Endoscopic full-thickness biopsy of the gastric wall with defect closure by using an endoscopic suturing device: survival porcine study

Elizabeth Rajan, MD,[1] Christopher J. Gostout, MD,[1] Eduardo Aimore Bonin, MD,[1] Erica A. Moran, MD,[1] Richard G. Locke, MD,[2] Lawrence A. Szarka, MD,[2] Nicholas J. Talley, MD,[2] Jodie L. Deters, LPN,[1] Charles A. Miller, LPN,[1] Mary A. Knipschield, MS,[1] Matthew S. Lurken, MS,[2] Gary J. Stoltz,[2] Cheryl E. Bernard,[2] Madhusudan Grover, MD,[2] Gianrico Farrugia, MD[2]

Rochester, Minnesota, USA

Background: The pathogenesis of several common gastric motility diseases and functional GI disorders remains essentially unexplained. Gastric wall biopsies that include the muscularis propria to evaluate the enteric nervous system, interstitial cells of Cajal, and immune cells can provide important insights for our understanding of the etiology of these disorders.

Objectives: To determine the technical feasibility, reproducibility, and safety of performing a full-thickness gastric biopsy (FTGB) by using a submucosal endoscopy with mucosal flap (SEMF) technique; the technical feasibility, reproducibility, and safety of tissue closure by using an endoscopic suturing device; the ability to identify myenteric ganglia in resected specimens; and the long-term safety.

Design: Single center, preclinical survival study.

Setting: Animal research laboratory, developmental endoscopy unit.

Subjects: Twelve domestic pigs.

Interventions: Animals underwent an SEMF procedure with gastric muscularis propria resection. The resultant offset mucosal entry site was closed by using an endoscopic suturing device. Animals were kept alive for 2 weeks.

Main Outcome Measurements: The technical feasibility, reproducibility, and safety of the procedure; the clinical course of the animals; the histological and immunochemical evaluation of the resected specimen to determine whether myenteric ganglia were present in the sample.

Results: FTGB was performed by using the SEMF technique in all 12 animals. The offset mucosal entry site was successfully closed by using the suturing device in all animals. The mean resected tissue specimen size was 11 mm. Mean total procedure time was 61 minutes with 2 to 4 interrupted sutures placed per animal. Histology showed muscularis propria and serosa, confirming full-thickness resections in all animals. Myenteric ganglia were visualized in 11 of 12 animals. The clinical course was uneventful. Repeat endoscopy and necropsy at 2 weeks showed absence of ulceration at both the mucosal entry sites and overlying the more distal muscularis propria resection sites. There was complete healing of the serosa in all animals with minimal single-band adhesions in 5 of 12 animals. Retained sutures were present in 10 of 12 animals.

Limitations: Animal experiment.

Conclusions: FTGB by using the SEMF technique and an endoscopic suturing device is technically feasible, reproducible, and safe. Larger tissue specimens will allow improved analysis of multiple cell types. (Gastrointest Endosc 2012;76:1014-9.)

Abbreviations: FTGB, full-thickness gastric biopsy; ICC, interstitial cells of Cajal; PGP9.5, protein gene product 9.5; SEMF, submucosal endoscopy with mucosal flap; SFC, submucosal fluid cushion.

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Current affiliations: Developmental Endoscopy Unit (1) and Enteric NeuroScience Program (2), Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine, Rochester, Minnesota, USA.

Reprint requests: Elizabeth Rajan, MD, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905.

The etiology of GI neuromuscular diseases, including functional GI disorders, remains largely unknown. There is recent evidence to support underlying neuromuscular pathological changes that are heterogeneous and include the loss of interstitial cells of Cajal (ICC) and enteric nerves and the presence of inflammatory infiltrates.[1-5] For example, surgically obtained full-thickness gastric biopsy (FTGB) samples from patients with gastroparesis show a decrease in ICC in 50% of patients, an immune infiltrate in 45%, and a decrease in nerve fibers.[6] The presence of an immune infiltrate correlated with nausea and vomiting.[7] Nonsurgically obtained FTGB samples that include the muscularis propria to evaluate the enteric nervous system, ICC, immune cells, and other related cells are essential to further our understanding of the pathophysiology of these disorders and intervene earlier in the disease process. Mucosa-based biopsies are insufficient as they do not allow evaluation of the deep muscle layers as well as the myenteric plexus present between the inner circular and outer longitudinal muscle layers. Our earlier work with experimental endoscopic techniques was limited by a combination of poor safety data and inadequate tissue sampling.[8,9] Endoscopic acquisition of FTGB samples that is safe, effective, and minimally invasive would contribute to accurate diagnosis and identification of patients who would benefit from targeted therapy.

The aims of this study were to determine the technical feasibility, reproducibility, and safety of performing an FTGB by using a submucosal endoscopy with mucosal flap (SEMF) technique; reliable tissue closure by using endoscopic suturing; the ability to identify myenteric ganglia in resected specimens; and long-term safety.

Take-home Message

- Full-thickness gastric biopsy by using the submucosal endoscopy with mucosal flap technique with endoscopic suturing is feasible, reproducible, and safe. Ample tissue samples can be obtained by using this technique to allow analysis of multiple cell types including myenteric ganglia and interstitial cells of Cajal.
- Such an endoscopic approach reflects an important directional shift in diagnostic procedures that may lead to invaluable insights into the pathophysiology and potential novel targeted therapy of GI neuromuscular disorders.

MATERIALS AND METHODS

Experimental design

This preclinical survival study in a pig model was approved by the Institutional Animal Care and Use Committee. Twelve pigs were studied. Each animal underwent an SEMF procedure with an FTGB followed by closure of the offset mucosal entry point by using an endoscopic suturing device. Animals were kept alive for 2 weeks at which time a repeat endoscopy was performed, followed by necropsy. The main study outcome measurements were the clinical course of animals, technical feasibility, reproducibility, and short- and long-term (2 weeks) safety of the procedure. Data on the procedure, clinical course, and follow-up endoscopy with necropsy were recorded. Data analysis was descriptive for this feasibility study.

Animals

Fourteen domestic 35-kg pigs were approved for the study with the intent of using 12 of them. With the animals under general anesthesia, endoscopy was performed. Animals were allowed a liquid diet 48 hours before the procedure and water only ad libitum 24 hours before the procedure. Antibiotics were administered for 5 days after the procedure (ceftiofur 5 mg/kg IM daily and metronidazole 1 g bid PO). Analgesia (buprenorphine hydrochloride 0.03 mg/kg IM) was given immediately after the procedure. Animals were placed on a liquid diet for 1 day after the procedure, fed softened food on the second day, and by the third day, the animals resumed regular feed if tolerated. Each animal received oral proton pump inhibitors (Nexium [esomeprazole magnesium] 40 mg bid PO) for 7 days after the procedure. After a 2-week survival period, repeat endoscopy was performed. Animals were chemically euthanized (pentobarbital 100 mg/kg IV) immediately after endoscopy, and this was followed by necropsy.

Procedure

Step 1. SEMF. The intent was to create a submucosal tunnel within which a full-thickness biopsy specimen that included the muscularis propria would be obtained. The resection site was offset from the mucosal entry point to the submucosal tunnel by approximately 4 to 5 cm. The overlying mucosal flap created by tunneling through the submucosa was used as a sealant flap protecting the peritoneum from contamination by the gastric contents.

A large submucosal fluid cushion (SFC) was initially formed by using saline solution (~40 mL) injected via a standard needle injection catheter (23-gauge Injector Force; Olympus America, Center Valley, Pa). A small incision (<5 mm) was made on the proximal aspect of the SFC by using a needle-knife, which served as the mucosal entry point. A tunneling balloon 18 mm in diameter (Apollo Endosurgery Inc, Austin, Tex) was inserted in the SFC, and as the balloon was inflated (Fig. 1), the unique progressive unfurling of this dilation balloon created a submucosal tunnel revealing muscularis propria. The length of the submucosal tunnel varied and depended on the length of the balloon used (5-8 cm) and degree of balloon inflation.

After the submucosal tunnel was created, a double-channel endoscope (2T 160; Olympus America) with an

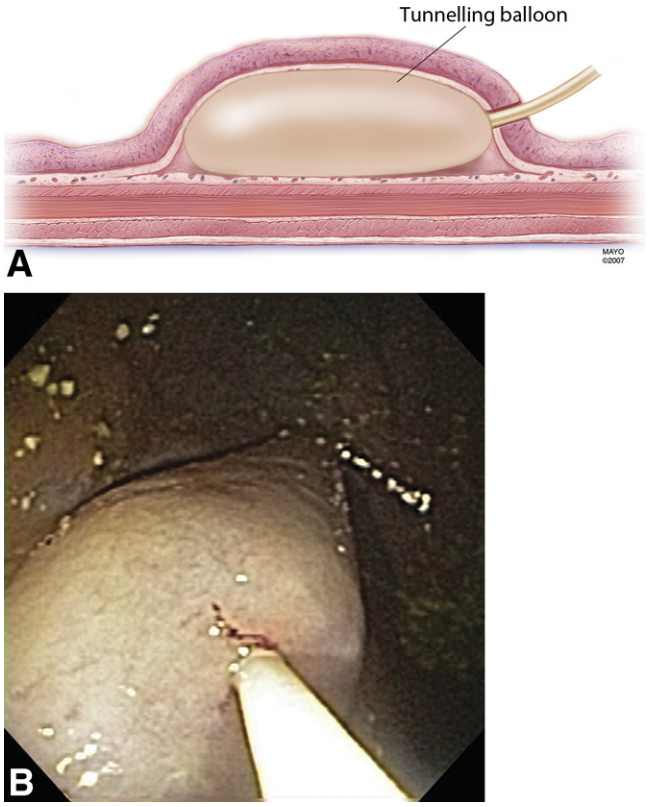

Figure 1. A, Dissection of submucosal space with a tunneling balloon. **B,** Endoscopic view of a tunneling balloon dilated in the submucosal space.

EMR-type cap attached was advanced through the submucosal tunnel. The EMR clear cap maintained tunnel patency and allowed improved visualization. An endoscopic Doppler probe (VTI Vascular Technology, Nashua, NH) was advanced through the endoscope working channel and placed within this submucosal space to identify any underlying blood vessels. Then a spiral tissue helix (Apollo Endosurgery Inc) or rat-tooth grasping forceps (Olympus America) was used to tent the muscularis propria toward the endoscope and into the cap. By using electrocautery, the muscularis propria was resected by using a spiral snare (Olympus America) or hexagonal snare (Traxtion US Endoscopy, Mentor, Ohio). Tissue was retrieved and submitted for analysis.

Step 2. Endoscopic suturing. The suturing device (Overstitch; Apollo Endosurgery Inc) was attached to the endoscope and advanced into the stomach through an overtube (US Endoscopy) placed within the esophagus. The Overstitch suturing device simulates free-hand suturing and allows controlled suture placement. The offset mucosal entry point was closed by interrupted polypropylene 3-0 sutures. Closure was considered adequate if the entry site was visibly closed without gaps and there was sustained distention of the gastric lumen with air insufflation suggesting no air leak.

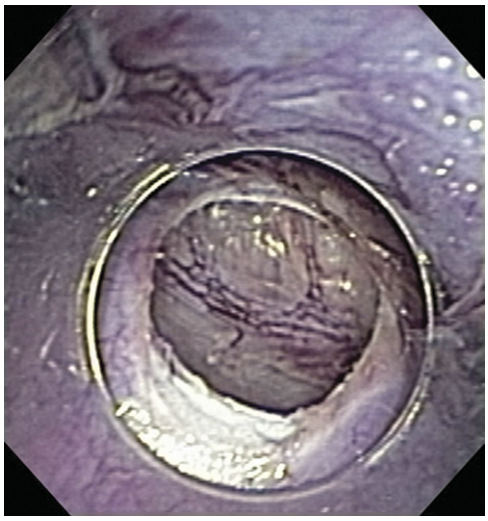

Figure 2. Visualization of the peritoneum after full-thickness gastric biopsy.

Tissue samples

The resected tissues were transported over ice to the laboratory in Ham F12 media (Invitrogen, Carlsbad, Calif). Resected tissue was measured and sectioned.

Hematoxylin and eosin staining was used to determine which muscle layers were included in the resected specimen, and an antibody to protein gene product 9.5 (PGP9.5) was used as a general neuronal marker to determine whether myenteric neurons were present in the sample.[8,9]

RESULTS

To study 12 animals, 14 pigs were enrolled. Two were excluded early in the study after 1 death caused by anesthesia-related complications and the other had a superficial mucosal tear over the tunnel. In the former, necropsy was performed and no abnormality was detected within the peritoneal cavity with an unremarkable postbiopsy site. In the latter, a muscularis propria resection was not performed, but the animal recovered well. In this setting, the procedure could be hypothetically repeated after mucosal healing in 4 to 6 weeks.

An FTGB was performed by using the SEMF technique in all 12 animals. The peritoneal cavity was visualized in each animal, providing endoscopic confirmation of a full-thickness resection (Fig. 2). The offset mucosal entry site was successfully closed in all animals by using the endoscopic suturing device (Fig. 3). No immediate procedure-related complications occurred. Histology showed muscularis propria and serosa, confirming full-thickness resections in all animals (Fig. 4). Multiple myenteric ganglia were visualized in 11 of 12 animals by using PGP9.5 antibodies (Fig. 5). In 1 animal, the snare slipped during resection, resulting in a smaller sample that was full thickness but without

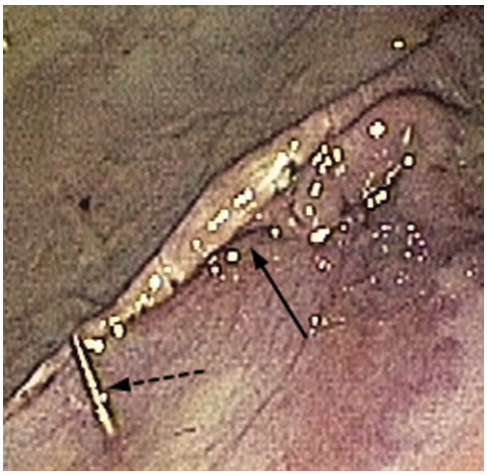

Figure 3. Closure of the offset mucosal entry point (*solid arrow*) after endoscopic suturing with suture tag visible (*broken arrow*).

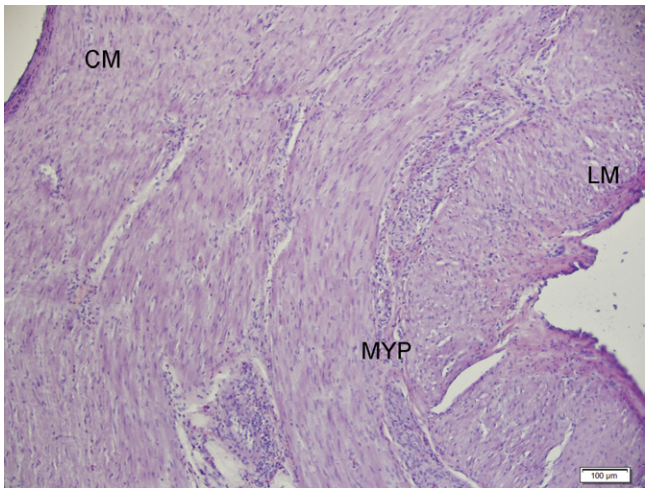

Figure 4. Hematoxylin and eosin–stained section showing the presence of circular muscle (CM), myenteric plexus (MYP), and longitudinal muscle (LM) in the sample. Scale bar = 100 μm.

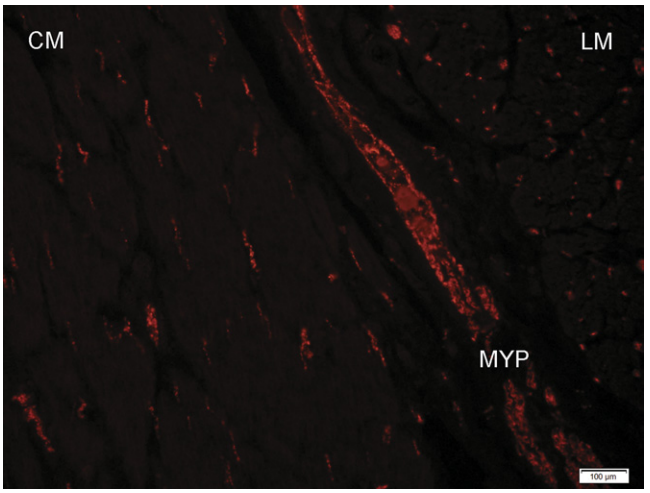

Figure 5. Resected tissue showing anti–protein gene product 9.5 immunopositive myenteric ganglia. Circular muscle (CM), myenteric plexus (MYP), longitudinal muscle (LM). Scale bar = 100 μm.

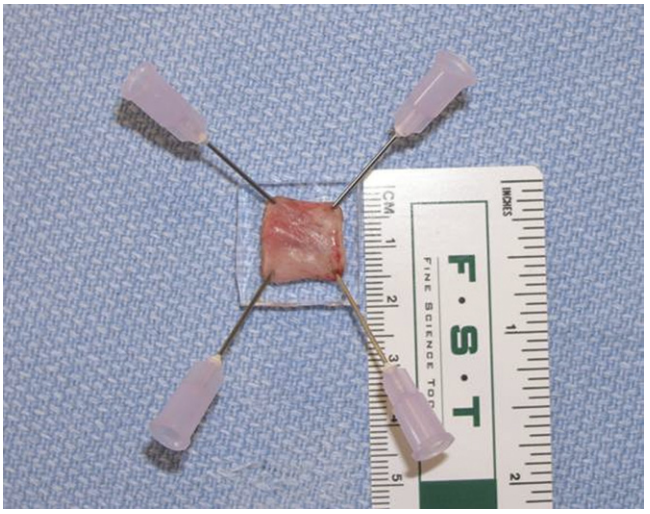

Figure 6. Resected tissue.

identifiable myenteric ganglia. The mean total procedure time from submucosal injection to completion of suturing was 61 minutes (range 40-95 minutes). In the latter 6 animals, the resected tissues were measured before fixation with a recorded mean long-axis length of 11 mm (range 7-13 mm) (Fig. 6). Resections were performed from either the anterior or posterior gastric body. Two to 4 interrupted sutures were placed per animal. Procedure feasibility and safety did not differ with the use of rat-tooth grasping forceps (n = 6) versus a spiral tissue helix (n = 6) and a spiral snare (n = 6) versus hexagonal snare (n = 6). The clinical course was uneventful in all animals. Repeat endoscopy at 2 weeks showed stellate scarring at the mucosal entry sites and the absence of mucosal ulceration at the entry sites and overlying the more distal muscularis propria resection sites (Fig. 7). Necropsy after endoscopy showed complete healing of the serosa in all animals with minimal single-band adhesions in 5 of 12 animals (Fig. 8). Retained sutures were present in 10 of 12 animals.

DISCUSSION

This preclinical survival study evaluated the technical feasibility, reproducibility, and safety of an FTGB by using the SEMF technique and endoscopic suturing. By using this novel technique, a full-thickness biopsy of the entire muscularis propria that included oblique, circular, and longitudinal muscle layers could be technically achieved with sufficient tissue obtained from the intermuscular layer to identify multiple myenteric ganglia by using PGP9.5 antibodies. This is important because myenteric ganglia do not form a continuous layer, and therefore the sample needs to be sufficiently large to capture several ganglia. The significant benefit of the SEMF technique is the pres-

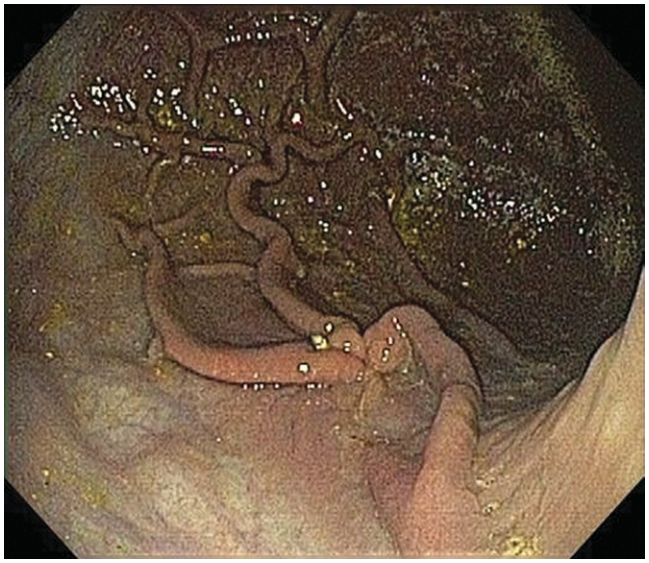

Figure 7. Stellate scar at mucosal entry site seen at endoscopy.

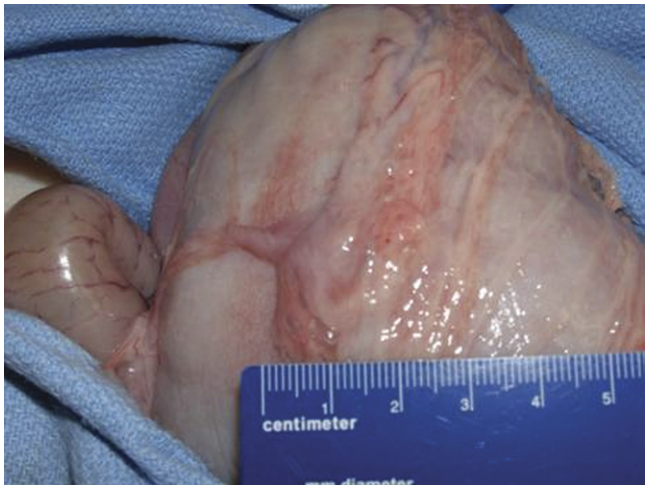

Figure 8. Serosa with single-band adhesion.

ence of the overlying mucosal flap that serves as a safety valve to seal the gastric wall perforation. Effective closure of the mucosal entry point was achieved in all animals by using the endoscopic suturing device. All 12 animals had an uncomplicated clinical course with complete healing of the mucosal and serosal aspects of the resection sites at follow-up endoscopy and necropsy. There was acquisition of ample tissue samples comparable to surgical specimens and in close accordance with the guidelines of the Gastro 2009 International Working Group on histological techniques.[10,11] In human trials, the target site will be the anterior gastric body, approximately 9 cm proximal to the pylorus, as recommended by the guidelines of the Gastro 2009 International Working Group.[10] We anticipate that the resection technique, in theory, should be easier in a human study because of the improved endoscope position within the stomach compared with the near-retroflexed position of the endoscope when working in the porcine stomach.

This procedure reflects an important directional shift in approaching invasive and complex endoscopic techniques. We previously reported on the evaluation of different existing endoscopic approaches for acquisition of deep biopsy samples of the gastric muscle wall to include the intermuscular layer. However, all of the studied techniques including the innovative "no-hole" double EMR were limited by the lack of adequate tissue and/or safety.[8,9] The no-hole EMR technique involved an initial gastric EMR followed by creating a pseudopolyp of the exposed muscularis propria by using endoloops and T-tag tissue anchors. The pseudopolyp was then resected. This study explored the concept of obtaining deep muscle wall biopsies by using a unique approach of resection without perforation. The SEMF technique was pioneered by research in our Developmental Endoscopy Unit as a concept to use the submucosa as an intramural working space for endoscopic interventions into or beyond the gut wall.[12-15] The offset mucosal entry into the submucosal space and exit into the peritoneal cavity minimizes peritoneal contamination, and the overlying mucosa serves as a sealant flap valve. This unique SEMF approach has been successfully performed clinically to access the peritoneal cavity for natural orifice transluminal endoscopic surgery applications and for the performance of myotomy in achalasia.[16,17]

Percutaneous endoscopically assisted transenteric full-thickness biopsy is a novel clinically applied method for assessing histopathological abnormalities in GI neuromuscular disease patients. Initial experience showed abnormalities identified in 44% of patients such as possible degenerative leiomyopathy.[18,19] The limitation of this technique compared with the SEMF technique or that obtained by standard laparoscopy is the small sample size, which is less than the size recommended by the Gastro 2009 International Working Group guidelines and does potentially reduce diagnostic yield.[20] Another approach that was used in a nonsurvival study evaluated colonic endoscopic full-thickness biopsies by using an EMR-based technique.[21] Future studies are needed to assess the safety of this procedure.[21] Other options for evaluating myenteric ganglia are also being investigated. The use of innovative submucosal probe–based confocal laser endomicroscopy that provides optical histological imaging is currently being evaluated in preclinical studies with promising results.[22,23] Studies identifying a neuron-specific fluorescent stain for human use and addressing any potential toxicity or long-term effects of these neuronal probes are under way. However, it is likely that subtyping neurons, immune cells, and ICC will continue to require tissue acquisition. In this context, our study technique using an invasive endoscopic approach allows the acquisition of sufficient tissue to facilitate quantitative and qualitative analysis of multiple cell types. The ready availability of such an endoscopic

technique may lead to invaluable insights into the pathophysiology and potential novel targeted therapy of GI neuromuscular disorders.

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