High-energy pacing in the jejunum elicits pulsatile segmental contractions

Nipuni D. Nagahawatte; Recep Avci; Timothy R. Angeli-Gordon; Niranchan Paskaranandavadivel; Leo K. Cheng

Abstract*— *Objective: **Compromised bowel function is associated with a range of motility disorders such as post-operative ileus and chronic intestinal pseudo-obstruction. Disordered or weak motility compromise the efficient movement of luminal contents necessary for digestion and nutrient absorption. This study investigated the potential of high-energy pacing to enhance contractions in the proximal jejunum of the small intestine.** ***Methods:*** **Pacing pulse parameters (pulse-width: 100 ms, 200 ms, 400 ms, pulse-amplitude: 4 mA, 6 mA, 8 mA) were systematically varied in the *in vivo* porcine jejunum (n=7) and the induced contractile responses were evaluated using a video mapping system. Localized segmental contractions were quantified by measuring the intestinal diameter and thereby computing the strain. The impact of pacing parameters on contractile strain was investigated. Finally, histological studies were conducted on paced tissue to assess for potential tissue damage.** ***Results:*** **Segmental contractions were successfully induced at all pulse-settings and evaluated across 67 pacing sessions. In response to pacing, the intestine segment at the site of pacing contracted, with diameter reduced by 6-18%. While contractile response increased with increasing pulse-amplitude, there was no significant increase in contractions beyond 6 mA. While the contractile response was enhanced with increasing pulse-width, the increase was significant only between 100 ms and 400 ms. Histology showed no tissue damage occurred when maximal pacing energy (pulse-amplitude=4-8 mA, pulse-width=400 ms, 5 minute duration) was applied.** ***Conclusion:*** **High-energy pacing induced periodic segmental contractions in response to pacing pulses and the contractile strain was proportional to the energy applied on the intestine. The ability to enhance motility through pacing may hold promising therapeutic potential for bowel disorders and awaits clinical translation.** ***Significance:*** **Small intestine pacing elicits localized segmental contractions which increase in magnitude with increasing pulse settings. This study marks the first adaptation of video mapping techniques to track the pacing response in the small intestine.**

***Index Terms*— Motility, Contractions, Electroceuticals, Jejunum, Pacing, Small intestine, Stimulation, Video mapping**

I. Introduction

GASTROINTESTINAL (GI) motility disorders are associated with increased morbidity and affect over 40% of the world population [1]. While nutritional support and pharmaceuticals are common treatment options for GI motility disorders, surgical intervention is often necessary for patients that fail conventional treatments. Post-operative ileus is one such motility disorder which occurs after surgery where small bowel motility is disrupted due to anesthesia, manipulation and handling of the bowel. The bowel function of 10-30% of patients is compromised after abdominal surgery [2]. Chronic intestinal pseudo-obstruction is a rare, yet critical disorder characterized by altered and inefficient propulsive motility that prevents the movement of luminal content in the absence of any obstruction [3]. This condition is a result of neuronal and muscular abnormalities and has a prevalence of 0.9 per 100,000 of the population [4], [5]. While conventional treatment options are effective for some chronic intestinal pseudo-obstruction and post-operative ileus patients, the symptoms remain unresolved for a large patient population and results in prolonged hospital stays and increased healthcare costs, causing a substantial clinical and economic burden [3], [6]. Therefore, more effective alternative treatment options are required.

High-energy pacing and low-energy stimulation are 2 types of electroceuticals that involve the application of extrinsic electrical pulses at low and high frequencies, respectively. Low-energy stimulation targets neural pathways and has had success in relieving symptoms [7], [8]. On the other hand, high-energy pacing targets the interstitial cells of Cajal – the pacemaker cells of the GI tract – and therefore, can induce contractions if sufficient energy levels are applied [9], [10]. High-energy pacing has been shown to modulate GI function including: motility, absorbency, and bio-electrical activity [11]–[13]. The ability to modulate the contractile response of the small intestine via pacing is of importance to develop novel therapies for disorders such as post-operative ileus and chronic intestinal pseudo-obstruction. The motility response during pacing has previously been studied in the intestine by

Submitted for review 6 March 2023.

This work was supported by a University of Auckland Doctoral Scholarship, Marsden Fund Council, and Riddet Institute Centre of Research Excellence. TRA-G was supported by a Rutherford Discovery Fellowship from the Royal Society Te Apārangi.

Nipuni D. Nagahawatte, Recep Avci, Timothy R. Angeli-Gordon, Niranchan Paskaranandavadivel and Leo K. Cheng are with the Auckland Bioengineering Institute, University of Auckland, Auckland, New Zealand. Timothy R. Angeli-Gordon is also affiliated with the Department of Surgery, University of Auckland, Auckland, New Zealand. *Leo K. Cheng is also with the Department of Surgery, Vanderbilt University, Nashville, Tennessee, USA and the Riddet Institute Centre of Research Excellence, Palmerston North, New Zealand (correspondence e-mail: l.cheng@auckland.ac.nz).

measuring the direction of the chyme flow, and the rate of emptying and contractions [14]–[16]. Literature reports that the contractile rate and emptying rates can be enhanced by antegrade pacing (where the positive pacing electrode is located proximal to the negative electrode) or inhibited by retrograde pacing (where the negative pacing electrode is located proximal to the positive electrode) [17]–[19].

In comparison to gastric motility, small intestine motility quantification metrics are in its infancy and are actively being researched. Clinical tools such as magnetic resonance imaging, x-rays and endoscopes have enabled investigation of motility patterns in the small intestine through visual deformations [20]–[22]. Specifically, magnetic resonance imaging has been useful in overcoming the anatomical and functional complexities and thereby estimating the small bowel motility. While the non-invasive and non-ionizing character is its key advantage, the ability to perform global assessments by visualizing the entire GI tract makes magnetic resonance imaging a favorable approach [21], [23], [24]. However, it is expensive and requires expert training. The adoption of more invasive techniques such as video recordings and manometry has enabled an improved understanding of passive deformations in the intestine such as those caused by tissue elasticity [25], [26]. The investigation of the small intestine motility in response to pacing has been largely limited to and conducted using manometry and by measuring the recovered amount of known luminal contents [15], [27]. Fluoroscopy has also been used to determine the direction of intestinal contents [14]. While these techniques have enabled a clear understanding of the ability of pacing the small intestine, they remain invasive, expensive and complex to implement compared to video mapping. Video mapping primarily focuses on mapping the change in diameter of the intestinal wall and tracking bio-markers [25], [28], [29]. This technique, which is typically applied to *in vitro* or *ex vivo* investigations, requires 'line of sight' and is yet to be translated to study the pacing response of the small intestine. Reliable quantitative metrics can be derived by adopting such techniques to measure the contractile response induced via pacing.

This study presents a novel framework that enabled visualization and quantification of the pacing response in the small intestine. Pacing parameters were systematically varied to determine the most effective pulse input to maximize contractile response. Histological analysis was performed to assess for potential tissue damage at the pacing site. Thereby, this study investigated the use of small intestine pacing in a pre-clinical animal model to induce and enhance segmental contractions as an alternative therapy for post-operative ileus and chronic intestinal pseudo-obstruction.

II. Methods

A. Contraction mapping and pacing

1) Animal preparation

Ethical approval for all studies was obtained from the University of Auckland Animal Ethics Committee. Experiments were conducted *in vivo* on female weaner cross-breed pigs (n=7, 41.2 ± 1.7 kg) that were fasted overnight. Animal care and surgical procedures have previously been described [30], [31]. In summary, pigs were subjected to general anesthesia induced with Zoletil and maintained using isoflurane. A midline laparotomy was performed to provide access to the small intestine. At the conclusion of the studies, the animals were euthanized while still under anesthesia with a bolus injection of sodium pentobarbital.

2) Experimental protocol

A saline-soaked layer of gauze was placed on the surface of abdomen on which a pair of bipolar pacing electrodes was placed with the electrodes facing up. The electrodes were 3 mm in diameter with 5 mm inter-electrode spacing. A jejunal loop of approximately 20 cm was exteriorized and placed on the electrodes such that the bottom serosal surface of the intestine was in contact with the electrodes. Output from a DS 8000 stimulator (World Precision Instruments, Inc., Sarasota, FL, USA) was connected to the pacing electrodes for serosal pacing [31]. Plastic film (Glad, Clorox New Zealand Ltd., Auckland, New Zealand) was laid on top of the intestinal segment to prevent drying and cooling. Air bubbles beneath the plastic film were removed by applying light pressure to ensure clarity of contractions along the intestinal borders. A video camera (Logitech HD Webcam C525, Logitech International S.A., Lausanne, Switzerland) was positioned directly above the exteriorized segment of jejunum and was used to track the motion (see Fig. 1). The isolator of the stimulator, which illuminated a light-emitting diode (LED) at the output of a stimulus, was placed within the view of the video. The blink of the LED synchronized the pacing pulse with the video recording. The camera was controlled by a microcontroller (Arduino UNO, Arduino, Somerville, MA, USA) and video recordings were captured at a framerate of 30 frames per second.

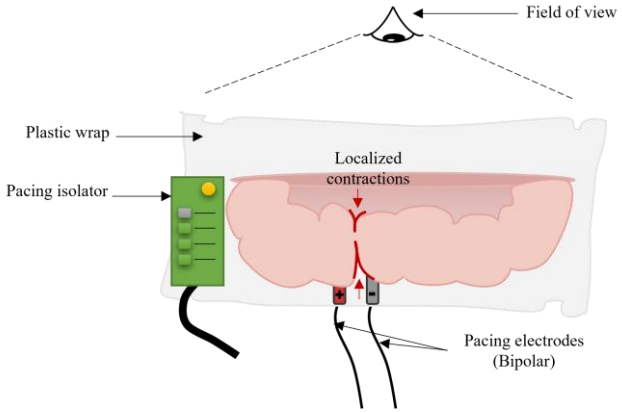

Fig. 1. Experimental setup for mapping the contractile response. The field of view of the mounted camera included the exteriorized jejunal segment, the pacing electrodes (positive and negative), and the pacing isolator which indicates the onset of each pacing pulse by a blinking light indicated in yellow.

Each experimental study evaluated the effects of 9 pacing sessions comprised of all the combinations of 3 pacing pulse-widths (100, 200, and 400 ms) and 3 pacing pulse-amplitudes (4, 6, and 8 mA). Pulse-widths below 100 ms and pulse-amplitudes below 4 mA were not investigated as a previous study has shown that the use of pacing parameters

below these levels were less reliable at entraining slow wave activity [31]. Each pulse combination was applied for 40 s at an interval of 10 s with a resting period of 30 s in between each setting. A baseline recording of 60 s was obtained prior to each pacing session. Pacing was applied in the antegrade direction as our previous studies have shown this orientation was most reliable for entraining the underlying slow wave activity [31].

3) Strain measurement

The intensity of the contractions induced in response to pacing was quantified by determining the strain of the intestinal segment at the localized contraction. Strain computations were based on the change in diameter of the intestine, which was measured using the video frames obtained during baseline and pacing. The diameter of the intestine was measured by tracing the mesenteric and anti-mesenteric borders (Fig. 2(a)) of the intestine, which were then fitted with a piecewise cubic polynomial interpolation using the 'pchip' function in MATLAB 2020a (MathWorks, Natick, MA, USA). The diameter of the intestine was calculated between 20 evenly spaced points located on both borders. The minimum distance between the mesenteric and anti-mesenteric borders (refer to the yellow line in Fig. 2(b)) was defined as the contracted diameter, and the undeformed state at the same location (determined based on anatomical landmarks, e.g., blood vessels) during baseline was measured for comparison. For each pacing setting, a baseline and a pacing-induced contraction were analyzed. The dimensions of a reference object (e.g., the pacing electrodes) in the videoframe were used to calculate the intestinal diameter in mm. This automatically accounted for large rigid-body movement within the mapped frame due to respiration (at inspiration vs expiration) as all diameter measurements were scaled based on the reference object within that frame in mm.

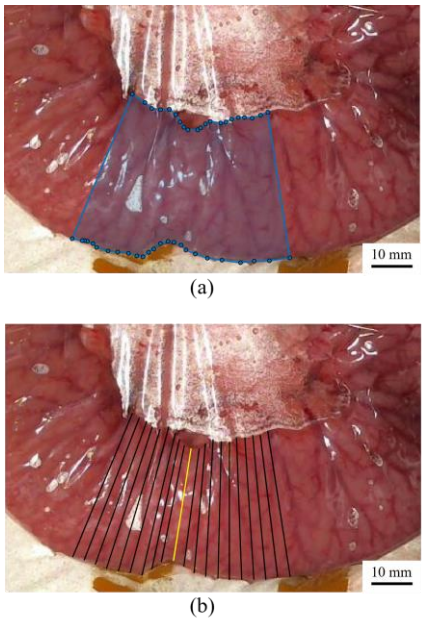

Fig. 2. Steps to determine the diameter of the contracted intestinal segment. (a) An envelope around the region of interest was created by tracing the mesenteric and anti-mesenteric borders of the intestine. (b) The envelope was fitted with 2 polynomials along the mesenteric and anti-mesenteric borders of the intestine. Equally spaced lines between the 2 polynomials were used to determine the intestinal diameter (black lines), and the shortest line was identified as the point of maximum contraction (yellow line).

Each diameter computation was an average of multiple contractions at peak contractility of the same protocol. Thereby, the strain induced during pacing was calculated using (1) [32].

$$Strain = (C_d - B_d)/B_d \quad (1)$$

where C_d is the diameter of the intestine at the time of maximum contraction induced by pacing and B_d is the diameter of the intestine during the baseline session at the same localized point corresponding to the contracted diameter. A negative strain corresponds to a contraction.

B. Tissue Histology

1) Experimental procedures

Histological analysis was performed to determine whether the pacing resulted in tissue damage. Pacing was applied at the highest energy levels with the maximum pulse-width (400 ms) at pulse-amplitudes of: i) 4 mA, ii) 6 mA, and iii) 8 mA. These energy levels were applied in separate pacing sessions for a duration of 5 minutes with a period of 10 s along the antegrade direction. The sessions were sequentially applied 5 cm apart with the first site located approximately 10 cm distal to the original site of video mapping (Section II.A). While contractions were analyzed by applying 4 pacing pulses (over 40 s), the pacing applied for histological analysis consisted of pacing over 5 minutes at 10 s intervals. Therefore, pacing was applied at the highest energy levels for longer duration prior to histological assessment. Following each pacing session, the pacing sites were marked with 2 sutures immediately above the locations of the pacing electrodes.

Immediately prior to euthanasia, the intestinal segment consisting of the 3 marked pacing sites and control tissue was excised. The intestinal segment was then washed with warm saline and fixed in 20 % natural buffered formalin solution for at least 48 hours. The cutlines, slicing and staining procedures were explained in a previous study [31]. In brief, 8 segments approximately 10 mm long - 6 located across the pacing sites and 2 across a control segment - were cut and embedded in paraffin blocks. Tissue sections of 5 µm thickness were sliced from the paraffin blocks, placed on glass slides, and stained with hematoxylin and eosin (H&E). The stained tissue slices were then imaged to determine any tissue damage at the pacing sites. The qualitative assessment included observation for damage to the tissue structure, blood congestion and swelling of nucleus.

2) Energy quantification

To determine the total energy applied to the tissue during the 5 minute pacing session conducted for histological analysis, the voltage across the 2 pacing electrodes were measured using an oscilloscope (PicoScope 2000, Pico Technology, Saint Neots, UK). The voltages measured during pacing pulses were averaged, and (2) was used to compute the total duration over which pacing energy was applied.

$$ON\ time = Paced\ duration \times (Duty\ cycle)/100 \quad (2)$$

where the paced duration was 5 minutes, and the duty cycle was based on a paced period of 10 s and a pulse-width of 400 ms.

Thereby, the total energy applied was determined using (3) [33].

$$Applied\ energy = Voltage \times Applied\ Current \times ON\ time \quad (3)$$

where the applied current was 4 mA, 6 mA or 8 mA.

C. Statistical Analysis

All data processing, including statistical analyses, were performed using MATLAB R2020a (MathWorks, Natick, MA, USA), and all metrics were expressed as mean ± standard deviation. Normality of data was tested using a Shapiro-Wilk test. P<0.05 was considered statistically significant in all statistical tests.

Two-way analysis of variance (ANOVA) was used to investigate main effects and the interaction effect of pulse-width and amplitude on strain values. The effect of pacing on the intestinal tissue was evaluated by comparing the voltage, tissue resistance and applied pacing energy across different pulse-amplitudes (4, 6 and 8 mA) for a pulse-width of 400 ms using a one-way ANOVA test followed by a Bonferroni test on the statistically significant groups.

III. Results

No visible contractions were observed during any of the baseline periods, and visible segmental contractions were immediately induced in response to all combinations of pulse-settings (4-8 mA, 100-400 ms). Contractions occurred 1:1 with the extrinsic pacing pulses and increased in intensity with increasing pulse-settings (energy levels). For each of the 9 energy levels, 7 or 8 pacing sessions were performed, resulting in a total of 67 pacing sessions. No other segmental contractions were observed during the quiescent period in between pulses.

A. Relationship between pacing parameters and contractions

The maximum strain measurements which resulted from pulses in each session were averaged to obtain the strain of jejunal contractions at each pulse-setting. Maximum contraction was observed approximately 1 s after applying each pacing pulse, and the contracted region was fully relaxed within approximately 2 s after the pacing pulse.

Fig. 3 illustrates the strain measurements at all settings comparing the effect of pulse-width and pulse-amplitude on the induced contractions. For the pulse-amplitude of 4 mA, the mean strain was less than 0.10 in magnitude for all 3 pulse-widths (100 ms: -0.06 ± 0.03, 200 ms: -0.07 ± 0.03, and 400 ms: -0.09 ± 0.04). The mean strain was between -0.10 and -0.14 for the pulse-amplitude of 6 mA (100 ms: -0.10 ± 0.04, 200 ms: -0.12 ± 0.05, 400 ms: -0.14 ± 0.04). The highest strains were observed with the pulse-amplitude of 8 mA (pulse-width 100 ms: 0.14 ± 0.04, 200 ms: -0.15 ± 0.04, and 400 ms: -0.18 ± 0.04). Therefore, the intestine locally contracted by 6-18% with the investigated jejunal pacing parameters.

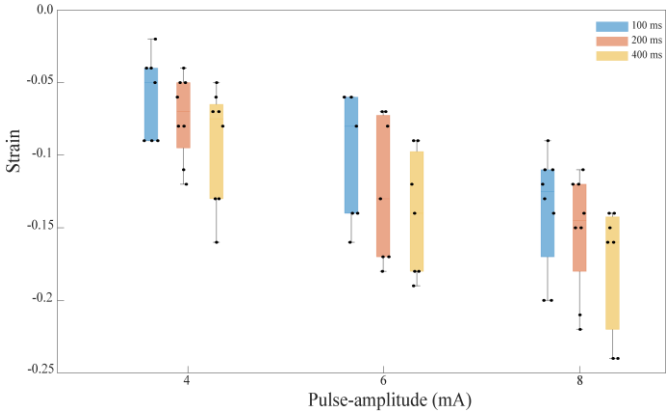

Fig. 3. The relationship between pulse-amplitude (4, 6, 8 mA), pulse-width (100, 200, 400 ms), and strain associated with segmental contractions (8 recordings from 7 female pigs). A negative strain indicates contraction in the circumferential axis.

The effect of both the pulse-width and pulse-amplitude on strains were found to be significant (pulse-width, p<0.001; pulse-amplitude, p<0.001), while the interaction between the two factors had no significant influence. The strain of contractions induced when paced with 4 mA was significantly lower than those induced by 6 mA (p<0.001) and 8 mA (p<0.001), and the contractions induced when paced with 6 mA were also significantly lower than those induced by 8 mA (p=0.007). While pacing with a pulse-width of 100 ms induced significantly lower contractile strains compared to 400 ms (p=0.002), the strain initiated at 200 ms was higher than at 100 ms and lower than that at 400 ms. However, the contractile strain at 200 ms was statistically comparable to the response initiated with 100 ms (p=0.143) and 400 ms (p=0.092).

Fig. 4 illustrates a representative case of segmental contractions induced when paced with a pulse-amplitude of 8 mA with varying pulse-widths. Fig. 4(a) shows the uncontracted intestine at baseline. The region where contractions occur has been identified by black arrows. When paced with a pulse-width of 100 ms (Fig. 4(b)), the anti-mesenteric border of the jejunal segment adjacent to the pacing site contracted in the circumferential axis resulting in a strain of -0.14. The intensity of the contraction on the anti-mesenteric border increased when paced with a pulse-width of 200 ms and a small contractile response was observed on the mesenteric border as well, as depicted in Fig. 4(c) (strain = -0.15). When the pulse-width was increased to 400 ms, the intensity of the contractions increased on both the mesenteric and anti-mesenteric borders resulting in a strain of -0.16. Segmental contractions induced at all pulse-settings were centered over the negative pacing electrode.

B. Correlation between pulse parameters and energy

When pacing with a constant pulse-width of 400 ms, the voltage measured across the pacing site increased when the pulse-amplitude was increased in the order of 4 mA (5.0 ± 0.9 V), 6 mA (6.7 ± 1.2 V) and 8 mA (7.7 ± 0.7 V). While the pacing voltages were significantly different between 4 mA and 8 mA (p=0.010), the consecutive voltage increments were statistically comparable (p=0.122 between 4 mA and 6 mA,

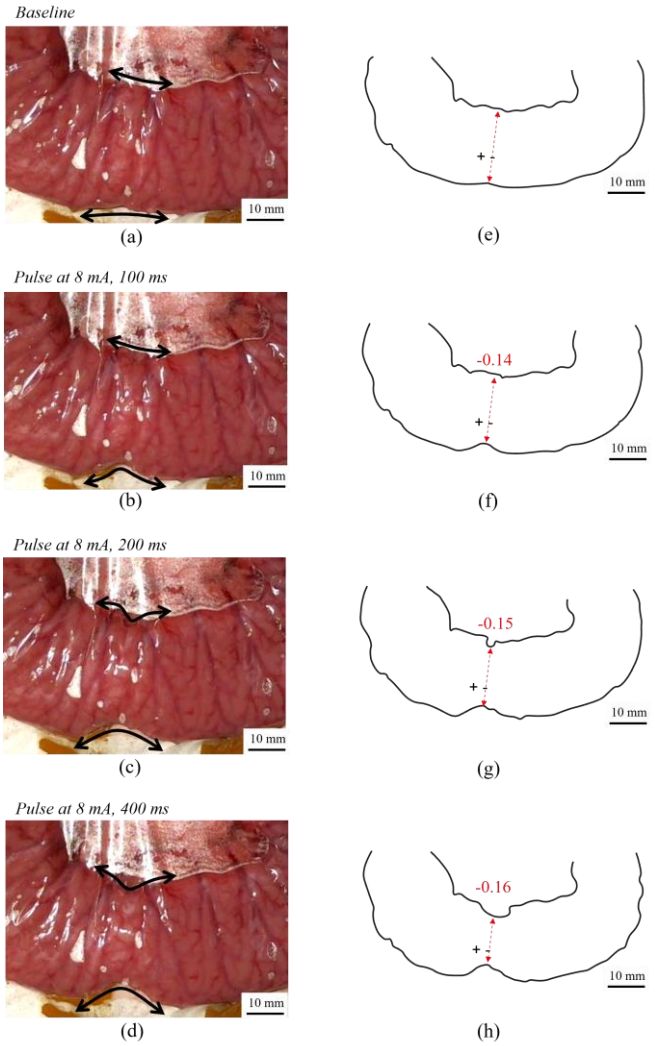

Fig. 4. Segmental contractions in the jejunum in response to high-energy pacing. Shown are (a) baseline activity compared against the segmental contractions induced in response to pacing with a pulse-amplitude of 8 mA and pulse-widths of (b) 100 ms, (c) 200 ms, and (d) 400 ms. The activity area is highlighted with black arrows along both the mesenteric and antimesenteric borders. Contractions along the mesenteric borders were initiated only at (c), and therefore, an air pocket was created at the contraction points of (c) and (d). The outlines of the intestinal borders are given in (e) - (h) with relative location of the pacing electrodes indicated by (+) and (-) signs. The diameter of the contracted intestine is indicated by red arrows.

p=0.476 between 6 mA and 8 mA). The corresponding tissue resistance computed from the measured voltages were essentially constant, with no statistically significant difference ($p>0.05$), and were in the range 1.0 ± 0.1 to 1.3 ± 0.2 kΩ. Based on the tissue resistance and pacing voltage, the applied pacing energy was determined and visualized in Fig. 5. For a pulse-width of 400 ms, the pacing energy applied on the tissue site linearly increased from 0.2 ± 0.1 to 0.7 ± 0.1 J for pulse-amplitudes between 4-8 mA. The energy levels were significantly different for each pulse-amplitude ($p=0.003$ between 4 mA and 6 mA, $p=0.002$ between 6 mA and 8 mA, and $p<0.001$ between 4 mA and 8 mA).

C. Histological analysis

Histological analysis of the tissue samples located beneath the pacing electrodes showed normal cellular integrity of the

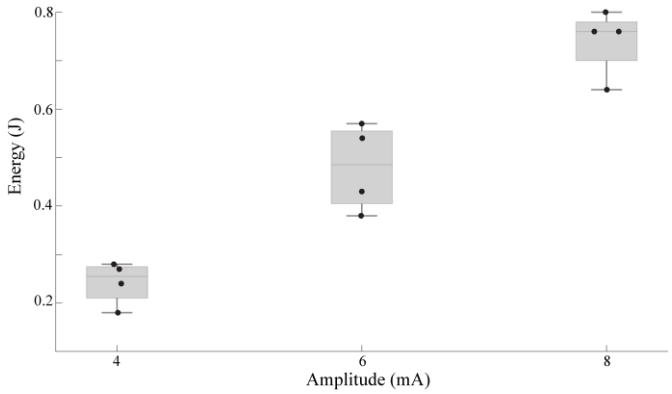

Fig. 5. Resultant applied energy at increasing pulse-amplitudes (4, 6, 8 mA) for a pulse-width of 400 ms. The energy increased linearly with increasing pulse-amplitude. Individual experimental results are indicated by the black markers (4 recordings from 4 female pigs).

serosa and subsequent muscle layers through to the mucosa. The tissue structure was compared against the control tissue specimens and no signs of swollen nuclei or blood congestions were observed. Fig. 6 illustrates representative specimens from each energy level (Figs. 6(a)-(c)) along with a control specimen (Fig. 6(d)). The gray arrows placed alongside the serosal layer in Figs. 6(a)-(c) indicate the location of pacing sites. Magnified views of the pacing locations and a control specimen highlighted by the black squares in Figs. 6(a)-(d) are shown in Figs. 6(e)-(h).

IV. Discussion

This study introduces a framework that enables quantification of *in vivo* small intestine contractile response to high-energy pacing. Pulsatile segmental contractions were induced with the onset of periodic pacing pulses and the contractile response was correlated with different levels of pacing energy achieved by systematically varying the pacing parameters. This framework allows investigation of the effect of small intestinal pacing on contractility with minimal handling of the small intestine as opposed to manometry and optical mapping, which require placement of sensors, markers and dye injection that may disturb the intrinsic behavior [25], [26], [34]. In addition, this framework does not require any motion correction to account for the frame shift due to respiration. The intensity of the visual deformation of the contractions was correlated with the pacing parameters by computing their strain. Histological analysis showed no presence of tissue damage.

Antegrade pacing was used in this study as it has a higher chance of entraining the slow wave activity [31]. The contractile response significantly increased when the jejunum was paced with increasing pulse-amplitudes (4-8 mA) resulting in significant differences in the strain of the contractions. While increasing pulse-widths enhanced the strain of the contractions, the difference was only significant between the extreme pulse-widths (100 ms vs 400 ms). Therefore, the pulse-amplitude plays a more critical role in regulating the contractile strength in comparison to the pacing pulse-width.

Significant research has been conducted on the small intestine to determine the effect of pacing on the GI

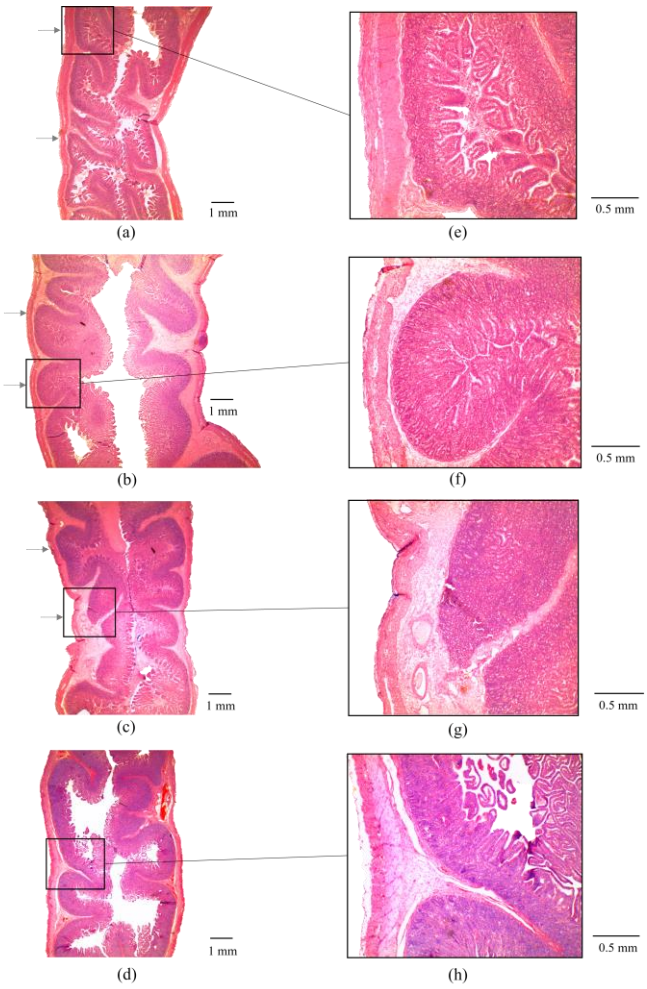

Fig. 6. Histological analysis from 4 representative specimens which were used to assess for tissue damage (pacing was applied for 5 minutes at 10 s intervals). The intestinal diameter was reduced by 9-18% during pacing. The structure of ((a)-(c)) paced tissue specimens was comparable to (d) a control tissue specimen. For a fixed pacing pulse-width of 400 ms, the pulse-amplitude was increased through (a) 4 mA, (b) 6 mA to (c) 8 mA. The gray arrows indicate the location of pacing sites, and magnified views of the areas highlighted by black squares in (a)-(d) are provided in (e)-(h).

motility [13]. However, investigators primarily mapped the rate of emptying [14], [35], [36], or used low-resolution manometry to determine the motility response [15], [19], [37]. Only a few studies focused on the response of localized contractions that were quantified based on pressure deflections [19], [38]. While one study inhibited the motility response by pacing, the effect was reported to be uninfluenced by the direction of pacing (antegrade or retrograde) [19]. Another study successfully induced pulsatile contractions with high pulse-amplitudes (25-30 mA) in both anesthetized and awake animals [38]. These results agree with our findings, but we were able to successfully induce periodic contractions with significantly less energy which corresponded to pulse-amplitudes of 4-8 mA.

The results of our present study demonstrated that pulsatile contractions were elicited in response to pacing pulses and their rate was regulated by the period of the pacing pulses, whereas the strength was defined by the pulse-amplitude and pulse-width. This phenomenon is potentially therapeutic in treating intestinal disorders with compromised motility functions such as post-operative ileus and chronic intestinal pseudo-obstruction. The ability to induce coordinated contractions, especially in an obstructed intestinal segment is critical to reinstate the healthy state of intestinal function [39]. Therefore, pacing has potential as a new emerging technique that could offer therapeutic potential for post-operative ileus in the future. On the other hand, segmental contractions can be induced in the absence of any obstruction through pacing to treat chronic intestinal pseudo-obstruction [40]. Segmental contractions aid digestion by breaking down food into smaller pieces and mixing chyme to promote absorption, whereas peristaltic contractions transport luminal contents along the length of the intestine [41]. Therefore, enhancing segmental contractions through pacing may be beneficial for managing conditions like short bowel syndrome [18]. Therefore, the ability to elicit pulsatile segmental contractions is of paramount importance in treating a range of disorders in the GI tract.

Histological analysis was performed at maximum pacing energy settings with pulse-widths of 400 ms and pulse-amplitudes varying from 4-8 mA over a 5 minute duration. Although no tissue damage was observed during these acute studies, chronic studies will be needed to confirm if tissue damage may result from sustained pacing over longer periods. Similar findings were reported from a series of chronic high frequency stimulation studies conducted on the proximal jejunum where localized intestinal contractions were induced in response to each stimulus [42]. Based on histological analysis reported in that study, electrical stimulation did not damage the stimulation site while suturing the electrodes caused mild inflammation and hemorrhage, which were not clinically significant [42]. Future studies can enhance the evaluation of tissue integrity by extending the analysis to longer pacing durations and examining tissue inflammation using histological indices like the Geboes score and peripheral blood biomarkers, as well as measuring cellular stress through assessing damage to biomolecules such as DNA and RNA [43], [44]. It is important to note that long-term pacing may lead to corrosion and metal deposit at the pacing site. Therefore, optimizing electrode characteristics for chronic environments, employing biphasic pacing and using biocompatible adhesives to attach the electrodes to the intestinal tissue are critical measures to be taken to minimize tissue damage due to pacing.

The contractile response was analyzed while fasted, which may be different to the response at a fed state. In addition, the observed contractions were localized and did not exhibit peristaltic propagation, but this may also be due to the fact that the intestine contained no digesta. A key limitation of this framework is the manual tracing of the active intestinal segment to compute strain, which could introduce observer bias. Automated methods that quantify regional deformations using strain fields can be adopted in future to mitigate this effect [29], [45]. Furthermore, pendular contractions were observed occasionally at higher pulse-amplitudes (6 and 8 mA), but the analysis was restricted to segmental contractions as pendular contractions occurred sporadically only in 10 % of the higher pacing energy studies. The localized segmental contractions

were possibly associated with sites of bioelectrical slow wave initiation where slow waves propagated radially outwards based on our previous investigations [31]. Future studies with simultaneous electrical and contractile mapping are required to confirm this hypothesis.

Conclusions from this study were derived from limited pacing durations and the reliability of these findings can be improved by pacing for extended durations and allowing longer rest periods to minimize residual effects in future studies. Wireless implantable devices optimized for pacing parameters are a critical advancement necessary for the progression of chronic studies. In addition, the analysis of the contractile response was limited to the intestinal region within the field of view of the camera, but the entire exteriorized segment was within the view and included approximately 20 cm around the paced site. Local and propagating contractions have been induced over longer lengths of the isolated colon using optogenetics and electrical stimulation resulting in increased motility and fecal output [46], [47]. Such techniques can now be integrated and compared in future studies. Mapping contractions based on visibility do not account for isometric contractions that do not induce visible deformations [48], as well as passive deformations which are affected by factors such as tissue elasticity [49]. The presented framework could be integrated with high-resolution manometry in the future for improved understanding of the induced contractility [26].

The developed techniques can now be applied to map the contractile response during pacing on other parts of the small intestine, and to also investigate other types of contractions such as peristaltic contractions [50]. The motility and electrical response of the small intestine during pacing was previously correlated, but the response of localized contractions were not investigated, and the studies were conducted using low-resolution techniques [51], [52]. High-resolution mapping techniques have been previously used to map the intrinsic electrical activity of the small intestine in both animals and humans [30], [31], [53], [54]. Therefore, similar techniques can be adopted in future research for simultaneous high-resolution measurement and correlation of contractile and electrical responses during pacing. A robust understanding of the pathways involved in pacing-induced coordinated contractions is of utmost importance in developing clinical solutions for a range of functional motility disorders.

V. Conclusion

Localized segmental contractions were induced in response to jejunal pacing and were defined based on visible deformations. Rhythmic contractions were initiated at an interval that corresponded with the pacing pulses. Contractile strength was increased with increasing pulse-width, and most importantly, the pulse-amplitude. A novel video-based framework was introduced with minimal intestinal handling to investigate the contractility during small intestine pacing. Triggering and enhancing motility response is of therapeutic potential for a range of critical bowel disorders, and therefore, anticipate clinical translation of this technique in the future.

Acknowledgment

The authors thank Linley Nisbet, Satya Amirapu and Zahra Aghababaie for their assistance.

Disclosure

No commercial support was received for any material presented in this publication. TRA-G, NP, and LKC hold intellectual property and/or patent applications on gastrointestinal electrophysiology. TRA-G, NP, and LKC are shareholders in FlexiMap Ltd.

References

[1] A. D. Sperber *et al.*, “Worldwide prevalence and burden of functional gastrointestinal disorders, Results of Rome Foundation global study,” *Gastroenterology*, vol. 160, no. 1, pp. 99-114.e3, Jan. 2021, doi: 10.1053/j.gastro.2020.04.014.

[2] A. Venara *et al.*, “Postoperative ileus: Pathophysiology, incidence, and prevention.,” *J. Visc. Surg.*, vol. 153, no. 6, pp. 439–446, Dec. 2016, doi: 10.1016/j.jviscsurg.2016.08.010.

[3] A. Antonucci *et al.*, “Chronic intestinal pseudo-obstruction.,” *World J. Gastroenterol.*, vol. 14, no. 19, pp. 2953–61, May 2008, doi: 10.3748/wjg.14.2953.

[4] R. De Giorgio, G. Sarnelli, R. Corinaldesi, and V. Stanghellini, “Advances in our understanding of the pathology of chronic intestinal pseudo-obstruction.,” *Gut*, vol. 53, no. 11, pp. 1549–52, Nov. 2004, doi: 10.1136/gut.2004.043968.

[5] H. Iida, H. Ohkubo, M. Inamori, A. Nakajima, and H. Sato, “Epidemiology and clinical experience of chronic intestinal pseudo-obstruction in Japan: a nationwide epidemiologic survey.,” *J. Epidemiol.*, vol. 23, no. 4, pp. 288–294, 2013, doi: 10.2188/jea.je20120173.

[6] S. L. Gortmaker, A. Must, J. M. Perrin, A. M. Sobol, and W. H. Dietz, “Social and economic consequences of overweight in adolescence and young adulthood,” *N. Engl. J. Med.*, vol. 329, no. 14, pp. 1008–1012, Sep. 1993, doi: 10.1056/NEJM199309303291406.

[7] G. O’Grady, J. U. Egbuji, P. Du, L. K. Cheng, A. J. Pullan, and J. A. Windsor, “High-frequency gastric electrical stimulation for the treatment of gastroparesis: A meta-analysis,” *World J. Surg.*, vol. 33, no. 8, pp. 1693–1701, 2009, doi: 10.1007/s00268-009-0096-1.

[8] P. Ducrotte *et al.*, “Gastric electrical stimulation reduces refractory vomiting in a randomized crossover trial,” *Gastroenterology*, vol. 158, no. 3, pp. 506–514, 2020, doi: 10.1053/j.gastro.2019.10.018.

[9] M. Bortolotti, “Gastric electrical stimulation for gastroparesis: A goal greatly pursued, but not yet attained,” *World J. Gastroenterol.*, vol. 17, no. 3, p. 273, Apr. 2011, doi: 10.3748/wjg.v17.i3.273.

[10] E. E. Soffer, “Gastric Electrical Stimulation for Gastroparesis,” *J. Neurogastroenterol. Motil.*, vol. 18, no. 2, pp. 131–137, Apr. 2012, doi: 10.5056/jnm.2012.18.2.131.

[11] J. Collin, K. A. Kelly, and S. F. Phillips, “Increased canine jejunal absorption of water, glucose, and sodium with intestinal pacing,” *Am. J. Dig. Dis.*, vol. 23, no. 12, pp. 1121–1124, 1978, doi: 10.1007/BF01072888.

[12] O. Akwari, K. Kelley, J. Steinbach, and C. Code, “Electric pacing of intact and transected canine small intestine and its computer model,” *Am. J. Physiol. Content*, vol. 229, no. 5, pp. 1188–1197, Nov. 1975, doi: 10.1152/ajplegacy.1975.229.5.1188.

[13] N. D. Nagahawatte, L. K. Cheng, R. Avci, T. R. Angeli-Gordon, and N. Paskaranandavadivel, “Systematic review of small intestine pacing parameters for modulation of gut function,” *Neurogastroenterol. Motil.*, vol. 35, no. 1, p. e14473, Jan. 2023, doi: 10.1111/nmo.14473.

[14] K. A. Kelly and C. F. Code, “Duodenal-gastric reflux and slowed gastric emptying by electrical pacing of the canine duodenal pacesetter potential,” *Gastroenterology*, vol. 72, no. 3, pp. 429–433, 1977, doi: 10.1016/S0016-5085(77)80252-7.

[15] X. Zhao *et al.*, “Inhibitory effects and mechanisms of intestinal electrical stimulation on gastric tone, antral contractions, pyloric tone, and gastric emptying in dogs,” *Am. J. Physiol. - Regul. Integr. Comp. Physiol.*, vol. 296, no. 1, pp. 36–42, 2009, doi:

10.1152/ajpregu.90627.2008.

[16] M. G. Sarr, K. A. Kelly, and H. E. Gladen, "Electrical control of canine jejunal propulsion.," *Am. J. Physiol.*, vol. 240, no. 5, pp. G355-60, May 1981, doi: 10.1152/ajpgi.1981.240.5.G355.

[17] A. Sawchuk *et al.*, "Electrical pacing of the Roux limb resolves delayed gastric emptying," *J. Surg. Res.*, vol. 42, no. 6, pp. 635–641, Jun. 1987, doi: 10.1016/0022-4804(87)90007-2.

[18] T. Layzell and J. Collin, "Retrograde electrical pacing of the small intestine—a new treatment for the short bowel syndrome?," *Br. J. Surg.*, vol. 68, no. 10, pp. 711–713, 1981, doi: 10.1002/bjs.1800681012.

[19] X. Zhao, J. Yin, L. Wang, and J. D. Z. Chen, "Diffused and sustained inhibitory effects of intestinal electrical stimulation on intestinal motility mediated via sympathetic pathway," *Neuromodulation*, vol. 17, no. 4, pp. 373–380, 2014, doi: 10.1111/ner.12099.

[20] W. C. Alvarez and A. Zimmermann, "The absence of inhibition ahead of peristaltic rushes," *Am. J. Physiol. Content*, vol. 83, no. 1, pp. 52–59, Dec. 1927, doi: 10.1152/ajplegacy.1927.83.1.52.

[21] C. S. de Jonge, A. J. P. M. Smout, A. J. Nederveen, and J. Stoker, "Evaluation of gastrointestinal motility with MRI: Advances, challenges and opportunities.," *Neurogastroenterol. Motil.*, vol. 30, no. 1, Jan. 2018, doi: 10.1111/nmo.13257.

[22] W. Selby, "Complete small-bowel transit in patients undergoing capsule endoscopy: determining factors and improvement with metoclopramide.," *Gastrointest. Endosc.*, vol. 61, no. 1, pp. 80–5, Jan. 2005, doi: 10.1016/s0016-5107(04)02462-9.

[23] A. Menys *et al.*, "Global small bowel motility: assessment with dynamic MR imaging.," *Radiology*, vol. 269, no. 2, pp. 443–50, Nov. 2013, doi: 10.1148/radiology.13130151.

[24] S. Hosseini, R. Avci, N. Paskaranandavadivel, V. Suresh, and L. K. Cheng, "Quantification of the Regional Properties of Gastric Motility Using Dynamic Magnetic Resonance Images," *IEEE Open J. Eng. Med. Biol.*, vol. 4, pp. 38–44, 2023, doi: 10.1109/OJEMB.2023.3261224.

[25] G. W. Hennig, M. Costa, B. N. Chen, and S. J. H. Brookes, "Quantitative analysis of peristalsis in the guinea-pig small intestine using spatio-temporal maps," *J. Physiol.*, vol. 517, no. 2, pp. 575–590, Jun. 1999, doi: 10.1111/j.1469-7793.1999.0575t.x.

[26] P. G. Dinning *et al.*, "Quantification of in vivo colonic motor patterns in healthy humans before and after a meal revealed by high-resolution fiber-optic manometry.," *Neurogastroenterol. Motil.*, vol. 26, no. 10, pp. 1443–57, Oct. 2014, doi: 10.1111/nmo.12408.

[27] Y. Sun and J. Chen, "Intestinal electric stimulation decreases fat absorption in rats: therapeutic potential for obesity," *Obes. Res.*, vol. 12, no. 8, pp. 1235–42, Aug. 2004, doi: 10.1038/oby.2004.157.

[28] T. Benard, M. Bouchoucha, M. Dupres, and P. H. Cugnenc, "In vitro analysis of rat intestinal wall movements at rest and during propagated contraction: a new method.," *Am. J. Physiol.*, vol. 273, no. 4, pp. G776-84, 1997, doi: 10.1152/ajpgi.1997.273.4.G776.

[29] S. Kuruppu *et al.*, "High-resolution spatiotemporal quantification of intestinal motility with free-form deformation.," *IEEE Trans. Biomed. Eng.*, vol. 69, no. 6, pp. 2077–2086, 2022, doi: 10.1109/TBME.2021.3135855.

[30] T. R. Angeli *et al.*, "Experimental and automated analysis techniques for high-resolution electrical mapping of small intestine slow wave activity," *J. Neurogastroenterol. Motil.*, vol. 19, no. 2, pp. 179–191, Apr. 2013, doi: 10.5056/jnm.2013.19.2.179.

[31] N. D. Nagahawatte, R. Avci, N. Paskaranandavadivel, T. R. Angeli-Gordon, and L. K. Cheng, "Spatial response of jejunal pacing defined by a novel high-resolution multielectrode array," *Am. J. Physiol. Liver Physiol.*, vol. 324, no. 5, pp. G329–G340, May 2023, doi: 10.1152/ajpgi.00258.2022.

[32] J. M. Gere and B. J. Goodno, *Mechanics of Materials*, 8th Editio. Cengage Learning, 2012.

[33] H. Crew, *General Physics An Elementary Text-Book For Colleges*, 2nd editio. New York: The Macmillan Company, 1908.

[34] H. Zhang *et al.*, "High-resolution optical mapping of gastric slow wave propagation.," *Neurogastroenterol. Motil.*, vol. 31, no. 1, p. e13449, 2019, doi: 10.1111/nmo.13449.

[35] J. M. Becker, P. Sava, K. A. Kelly, and L. Shturman, "Intestinal pacing for canine postgastrectomy dumping," *Gastroenterology*, vol. 84, no. 2, pp. 383–387, 1983, doi: 10.1016/S0016-5085(83)80137-1.

[36] B. Cranley, K. A. Kelly, V. L. W. Go, and L. A. McNichols, "Enhancing the anti-dumping effect of Roux gastrojejunostomy with intestinal pacing," *Ann. Surg.*, vol. 198, no. 4, pp. 516–524, 1983, doi: 10.1097/00000658-198310000-00011.

[37] P. D. Morrison and K. A. Kelly, "Increasing antidumping effect of intestinal pacing with motor-active agents," *Dig. Dis. Sci.*, vol. 31, no. 4, pp. 422–427, 1986, doi: 10.1007/BF01311680.

[38] A. Moritz *et al.*, "Electrical pulse train and single pulse stimulation of the small intestine: acute and chronic studies in the dog," *Artif. Organs*, vol. 13, no. 2, pp. 116–122, 1989, doi: 10.1111/j.1525-1594.1989.tb02846.x.

[39] B. W. Miedema and J. O. Johnson, "Methods for decreasing postoperative gut dysmotility.," *Lancet. Oncol.*, vol. 4, no. 6, pp. 365–72, Jun. 2003, doi: 10.1016/s1470-2045(03)01118-5.

[40] S. L. Gabbard and B. E. Lacy, "Chronic intestinal pseudo-obstruction.," *Nutr. Clin. Pract.*, vol. 28, no. 3, pp. 307–16, Jun. 2013, doi: 10.1177/0884533613485904.

[41] J. D. Huizinga *et al.*, "The origin of segmentation motor activity in the intestine," *Nat. Commun.*, vol. 5, no. 1, p. 3326, May 2014, doi: 10.1038/ncomms4326.

[42] P. Wang, G. Dubrovsky, J. C. Y. Dunn, Y. Lo, and W. Liu, "A wireless implantable system for facilitating gastrointestinal motility," *Micromachines*, vol. 10, no. 525, pp. 1–11, 2019.

[43] M. P. Murphy *et al.*, "Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo," *Nat. Metab.*, vol. 4, no. 6, pp. 651–662, Jun. 2022, doi: 10.1038/s42255-022-00591-z.

[44] L. Rosenberg *et al.*, "Histologic Markers of Inflammation in Patients With Ulcerative Colitis in Clinical Remission," *Clin. Gastroenterol. Hepatol.*, vol. 11, no. 8, pp. 991–996, Aug. 2013, doi: 10.1016/j.cgh.2013.02.030.

[45] S. Kuruppu, L. K. Cheng, R. Avci, T. R. Angeli-Gordon, and N. Paskaranandavadivel, "Relationship between intestinal slow-waves, spike-bursts, and motility, as defined through high-resolution electrical and video mapping," *J. Neurogastroenterol. Motil.*, vol. 28, no. 4, pp. 664–677, Oct. 2022, doi: 10.5056/jnm21183.

[46] N. J. Spencer and R. A. Bywater, "Enteric nerve stimulation evokes a premature colonic migrating motor complex in mouse," *Neurogastroenterol. Motil.*, vol. 14, no. 6, pp. 657–665, Dec. 2002, doi: 10.1046/j.1365-2982.2002.00367.x.

[47] T. J. Hibberd *et al.*, "Optogenetic Induction of Colonic Motility in Mice," *Gastroenterology*, vol. 155, no. 2, pp. 514-528.e6, Aug. 2018, doi: 10.1053/j.gastro.2018.05.029.

[48] M. Costa *et al.*, "An experimental method to identify neurogenic and myogenic active mechanical states of intestinal motility," *Front. Syst. Neurosci.*, vol. 7, p. 7, 2013, doi: 10.3389/fnsys.2013.00007.

[49] R. G. Lentle and C. M. Hulls, "Quantifying Patterns of Smooth Muscle Motility in the Gut and Other Organs With New Techniques of Video Spatiotemporal Mapping," *Front. Physiol.*, vol. 9, p. 338, Apr. 2018, doi: 10.3389/fphys.2018.00338.

[50] G. Dubrovsky, Y. Lo, P. Wang, M. Wu, N. Huynh, and W. Liu, "Intestinal electrical stimulation to increase the rate of peristalsis," *J. Surg. Res.*, vol. 236, pp. 153–158, 2018, doi: 10.1016/j.jss.2018.11.044.

[51] L. H. Karlstrom, N. J. Soper, K. A. Kelly, and S. F. Phillips, "Ectopic jejunal pacemakers and enterogastric reflux after Roux gastrectomy: effect of intestinal pacing," *Surgery*, vol. 106, no. 3, pp. 486–95, Sep. 1989, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/2672401.

[52] J. Collin, K. A. Kelly, and S. F. Phillips, "Absorption from the jejunum is increased by forward and backward pacing," *Br. J. Surg.*, vol. 66, no. 7, pp. 489–492, 1979, doi: 10.1002/bjs.1800660712.

[53] K. J. W. Miller, L. K. Cheng, T. R. Angeli-Gordon, R. Avci, and N. Paskaranandavadivel, "The bioelectrical conduction system around the ileocecal junction defined through in vivo high-resolution mapping in rabbits.," *Am. J. Physiol. Gastrointest. Liver Physiol.*, vol. 323, no. 4, pp. G318–G330, Oct. 2022, doi: 10.1152/ajpgi.00329.2021.

[54] T. R. Angeli, G. O'Grady, R. Vather, I. P. Bissett, and L. K. Cheng, "Intra-operative high-resolution mapping of slow wave propagation in the human jejunum: Feasibility and initial results," *Neurogastroenterol. Motil.*, vol. 30, no. 7, p. e13310, Jul. 2018, doi: 10.1111/nmo.13310.