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Submucosal Hydrogel for Spring-Mediated Intestinal Lengthening

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ABSTRACT

Spring-mediated distraction enterogenesis has shown success in intestinal lengthening, with spring confinement achieved by external plication with sutures to reduce the lumen diameter at both ends of the intestinal segment. Endoscopic spring placement would minimize the morbidity associated with device insertion. This study investigates the use of submucosal injection of engineered hydrogel to temporarily confine a compressed spring within an intestinal segment. Engineered hydrogels were composed of hyaluronic acid (HA) alone or HA with elastin-like protein (HELP). To simulate endoscopic injection in six juvenile pigs, hydrogel was injected into the submucosa in everted jejunum, followed by the placement of a gelatin-encapsulated, compressed nitinol spring. The jejunum was then unfolded over the spring, and hydrogel was injected distally into the submucosa. Sutures were placed as fiducial markers. After 7 days on a liquid diet, the pigs were euthanized, and their intestinal segments were analyzed for lengthening and histological changes. The spring-containing jejunal segments expanded in all animals, lengthening to 132% in the HA group and 188% in the HELP group. HELP hydrogels exhibited slower biodegradation than HA-only hydrogels. Histological analysis showed increased crypt width and decreased crypt density in the spring-containing segments compared to controls. Hydrogel effectively provides temporary spring confinement within intestinal segments without adverse effects. The mechanical stimulation from the spring induces crypt fission, expanding the intestinal epithelium. These results support the feasibility of gel-enabled, spring-mediated distraction enterogenesis for intestinal lengthening.

1 | Introduction

Short bowel syndrome (SBS) is a highly morbid disease in infants and children with high mortality and morbidity rates worldwide [1]. This syndrome occurs due to congenital or acquired loss of a significant portion of the bowel, resulting in nutrient malabsorption, excessive fluid loss, and poor growth in children [1, 2]. SBS is the most frequent cause of chronic intestinal failure and is fatal without treatment [3–6]. Current treatment options are limited and mainly supportive, relying heavily

Abbreviations: 3D, three-dimensional; HA, hyaluronic acid; HELP, hyaluronan and elastin-like protein; POD, postoperative day; SBS, short bowel syndrome.
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on long-term parenteral nutrition (PN) [3]. In the United States, estimates suggest that about 35% of adults receiving home PN suffer from SBS [5, 7]. Unfortunately, long-term PN is associated with many complications, ranging from central line infections and catheter thrombosis to sepsis and liver and kidney diseases [2, 8]. Such limited treatment options have led to strategies to surgically increase the length of the existing intestine, as even a 1% increase in intestinal length increases the chance of weaning patients from PN by 3% [9].

Nonetheless, current surgical techniques for intestinal lengthening have high rates of complications, including death [10–12]. As an alternative surgical approach, our group focuses on a novel treatment for SBS through spring-mediated distraction enterogenesis (SMDE). We have successfully demonstrated intestinal lengthening using intraluminal, self-expanding springs in several animal models, including mice, rats, and pigs [13–21]. SMDE led to multifold lengthening of the intestine with normal function, which was sustained after device removal [20, 22]. Both internal and external plication of the intestine with sutures has been performed for spring confinement during distraction enterogenesis, providing permanent anchorage of the spring [16, 23]. Although SMDE has shown promising functional results, the insertion and removal of the spring still require two invasive surgeries. Furthermore, while internal plication has been experimented with in an open manner to mimic endoscopic suturing with comparable results, endoscopic internal suturing is technically challenging [23], hindering widespread clinical translation. Thus, our long-term goal is to develop a technique for transient endoscopic spring confinement to minimize the morbidity associated with the surgical device insertion and removal. Towards this goal, here in this proof-of-concept study, we demonstrate the feasibility of providing temporary confinement to a compressed spring within the bowel through a submucosal injection of a biodegradable hydrogel, Figure 1A–C. The gel can be injected proximal and distal to the compressed spring, narrowing the lumen by creating a localized stiff object that anchors the device, Figure 1D. Once deployed, the spring applies tension against the injected gel to mechanically stimulate and lengthen the bowel. As the hydrogel biodegrades in vivo, the spring will be displaced for natural evacuation without further surgical intervention. The concept of hydrogel anchoring of an

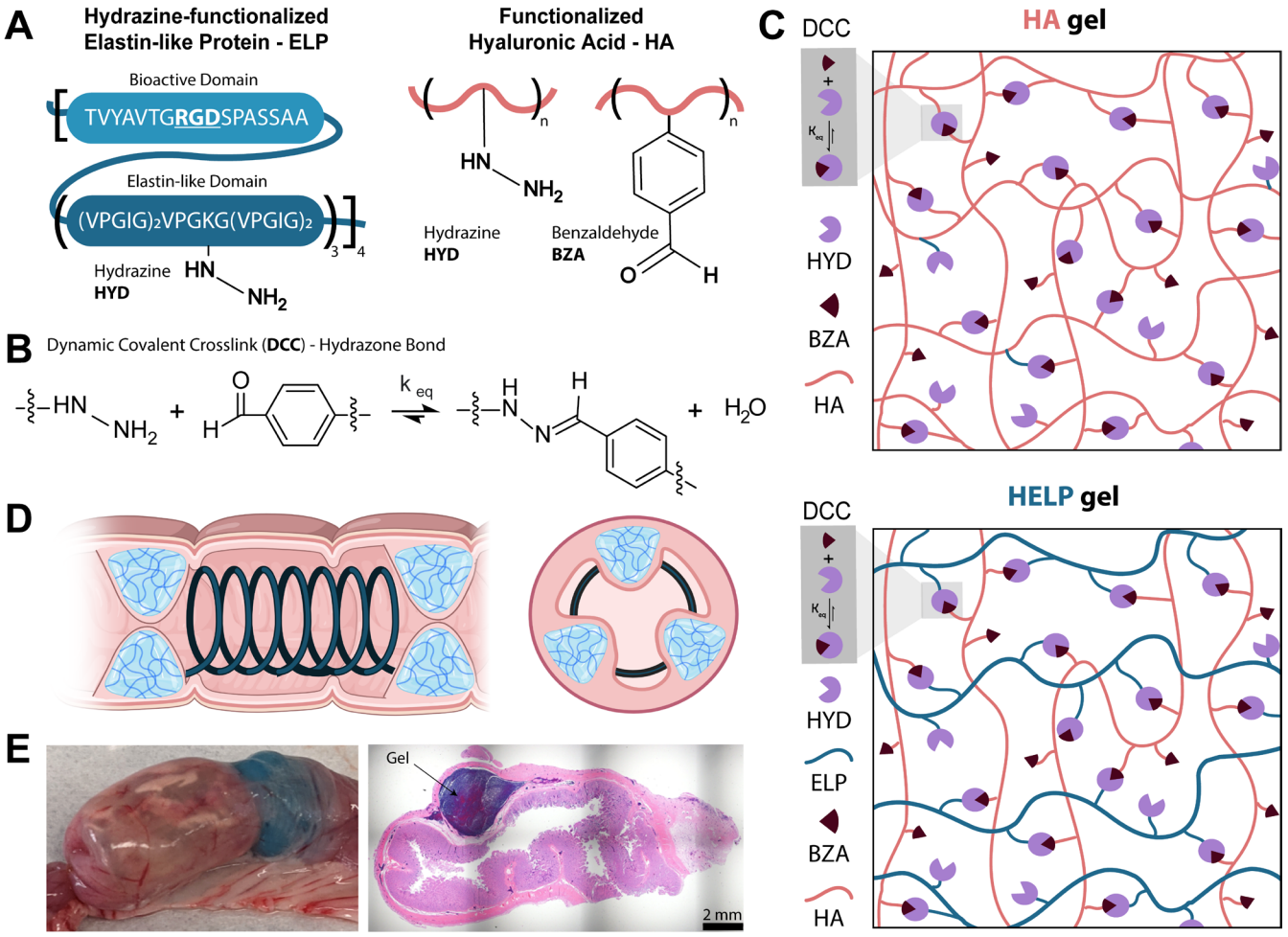

FIGURE 1 | Schematic of gel-enabled spring immobilization. (A) Schematic of elastin-like protein (ELP) functionalized with hydrazine (HYD), and hyaluronic acid functionalized with HYD or benzaldehyde (BZA). (B) Dynamic covalent chemistry (DCC) reaction responsible for crosslinking the polymers together with a hydrazone bond. (C) Schematic of HA (top) and HELP (bottom) gels. (D) Representation of spring confined with hydrogel injections, longitudinal section (left) and cross section (right). (E) Encapsulated, compressed spring contained within an intestinal segment using sutures at one end and colored gel at the other end (left); and histologic examination of a bowel section with H&E stain showing submucosal gel in dark purple (right).

intestinal device has not been tested previously. Thus, in this first study, we aimed to assess two important aspects: (1) efficacy of hydrogel confinement, and (2) suitability of degradation rate to allow for both effective lengthening and the onset of natural evacuation.

To achieve these goals, our hydrogel must be injectable (to allow for minimally invasive delivery), stiff (to withstand the mechanical tension applied by the spring), and biodegradable (to allow for both sufficiently long confinement and the natural evacuation of the spring). However, designing hydrogels that are simultaneously injectable, stiff, and biodegradable is still a challenge in the biomaterials field [24–28]. A rising solution to create mechanically robust yet injectable hydrogels is dynamic covalent chemistry (DCC) crosslinks [24]. The covalent nature of these bonds strengthens the hydrogel, whereas their reversible behavior allows the polymer chains to associate and dissociate [28–32]. These properties enable the hydrogel to fluidize upon application of hand-force to allow injection, then rapidly stiffen in situ, retaining its original mechanical properties (self-healing) and significantly improving gel retention within the tissue [33].

Aiming to mimic native intestinal extracellular matrix (ECM), we have developed easily injectable yet stiff DCC crosslinked hydrogels based on hyaluronic acid (HA), with or without the addition of an elastin-like protein (ELP), Figure 1A–C. HA is a natural glycosaminoglycan essential for normal intestinal growth [34–36]. It is amenable to chemical modification for hydrogel formation, biocompatible, biodegradable [30, 33], and has been FDA approved for several biomedical applications. Elastin protein is also a key component of the native intestinal ECM [37], providing essential elastic mechanical properties to the tissue. ELP is a genetically engineered protein that has been widely used in bioengineering, partially due to its biocompatibility and slow degradation rate [34, 38]. Specifically, ELP is known to form protein aggregates at physiological temperatures, which act as secondary crosslinks within the hydrogel network, increasing erosion stability [39, 40].

Given the importance of controlling gel degradation to confine the spring for a sufficient time to promote intestinal lengthening effectively, we investigated two DCC hydrogel compositions: (1) HA only (HA gel), and (2) HA blended with ELP (HELP gel), Figure 1C. As it is essential for successful spring confinement, hydrogel stiffness was kept constant in both gel formulations. We hypothesized that both formulations would enable spring confinement, whereas the higher stability of HELP would lead to slower biodegradation and hence more intestinal lengthening prior to the onset of natural evacuation. This study represents a first step toward the development of a minimally invasive, hydrogel-enabled SMDE as a future treatment for SBS.

2 | Materials and Methods

2.1 | Spring Production

Springs were made by shape-setting heat treatment as described in previous studies [41–43]. Previously, we have shown that spring diameter does not significantly impact bowel lengthening results through SMDE in this model [17]. Juvenile pigs have an average internal jejunal diameter of 11 mm, and each individual has a slightly different diameter. Thus, here the surgeon selected either a 10- or 12-mm diameter spring for each surgery, based on the dimension of the intestine receiving the spring. Based on our previous studies, springs with a force constant of around 5 N/m result in adequate intestinal lengthening [44]. The amount of applied force from springs was kept in this range by changing the number of active coils according to the equation of Swieskowski [43, 44].

$$k = \frac{G \times d^4}{8 \times n \times D^3}$$

D, diameter of spring; d, diameter of wire; G, shear modulus; k, force constant; n number of active rotations.

Steel rods with 10- or 12-mm diameter were used as molds to heat-set helical springs. Nickel-titanium (nitinol) wire (0.015 in. gage, McMaster-Carr, Santa Fe Springs, CA) was wrapped around the mandrel on the grooved threads in a helical fashion, secured in place with hose clamps, and configured by heat-shape setting treatment at 500°C for 30 min. The wrapped mandrel was rapidly cooled down by submerging it in ice-cold water. The force constants of the springs were measured using the compression formula of Hooke's law. All springs measured 7.5 cm in length before compression. Springs were compressed to 2.0 cm within gelatin capsules (Electron Microscopy Sciences, Hatfield, PA). The spring-loaded capsules were coated thrice with cellulose acetate phthalate solution (Eastman Chemicals, Kingsport, TN) to slow the dissolution of the gelatin from minutes to hours [22]. Before the surgery, coated capsules were sterilized under UV light overnight.

2.2 | Hydrogel Properties

A hydrogel is a three-dimensional (3D) network of hydrophilic polymers that can hold a large amount of water while maintaining its structure due to the chemical or physical crosslinking of individual polymer chains [24]. The hydrogel properties that we sought for the purpose of our study included biocompatibility, easy injectability by hand, rapid self-healing to form a solid post-injection, and biodegradability rates that allowed for gel retention of at least 1 week. To create a gel that is injectable, we selected gel formulations that are crosslinked using hydrazone bonds, a type of dynamic covalent chemistry (DCC) that forms reversible crosslinks between the polymers [45], Figure 1A,B. To create a gel that is biocompatible and biodegradable, we selected formulations composed of recombinant biopolymers that degrade in response to enzymes present in the intestinal submucosa. Two hydrogels that met our design criteria were chosen. The first was an engineered HA-based hydrogel, which is formed by mixing HA chemically modified with benzaldehyde (HA-BZA, 500 kDa HA molecular weight, 1 wt% solution in 10× isotonic phosphate buffered saline (10× iPBS: 81 mM sodium phosphate dibasic, 19 mM sodium phosphate monobasic, 60 mM sodium chloride in Milli-Q water; pH adjusted to 7.4; 0.22 μm filtered)) and HA modified with hydrazine (HA-HYD, 100 kDa HA molecular weight, 1 wt% solution in 10× iPBS). The HA polymers were chemically modified and purified as previously reported

[39, 45]. Mixing of the two HA polymers leads to the spontaneous crosslinking, creating an infinite, continuous network, and forming stiff gels with a plateau shear storage modulus (G′) of ~1000 Pa that are injectable and self-healing (Figure S1). HA is biodegradable in response to hyaluronidase [39]. The second gel was formulated by mixing HA-BZA (60 kDa HA molecular weight, 1 wt% solution in 10× iPBS) with an ELP modified with hydrazine groups (ELP-HYD), which we term HELP gel [45, 46]. The ELP was recombinantly expressed in *Escherichia coli*, purified, and chemically modified as previously reported [47, 48]. The HELP gel system leads to the formation of gels with similar mechanical properties (G′ ~1000 Pa, Figure S1) that are also injectable but are predicted to be more slowly biodegradable due to the inclusion of the ELP component [33].

2.3 | In Vitro Study

Initially, an in vitro study was done on harvested pig intestines to determine the appropriate method and amount of gel for injection. Fresh pig jejunum was used for the study. To mimic endoscopic injection, the transected bowel was everted, and the hydrogel was injected into the submucosa at 3–4 points on the inner section of the bowel using a 26-gauge needle. A total of 1.5–2 cc of hydrogel was adequate to cause luminal narrowing to hold the gelatin-encapsulated, compressed spring in place. The specimen was formalin-fixed and examined under microscopy to visualize the hydrogel in the submucosa (Figure 1E).

2.4 | Animal Study

The study was approved by the Animal Research Committee (assurance number D16-00134 (A3213-01), protocol number 32278). The study was conducted between February 2022 and December 2023. Female juvenile miniature Yucatan pigs ($n = 6$) weighing 8.6–10.4 kg and aged 7–10 weeks (*Sus scrofa*, S&S Farms, Ramona, CA) were anesthetized with inhaled isoflurane and intubated. Three pigs received HA hydrogel, and three received HELP hydrogel. In this study, we performed the submucosal hydrogel injection in an open manner to avoid technical challenges associated with injection through a long endoscope. Male pigs were not used to avoid possible complications associated with their urethral anatomy [44]. Subjects were prepped and draped in a sterile fashion. A midline laparotomy incision was made. A segment of the jejunum, approximately 60 cm distal to the ligament of Treitz, was eviscerated. The jejunum was transected, and the proximal end was everted using tissue forceps to expose the mucosal surface. Approximately 1.5–2 cc of hydrogel was injected into the submucosa of the everted jejunum at 3–4 points. A gelatin-encapsulated, compressed nitinol spring was placed next to the injected hydrogel, and a portion of the everted bowel was unfolded over the capsule, followed by injection of another 1.5–2 cc of hydrogel distally to confine the spring within the lumen. The remaining portion of the everted bowel was reduced completely, and the length of the spring segment (2.5 cm) was marked with 4–0 Prolene sutures on the serosal side for visual identification of length at the time of sacrifice. The jejunal continuity was restored with an end-to-end anastomosis. Another 2.5 cm jejunal segment distal to the spring site was also marked with Prolene sutures to serve as the control segment. The small intestine was returned to the abdominal cavity, and the incision was closed in two layers. Postoperatively, pigs were placed on a liquid diet and were euthanized on postoperative day (POD) 7. Jejunal segments were evaluated for length between the marking sutures and were processed for histological examination.

2.5 | Histology and Inflammation Analysis

The samples were fixed in 10% formalin for 48 h and embedded in paraffin. The tissue blocks were cut into 5-μm sections and stained with hematoxylin–eosin (H&E). Slides were imaged under bright field microscopy (IX73 Olympus, Tokyo) at 400× magnification. The radial thickness of intestinal layers and the width and density of crypts were measured. Micrographs were quantified with the software cellSens (Olympus, Tokyo), and measurements were pooled in their experimental groups for statistical analysis.

All micrographs were analyzed for inflammation by a blinded pathologist from Stanford's Veterinary Service Center. Regions with the spring, as well as the injected gel, were analyzed for the presence of neutrophils, macrophages, and lymphocytes to assess the type of inflammation response (Figure S2).

2.6 | Onset of Spring Evacuation and Hydrogel Biodegradation

The onset of spring evacuation was measured as the distance of spring slippage past the site of hydrogel injection, as seen in Figure 2A. As a measure of biodegradation, the residual hydrogel at POD 7 was measured through histological analysis. Briefly, the remaining hydrogel in the H&E section images was traced, creating a black mask (Figure S3A,B). Then, the masked image was thresholded through (FIJI, ImageJ) to calculate the area of both the gel (A_{gel}, Figure S3C) and the full area of the intestine section (A_{total}, Figure S3D). The relative area of residual gel was calculated as the ratio of gel area to the section area (A_{gel}/A_{total}).

2.7 | Statistical Analysis

Statistical analysis for the lengthening of tissue and histological sections was performed on Prism 9 software (GraphPad, San Diego, CA) using normality of distribution tests, unpaired and paired Student *t*-tests, and ordinary parametric one-way ANOVA for multiple comparisons with Tukey correction with an alpha risk of 5%. Numerical values were formatted as mean ± standard deviation and expressed in units of μm or %.

3 | Results

3.1 | Spring Properties

The Swieskowski equation predicted 13 and 7 active coils for the 10- and 12-mm diameter springs, respectively, to achieve a force constant close to 5 N/m. After fabrication, springs were characterized to have a force constant of 4.4 ± 0.4 N/m. Springs

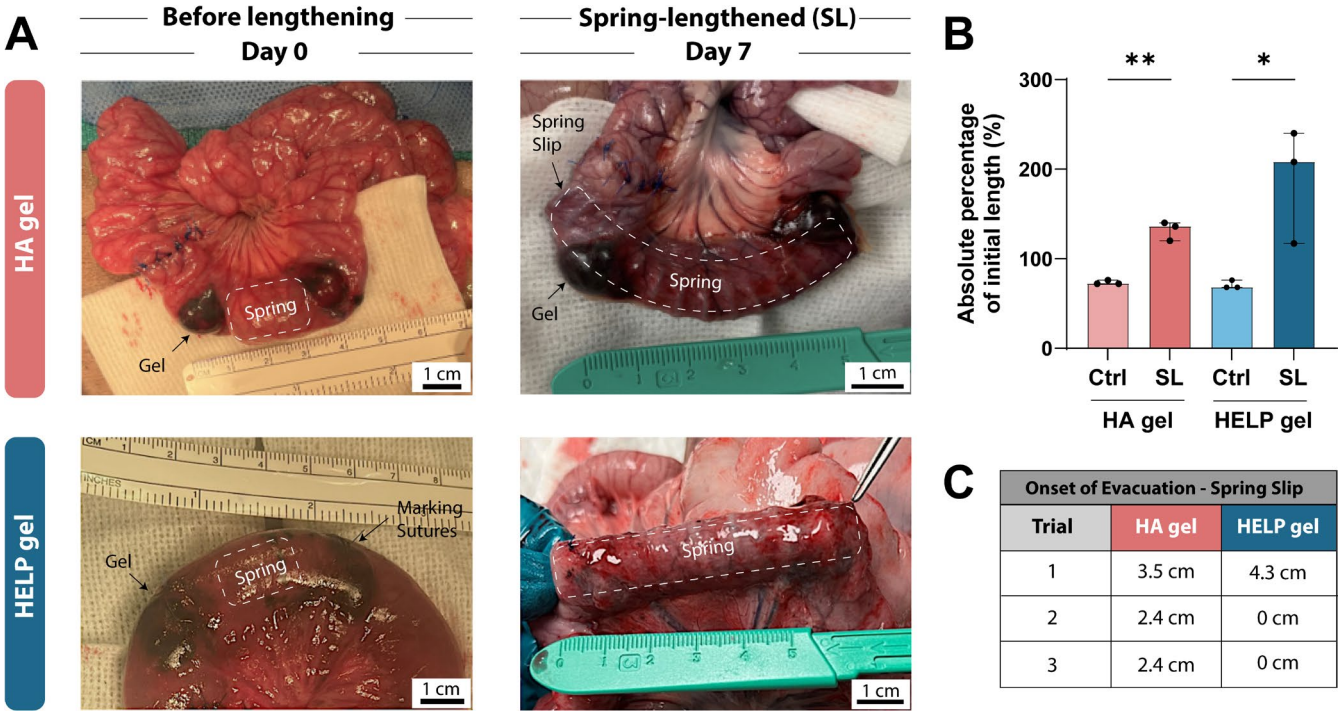

FIGURE 2 | Significant intestinal lengthening was observed after insertion of compressed springs on POD 7 in both hydrogel groups compared to controls. (A) Gross histology of bowel at the time of spring insertion, before lengthening at Day 0, and at euthanasia, spring-lengthened tissue at Day 7, HA gel (top) and HELP gel (bottom). Legends identifying the spring, gel injection marking sutures, and spring slip. (B) Absolute percentage of initial length increase of intestinal segments at POD 7. Ctrl, control segment, and SL, spring-lengthened segment. (C) Quantification of spring slip at POD 7 in the three animals of each group (trials, $n=3$). Paired Student's t tests, $^{*}p<0.05$, $^{**}p<0.01$.

were compressed a set distance within a soluble gelatin capsule to exert a maximal load of 0.24 ± 0.02 N at the beginning of deployment (i.e., upon gelatin capsule dissolution, which occurs within a few hours [22]).

3.2 | Surgical Outcomes

All pigs tolerated the surgery without complications. The post-operative course was uneventful. They tolerated the liquid diet without bowel perforation or obstruction. No significant change in pigs' body weight was observed. At least some residual hydrogel was observed in the submucosa, both proximal and distal to the spring, in all animals.

3.3 | Bowel Lengthening

The length of the spring-containing and control segments was measured on post-operative day (POD) 7 and was calculated as a percentage of the initial length. Consistent with our previous reports, the control segment shrunk to $72\%\pm4\%$ of the initial length. Shrinkage of the control segments has been a consistent pattern observed in our previous studies, attributed to surgical manipulation and postoperative adhesions [49, 50]. Next, we compared the segments treated with either HA and HELP confined springs. On POD 7, the two spring-containing HA and HELP segments exhibited significant lengthening of $132\%\pm11\%$ and $188\%\pm64\%$ of the initial length, Figure 2B, respectively ($p<0.01$ and $p=0.04$, respectively, in comparison to control segments). There was a trend towards more lengthening in the HELP group as compared to the HA group, although this was not statistically significant ($p=0.10$), Figure 2B.

3.4 | Onset of Spring Evacuation and Hydrogel Biodegradation

Spring slippage, defined as the spring passing through the injected hydrogel (Figure 2A), was observed in 4 out of 6 animals, with values ranging from 2.4 to 4.3 cm, Figure 2C. All animals in the HA gel group ($n=3$) showed evidence of spring slippage at POD 7. For the HELP gel group, spring slippage was observed in only 1 out of 3 animals. These results suggest that the presence of the ELP slowed down the biodegradation rate of the gel, delaying the onset of evacuation and potentially prolonging the application of mechanical stimulation.

When comparing the in vitro degradation rates of the two gels, we observed that formulations of HELP gels had significantly more gel remaining after 30 days of exposure to saline compared to HA gel formulations (Figure S4). As we are unable to perform longitudinal in vivo tracking of hydrogel biodegradation through In Vivo Imaging Systems (IVIS) due to the depth of the abdominal cavity in large animals, we quantified the relative area of remaining hydrogel using histology at the endpoint, POD 7 (Figure S3). The relative area of residual hydrogel normalized to the bowel cross-section was $16.66\%\pm6.44\%$ for HELP hydrogels and $3.96\%\pm2.96\%$ for HA hydrogels, Figure 3B.

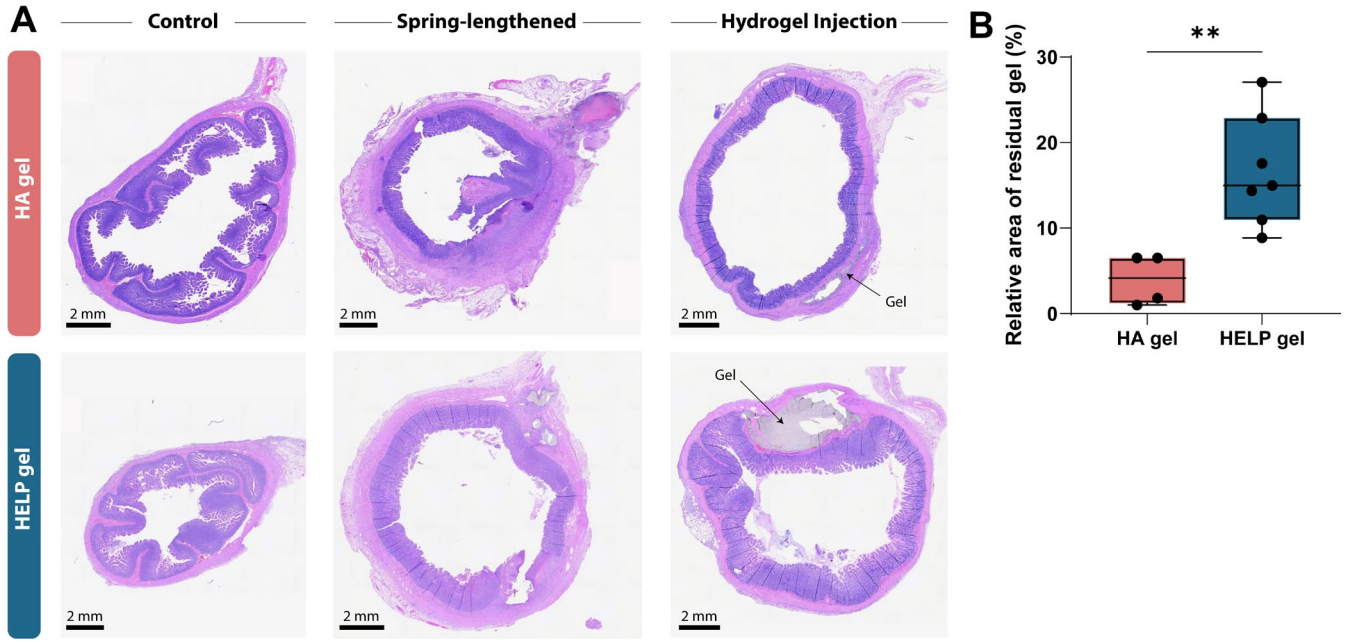

FIGURE 3 | (A) Histological adaptation of the lengthened jejunum on POD 7 in HA (top) and HELP gel (bottom). H&E histology is shown for intestinal sections a few inches removed from the site of spring implantation (left, Control Jejunum), within the site of spring lengthening (middle, Spring-lengthened Jejunum), and within the site where the hydrogel was injected (right, Jejunum with Hydrogel). (B) Quantification of the relative area of residual hydrogel at the hydrogel injection site. Unpaired Student's t tests, $^{**}p < 0.01$.

3.5 | Intestinal Histology and Inflammation Analysis

Histologic adaptation of the lengthened jejunum was similar between the two hydrogel groups (Figure 3A). In both groups, compared to the control segment, an increase in the crypt width was noted in the spring-containing segments, with an average crypt width of $46.4 \pm 7.7\,\mu m$ and $44.7 \pm 6.4\,\mu m$ in the control segments of the HA and HELP groups versus crypt widths of $62.5 \pm 11.6\,\mu m$ ($p < 0.001$) and $64.0 \pm 12.0\,\mu m$ ($p < 0.0001$) in the spring-containing segments, Figure 4A,B, respectively. Crypt widths were similar across control groups ($p = 0.54$). Similarly, there was no significant difference in the crypt width of the spring-containing segments across the hydrogel groups ($p = 0.98$).

In both groups, compared to the control segments, a decrease in the crypt density was noted in the spring-containing segments, with average crypt densities of 16.8 ± 2.5 and 18.5 ± 2.2 crypts per mm in the control segments of HA and HELP groups versus crypt densities of 12.2 ± 2.2 ($p < 0.0001$) and 12.5 ± 2.3 crypts per mm ($p < 0.0001$) in the spring-containing segments, Figure 4A,C, respectively. Crypt densities were similar across control groups ($p = 0.08$). Similarly, there was no significant difference in the crypt density of the spring segments detected across the hydrogel groups ($p = 0.85$) (Figure 4).

Although no animals presented any symptoms, weight loss, or signs of discomfort, inflammation markers were seen in all conditions in histological analysis. In the HA gel group, animals presented with mild to moderate inflammation composed mainly of macrophages, fewer neutrophils, and multinucleated macrophages, indicating a foreign body reaction around the residual hydrogel (Figure S2A). In this group, the gel leaked into the intestinal lumen in only one animal, leading to the presence of an inflammatory infiltrate mainly composed of neutrophils. In the HELP gel group, the gel leaked into the intestinal lumen in all animals, leading to an inflammatory response predominantly neutrophilic with varying numbers of intralesional bacteria (Figure S2B). A secondary invasion of intestinal commensal bacteria into the hydrogel is most likely the cause of the prominent neutrophilic inflammation. Finally, mural granulation tissue formation was present in all spring sites with moderate to severe ulceration, a common finding in previous studies [42, 51–54].

4 | Discussion

4.1 | DCC Gel Formulations Are Suitable for Spring Confinement

Our group previously showed successful intestinal lengthening through SMDE as a treatment option for short bowel syndrome [13–21]. Our long-term goal is to develop an endoscopic method for spring implantation to minimize the morbidity associated with device insertion. Here we evaluated the hypothesis that intestinal lumen narrowing by circumferential injection of hydrogel into the submucosa could serve as an effective substitute for endoscopic suturing. We showed that both HA and HELP hydrogels were suitable for temporary spring confinement within an intestinal segment. In both groups, the hydrogels were pre-formed by mixing the individual polymers and then injected using a small-gauge needle [28]. The dynamic nature of hydrazone-crosslinked gels enabled them to shear-thin during injection and then self-heal to re-form a solid in situ [28, 55]. Histologic examination of the retrieved intestine showed residuals of both hydrogels within the submucosa, causing inward

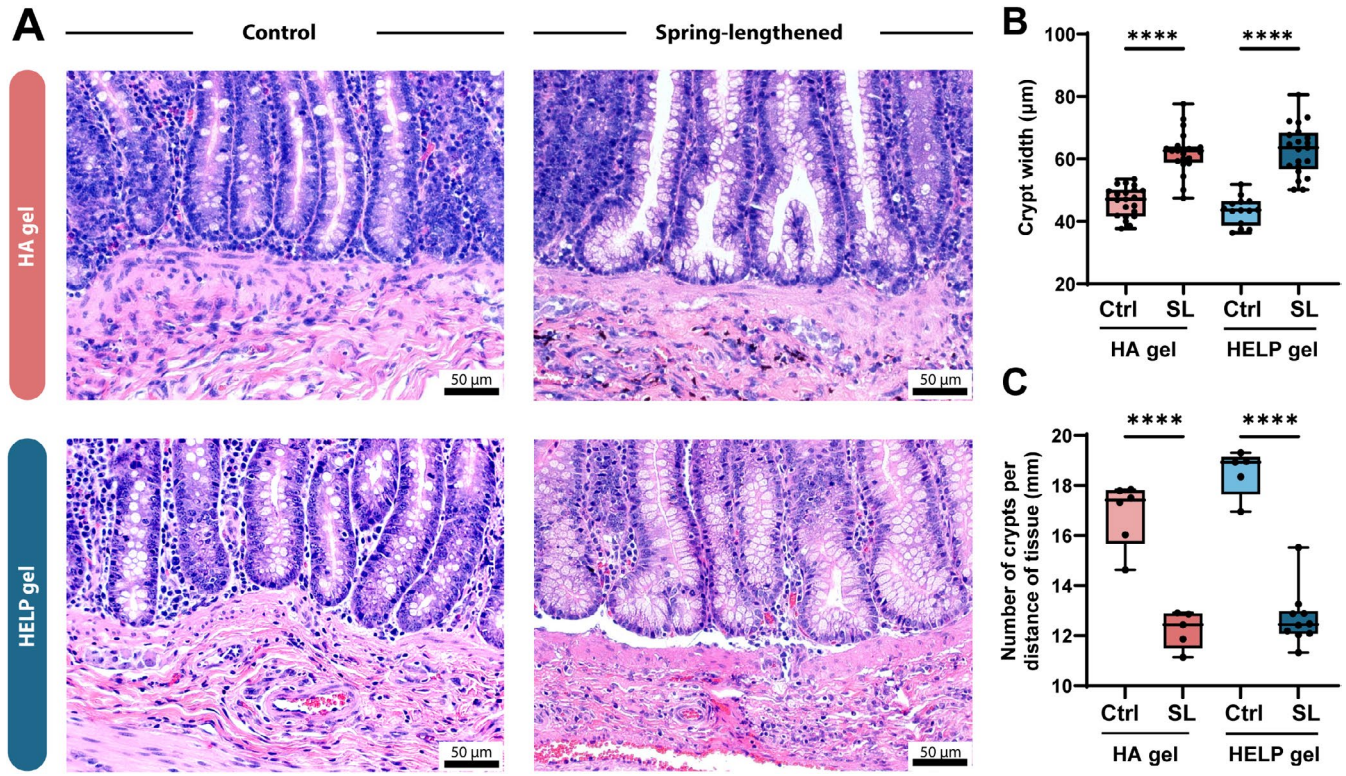

FIGURE 4 | (A) H&E Histology of control and spring-lengthened jejunum at 400× magnification, HA (top) and HELP gel (bottom) groups. (B, C) Crypt width (B) and Crypt density (C) (defined as the number of crypts per distance of tissue in mm) changes in spring-lengthened jejunum (SL) and control jejunum (Ctrl) at POD 7 in both hydrogel groups. One-way ANOVA, ****$p < 0.0001$.

bulging of the intestinal wall, which provided spring confinement. Both hydrogels had started to biodegrade within a week after injection, which would allow for eventual spring evacuation downstream after intestinal lengthening without requiring a secondary surgery [28, 33].

Given the proof-of-concept nature of this project, we compared our results to previous suture-based studies performed by our group in the same animal model. The bowel lengthening achieved after 1 week using HELP gel as a confinement method, 188% ± 64% relative to the initial length, was similar to that previously seen using internal plications to confine the spring, 172% ± 36% [23].

4.2 | Addition of ELP Results in Slower Gel Biodegradation

Although both hydrogel groups displayed successful intestinal lengthening compared to the control segments, a statistically not significant trend of more lengthening was seen in the HELP group. This might be associated with differing biodegradation times of the HA and HELP gels. Previous studies have demonstrated that in vitro degradation times of hydrazone-crosslinked gels can be significantly extended through the addition of ELP [39]. For example, while similar HA gels displayed ~70% retention after 10 days in vitro [45], similar HELP gel formulations had over 90% of the gel still present after 10 days [46]. Consistent with these previous reports, we observed that formulations of HELP gels degraded four times more slowly than the formulations of HA gels in vitro (Figure S4).

Although we were unable to perform longitudinal in vivo biodegradation tracking due to the depth of the abdominal cavity in juvenile pigs, the analysis of the remaining gel at the endpoint also supports our hypothesis that the addition of ELP significantly slows down gel degradation (Figure 3B). Although the HA and ELP biopolymers can both be degraded by enzymes present in the body, HA is well known for having a relatively short half-life compared to elastin [56, 57].

An interesting finding was the presence of spring slippage in 4 out of 6 animals, suggesting the onset of natural evacuation. We had not observed this in our previous suture-based studies, as those used permanent ligations that fully occlude the lumen and require surgical removal of the spring after treatment. The presence of slippage in our gel-confined animals is likely due to the biodegradation of the gel, both due to the mechanical forces applied by the spring as well as by the enzymatic and mechanical cues present in the intestinal environment. Thus, in contrast to suture-based approaches, we have successfully demonstrated that our gel-confined springs are intentionally transiently anchored. As the gel degrades, the spring can move past the injection site and be naturally evacuated. Further, the absence of slippage in 2 out of 3 animals in the HELP gel group suggests that the incorporation of ELP was able to delay the onset of natural evacuation. Taken together with the longer lengthening observed in the HELP group, these data indicate that the HELP gel is a better candidate to achieve our goal of simultaneously providing effective lengthening and natural spring evacuation.

Given the proof-of-concept nature of these studies, the animal model was ended after 1 week of treatment, which was a sufficient time to demonstrate intestinal lengthening. This endorses future studies on HELP gel-confined springs for SMDE with longer durations, larger cohorts, and multiple time points to investigate gel biodegradation in vivo, paving the way for clinical translation of this technology. In our previous studies of HELP gel in an alternative disease model of myocardial infarction, we observed that similar formulations of HELP gel remained in vivo for up to 1 month [58].

4.3 | Inflammation of the Tissue Was Within Expected Parameters

Histology analysis showed the presence of local inflammation markers in all animals. In the HA gel group, there was a mild local foreign body response, which was expected with the use of biomaterials and did not cause any systemic symptoms in the animals. In the HELP group, there was mainly a neutrophilic response, most likely due to the invasion of intestinal bacteria into the submucosal gel, which leaked into the intestinal lumen in all animals. This gel leak was also seen in one animal in the HA group, which also presented a neutrophilic inflammatory response. As this is a novel submucosal gel injection surgical procedure, the gel leaks are likely caused by procedural challenges, as the surgeon could have injected through the submucosa and into the lumen. Thus, we cannot conclude that the addition of ELP led to a change in inflammatory response, as the invasion of commensal bacteria into the leaked gel most likely elicited the observed immune response. However, while there were some inflammatory responses to the hydrogel, they did not cause any symptoms or weight loss in the animals. These results support the importance of future optimization of the hydrogel injection procedure to prevent gel leaks into the intestinal lumen. Further, the presence of a foreign body response in the first 7 days endorses the need for extended studies to analyze the progression of this initial inflammatory response. Finally, the presence of ulceration at the spring sites is a common finding in previous studies, which heals after the removal of the spring from the intestine [42, 51–54].

4.4 | Histological Adaptations Suggest the Onset of the Formation of Functional Bowel

Histologic adaptation of the lengthened jejunum was similar among the two hydrogel groups and consistent with our results in previous studies [17, 19]. On close observation, we noticed an increase in the crypt width and a decrease in the crypt density along the spring-lengthened segments compared to the control, which suggests that the applied force first stretched out the epithelium, leading to decreased density, and this induced crypt widening and subsequent fission. This observation is consistent with previous reports by others that demonstrated mechanical stretch leads to epithelial differentiation and loss of Lgr5 expression through the process of type II crypt fission [59]. Over time, the remaining patches of stem cell zones will form new crypts [59], achieving functionally viable lengthened bowel [49]. Histologic changes in our study in the first 7 days suggest we are observing the early stage of type II fission. In light of these promising findings, a longer course of study will be necessary to observe further changes, including the possible formation of new crypts from the widened crypts, as well as the analysis of the functionality of the lengthened bowel.

Having demonstrated the feasibility of using a hydrogel to confine springs within an intestinal segment through an open surgery model, our next step is to develop an innovative surgical procedure to endoscopically deliver the hydrogel and transiently anchor the spring, using a commercial endoscope for clinical applications in patients with short bowel syndrome.

5 | Conclusion

This was the first proof-of-concept study towards achieving endoscopic, suture-less spring insertion using a hydrogel for bowel lengthening. We successfully demonstrated formulation of a recombinant hydrogel that is injectable, self-healing to effectively confine a spring device, and biodegradable to allow for onset of natural evacuation without requiring a second surgery. Although we have demonstrated the gel's ability to securely anchor the spring for a temporary time period, this initial study did not include endoscopic injection to achieve confinement of the spring. Given our promising results, further experiments are necessary to explore endoscopic hydrogel injection and spring placement, which are crucial for clinical translation. Taken together, our data suggest that gel-enabled endoscopic spring-mediated distraction enterogenesis has the potential to become an effective minimally invasive treatment for SBS.

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Conflicts of Interest

Dr. James C. Y. Dunn is the Co-founder of Eclipse Regenesis and reported his patent on "Expandable distension device for hollow organ growth." Dr. James C. Y. Dunn, Dr. Sarah C. Heilshorn, Dr. Fereshteh Salimi-Jazi, Anne-Laure Thomas, Narelli de Paiva Narciso, Dr. Riley Suhar, and Dr. Renato Navarro are listed as co-inventors on a provisional patent titled "Hydrogel Injection for Intestinal Anchoring". All other authors report no disclosures related to this work.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. R. Chandra and A. Kesavan, "Current Treatment Paradigms in Pediatric Short Bowel Syndrome," *Clinical Journal of Gastroenterology* 11, no. 2 (2018): 103–112, https://doi.org/10.1007/s12328-017-0811-7.

2. B. A. Shakhsheer and B. W. Warner, "Short Bowel Syndrome," *Current Treatment Options in Pediatrics* 5, no. 4 (2019): 494–505, https://doi.org/10.1007/s40746-019-00179-y.

3. F. Bioletto, C. D'eusebio, F. D. Merlo, et al., "Efficacy of Teduglutide for Parenteral Support Reduction in Patients With Short Bowel Syndrome: A Systematic Review and Meta-Analysis," *Nutrients* 14, no. 4 (2022): 796, https://doi.org/10.3390/NU14040796/S1.

4. H. K. Sabra, G. S. Remeih, I. M. Kereet, et al., "Efficacy and Safety of Glucagon-Like Peptide 2 in Patients With Short Bowel Syndrome: A Systematic Review and Network Meta-Analysis," *Journal of Gastrointestinal Surgery* 28, no. 7 (2024): 1194–1205, https://doi.org/10.1016/J.GASSUR.2024.04.009.

5. N. N. Youssef, A. G. Mezoff, B. A. Carter, and C. R. Cole, "Medical Update and Potential Advances in the Treatment of Pediatric Intestinal Failure," *Current Gastroenterology Reports* 14, no. 3 (2012): 243–252, https://doi.org/10.1007/S11894-012-0262-8.

6. D. Wendel, C. R. Cole, and V. C. Cohran, "Approach to Intestinal Failure in Children," *Current Gastroenterology Reports* 23, no. 6 (2021): 8, https://doi.org/10.1007/s11894-021-00807-4.

7. S. J. D. O'Keefe, A. L. Buchman, T. M. Fishbein, K. N. Jeejeebhoy, P. B. Jeppesen, and J. Shaffer, "Short Bowel Syndrome and Intestinal Failure: Consensus Definitions and Overview," *Clinical Gastroenterology and Hepatology* 4, no. 1 (2006): 6–10, https://doi.org/10.1016/J.CGH.2005.10.002.

8. G. Dubrovsky, J. S. Taylor, A. L. Thomas, S. Shekherdimian, and J. C. Y. Dunn, "Optimization of In-Continuity Spring-Mediated Intestinal Lengthening," *Journal of Pediatric Surgery* 55, no. 1 (2020): 158–163, https://doi.org/10.1016/j.jpedsurg.2019.09.072.

9. C. Belza, K. Fitzgerald, N. de Silva, et al., "Predicting Intestinal Adaptation in Pediatric Intestinal Failure: A Retrospective Cohort Study," *Annals of Surgery* 269, no. 5 (2019): 988–993, https://doi.org/10.1097/SLA.0000000000002602.

10. H. S. Hosseini, J. S. Taylor, L. S. Y. Wood, and J. C. Y. Dunn, "Biomechanics of Small Intestine During Distraction Enterogenesis With an Intraluminal Spring," *Journal of the Mechanical Behavior of Biomedical Materials* 101 (2020): 103413, https://doi.org/10.1016/j.jmbbm.2019.103413.

11. M. F. Winkler and C. E. Smith, "Clinical, Social, and Economic Impacts of Home Parenteral Nutrition Dependence in Short Bowel Syndrome," *JPEN Journal of Parenteral and Enteral Nutrition* 38, no. 1 (2014): 32S–37S, https://doi.org/10.1177/0148607113517717.

12. R. H. Squires, C. Duggan, D. H. Teitelbaum, et al., "Natural History of Pediatric Intestinal Failure: Initial Report From the Pediatric Intestinal Failure Consortium," *Journal of Pediatrics* 161, no. 4 (2012): 723–728.e2, https://doi.org/10.1016/J.JPEDS.2012.03.062.

13. H. S. Hosseini, L. S. Y. Wood, J. S. Taylor, et al., "Biomechanical Signaling and Collagen Fiber Reorientation During Distraction Enterogenesis," *Journal of the Mechanical Behavior of Biomedical Materials* 101 (2020): 101, https://doi.org/10.1016/j.jmbbm.2019.103425.

14. S. Shekherdimian, A. Scott, A. Chan, and J. C. Y. Dunn, "Intestinal Lengthening in Rats After Massive Small Intestinal Resection," *Surgery* 146, no. 2 (2009): 291–295, https://doi.org/10.1016/j.surg.2009.03.034.

15. V. F. Sullins, A. Scott, J. P. Wagner, et al., "Intestinal Lengthening in an Innovative Rodent Surgical Model," *Journal of Pediatric Surgery* 49, no. 12 (2014): 1791–1794, https://doi.org/10.1016/j.jpedsurg.2014.09.022.

16. G. Dubrovsky, N. Huynh, A. L. Thomas, S. Shekherdimian, and J. C. Dunn, "Double Plication for Spring-Mediated In-Continuity Intestinal Lengthening in a Porcine Model," *Surgery* 165, no. 2 (2019): 389–392, https://doi.org/10.1016/j.surg.2018.07.028.

17. F. Salimi-Jazi, A. L. Thomas, T. Rafeeqi, M. Diyaolu, L. S. Y. Wood, and J. C. Y. Dunn, "The Effect of Spring Diameter on Porcine Ileal Distraction Enterogenesis," *Pediatric Surgery International* 39, no. 1 (2022): 19, https://doi.org/10.1007/s00383-022-05300-1.

18. F. Salimi-Jazi, A.-L. Thomas, T. A. Rafeeqi, L. S. Y. Wood, K. Portelli, and J. C. Y. Dunn, "Stem Cell Activation During Distraction Enterogenesis in the Murine Colon," *Pediatric Surgery International* 39, no. 1 (2023): 172, https://doi.org/10.1007/s00383-023-05455-5.

19. T. A. Rafeeqi, M. Diyaolu, A. L. Thomas, F. Salimi-Jazi, L. S. Y. Wood, and J. C. Y. Dunn, "Generation of Porcine Ileum Through Spring-Mediated Mechanical Distraction," *Journal of Surgical Research* 280 (2022): 371–378, https://doi.org/10.1016/j.jss.2022.07.043.

20. T. Rafeeqi, V. F. Sullins, A. L. Thomas, et al., "Long-Term Safety of Intraluminal Spring-Mediated Bowel Lengthening," *Journal of Pediatric Surgery* 58, no. 1 (2023): 89–93, https://doi.org/10.1016/j.jpedsurg.2022.09.034.

21. G. Dubrovsky and J. C. Y. Dunn, "Mechanisms for Intestinal Regeneration," *Current Opinion in Pediatrics* 30, no. 3 (2018): 424–429.

22. F. R. Demehri, B. Utter, J. J. Freeman, et al., "Development of an Endoluminal Intestinal Attachment for a Clinically Applicable Distraction Enterogenesis Device," *Journal of Pediatric Surgery* 51, no. 1 (2016): 101–106, https://doi.org/10.1016/j.jpedsurg.2015.10.026.

23. T. A. Rafeeqi, A.-L. Thomas, F. Salimi-Jazi, M. Diyaolu, and J. C. Y. Dunn, "Internal Plication for Spring Confinement to Lengthen Intestine in a Porcine Model," *PLoS One* 17, no. 9 (2022): 1–8, https://doi.org/10.1371/journal.pone.0274612.

24. S. C. Tang, B. M. Richardson, and K. S. Anseth, "Dynamic Covalent Hydrogels as Biomaterials to Mimic the Viscoelasticity of Soft Tissues," *Progress in Materials Science* 120 (2021): 100738, https://doi.org/10.1016/j.pmatsci.2020.100738.

25. K. S. Anseth and H. A. Klok, "Click Chemistry in Biomaterials, Nanomedicine, and Drug Delivery," *Biomacromolecules* 17, no. 1 (2016): 1–3, https://doi.org/10.1021/acs.biomac.5b01660.

26. C. B. Rodell, N. N. Dusaj, C. B. Highley, and J. A. Burdick, "Injectable and Cytocompatible Tough Double-Network Hydrogels Through Tandem Supramolecular and Covalent Crosslinking," *Advanced Materials* 28, no. 38 (2016): 8419–8424, https://doi.org/10.1002/adma.201602268.

27. M. H. Chen, L. L. Wang, J. J. Chung, Y. H. Kim, P. Atluri, and J. A. Burdick, "Methods to Assess Shear-Thinning Hydrogels for Application as Injectable Biomaterials," *ACS Biomaterials Science & Engineering* 3, no. 12 (2017): 3146–3160, https://doi.org/10.1021/acsbiomaterials.7b00734.

28. N. de Paiva Narciso, R. S. Navarro, A. E. Gilchrist, M. L. M. Trigo, G. Aviles Rodriguez, and S. C. Heilshorn, "Design Parameters for Injectable Biopolymeric Hydrogels With Dynamic Covalent Chemistry Crosslinks," *Advanced Healthcare Materials* 12 (2023): e2301265, https://doi.org/10.1002/adhm.202301265.

29. M. Dinkgreve, J. Paredes, M. M. Denn, and D. Bonn, "On Different Ways of Measuring "the" Yield Stress," *Journal of Non-Newtonian Fluid Mechanics* 238 (2016): 233–241, https://doi.org/10.1016/j.jnnfm.2016.11.001.

30. A. C. Gaffey, M. H. Chen, C. M. Venkataraman, et al., "Injectable Shear-Thinning Hydrogels Used to Deliver Endothelial Progenitor Cells, Enhance Cell Engraftment, and Improve Ischemic Myocardium," *Journal of Thoracic and Cardiovascular Surgery* 150, no. 5 (2015): 1268–1276, https://doi.org/10.1016/j.jtcvs.2015.07.035.

31. A. K. Grosskopf, O. A. Saouaf, H. Lopez Hernandez, and E. A. Appel, "Gelation and Yielding Behavior of Polymer-Nanoparticle Hydrogels," *Journal of Polymer Science* 59, no. 22 (2021): 2854–2866, https://doi.org/10.1002/pol.20210652.

32. Y. Han, Y. Cao, and H. Lei, "Dynamic Covalent Hydrogels: Strong Yet Dynamic," *Gels* 8, no. 9 (2022): 577, https://doi.org/10.3390/gels8090577.

33. R. A. Suhar, V. M. Doulames, Y. Liu, et al., "Hyaluronan and Elastin-Like Protein (HELP) Gels Significantly Improve Microsphere Retention in the Myocardium," *Biomaterials Science* 10, no. 10 (2022): 2590–2608, https://doi.org/10.1039/d1bm01890f.

34. D. R. Hunt, K. C. Klett, S. Mascharak, et al., "Engineered Matrices Enable the Culture of Human Patient-Derived Intestinal Organoids," *Advanced Science* 8, no. 10 (2021): 2004705, https://doi.org/10.1002/advs.202004705.

35. T. E. Riehl, L. Foster, and W. F. Stenson, "Hyaluronic Acid Is Radioprotective in the Intestine Through a TLR4 and COX-2-Mediated Mechanism," *American Journal of Physiology. Gastrointestinal and Liver Physiology* 302, no. 3 (2012): G309–G316, https://doi.org/10.1152/ajpgi.00248.2011.

36. C. A. de la Motte and S. P. Kessler, "The Role of Hyaluronan in Innate Defense Responses of the Intestine," *International Journal of Cell Biology* 2015 (2015): 481301, https://doi.org/10.1155/2015/481301.

37. L. Wang, S. K. Murthy, W. H. Fowle, G. A. Barabino, and R. L. Carrier, "Influence of Micro-Well Biomimetic Topography on Intestinal Epithelial Caco-2 Cell Phenotype," *Biomaterials* 30, no. 36 (2009): 6825–6834, https://doi.org/10.1016/j.biomaterials.2009.08.046.

38. D. C. Gowda, C. H. Luan, R. L. Furner, et al., "Synthesis and Characterization of the Human Elastin W4 Sequence," *International Journal of Peptide and Protein Research* 46, no. 6 (1995): 453–463, https://doi.org/10.1111/j.1399-3011.1995.tb01600.x.

39. H. Wang, D. Zhu, A. Paul, et al., "Covalently Adaptable Elastin-Like Protein—Hyaluronic Acid (ELP – HA) Hybrid Hydrogels With Secondary Thermoresponsive Crosslinking for Injectable Stem Cell Delivery," *Advanced Functional Materials* 27, no. 28 (2017): 1605609, https://doi.org/10.1002/ADFM.201605609.

40. H. Wang, L. Cai, A. Paul, A. Enejder, and S. C. Heilshorn, "Hybrid Elastin-Like Polypeptide–Polyethylene Glycol (ELP-PEG) Hydrogels With Improved Transparency and Independent Control of Matrix Mechanics and Cell Ligand Density," *Biomacromolecules* 15, no. 9 (2014): 3421–3428, https://doi.org/10.1021/bm500969d.

41. N. Huynh, G. Dubrovsky, J. D. Rouch, et al., "Feasibility and Scalability of Spring Parameters in Distraction Enterogenesis in a Murine Model," *Journal of Surgical Research* 215 (2017): 219–224, https://doi.org/10.1016/j.jss.2017.04.009.

42. J. Park, D. P. Puapong, B. M. Wu, J. B. Atkinson, and J. C. Y. Dunn, "Enterogenesis by Mechanical Lengthening: Morphology and Function of the Lengthened Small Intestine," *Journal of Pediatric Surgery* 39, no. 12 (2004): 1823–1827, https://doi.org/10.1016/j.jpedsurg.2004.08.022.

43. H. P. Swieskowski, *Helical Springs, Stresses, Mathematical Models, Coils, Wire, Compressive Properties, Nomographs, Loads, Numerical Analysis, Deflection* (Dover (NJ): Army Armament Research and Development Center, 1979).

44. L. S. Wood, H. S. Hosseini, M. Diyaolu, A.-L. Thomas, J. S. Taylor, and J. C. Dunn, "Mechanical Lengthening of Porcine Small Intestine With Decreased Forces," *Journal of Pediatric Surgery* 56, no. 7 (2021): 1192–1198.

45. J. Lou, F. Liu, C. D. Lindsay, O. Chaudhuri, S. C. Heilshorn, and Y. Xia, "Dynamic Hyaluronan Hydrogels With Temporally Modulated High Injectability and Stability Using a Biocompatible Catalyst," *Advanced Materials* 30, no. 22 (2018): e1705215, https://doi.org/10.1002/adma.201705215.

46. S. M. Hull, J. Lou, C. D. Lindsay, et al., "3D Bioprinting of Dynamic Hydrogel Bioinks Enabled by Small Molecule Modulators," *Science Advances* 9, no. 13 (2023): eade7880, https://doi.org/10.1126/sciadv.ade7880.

47. B. L. Lesavage, N. A. Suhar, C. M. Madl, and S. C. Heilshorn, "Production of Elastin-Like Protein Hydrogels for Encapsulation and Immunostaining of Cells in 3D," *Journal of Visualized Experiments: Jove* 2018, no. 135 (2018): e57739, https://doi.org/10.3791/57739.

48. M. E. Hefferon, M. S. Huang, Y. Liu, et al., "Cell Microencapsulation Within Engineered Hyaluronan Elastin-Like Protein (HELP) Hydrogels," *Current Protocols* 3, no. 11 (2023): e917, https://doi.org/10.1002/cpz1.917.

49. V. F. Sullins, J. P. Wagner, C. M. Walthers, et al., "Function of Mechanically Lengthened Jejunum After Restoration Into Continuity," *Journal of Pediatric Surgery* 49, no. 6 (2014): 971–975, https://doi.org/10.1016/j.jpedsurg.2014.01.033.

50. P. C. Y. Chang, J. Mendoza, J. Park, et al., "Sustainability of Mechanically Lengthened Bowel in Rats," *Journal of Pediatric Surgery* 41, no. 12 (2006): 2019–2022, https://doi.org/10.1016/j.jpedsurg.2006.08.014.

51. K. I. Portelli, J.-B. Park, J. S. Taylor, et al., "Intestinal Adaptation Following Spring Insertion Into a Roux Limb in Mice," *Journal of Pediatric Surgery* 56, no. 2 (2021): 346–351, https://doi.org/10.1016/j.jpedsurg.2020.06.033.

52. M. Diyaolu, A.-L. Thomas, L. S. Wood, J. Taylor, and J. C. Dunn, "Mesenteric Neovascularization During Spring-Mediated Intestinal Lengthening," *Journal of Pediatric Surgery* 56, no. 1 (2021): 5–10, https://doi.org/10.1016/j.jpedsurg.2020.09.042.

53. Z. Jabaji, R. Stark, and J. C. Y. Dunn, "Regeneration of Enteric Ganglia in Mechanically Lengthened Jejunum After Restoration Into Intestinal Continuity," *Journal of Pediatric Surgery* 48, no. 1 (2013): 118–123, https://doi.org/10.1016/j.jpedsurg.2012.10.027.

54. D. P. Puapong, B. M. Wu, M. M. Lam, J. B. Atkinson, and J. C. Y. Dunn, "Distension Enterogenesis: Increasing the Size and Function of Small Intestine," *Journal of Pediatric Surgery* 41, no. 4 (2006): 763–767, https://doi.org/10.1016/j.jpedsurg.2006.02.017.

55. B. M. Richardson, D. G. Wilcox, M. A. Randolph, and K. S. Anseth, "Hydrazone Covalent Adaptable Networks Modulate Extracellular Matrix Deposition for Cartilage Tissue Engineering," *Acta Biomaterialia* 83 (2019): 71–82, https://doi.org/10.1016/j.actbio.2018.11.014.

56. K. Wang, X. Meng, and Z. Guo, "Elastin Structure, Synthesis, Regulatory Mechanism and Relationship With Cardiovascular Diseases," *Frontiers in Cell and Development Biology* 9 (2021): 596702, https://doi.org/10.3389/FCELL.2021.596702.

57. C. A. de la Motte, "Hyaluronan in Intestinal Homeostasis and Inflammation: Implications for Fibrosis," *American Journal of Physiology. Gastrointestinal and Liver Physiology* 301, no. 6 (2011): 945–949.

58. H. Yang, X. Qin, H. Wang, et al., "An In Vivo miRNA Delivery System for Restoring Infarcted Myocardium," *ACS Nano* 13, no. 9 (2019): 9880–9894, https://doi.org/10.1021/acsnano.9b03343.

59. G. M. Jowett and E. Gentleman, "Inflation Comes Before the Fall: How Epithelial Stretch Drives Crypt Fission," *Cell Stem Cell* 28, no. 9 (2021): 1505–1506.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** jbma37986-sup-0001-supinfo.pdf.