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Double plication for spring-mediated in-continuity intestinal lengthening in a porcine model ☆

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A R T I C L E I N F O

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A B S T R A C T

Background: Short bowel syndrome is a condition with substantial morbidity and mortality, yet definitive therapies are lacking. Distraction enterogenesis uses mechanical force to “grow” new intestine. In this study, we examined whether intestinal plication can be used to safely achieve spring-mediated intestinal lengthening in a functioning segment of jejunum in its native position.
Methods: A total of 12 juvenile, miniature Yucatan pigs underwent laparotomy to place either compressed springs or expanded springs within a segment of jejunum ($n = 6$ per group). The springs were secured within the jejunum by performing intestinal plication to narrow the intestinal lumen around the spring. After 3 weeks, the jejunum was retrieved and examined for lengthening and for histologic changes.
Results: There were no intraoperative or postoperative complications, and the pigs tolerated their diets and gained weight. Segments of jejunum containing expanded springs showed no significant change in length over the 3 weeks. In contrast, jejunum containing compressed springs showed nearly a 3-fold increase in length ($P < .001$). Histology of the retrieved jejunum showed a significant increase in thickness of the muscularis propria and in crypt depth relative to normal jejunum.
Conclusion: Intestinal plication is effective in securing endoluminal springs to lengthen the jejunum. This approach is a clinically relevant model because it allows for normal GI function and growth of animals during intestinal lengthening, which may be useful in lengthening intestine in patients with short bowel syndrome.

Introduction

Although a relatively rare disorder that affects 24.5 infants in 100,000 live births, short bowel syndrome (SBS) carries considerable morbidity and mortality.[1] This condition refers to the inability of the digestive system to meet the nutritional needs of the body secondary to a critical loss of intestinal length and thus absorptive surface area.[2] The causes may be either congenital or acquired and include necrotizing enterocolitis, intestinal atresia, gastroschisis, and midgut volvulus.[3,4] The mortality is high at 20%–40% as a result of numerous complications, such as electrolyte and metabolic derangements, catheter-associated infections, renal failure, and liver failure.[5,6] This rare condition also poses an incredible financial burden because the median cost is estimated to be $1.6 million per child over a 5-year period.[7]

Treatment of SBS is multifaceted and includes dietary supplements, total parenteral nutrition, and medications to slow gut transit and improve intestinal adaptation.[8,9] Some patients may require operative intervention to maximize bowel length, but unfortunately these procedures are not always successful in weaning patients from total parenteral nutrition.[10–13] Ultimately, patients may need an intestinal transplantation, but the 5-year survival rate for pediatric patients after intestinal transplant is only 75%.[14] As such, new and improved therapies are being sought to help patients with SBS.

One area of active research is distraction enterogenesis, or the use of mechanical force to induce novel tissue growth. In numerous animal models using various devices, this has been shown to be successful in increasing intestinal length.[15–22] These methods, however, are limited because they typically require the intestine to be first isolated or taken out of continuity from the rest of the bowel. We have employed small, implantable, helical springs to achieve intestinal lengthening, and we have recently developed

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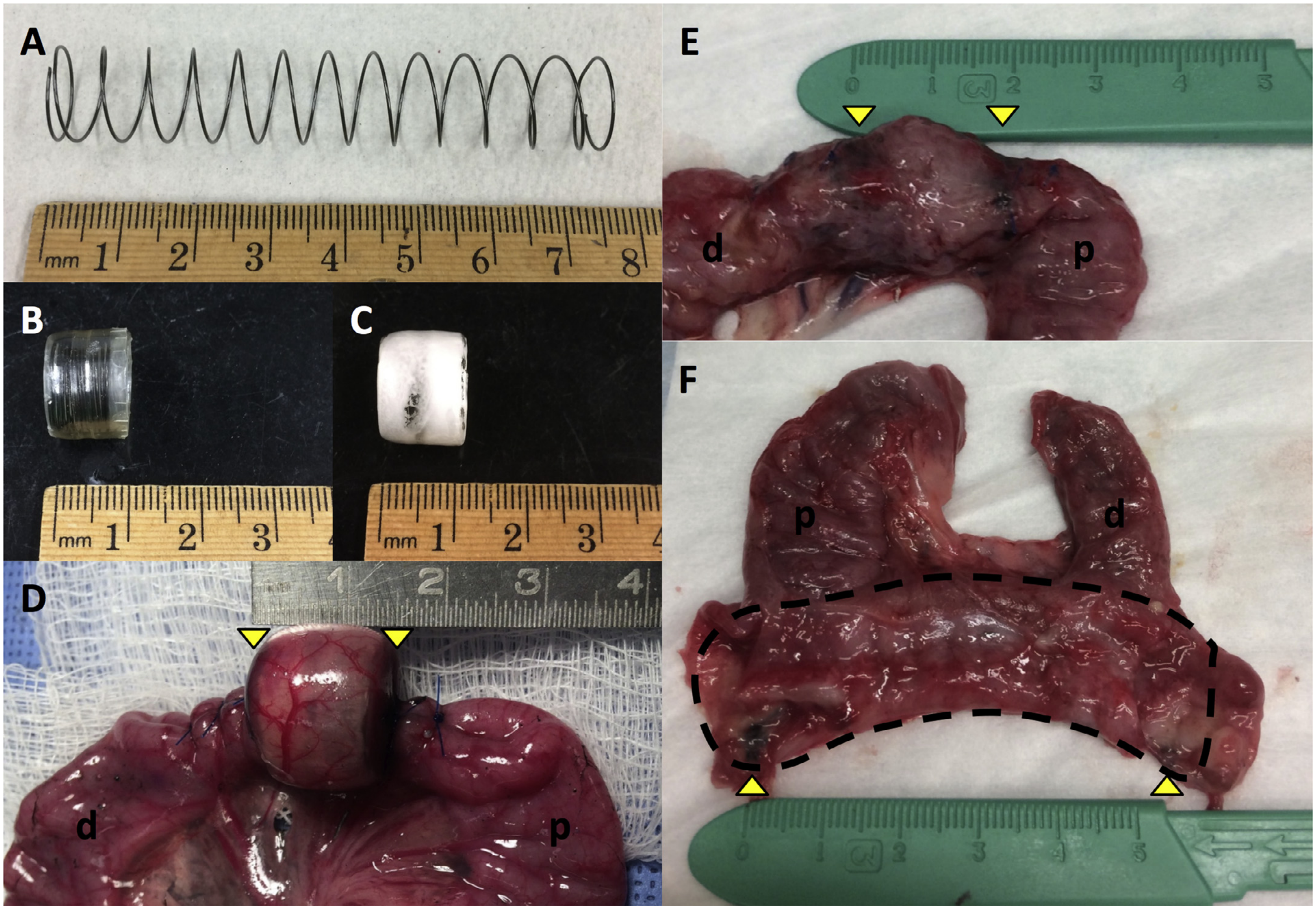

Fig. 1. (A) 7.5 cm experimental nitinol spring. (B) Gelatin capsule with a compressed spring inside. (C) CAP-coated gelatin capsule with a compressed spring inside. (D) In-continuity segment of jejunum with a spring-containing gelatin capsule placed inside; plication sutures surround the capsule and measure 1.5 cm apart. (E) Jejunum containing an initially expanded spring, with spring removed 3 weeks after spring placement. (F) Jejunum containing an initially compressed spring 3 weeks after spring placement, with spring removed and ends cut open to show spots of India ink. Arrowheads mark ends of segments, and dashed lines show contours of intestine containing the spring. *P* = intestine proximal to segment containing the spring; *d* = intestine distal to segment containing the spring.

a new method of securing these springs within the intestinal lumen using plication to narrow the lumen of the defunctionalized intestine around the spring.[23] In this current study, our aim was to build on this previous work to show that spring-mediated intestinal lengthening can be safely achieved in a functional segment of intestine left in its native configuration.

Materials and methods

Animal use was approved by the Animal Research Committee (institutional review board number 2014-142-03). All materials were FDA-approved for use in humans. The 12 pigs were female, juvenile, miniature Yucatans weighing 8–11 kg and aged 4–8 weeks (S&S Farms, Ramona, CA). Nickel-titanium (nitinol) wire (0.02 inch in diameter, McMaster-Carr, Santa Fe Springs, CA) was used to create helical springs. These springs were prepared for intestinal insertion by placing them inside size 13 gelatin capsules (Fisher Scientific, Pittsburgh, PA), and then coating the capsules in cellulose acetate phthalate ([CAP], Eastman Chemicals, Fairfield, NJ). Histologic slides were prepared with 10% buffered formalin (Fisher Scientific, Pittsburgh, PA).

Spring production

A threaded mandrel with a diameter of 1.3 cm was used as a mold to create springs with a pitch of 0.625 cm and 12 active coils over 7.5 cm (Fig. 1). The nitinol wire was wrapped around the mold and heated to 500°C for 25 min to set the shape. The springs were then cooled rapidly under water. Final springs measured 7.5 cm in length, and spring constants were 6–7 N/m ($n = 6$). Expanded springs ($n = 6$) were made the same way but were cut to be shorter in length so they would not be compressed and would not exert any axial force. These served as controls. Size 13 gelatin capsules were trimmed and the ends folded in to create a more compact capsule of 1.0 cm in length. Springs were placed inside, and the capsules were coated in CAP to delay capsule breakdown until after operative insertion was completed (Fig. 1, *A–C*).

Surgical procedure

The pigs ($n = 12$) were premedicated with ketamine and midazolam and were then intubated and placed under general anesthesia with inhaled isoflurane. Via a midline incision, the ligament of Treitz was identified, and the jejunum was measured 50 cm distally. The intestine was then transected at this point. A 1.5 cm length of intestine was identified 20 cm proximal to this transection. This distance was marked by injecting 2 spots of India ink to indicate where the springs (either the expanded springs in the control group [$n = 6$] or the compressed springs in the experimental group [$n = 6$]) would be placed so that the segment could be evaluated later for lengthening.

Intestinal plication was performed by placing 4-0 polypropylene sutures in series, and thus narrowing the diameter of the bowel in both the control and the experimental group. This was first done at the proximal spot of India ink. A 20 French Foley catheter was passed into the intestine via the transected end, and 2 interrupted 4-0 polypropylene sutures in series were used to plicate the bowel down to the diameter of the Foley catheter at the proximal spot of India ink. The Foley was removed, and a capsule containing either a compressed spring ($n = 6$) or an expanded spring ($n = 6$) was placed within the marked segment of intestine. The Foley catheter was then again replaced, and 4 more plication sutures were placed in series at the distal spot of India ink (Fig. 1, *D*). The Foley was removed, and the transected bowel was re-anastomosed in its original configuration using interrupted 4-0 polypropylene sutures. The location of the spring was also marked by placing small clips in the mesentery leading to that jejunal segment to identify the location on radiographs. The intestine was returned into the abdomen, and the incision was closed in multiple layers.

Radiographic evaluation

Expansion of the springs and their locations relative to the mesenteric clips were evaluated with serial radiographs. The pigs had abdominal radiographs performed approximately 1 and 2 weeks after operation.

Gross and histologic evaluation

The pigs were killed 3 weeks (post-op day 21–24) after the operation for spring placement. At this time, a normal segment of jejunum was retrieved distal to the implanted spring and proximal to the bowel anastomosis. Jejunum containing expanded or compressed springs was also retrieved. Springs were removed, and the segments were then measured for length based on India ink location and plication suture location. The segments of jejunum were placed in 10% buffered formalin overnight. These samples of jejunum were then cut into cross sections, and slides were prepared by imbedding the cross sections in paraffin. Five µm sections were cut, stained with hematoxylin and eosin, and examined under bright-field microscopy at 4x magnification. Measurements were taken to evaluate the thickness of the muscularis propria and crypt depth.

Statistical analysis

Data were expressed and reported as means ± standard deviations; 2-tailed paired Student's t-tests were used for analysis of lengthening data, whereas 2-tailed unpaired Student's *t*-tests were used for analysis of weight change and histologic data.

Results

There were no intraoperative complications. Postoperatively, all pigs tolerated liquid diets, and there were no cases of perforation or obstruction. Both control and experimental groups showed average weight gains of 2.9 kg over the 3 weeks after operation.

X-ray imaging

All springs were seen to remain in place based on radiographs for the duration of the study. Whereas expanded springs (control group) showed no change from their starting length, compressed springs (experimental group) showed a gradual expansion over the 3 weeks. On average, 1 week after implantation, the compressed springs measured 4.8 cm on radiographs, and 2 weeks after implantation they measured 5.7 cm on radiographs.

Intestinal lengthening

Jejunum that contained expanded springs showed no significant change in length ($P = 0.29$). In contrast, jejunum that contained compressed springs increased to an average length of 4.2 ± 0.8 cm ($P < .001$). Based on the starting length of 1.5 cm, this represented a 2.8-fold increase in length (Fig. 1, *E–F*).

Histologic analysis

Normal jejunum collected distal to the implanted springs showed an average thickness of the muscularis propria of 225 ± 51 µm and an average crypt depth of 243 ± 50 µm. Relative to normal jejunum, jejunum containing both expanded springs and compressed springs showed a significant increase in the thickness of the muscularis propria and crypt depth. Jejunum with expanded springs had an average muscularis propria thickness of 354 ± 62 µm ($P < .001$), and an average crypt depth of 417 ± 113 µm ($P < .001$). Jejunum with compressed springs had an average thickness of the muscularis propria of 487 ± 87 µm ($P < .001$) and an average crypt depth of 476 ± 74 µm ($P < .001$). Comparing the control group and the experimental group, both thickness of the muscularis and crypt depth were increased in the group with compressed springs ($P < .05$).

Discussion

In this study, we showed that small endoluminal springs can be used to increase the length of a segment of intestine by nearly 3-fold in just 3 weeks in pigs. Most importantly, the lengthening was achieved in a segment of intestine that was in continuity with the remainder of the GI tract, and neither our endoluminal device nor our intestinal plication appeared to interfere with the natural enteric flow of GI contents. All the pigs were able to tolerate liquid diets postoperatively without obstruction, and they showed adequate weight gain with the spring in place. Histology of the lengthened segments revealed that both the muscle layer and the crypts showed significant hypertrophy relative to normal jejunum. This supports the idea that spring-mediated lengthening is not simply stretching an existing piece of intestine and making it thinner, but rather that the mechanical stress from the spring prompted a cellular response in the tissue that resulted in novel growth. Although the spring-lengthened intestine had a significantly thicker muscle layer relative to the control segment, even the expanded spring caused substantial histologic changes compared to normal jejunum. This observation can be explained by the fact that during spring placement, the gelatin capsule distends the intestine radially and causes some radial expansion although no longitudinal lengthening. It is likely this radial force precipitates the cellular changes. These results are also consistent with our past experiments, where a sham device with no spring caused thickening of the muscle layer but no lengthening.[22,23]

In-continuity lengthening may prove to be a major improvement on past models of distraction enterogenesis. Besides endoluminal springs, a number of different devices have been used for distraction enterogenesis, including polyethylene glycol, an extraluminal shape memory polymer, and a hydraulic piston.[15–17] So far, the use of these devices has been limited because they may create an intestinal blockage during lengthening or they may require multiple operations to place and retrieve them. Having to reoperate and to reconfigure the anatomy of the bowel to create a defunctionalized segment leads to loss of crucial intestinal length and is not realistic in the clinical setting. As a result, these past models served an important role as a proof of concept, but they are not ready to be implemented in a patient population. In contrast, our current approach may be relevant and may be adapted to patients with SBS.

Challenges remain with our current model of intestinal lengthening. First, the metal springs we used in this study remained in the intestine for the duration of the study, and thus in a human trial they would need to be retrieved with a second operation, which would be less desirable. To overcome this challenge, we see the following possible solutions. In the past, we have used springs made of polycaprolactone, which is a dissolvable material also used in monocryl sutures.[18] Using springs made of this material allows for lengthening to occur first, and then the spring is able to slowly dissolve over time without needing removal. Alternatively, we have considered using dissolvable sutures to create the intestinal plication so that after lengthening, dissolution of the sutures might allow the plicated segment of intestine to be released and thereby allow the spring to naturally pass through the intestine. These are both future alterations that we will consider implementing in this model.

Another challenge of this model is that although a 2.8-fold increase in bowel length would pose a substantial clinical benefit to patients, the absolute lengthening we saw in this study was an increase on average of only 2.7 cm. There are 2 ways that this small gain can be amplified to become clinically meaningful. First, it may be possible to use multiple springs in series in the same patient. For example if 10 springs were placed sequentially, this might yield a total gain of 27 cm, which would be clinically relevant. Second, it may be possible to lengthen the same segment of intestine multiple times to keep magnifying the amount of length gained. Both of these strategies have been effective in a rat model.[20,21]

Another important consideration in bowel lengthening is the stability and functionality of the lengthened intestine. Past studies in pigs have shown that once a segment of intestine is lengthened, the amount of lengthening is preserved even after the force being exerted on the intestine is removed.[22] In rats, intestine lengthened via springs is also capable of normal peristalsis under fluoroscopy and shows increased absorption of glucose.[24] This observation gives credence to the idea that distraction enterogenesis results in tissue growth that is clinically relevant and that this method could provide long-term benefits to patients.

Finally, our current model has the disadvantage of necessitating an operation to place the spring and to perform an intestinal plication. Although this does not completely preclude its clinical suitability, a less invasive lengthening model would be preferred. One possible solution would be to deploy the springs endoscopically. Double-balloon enteroscopy can be performed safely in pediatric patients and allows for access to the small bowel orally, anally, or via an ostomy if present.[25] It also is possible to perform endoscopic suturing, as has been done to close mucosal defects or to perform a gastroplasty,[26–28] but performing endoscopic suturing inside the small bowel of a child would be much more challenging given the small size. We believe, however, it may be possible to make use of these existing tools to insert a spring inside the intestine and to secure it with plication sutures all via an endoscope, which would obviate the need for a laparotomy altogether.

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Conflicts of interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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