Mechanical Enterogenesis – A Review

Rebecca Stark[1] and James C.Y. Dunn[1,2]*
[1]Division of Pediatric Surgery, [2]Department of Bioengineering,
University of California, Los Angeles (UCLA), Los Angeles, California, USA

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ABSTRACT
Mechanical enterogenesis is a novel method of lengthening pre-existing intestine with distractive force. The application of mechanical force on small intestine aims to induce cellular proliferation and ultimately increase bowel length. This has been investigated primarily for the treatment of short bowel syndrome (SBS). Research has been ongoing for well over a decade in this arena and a multitude of advances have been made, both in the understanding of the biology behind force induced cellular proliferation and in the basic mechanics of force delivery systems. Important experimental models have been developed for studying this phenomenon and the collaboration of engineers and medical researchers has lead to the design of several devices that successfully lengthen small intestine. This has catapulted the field forward and there may soon be a device suitable for medical use in humans. This review analyses the past, present and future of mechanical enterogenesis.

Keywords: mechanical enterogenesis, distraction enterogenesis, intestinal lengthening, short bowel syndrome

1. INTRODUCTION
Mechanical enterogenesis is a novel method of lengthening pre-existing intestine with distractive force. This concept, as applied to other organs (bone, breast, bladder, etc.), has already reached clinical significance. The application of distractive forces on small intestine aims to induce cellular proliferation and ultimately increase bowel length. This has been investigated primarily for the treatment of short bowel syndrome (SBS). Research has been ongoing for well over a decade in this arena and a multitude of advances have been made, both in the understanding of the biology behind force induced cellular proliferation and in the basic mechanics of force delivery systems. This review analyses the evolution of this bioengineering niche.

*Corresponding author: James Dunn, MD, PhD, Division of Pediatric Surgery, David Geffen School of Medicine at UCLA, Box 709818, 10833 Le Conte Avenue, Los Angeles, CA 90095-7098. Telephone: (310) 206-242 Fax: (310) 206-1120 Email: jdunn@mednet.ucla.edu.

2. SHORT BOWEL SYNDROME

Short bowel syndrome (SBS) is a highly morbid disorder caused by inadequate length of functional small intestine. It is characterized by malnutrition, malabsorption and dehydration [1]. Although the diagnosis is both clinical and highly variable, it is commonly associated with the loss of 70% or more of small intestinal length. The etiology of SBS varies from congenital disorders to surgical resection of large amounts of small intestine. Common causes of SBS in the pediatric population are necrotizing enterocolitis, aganglionosis, intestinal atresias, midgut volvulus, and abdominal wall defects [2]. In the adult population, the most common cause is surgical resection for trauma or intestinal ischemia. Mortality rate of SBS in the pediatric population approaches 30% and the surviving children face a barrage of morbidities [3, 4]. Primary nutritional support is based on central venous parenteral nutrition, which inevitably leads to liver disease and central catheter complications. Other common complications include malabsorptive diarrhea, fluid and electrolyte imbalances, and micronutrient deficiencies. The incidence of SBS has been reported to be as high as 1,200/100,000 live births, and currently it is estimated that 40,000 patients in the U.S. require parenteral nutrition due to SBS [2]. The costs of SBS have been studied, and one recent study found that the average cost per patient exceeds $200,000 per year [4].

Current management of SBS includes supportive therapies, hormone administration, bowel lengthening surgeries, and small bowel transplantation. Supportive therapies include parenteral nutrition, anti-motility agents, ursodiol, and vitamin supplementation [1, 5]. Recently, human growth hormone has been used as treatment for SBS with initial response demonstrated by weight gain and decreased need for parenteral nutrition. However, these benefits are short lived with regression within 3 months of treatment [6]. Surgical therapies for SBS involve interposition of reversed intestine, longitudinal intestinal lengthening and tailoring and serial transverse enteroplasty procedure (STEP). All these techniques have varying results and are rarely curative [7]. The final treatment for SBS is small bowel transplantation; problems thereof include five-year survival rates of approximately 50%, the requirement of lifelong immune suppression and the paucity of available donors [8].

There are two facets of bioengineering research currently underway to develop a solution to this highly morbid condition. The goal of both is the same, to increase overall bowel surface area and thus absorptive capacity. One approach is the creation of tissue-engineered bowel. This entails growing new bowel on an engineered scaffold using growth factors and other stimulants to cause cellular proliferation. While this approach is promising, there are still a number of significant obstacles to overcome [9]. The other method for generating additional intestinal surface area is to lengthen existing bowel with mechanical force, which has been shown in some cases to dramatically increase intestinal dimensions as well as cellularity [10, 11]. This review will focus on the latter technique.

3. THE MOLECULAR BIOLOGY OF MECHANICAL ENTEROGENESIS

The concept of using mechanical force to induce cellular growth has been explored for decades on virtually every tissue type. Tissue expanders have been used in plastic

surgery for breast augmentation, skin flaps, and muscle expansion [12]. Distraction osteogenesis is used to lengthen bones for a variety of diseases [13–15]. Urologists stretch bladder and dilate ureters after radical resection [16, 17]. Esophageal atresia is often treated with serial bougenage to stretch the proximal blind end, eventually encouraging tissue growth and lengthening the pouch for reconnection of the esophagus [18]. Another technique of esophageal lengthening for esophageal atresia was developed by Foker et al. [19]. This technique employs the use of traction sutures in long gap atresias to promote growth of either ends of the esophagus, making a primary anastamosis ultimately achievable. Recent research has focused on applying these principles to intestinal tissue. The underlying principle of mechanical enterogenesis is that the increased surface area achieved by mechanical strain results from new cellular regeneration or growth and is not secondary to deformations from stretch. One elegantly simple and intuitive theory is that, when put under tension, the cells proliferate to reduce the tension, maintaining homeostasis [12]. This so called mechanotransduction involves a multitude of signaling cascades and chemical pathways that ultimately result in cellular proliferation.

The extracellular matrix is critical for cellular proliferation. Mechanical strain has been shown to increase collagen synthesis, which aids in the development of extracellular matrix and influences cellular division [20]. This is mediated through phospholipase C, Ca++ mobilization, inositol phospate and integrins. Extracellular signals are transmitted through these proteins to the cytoplasm of surrounding cells inducing proliferation [12, 20–24].

Growth factors are also intimately linked with the extracellular matrix surrounding cells, and distortion of this extracellular architecture has been shown to cause the release of certain growth factors such as fibroblast growth factor (FGF), epithelial growth factor (EGF), and insulin-like growth factor (IGF) [25, 26]. Stretched rat jejunum has been shown to have a 6-fold increase in IGF expression [27]. Exogenous EGF given to rats was shown to increase overall surface area of intestine in another study [26]. These studies further support the role of growth factors in bowel lengthening.

Ion channels have a role in signaling via conformational changes that are mediated by mechanoreceptors, which are present on the surface of most cells in mammals. Stretch induces conformation changes to cell structure causing the influx of cations (K+, Ca++, and Na+) [12, 28]. These cations play a known role in cellular depolarization, but Ca++ has also been shown to be a potent actor in intracellular signaling cascades. Specifically, increased intracellular calcium concentrations activate phopholipase C which increases activity of protein kinase C, a crucial enzyme in cell proliferation [29].

Cellular proliferation is also influenced by intracellular signaling mechanisms that involve protein kinases and G proteins. The transcription of certain genes is upregulated and induced by mechanical stimuli, presumably as a result of protein kinase and G protein cascade activation [30]. These signaling cascades induce the DNA synthesis necessary for cellular division although the exact mechanism by which this occurs has not been fully elucidated.

4. CHALLENGES IN EXPERIMENTAL DESIGN

There are several important factors that need to be addressed in designing the model or device for bowel lengthening. For example, it is known that there is a range of force that is optimal for cellular proliferation, and that above that range, there is apoptosis as well as mechanical derangement of the vascular supply. Determining that critical force range is challenging, especially when different animal models are used and then finally, these force concepts are applied to human intestine.

In a paper by Miyasaka et al., the optimal force on human intestine was determined by measuring blood flow to the intestine via Doppler [31]. In this study, the authors first performed ex vivo experiments on pig small intestine to determine maximal limits of the distractive force, which could be applied. Using an apparatus that displaced both ends of the 10–15 cm length of small bowel with a weight and pulley system, they were able to assess gram force applied and tissue response. They studied the force necessary to cause macroscopic injury to the bowel and found it to be approximately > 235 gram-force, and also noted that bowel with mesentery was more resilient to macroscopic damage than that without mesentery. They then did several in vivo experiments in pigs utilizing an internal ratcheting device and laser Doppler to determine the level of force at which a decrease in blood flow to the bowel occurred. The internal expander was set to increase the force on the bowel at 30 gram-force increments. Mesenteric blood flow was most resistant to the force applied and could withstand up to 200 gram-force; however, some blanching was observed at forces above 120 gram-force. In contrast, at the ends of the bowel segments where the expanding device abuts the wall directly, a substantial decrease in blood flow was noted at 100 gram-force. Thus, the optimal experimental force was determined to be approximately 100 gram-force [31]. This information is critical in setting parameters for experimental design. However, force not only plays a role in blood flow to the affected cells but also induces various cellular signaling pathways including those causing apoptosis [12]. It is likely that the range for optimal cellular proliferation will be more sensitive than that of blood supply and further experiments are needed to determine this.

In addition, force applied to the intestine must be adequate to induce growth and at the same time render the new bowel functional. The resultant intestine must function mechanically as well as biologically. Several reports have shown successful lengthening of intestine in both rats and pigs with preservation of the mechanical properties of intestinal smooth muscle as well as enzymatic and absorptive properties [11, 32–34]. Muscle contraction strength and rate of stretched rat jejunum were measured in organ baths with cholinergic stimulation [32]. Stretched intestine has been shown to contract similarly to stimulation as non-stretched intestine. In a study by Mendoza et al., stretched rat jejunum was compared to both a normal control (harvested segment of rat jejunum) and a segment of rat jejunum that was isolated from intestinal continuity but not stretched [32]. Among all three segments, there was no significant difference in the presence of a response to stimulation with either potassium chloride or carbachol. However, the amount of response as measured by the change in tension from baseline was significantly higher in the normal jejunum as compared to both the isolated and stretched. There was no difference in the amount of response between the isolated and stretched bowel, suggesting that the difference is not due to the lengthening

itself but perhaps due to the isolation from intestinal continuity. In addition to stimulated contraction, basal contraction rates were also measured. Although basal contraction rates were higher in non-stretched bowel, the difference between the isolated bowel and stretched bowel was not significant [32]. The stretched bowel appears to be mildly dyskinetic, which may actually be beneficial in the setting of SBS by slowing transit and thus aiding in absorption.

Several studies have measured enzymatic activity and absorptive capacity in stretched small bowel with encouraging results. Park, et al. studied the activity of alkaline phosphatase and lactase in stretched versus non-stretched intestine [11]. They demonstrated an increase in alkaline phosphatase activity in stretched segments as compared to controls, and no difference in lactase activity between the two groups [11].

A study by Spencer et al., investigated barrier function and absorptive capacity of stretched small intestine, and demonstrated that barrier function of stretched bowel was intact by measuring transepithelial resistance and the transepithelial passage of ^{3}H-mannitol [33]. Using a buffered chamber which separated the mucosal side of the intestine from the serosal side, the authors applied tagged mannitol to the mucosal surface and a glucose solution to the serosal surface, then measured the diffusion rate of the mannitol across the mucosa, and compared the rated between stretched and normal jejunum with no significant difference found. There was no difference in transepithelial resistance. In addition, the authors determined absorptive capacity of stretched intestine by measuring glucose-mediated sodium transport, carbachol-induced chloride transport and alanine absorption [33]. Their results suggest that there is little to no difference between the stretched and non-stretched intestine in relation to barrier function and absorptive capacity.

An additional challenge with regard to mechanical enterogenesis is the sustainability of length once the stretch force is terminated. Chang et al. studied the ability of stretched bowel to maintain its length after removal of the expander by comparing two groups:(a) the lengthened rat jejunum was measured after achieving maximal expansion of the stretching device, and (b) the expander was removed after maximal stretch, but the segment was retrieved 3 weeks later, allowing for recoil [10]. Both groups showed greater than three fold lengthening of the jejunal segment, and there was no statistically significant difference between the two groups in terms of length. Data from a study by Safford et al. also suggests that stretched bowel retains most of its length [35]. They studied a subset of rats that underwent 30 days of intestinal stretch, followed by another 30 days of maintenance (expander in place). Thirty days after removal of expander, there was still significant lengthening of 149% compared to controls. However, there was a decrease in the length by 25% after removal of the device [35].

Another challenge in creating a mechanism for stretching intestinal tissue is designing an in situ expander. Ideally, an expander would be placed within the lumen of small intestine in continuity with the entire bowel. This has proved to be a difficult feat. However, current models have focused on surgically placing expanding devices within segments of isolated small intestine, which after expansion would be reinstated into continuity with the remaining intestine. Drawbacks to this method include the additional operation as well as the length of bowel lost with re-anastamosis.

5. CURRENT STUDY DESIGNS FOR MECHANICAL BOWEL LENGTHENING

There are several different approaches for bowel lengthening via mechanical force, all using the basic principle of stretch as the mode of applying mechanical force, as summarized in Table 1. One of the first designs for mechanical enterogenesis employed dilation of the small intestine with saline. An early study by Chen et al. in 1997 demonstrated lengthening intestine via mechanical stretch in a rabbit model by gradual saline dilation of an isolated small intestinal segment [36]. A blind end ostomy of terminal ileum 5 cm in length was created. Normal saline was then injected into the ostomy at a rate of 0.5 ml/12 hours until a final volume of 15 ml was reached. This volume was sustained at 15 ml for 2 weeks. The bowel was then measured, allowed to recoil and then remeasured. The authors demonstrated a stable 123% increase in the length of the isolated segment with no remarkable histological changes other than smooth muscle hypertrophy [36].

In a study by Puapong et al. using a rat model, a segment of jejunum was surgically isolated from the remaining small intestine [34]. The distal aspect of the segment was oversewn and the proximal aspect was sewn to the abdominal wall with a catheter placed inside. Saline was infused at regular intervals (0.2 ml/hr for one week). At the end of 7 days of expansion, the bowel length increased by 32%. The enzymatic activity in the stretched segment was similar to that of controls as shown by the alkaline phosphatase and lactase activity. Histologically, the segment showed atrophic villi and hypertrophied smooth muscle [34]. This is the first in vivo study demonstrating application of the concept of distraction to enterogenesis.

Several groups have employed an external screw to gradually lengthen an isolated segment of intestine in vivo, and demonstrated promising results that further substantiate the ability to lengthen intestinal segments. One early study by Printz et al. utilized a screw expander that was secured to the serosal aspect of an isolated segment of rabbit jejunum [37]. Distractive forces were applied to lengthen the segment at 1 mm/day, achieving a 100% increase in length over 3 weeks [37]. In other studies, the small intestinal segment is isolated and a blind end ostomy is created, as

Table 1. Summary of existing in vivo mechanical enterogenesis methods

Author	Year	Animal model	Device	Percent lengthened
Chen et al. [36]	1997	Rabbit	Saline dilation	126%
Printz et al. [37]	1997	Rabbit	Serosal screw	100%
Park et al. [11]	2004	Rat	Screw	210%
Puapong et al. [34]	2004	Rat	Saline dilation	32%
Safford et al. [35]	2005	Rat	Screw	149%
Chang et al. [10]	2006	Rat	Screw	100%
Mendoza et al. [32]	2006	Rat	Screw	130%
Spencer et al. [33]	2006	Pig	Hydraulic piston	45%
Luntz et al. [39]	2006	Pig	Hydraulic piston	69%
Shekherdimian et al. [40]	2009	Rat	Spring	250%

shown in Figure 1 [35]. A nut is tethered into the abdominal wall where the ostomy is matured and a screw is placed inside the lumen. The screw is gradually advanced (1-3 mm/day) until a maximal length is achieved, as shown in Figure 2 [35]. In most studies, a sustained length of approximately 150% is accomplished [10, 11, 37, 38]. This has been done in both rat and rabbit models. In addition, a study employed this technique with the creation of a SBS model in a rat with a massive small intestinal resection prior to screw lengthening [38]. Although the stretched bowel was not reimplanted in continuity, this proved that this method could be employed in the pathologic setting of SBS.

Another notable design employs a hydraulic piston as the mechanical expander [33, 39], shown schematically in Figure 3 and in the photograph in Figure 4 [39]. This was studied on Yorkshire pigs that underwent surgical isolation of a segment of jejunum similar to the previous models. The mechanical expander was surgically installed in the isolated segment, and fluid line and drain line were tunneled subcutaneously and brought out through the skin at the pig's scapula. This expander was designed with two telescoping

Figure 1. Schematic diagram of the lengthening device [35] (used with permission).

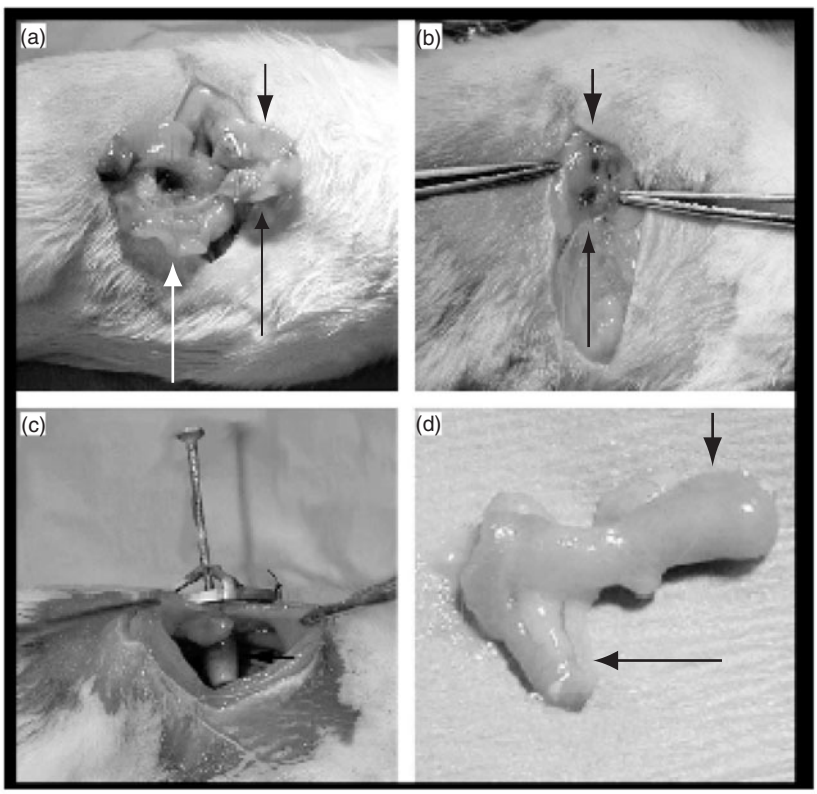

Figure 2. Surgical placement of the intestinal lengthening device. (A) A segment of small bowel was isolated, and an enterotomy was placed in the middle of the isolated loop of bowel. The long black arrow indicates the unlengthened limb, the short black arrow the lengthened limb, and the white arrow identifies the in situ bowel. (B) A double barrel ostomy was created with one loop for placement of the intestinal lengthening device, and the second as an internal control. (C) Intestinal lengthening device within the loop of bowel. (D) After 30 days, the isolated limb of small bowel is removed [35] (used with permission).

syringes enabling the device to approximately double its length (86% increase), as shown in Figures 3, 4. A radio-opaque marker was placed at either syringe end to enable radiologic tracking of the expansion in vivo. Original device was 11.8 cm in length and expanded to 22 cm. Bowel segment was lengthened 1.46 cm per day for seven days. Compared to controls, a 69% increase in sustained bowel length and 88% increase in surface area were observed [33, 39]. The design limitations include the need for an

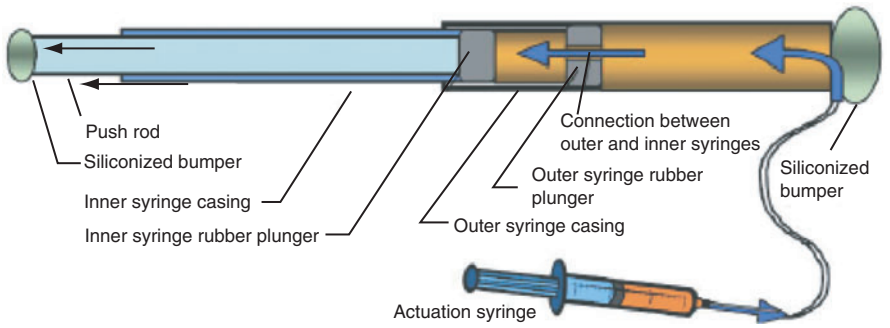

Figure 3. Schematic of the hydraulic bowel extender in the partially extended position [39] (used with permission).

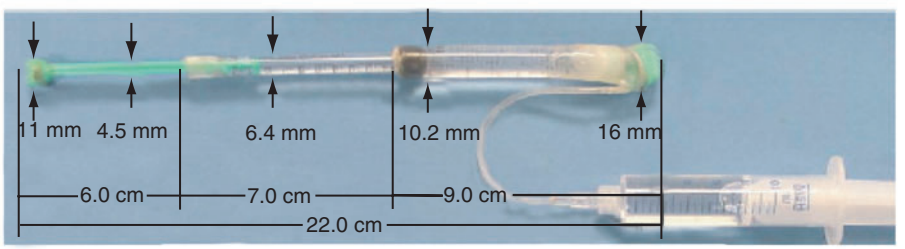

Figure 4. The prototype dual hydraulic piston fully extended [39] (used with permission).

external device for controlling expansion of the piston as well as the restriction on the maximal length of expansion inherent in the design. The piston can only expand to double its original length and because it is made of non-compliant material, it cannot bend to accommodate the abdomen once the maximal width of the animal's abdomen is achieved.

Recently, a smart metal alloy (SMA) wire spring design was used as a mechanical expander in rat jejunum [40]. This spring is made of nitinol, which is a biocompatible nickel titanium currently being used in vascular, hepatobiliary and urologic stents. This metal alloy has certain properties that make it uniquely suited for this design. First, an applied stress induces a phase conformation in this superelastic composition, that is completely recoverable upon removal of stress. Another beneficial property of nitinol is that the stress in the material remains relatively constant, even when stretched. Therefore, this expander device can deliver a constant force on the intestinal segment throughout the lengthening procedure (Figure 5) [41]. The spring constant of these springs was approximately 0.0010 N/mm. In this model, a segment of jejunum is isolated and the compressed spring is surgically inserted. The spring is deployed by release of a suture [41].

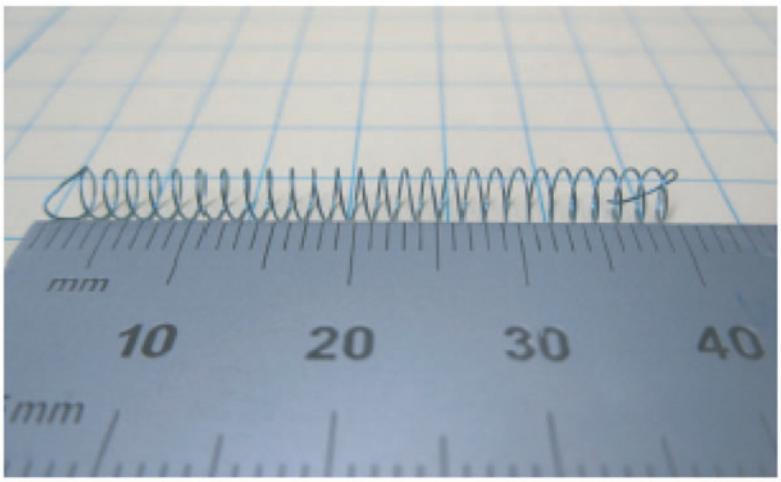

Figure 5. Nitinol spring [41] (used with permission).

Recent advancements have been made in the delivery method of the nitinol springs [42]. Prior to intraluminal placement of the spring, it is placed in a gelatin capsule and coated with an enteric coating designed to delay the release of the spring, as shown in Figure 6 [42]. Thus the timing of deployment can be adjusted by the dissolution rate of the dissolvable capsule. The compressed spring was approximately 1 cm and expanded to 4 cm on average. This design has the benefit of being completely intraluminal as well as the advantage of timed spring deployment. Isolated segments were lengthened on average four fold and demonstrated the common findings of villous atrophy and smooth muscle hyperplasia [40, 41, 42, 43]. Lastly, this design has been effectively used to both lengthen the isolated intestine and for restoration of the lengthened intestine back into intestinal continuity, Figure 7 [43].

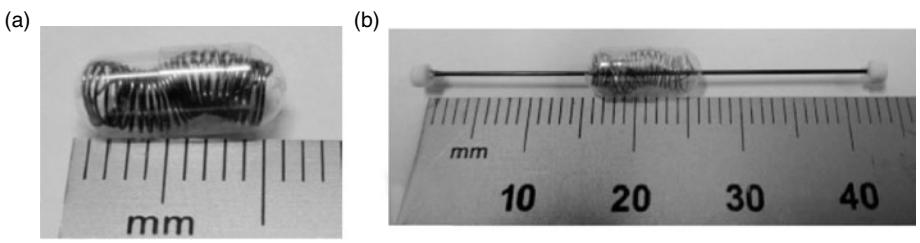

Figure 6. (A) Spring compressed in gelatin capsule. (B) Nitinol wire placed through capsule to prevent buckling of the spring [42] (used with permission).

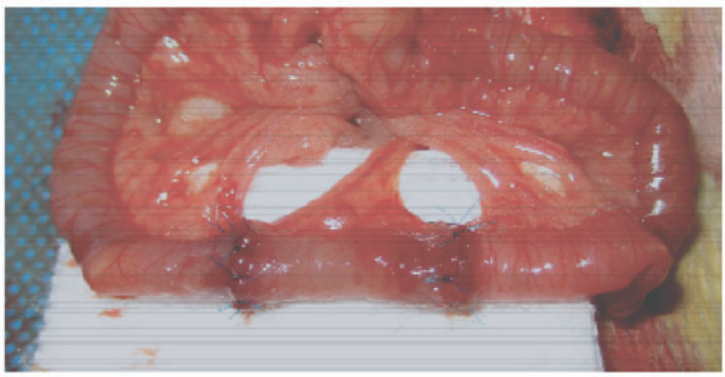

Figure 7. Stretched bowel restored back into continuity [43].

Preliminary research has been performed on ex vivo and, limitedly, in vivo pig intestine using an intraluminal ratcheting device [39, 44]. This device is battery powered and controlled via remote transceiver. The expansion device is approximately 10 cm in length and utilizes a ratcheting mechanism employing a piece of SMA wire, as shown in Figure 8. It expands linearly 0.8 mm with each actuation. There is a force transducer on one end to measure applied loads as well as a Hall Effect sensor and permanent magnet pair to monitor displacement. Ex vivo experimentation aided in exposing limitations of the device that have been further refined. The device must be able to withstand the fluid environment of the small intestine without decay or exposure of the battery. The battery must also have a life span long enough to achieve maximal extension of the device (86%). A recent presentation on this lab's work showed data gathered from in vivo experiments on 2 pigs [44]. This suggests that the device functions in vivo and currently, research is underway to validate this device as a feasible means for mechanical lengthening of bowel.

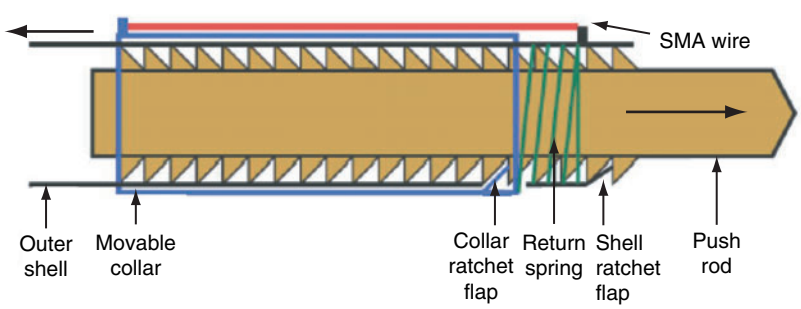

Figure 8. Schematic of SMA bowel extender device [39] (used with permission).

6. FUTURE RESEARCH

The afore-mentioned designs are in a constant state of refinement. Recent experiments have demonstrated that lengthened bowel can be re-implanted in continuity and functions normally. New devices are being created for use in large animal models and the continued collaboration of medical doctors and engineers is essential for furthering this field. Once the animal model for mechanical enterogenesis has been perfected, the arduous process of gaining approval for use in humans will begin. The safety of the device in normal human subjects will first be assessed, followed by assessment of its efficacy in treating SBS.

Eventually, it is anticipated that a device will be designed that can be deployed in humans via endoscopy. The bowel segment could therefore be lengthened in continuity. The precise placement of this device may be guided by capsule endoscopy. This expander will likely be a stenting device similar to what is now used for stenting colon and esophageal cancers, but it may be made from biodegradable material so that the retrieval of the device will not be necessary after lengthening. As research on the biochemical milieu of the growing intestine is advanced, growth factors and other stimulants to cellular proliferation can be added to the device, much like the drug eluting stents currently used in vascular lesions. With these bioengineering advancements, there is likely to be an effective treatment for SBS in the near future.

7. CONCLUSION

Mechanical enterogenesis is a promising solution to a devastating illness. Current research on the phenomenon of mechanical transduction is advancing our understanding of the complicated interplay between exogenous mechanical force and cellular signaling cascades. Use of this knowledge in combination with modern engineering designs may soon lead to the development of a device that successfully lengthens small intestine and improves the quality of life for those who suffer from short bowel syndrome.

CONFLICT OF INTEREST

The authors indicated no potential conflicts of interest.

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