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Extraluminal distraction enterogenesis using shape-memory polymer

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ABSTRACT

Purpose: Although a few techniques for lengthening intestine by mechanical stretch have been described, they are relatively complex, and the majority involve placement of an intraluminal device. Ideally, techniques applicable to humans would be easy to perform and extraluminal to avoid the potential for mucosal injury. This study of distraction enterogenesis used an extraluminal, radially self-expanding shape-memory polymer cylinder and a simple operative approach to both elongate intestine and grow new tissue.

Methods: Young Sprague Dawley rats (250–350 g) underwent Roux-en-Y isolation of a small intestinal limb and were divided in three groups: no further manipulation (Control 1, C1); placement of a nonexpanding device (Control 2, C2); or placement of a radially expanding device by the limb (Experimental, Exp). For C2 and Exp animals, the blind end of the limb was wrapped around the radially expanding cylindrical device with the limb-end sutured back to the limb-side. Bowel length was measured at operation and at necropsy (14 days) both in-situ and ex-vivo under standard tension (6 g weight). Change in length is shown as mean ± standard deviation. A blinded gastrointestinal pathologist reviewed histology and recorded multiple measures of intestinal adaptation. The DNA to protein ratio was quantified as a surrogate for cellular proliferation. Changes in length, histologic measures, and DNA:protein were compared using analysis of variance, with significance set at $P < 0.05$.

Results: The length of the Roux limb in situ increased significantly in Exp animals ($n = 8$, 29.0 ± 5.8 mm) compared with C1 animals ($n = 5$, −11.2 ± 9.0 mm, $P < 0.01$). The length of the Roux limb ex vivo under standard tension increased in the Exp group (25.8 ± 4.2 mm) compared with the C2 group ($n = 6$, −4.3 ± 6.0, $P < 0.01$). There were no differences in histologic measures of bowel adaptation between the groups, namely villous height and width, crypt depth, crypt density, and crypt fission rate (all $P \geq 0.08$). Muscularis mucosal thickness was also not different ($P = 0.25$). There was no difference in DNA:protein between groups ($P = 0.47$).

Conclusion: An extraluminally placed, radially expanding shape-memory polymer cylinder successfully lengthened intestine, without damaging mucosa. Lack of difference in muscularis thickness and a constant DNA:protein ratio suggests that this process may be related to actual growth rather than mere stretch. This study demonstrated a simple approach that warrants further study aiming at potential clinical applicability.

1. Background

Intestinal failure (IF) is an intrinsic bowel disease that results in the inability to sustain growth or maintain electrolyte and fluid homeostasis [1]. Medical advances, such as the development of intensive interdisciplinary intestinal rehabilitation programs have improved outcomes [2]. Though autologous intestinal reconstructive surgeries (AIRS) like longitudinal intestinal lengthening and tapering (LILT) [3] and serial transverse enteroplasty (STEP) [4] can improve bowel function, their use is limited to patients with significant intestinal dilation.

Mechanical stretch has been used for many years to stimulate growth in tissues such as bone and skin [5,6]. Experimentally, traction on bowel has been shown to produce additional tissue (enterogenesis) and a variety of devices and operative approaches have been used in animals, but none of these strategies have yet translated into clinical use [7–12]. Most of these devices must be placed within the lumen of the bowel, necessitating violation of the bowel wall, creation of a closed loop, and potentially damaging the mucosa. Some require complex activation mechanisms that require external activation. Further, none of the reported devices are FDA cleared or directly predicated on a cleared device.

Shape-memory polymers (SMPs) are plastics that have the ability to change configuration when activated [13]. SMPs can be produced in essentially any geometry, may be heat-activated, and recover their shape with relatively constant velocity, the degree of which can be specified

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by the designer [14–16]. Currently, there are FDA-cleared devices using SMPs, and they are indicated for orthopedic use.

This study used a radially self-expanding SMP device placed extraluminally using a simple operative approach to produce distraction enterogenesis in a rat model. The specific aims were to: (1) demonstrate elongation of intestine and (2) determine whether such elongation was a product of true tissue growth using histologic and biochemical markers.

2. Methods

The Institutional Animal Care and Use Committee at Boston Children's Hospital approved the study (#13-01-2356). Adolescent Sprague–Dawley rats were assigned to one of three groups: a control group without device placement (Control 1, C1), a control group with nonexpanding device placement (Control 2, C2), or the experimental group in which an expanding device was placed (Experimental, Exp).

2.1. Operative procedures

In all animals, a midline laparotomy was performed. Isolated, Roux-en-Y segments were created in each animal: the bowel was the eviscerated and a location approximately 30 cm from the ligament of Treitz (LOT) was selected for transection. An anastomosis was then created between the proximal cut end and a location 6–10 cm from the distal cut end [single layer, interrupted 6-0 PDS (Ethicon, Somerville, NJ)], leaving a length of isolated small intestine (the Roux limb). For C1 animals, the blind end of the Roux limb was ligated and the abdomen was closed with no further intervention. In both C2 and Exp animals, the blind end was wrapped around a coiled, cylindrical shape-memory polymer device. The blind end was then closed and sutured to the side of the roux limb (Figs. 1 and 2). In the C2 animals, this device did not expand, while in Exp animals, the device expanded radially (Fig. 2).

2.2. Device

A sheet of SMP was rolled into a cylinder, which was designed to expand radially over 7 days when activated by body heat and moisture.

To create the polymer, isobornyl acrylate, 2-hydroxyethylacrylate, and 1,6-hexanediol diacrylate were mixed in weight ratio of 75:20:5, respectively. 0.5 wt% of phenylbis(2,4,6-trimethylbenzoyl)phosphine oxide was used as a photoinitiator. All reagents were used as received (Sigma-Aldrich, St. Louis, MO). The mixture was injected into a glass mold with a 0.86 mm spacer and photopolymerized for 3 minutes at 405 nm using a LED flood lamp. After mold removal, the polymer was cut with a CO_2 laser (Gravograph LS-500, Gravotech, Inc, Duluth, GA) into 15 mm × 47 mm rectangles with rounded edges. In order to program the temporary shape, the thin rectangles were wrapped around a mandrel in 80 °C water to form a cylinder with a diameter of 8.5 mm and height of 15 mm. They were constrained with plastic ties while cooling to store the temporary shape.

2.3. Nutrition

All animals ate a standard solid chow diet until 24 hours before operation, during which they were provided a liquid diet ad libitum (Bio-Serv, Frenchtown, NJ). Liquid diet was continued postoperatively until the animals could tolerate at least 100 mL per day and had passed stool, at which time standard solid chow was restarted.

2.4. Measurements

The length of the Roux limb was measured off tension in duplicate along the antimesenteric border in situ at operation and again at necropsy. The portion that was wrapped around the device was measured in a similar fashion. At necropsy, a standard 6 g weight was hung from one end of the Roux limb and the length of the whole limb and wrapped (experimental) segment were each measured (tension).

2.5. Histologic evaluation

At necropsy, a segment of small bowel from the Roux limb (control 1) or wrapped segment (control 2, experimental) were preserved in 4% formalin. Care was taken to avoid areas adjacent to sites of anastomosis. They were then embedded for frozen section, sectioned, stained with hematoxylin and eosin, and reviewed by a gastrointestinal pathologist who was blinded to operative group. Mucosal thickness/crypt depth, thickness of internal muscularis propria (the circular layer), villous height and width were measured in each sample with cellSens digital imaging software (Olympus, USA) according to manufacturer's instruction. The parameters were evaluated in 10 distinct sites and the average was recorded. Crypt density and fission rate were determined using previously validated methodology [17].

2.6. Total DNA and protein

Measurements of total DNA and protein contents in the isolated bowel segment wall were performed in snap-frozen fresh samples based on methods as previously described [18]. Briefly, DNA and protein were isolated using the All Prep DNA/RNA/Protein Mini Kit (Qiagen, Gaithersburg, MD) per manufacturer's instructions. DNA was quantified using a NanoDrop 8000 (Thermo Scientific, Waltham, MA), with nucleic acid concentrations reported as mg/mL. Total protein was determined using the colorimetric DC Protein Assay (BioRad, Hercules, CA) per manufacturer's instructions, based on the reaction of protein with an alkaline copper tartrate solution and Folin reagent. Absorbances were read at 750 nm on a microplate reader (FLUOStar Omega; BMG Labtech, Cary, NC). A standard curve was obtained using known concentrations of bovine serum albumin provided by the manufacturer. Protein concentrations were also reported as mg/mL.

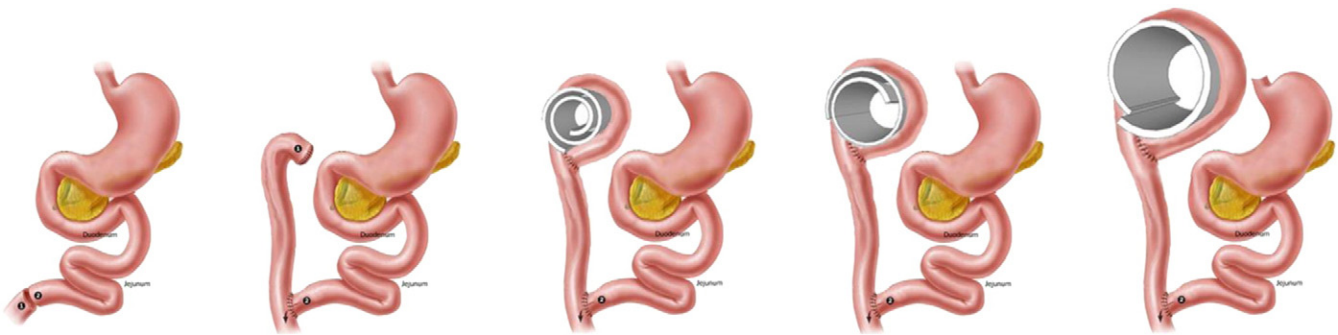

Fig. 1. Schematic for experimental animals; creation of Roux-en-Y limb, placement of cylindrical coil and radial expansion (over 7 days).