealed with N-butyl-2-cyanoacrylate. Like laparoscopic surgery, such endoscopic procedures are increasingly attractive alternatives to open surgery, because minimizing invasiveness can decrease postoperative stress, morbidity, and length of hospital stay. NOTES may offer patients benefits, such as reduced pain and less risk of complications, including wound infection, hernia, and adhesions.[1-3] A peroral transgastric approach to the abdominal cavity by using a flexible endoscope also might offer special advantages in patients with extensive scars, burns, or an infection of the abdominal wall, as well as those with severe obesity.

In transgastric surgery, we still need to optimize disinfection, acid control, incision making, air or carbon dioxide insufflation, maintenance of pneumoperitoneum, and, finally, incisional closure.

However, several problems need to be resolved before the clinical implementation of NOTES. First, the procedure should be reliable and safe, without early complications, such as infectious contamination, damage to adjacent organs, and bleeding. Second, any late complication, such as gastric dysfunction caused by adhesion resulting from a wide incision, should be managed effectively. Third, indications should be selected carefully. After Kaloo et al[1] reported liver biopsy and laparoscopic exploration as indications for transgastric endoscopic surgery, several investigators proposed possible additional indications for

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