A novel biodegradable device for intestinal lengthening

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text:

Abstract: Previous studies demonstrated successful mechanical lengthening of rat jejunum using an encapsulated Nitinol spring device over a stabilizing guidewire. We sought to improve the applicability of intestinal lengthening by creating a biodegradable device.

Methods: Using properties of the Nitinol spring device, polycaprolactone (PCL) springs with similar outer diameter and spring constant were created. After in vitro testing in dry and hydrated environments, they were used to lengthen 1-cm isolated segments of rat jejunum in vivo. Retrieved segments were analyzed histologically.

Results: Optimal PCL spring devices had an average spring constant 1.8 ± 0.4 N/m, pitch 1.55 ± 0.85 mm, and band width 0.825 ± 0.016 mm. In vitro testing demonstrated stable spring constants. Jejunal segments were lengthened from 1.0 cm to 2.7 ± 0.4 cm without needing a stabilizing guidewire. Histology demonstrated increased smooth muscle thickness and fewer ganglia compared to controls. Lengthened jejunum was successfully restored into intestinal continuity and demonstrated peristalsis under fluoroscopy.

Conclusions: A novel biodegradable spring device was successfully created and used to mechanically lengthen intestinal segments. Use of a biodegradable device may obviate the need for retrieval after lengthening. This improves device applicability and may be useful for the treatment of short bowel syndrome.

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Short bowel syndrome (SBS) is a devastating condition owing to loss of significant intestinal length thereby affecting the organ’s ability to absorb nutrients. This results in malnutrition, malabsorption and dehydration. The incidence of SBS is estimated to be 24.5 per 100,000 live births [1]. Despite improvements in medical and surgical management over the last few decades, mortality in the neonatal population has remained 20–40% [1,2]. The diagnosis of SBS is clinical, although it is typically associated with resection or loss of 70% or more of functional small intestine. Etiologies in the neonatal population include necrotizing enterocolitis, midgut volvulus, aganglionosis, intestinal atresias, abdominal wall defects and complicated meconium ileus [3]. While management strategies have changed significantly over the last few decades, morbidity owing to gastric hypersecretion, fluid and electrolyte abnormalities, osteoporosis, parenteral nutrition dependence, central venous catheter-related complications, and secondary hepatic dysfunction and failure remains high [3–6]. Even with a low prevalence in the pediatric population, health care costs are significant at an estimated $1.6M per patient [7]. Current surgical therapy includes transit-slowing and bowel lengthening procedures, and in the most severe cases small bowel and liver transplantation [3,8,9]. These procedures have significant complications [10], can only be performed in selected patients, and the 5-year survival rate of transplantation is 54% [11].

To address the fundamental issue of inadequate functional intestinal length in SBS, recent research has focused on distraction enterogenesis, a method of lengthening existing bowel. This concept has reached clinical success in multiple tissues including bone, breast, esophagus, urethra, and most recently in the aorta [12–16]. Multiple devices have been developed in recent years with encouraging results [17–21]. We previously showed that jejunum could be lengthened over 3-fold with an encapsulated Nitinol spring device and that this lengthened segment could successfully be restored into continuity [22,23]. In this model a stabilizing guidewire was necessary to prevent buckling of the spring during deployment. In addition, use of a non-biodegradable material may necessitate device retrieval if used in an endoscopic delivery system. We therefore sought to improve upon the applicability of the device by using a biodegradable material with the structural integrity to obviate the need for a stabilizing guidewire.

1. Materials and methods

Animal use was approved by the Animal Research Committee (Institutional Review Board Number 2002–037–22) and complied with all established institutional regulations. Adult female Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) were used.

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All materials were FDA approved for use in humans. Intestinal lengthening was achieved using springs made from polycaprolactone (PCL), a biodegradable polymer used in absorbable suture. PCL springs with a similar spring constant (1 N/m) and outer diameter (3.4 mm) to Nitinol springs were constructed. Springs were placed in cellulose acetate phthalate-coated (Eastman Chemicals, Kingsport, TN), size 5 gelatin capsules (Torpac Inc, Fairfield, NJ) for spring compression and delayed expansion. All operations were performed by a single surgeon.

1.1. Spring development

Polymer springs were fabricated using a 3.8% (w/w) PCL (Lactel, Birmingham, AL) solution in chloroform. PCL solutions were spray-coated onto a spinning 4 mm stainless steel mandrel to form polymer tubes with 300 and 500 μm radial thickness. Polymer spirals were hand cut from PCL tubes, elongated to 30 mm, and heat set at 50 °C for 1 hour to form final PCL springs (Fig. 1). Spring constants were measured using an Instron electromechanical testing system (Instron, Norwood, MA). The springs were tested in dry, hydrated and degraded conditions. Springs tested in dry conditions were measured in ambient air. Those tested in hydrated conditions were immersed in phosphate buffered saline (PBS) at 37 °C for 10 minutes prior to testing. Degraded PCL springs were incubated in 37 °C in PBS for 2 and 4 weeks then tested. Spring specifications producing spring constants most closely resembling previously used Nitinol springs were used to fabricate final springs. These were compressed into coated gelatin capsules as described above.

1.2. Surgical procedure

Rats were anesthetized with inhaled oxygen and isoflurane (n = 5). PCL spring devices were surgically placed into 1-cm isolated segments of jejunum approximately 10-cm from the ligament of Treitz as previously described [23]. Encapsulated 1-cm PCL tubes were placed into isolated jejunal segments to serve as controls (n = 4). Animals were explored 4–6 weeks postoperatively. After length measurements and tissue samples were collected, the lengthened segment was restored into continuity as previously described (n = 4) [24]. All animals survived. Of note, longer segments of jejunum from each end were sampled to obtain adequate tissue for histologic analysis. Thus, the restored segment length did not represent the total length of functional tissue. Animal weights were recorded weekly.

1.3. Radiographic evaluation

After the second procedure, animals underwent oral gavage with Omnipaque (GE Healthcare, Waukesha, WI) between 2 and 4 weeks. Small bowel follow through under fluoroscopy was performed with radiographs taken every 15 minutes until contrast was identified in the cecum. Gastrocecal transit times were recorded. Segments of restored jejunum were identified and visualized under fluoroscopy during the contrast study.

1.4. Histologic analysis

Normal and mechanically lengthened jejunal tissues were retrieved and fixed in 10% buffered formalin overnight. Tissue embedded in paraffin blocks was cut into 5 μm sections and stained with hematoxylin and eosin. Sections were viewed and recorded on a light microscope at 40× and 100× magnification. Thickness of the muscularis propria and circumference were measured. Unstained tissue sections were prepared and stained for S100 positive glial cells as previously described [25]. The number of ganglia was assessed at 100× magnification under fluorescent light microscopy in submucosal and myenteric plexuses and expressed as number of ganglia per mm circumference.

1.5. Statistical analysis

Data were expressed as mean values ± standard deviations. Two-tailed and paired Student’s t tests were used for statistical analyses where appropriate.

2. Results

2.1. Spring development

After testing 300 and 500 μm thickness springs, the devices most closely approximating the forces of the Nitinol spring had an average spring constant of 1.8 ± 0.4 N/m, outer diameter 3.34 ± 0.075 mm, pitch (distance between coils) 1.55 ± 0.085 mm, thickness 293 ± 8.17 μm, and band width 0.825 ± 0.016 mm (Table 1). Spring constants remained unchanged after testing in dry, hydrated and degraded environments (Fig. 2).

2.2. Lengthening

Jejunal segments were lengthened from 1 cm to 2.7 ± 0.4 cm (p < 0.001), a nearly 3-fold increase (Fig. 3). Isolated jejunal controls containing encapsulated 1-cm PCL tubes expanded from 1 cm to 1.6 ± 0.2 cm (p < 0.05). The change in length between experimental and control groups was statistically significant (p = 0.002). Lengthened segments were successfully restored back into intestinal continuity (Fig. 4).
2.3. Radiographic evaluation

Upper gastrointestinal contrast study with small bowel follow through revealed normal gastrocecal transit times (<105 minutes). The restored segment was identified on fluoroscopy by surgical clips. There was no evidence of bowel dilation and no delay in passage of contrast in restored segments. Peristalsis was seen throughout the restored jejunal segment and in segments of jejunum adjacent to the contrast in restored segments. Peristalsis was seen throughout the restored jejunal segment and in segments of jejunum adjacent to the restored segment (Fig. 5).

2.4. Histology

On histologic analysis lengthened jejunum had increased thickness of the muscularis propria compared to normal jejunum (263 ± 74 versus 93 ± 30 μm, p < 0.005). Control specimens also demonstrated an increase in muscularis propria thickness 4 weeks after PCL tube implantation. Mucosa with crypts and villi were present in each lengthened specimen. In comparison to normal jejunum, S100 staining demonstrated a marked decrease in the density of enteric ganglia in both the submucosal (0.3 ± 0.2 versus 3.0 ± 0.4 ganglia per mm, p < 0.001) and myenteric plexuses (0.7 ± 0.2 versus 3.4 ± 0.9 ganglia per mm, p < 0.005).

3. Discussion

Distraction enterogenesis, or intestinal lengthening in response to the application of linear forces, has been investigated in multiple ways to develop a feasible model for the treatment of SBS. A wide variety of lengthening devices including saline injections, hydraulic pistons, anchored screws, and Nitinol springs have been employed [17–22]. Improvements in development of the Nitinol spring device included modification of distractive forces to minimize tissue trauma, use of a guidewire to prevent spring buckling, and placement of the device into a polymer-coated gelatin capsule to more reliably control timing of deployment [23]. While these modifications have increased feasibility of the device there are still limitations to its applicability. Use of a non-biodegradable material necessitates either device retrieval with a second procedure or development of a method of temporarily anchoring the device to the jejunal wall when placed in a segment of bowel that is in continuity. In addition, a long guidewire was required to prevent the Nitinol spring from buckling during deployment. This protruded through the isolated jejunal segment and had the potential to injure surrounding tissues or cause intraperitoneal leakage of luminal contents. We therefore used specifications from the Nitinol spring to create a spring made from PCL, a biocompatible, biodegradable polymer used in multiple biomedical applications including absorbable suture [26]. The improved material strength of the expanded PCL spring combined with increased band width and thickness also allowed expansion of the device without buckling, thereby eliminating the need for a guidewire.

In vitro testing of the springs confirmed stable spring forces in varied environments. When placed in vivo to lengthen isolated segments of jejunum, we observed a nearly 3-fold increase in length. Although control segments demonstrated some lengthening, a significantly greater amount of lengthening was seen in experimental specimens. The lengthening seen in control segments was caused by mucus buildup and subsequent pressure on the intestinal wall, while the additional lengthening seen in experimental segments was owing to the mechanical force from the PCL spring. Although PCL will degrade over time in an aqueous environment, the PCL springs did not degrade in isolated intestinal segments during the short period of lengthening in these experiments. Tissue analysis of lengthened jejunum confirmed the presence of villi and crypts as well as muscularis propria thickening that is characteristic of mechanically lengthened small bowel. We also found a significant decrease in both myenteric and submucosal ganglia in lengthened segments compared to normal jejunum. These structural and histologic changes have been found in previous studies, suggesting the same mechanism of cell proliferation owing to mechanical stress [20,22,25].

The considerable decrease in density of enteric ganglia would suggest that motility is altered in the lengthened segment. An in vitro
study showed that isolated lengthened jejunal segments maintained contractility but at a decreased level \[27\]. However, we observed that after restoration back into continuity, peristalsis was seen in the lengthened segment and appeared to be in sync with adjacent bowel. There was no abnormal dilation and gastrocral transit times were normal. In order to corroborate our findings on fluoroscopy, histologic analyses of lengthened then restored segments will be studied and reported in the future. We previously demonstrated regeneration of enteric ganglia after lengthened jejunal segments were restored into continuity, although numbers of ganglia did not fully return to normal \[25\]. The time to tissue harvest in that particular study was only 3 weeks and one animal had normal numbers of ganglia after 5 weeks. Our fluoroscopic findings of peristalsis and normal intestinal transit times in combination with continued weight gain in all animals indicate that functional motility is regained. Although this suggests that ganglia are regenerated to normal or near normal numbers, the quantity of ganglia may not predict intestinal motor function. This is supported by the fact that some contrast small bowel studies were performed as early as 2 weeks postoperatively when there were likely to be fewer ganglia, yet clinically the animals were thriving. In a recent study by Koga, et al., smooth muscle contractility was decreased in mechanically lengthened jejunum, then increased 28 days after restoration back into continuity, but not to normal levels \[28\]. They also concluded that this measurable difference was not clinically relevant.

In summary, we created the first biodegradable intestinal lengthening device and it no longer requires a stabilizing guidewire. After nearly 3-fold lengthening, restored jejunal segments demonstrated peristalsis on contrast fluoroscopy. This supports the use of a biodegradable lengthening device as a treatment for SBS. Future studies are needed to examine the functional and absorptive capacity of mechanically lengthened, restored intestine.

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References


Fig. 4. Photograph of the lengthened, restored jejunal segment (arrow). P indicates proximal anastomosis.

Fig. 5. Contrast small bowel follow through under fluoroscopy showing relaxation (A) and contraction (B) during peristalsis in the restored segment (black arrow) demarcated by surgical clips (white arrowheads). Animal positioning is prone.


Discussion

Discussant: Dr. Daniel Teitelbaum (Ann Arbor, MI): I want to congratulate you. Beautiful talk and very innovative. I guess the next challenge would be, are you trying to move this to an in-continuity device as opposed to one that is isolated? We certainly seem to lose a lot of bowel by both the isolation as well as then replacing it back into continuity. We often lose a lot of what we’ve gained.

Response: Dr. Veronica Sullins: One of the major hurdles from the previous Nitinol spring was the fact that it was not absorbable. We felt like we overcome this hurdle; however, we are still working with the materials to try to find some way to couple it with the intestinal wall so that we can put it in continuity. We actually did—earlier this year our lab put it into a semi-continuous model and still there is some tissue loss when we take down the anastomosis proximally but that is something that we definitely are going to focus on in the future.

Discussant: Dr. Mary Brandt (Houston, TX): First of all, very well presented and congratulations on really exciting work. I may have not read it properly but it looked like you were starting with 1-cm pieces of bowel that went to 2.7 with the device but it looked like it went to 1.7 in the controls. I wonder if you would comment on that.

Response: Dr. Veronica Sullins: Right, I didn’t mention the control segments, but we put an encapsulated PCL tube that was not cut into a spring device into isolated segments of bowel and we’ve seen that happen where you see the same hypertrophy and histologic changes in the control segments; however, a lot of it has to do with the mucus production, lengthening of that intestinal segment. Our previous work as well as previous work in other labs has demonstrated some lengthening but it is not sustained and it also doesn’t lengthen as much.