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Repeated Mechanical Lengthening of Intestinal Segments in a Novel Model☆

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ABSTRACT

Purpose: Currently, animal models used for mechanical intestinal lengthening utilize a single lengthening procedure prior to analysis or restoration back into continuity. Here we developed a novel surgical model to examine the feasibility of repeated lengthening of intestinal segments. Methods: A Roux-en-Y jejunojejunostomy with a blind Roux limb was created in rats. An encapsulated polycaprolactone spring was placed into a 1 cm segment of the Roux limb. After 4 weeks, a second encapsulated PCL spring was inserted into a 1 cm portion of the lengthened segment. After another 4 weeks, the repeatedly lengthened segments were retrieved for histological analyses. Results: Jejunal segments of the Roux limb were successfully lengthened from 1.0 cm to 2.6 ± 0.7 cm. Four weeks after the second PCL spring placement, 1.0 cm of the previously lengthened segment increased to 2.7 ± 0.8 cm. Stronger mechanical force was required to achieve subsequent re-lengthening. Lengthened and re-lengthened segments had increased smooth muscle thickness and crypt depth when compared to normal jejunal mucosa. Conclusion: Using the Roux-en-Y model, previously lengthened segments of intestine can be successfully re-lengthened. Intestinal segments may be subjected to multiple lengthening procedures to achieve clinically significant length for the treatment of short bowel syndrome.

Despite decades of research, short bowel syndrome (SBS) remains a condition associated with high morbidity and mortality, ranging from 20% to 40% [1–4]. SBS in the neonatal population is linked to necrotizing enterocolitis, aganglionosis, intestinal atresias, midgut volvulus and abdominal wall defects [2,3]. Despite both medical and surgical advancements, patients with SBS continue to suffer significant long-term morbidity [5]. The use of TPN is associated with catheter-related morbidity, metabolic derangements, cholestasis, increase risk of liver failure and reduced quality of life [2,3]. Surgical treatment options are limited in overall results, rarely are curative and are associated with many complications [2,5,6].

A potential treatment of SBS is lengthening existing intestine using mechanical distraction [2,3,7]. Many groups have developed and studied intestinal lengthening using various devices [2,6–9]. Koga et al. showed that using a hydraulic-driven concentric piston, isolated segments of pig intestine can be lengthened through distraction [6]. Using polyethylene glycol (PEG), Sueyoshi et al. showed that PEG infused into isolated intestinal segments in mice resulted in elongation of segments [9]. Previously, we showed that isolated segments of intestine can be lengthened by the use of an expanding Nitinol spring and more recently a biodegradable polycaprolactone (PCL) spring [2,10]. In all models, lengthening was achieved in intestinal segments isolated from intestinal continuity. However, there have been no studies that have investigated re-lengthening previous lengthened segments. In our study, we used a novel surgical model to investigate the feasibility of repeated mechanical lengthening of the small intestine.

1. Materials and methods

The use of all animals was approved by the Animal Research Committee (Institutional Review Board no. 2002-037-22). All implanted and surgical materials used in this study were FDA approved for use in humans. Intestinal lengthening and re-lengthening was achieved using springs made from PCL, which is a biodegradable polymer used in many medical devices [10]. PCL springs were constructed as previously described [10], placed into size 5 gelatin capsules (Torpac Inc, Fairfield, NJ) that were coated with cellulose acetate phthalate (Eastman Chemicals, Kingsport, TN) to allow for delayed expansion. Springs used for the initial lengthening and re-lengthening had spring constants of at least 1 N/m and greater than 5 N/m, respectively.

1.1. Surgical procedure

Adult female Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing at least 220 g were anesthetized with

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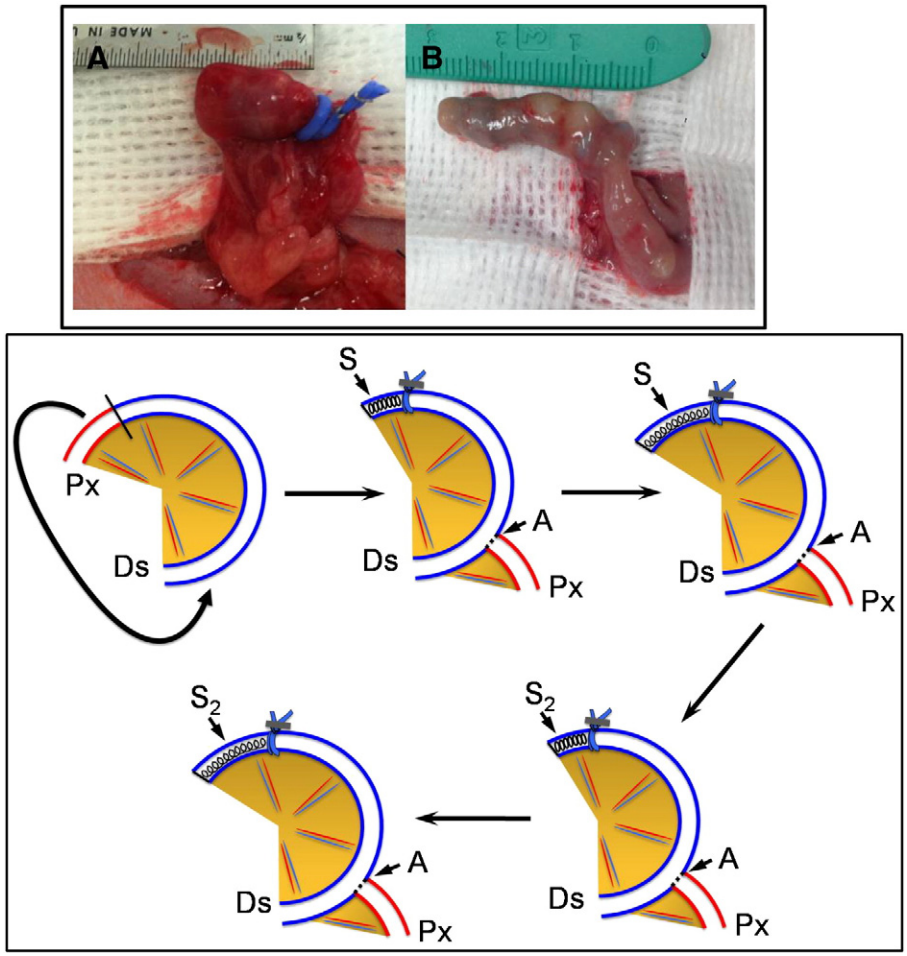

Fig. 1. Photographs and diagram of the Roux-en-Y procedure. (A) Lengthened segment trimmed to 1 cm with second PCL spring inserted and (B) re-lengthened intestinal segment 4 weeks after the second surgery. The bottom diagram is a schematic representation of the re-lengthening model [11]. Px = proximal, Ds = distal, S = spring, S_2 = second spring, A = anastomosis.

inhaled oxygen and vaporized isoflurane (n = 8). A midline laparotomy incision was used to enter the abdomen, and the jejunum was located and transected approximately 10 cm from the ligament of Treitz. A defunctionalized Roux limb was created as previously described [11]. A vessel loop was placed through a small opening in the mesentery 1 cm from the blind end of the Roux limb. An encapsulated PCL spring with a spring constant between 1 and 2 N/m was placed into the Roux limb and closed in an interrupted fashion using 6–0 Prolene suture (Ethicon, Johnson & Johnson; Somerville, NJ). The vessel loop was loosely secured with surgical clips, positioning the spring between the end of the defunctionalized limb and the vessel loop (Fig. 1). The bowel was carefully placed back into the abdomen, and the abdominal wall was closed in 2 layers using 3–0 Vicryl (Ethicon, Johnson & Johnson; Somerville, NJ) and 3–0 Monofilament (Ethicon, Johnson & Johnson; Somerville, NJ).

After 3–4 weeks, each animal underwent a second lengthening procedure. The Roux limb was carefully identified. The adherent tissue was carefully dissected from the lengthened segments while preserving the mesenteric blood supply. The lengthened segment of the Roux limb was then measured and trimmed back to a 1 cm segment from the initially placed vessel loop. A new encapsulated PCL spring with a spring constant between 5 and 10 N/m was placed back into the newly created 1 cm segment of the Roux limb. Again, the end was closed with 6–0 Prolene suture (Ethicon, Johnson & Johnson; Somerville, NJ) in an interrupted fashion (Fig. 1). Encapsulated 1 cm PCL tubes were placed into initially lengthened segments of the defunctionalized Roux limbs to serve as controls (n = 3). Re-lengthened and control segments were procured after 3–4 weeks and measured for length and used for histologic analysis.

1.2. Histologic analysis

Lengthened and normal jejunal tissues were fixed in 10% buffered formalin overnight followed by embedding in paraffin. Tissue blocks were cut into 5 μm sections followed by staining with hematoxylin and eosin. Histology was reviewed in a blinded fashion. Sections were examined and recorded at 40× and 100× magnification using light microscopy (Leica Microsystems, Buffalo Grove, IL). Muscularis propria thickness, villus height, crypt depth and circumference were measured for each specimen. Adjacent unstained tissue sections were prepared and stained for S100-positive glial cells as previously described [12]. Using fluorescent light microscopy, the number of ganglia was assessed at 100× magnification in submucosal and myenteric plexuses and expressed as the number of ganglia per cross section. The density of ganglia was calculated and expressed as number of ganglia per millimeter circumference.

1.3. Statistical analysis

The data were expressed as mean values ± SDs. Non-parametric testing (Wilcoxon rank-sum test) was used for statistical analyses.

2. Results

All animals continued to gain weight after the procedures. Intestinal segments of the Roux limb were initially lengthened from 1.0 cm to 2.6 ± 0.7 cm ($P < 0.05$, when compared to control segments). Lengthened segments were successfully re-lengthened from 1.0 cm to 2.7 ± 0.8 cm ($P = 0.056$, when compared to control segments) after the