








SHORT COMMUNICATION

A 3D-printable device allowing fast and reproducible longitudinal preparation of mouse intestines

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Abstract

Accurate and reproducible analysis of murine small and large intestinal tissue is key for preclinical models involving intestinal pathology. Currently, there is no easily accessible, standardized method that allows researchers of different skill levels to consistently dissect intestines in a time-efficient manner. Here, we describe the design and use of the 3D-printed “Mouse Intestinal Slicing Tool” (MIST), which can be used to longitudinally dissect murine intestines for further analysis. We benchmarked the MIST against a commonly used procedure involving scissors to make a longitudinal cut along the intestines. Use of the MIST halved the time per mouse to prepare the intestines and outperformed alternative methods in smoothness of the cutting edge and overall reproducibility. By sharing the plans for printing the MIST, we hope to contribute a uniformly applicable method for saving time and increasing consistency in studies of the mouse gastrointestinal tract.

KEYWORDS

adenoma, dissection, intestines, mice, printing, three-dimensional, reproducibility of results

1 | INTRODUCTION

In research, it is important to have uniform practices to attain reliable, high-quality results within and across institutes.^{1–4} Histological

evaluation of intestinal tissue is vital for assessing pathology in many different disease models. Consistent preservation of tissue samples allows for accurate assessment of biological replicates, and easier comparison between multiple groups. For example, in fields utilizing animal

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preclinical models of colorectal cancer, the enumeration and measurement of murine intestinal adenomas provide critical data.^{5,6} The ability to longitudinally open mouse intestines and evaluate their gross pathology is therefore key to gathering accurate adenoma data. While no standard method for dissection has been established, researchers commonly use a pair of offset scissors to longitudinally cut open intestines and reveal the lumen.⁷⁻¹¹ However, this time-consuming method leads to less-than-ideal visualization of adenomas.¹⁰ Rudling et al. developed an alternative to the scissors method by constructing a “gut cutting” device from several pieces of metal.¹⁰ This device consists of 4 blunt-end metal rods inserted through the lumens of the intestinal segments, which are then placed in a base unit. Next, a lid containing slanted cutting guides is placed on top, and the intestines are manually cut with a scalpel along the guides. Utilization of this gut cutting device took significantly less time and resulted in higher-quality preparation when compared with using scissors.¹⁰ Later versions of this device were machined out of a solid block of duralumin.¹² On the basis of these later designs,¹² we developed a similar, 3D-printed device named the “Intestinal Preparation Device” (IPD; Figure 1A,B). In utilizing our IPD, we encountered a few drawbacks that underscored areas in need of improvement. Through multiple redesigns and trials,

we optimized an easily 3D-printed tool named the “Mouse Intestinal Slicing Tool” (MIST; Figure 1C,D). To test the effectiveness and ease of the MIST in comparison with various mouse intestine preparation methods, we utilized intestines from C57BL/6 *Apc*^{Min/+} mice. These mice harbor the Min (multiple intestinal neoplasia) mutant allele in its *Apc* (adenomatous polyposis coli) locus,^{5,13} predisposing them to sporadic adenoma formation in both the small and large intestine. Finally, we compared colonic crypt orientation in Swiss rolls prepared using the scissors and MIST methods for colons from *Nod2*^{-/-} mice, a preclinical model for inflammatory bowel diseases. We propose that our 3D-printable MIST provides an easily accessible and reproducible method to standardize the longitudinal dissection of mouse intestinal tissue.

2 | METHODS

2.1 | Design of the IPD and MIST devices

The IPD was designed to efficiently handle up to 4 samples simultaneously. A shortcoming of this method was that the sample tissue was securely held only on the top, while free on the bottom.

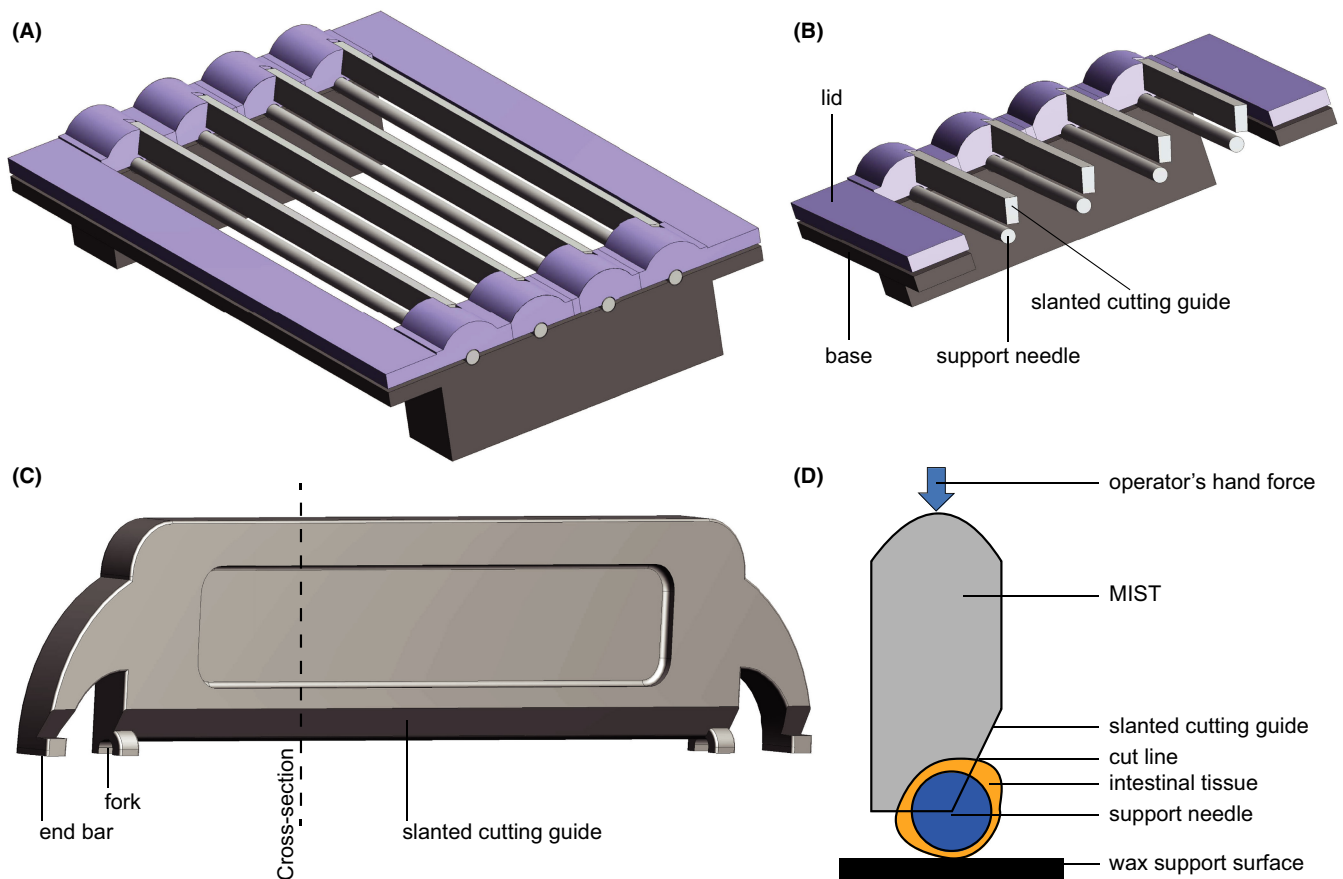


FIGURE 1 Schematic representations of the IPD and MIST. (A) Drawing of the IPD fully assembled with needles in place. (B) Cross-section of the IPD showing the metal slanted cutting guides that are used to secure the intestinal segment onto the needle. (C) Front view of the MIST without needle, showing the forks that prevent the needle from rolling and the end bars that prevent the needle from sliding out. (D) Schematic cross-section of the MIST technique. The intestinal tissue (orange) is loaded onto the needle (dark blue) and kept in place between the device and the wax surface (black) through the operator's downward hand force. The tissue is cut with a scalpel along the slanted cutting guide

In addition, the need to provide secure clamping of all 4 needles required increased fixture rigidity, leading to increased fixture weight. The MIST was designed to ensure that a needle containing an intestine sample can be secured between a wax support surface and the MIST using the clamping force provided by the operator's hand. Semi-circular needle holders at the bottom of the MIST secure the needle radially, while additional limiters on the MIST outside provide for axial stability. The MIST device used in this paper was 3D printed using Stratasys Vero material (<https://www.stratasys.com/materials/search/vero> Eden Prairie, MN, USA). Other materials could be used, depending on requirements for disinfection and sterilization. To assess the efficacy of the MIST, we benchmarked it against the widely used scissors method and compared it with 2 additional device-assisted methods, IPD and needle. We objectively quantified the effectiveness of these methods by measuring the amount of time it took to prepare intestines and the straightness of the cut edges. In addition, we did a comparison of the colonic Swiss-roll preparation for histology using the scissors and MIST methods.

2.2 | Animals

All animal procedures were approved by the Cleveland Clinic Institutional Animal Care and Use Committee, and all methods were performed in accordance with our approved protocols (approval number 2019-2302) and relevant institutional guidelines and regulations. Male C57BL/6 *Apc*^{Min/+} mice (C57BL/6J-*Apc*^{Min/J}, stock no. 002020, The Jackson Laboratory, Bar Harbor, ME, USA) and *Nod2*^{-/-} mice (B6.129S1-*Nod2*^{tm1Fiv/J}, stock no. 005763, The Jackson Laboratory) were housed under specific pathogen-free conditions and fed a standard breeder diet (Envigo Teklad Global Irradiated Rodent Diet 2018, Envigo, Indianapolis, IN, USA) in the Biological Resources Unit within the Cleveland Clinic Lerner Research Institute, Cleveland, OH. Mice between 5 and 6 months of age were killed by CO₂ asphyxiation, followed by cervical dislocation, and intestinal tissue was excised for device testing.

2.3 | Preparation of intestinal segments

The small intestine was cut into 3 equal segments: a proximal segment (SI-1), mid segment (SI-2), and distal segment (SI-3). Luminal contents of SI-1, SI-2, SI-3, and the colon (C) were removed by flushing with 0.9% saline. Cleaned intestinal segments were lined on a black wax dissection tray for further assessment.

2.4 | Scissors method

Intestinal segments were placed on paper tissue to remove excess saline. The tissue was placed vertically onto the working surface and a ~1 cm incision was made using a pair of sharp-ball tip spring scissors (Fine Science Tools, Foster City, CA, USA, item no.

15033-09). Using tweezers, the inner lumen was revealed and the intestinal segments were cut and spread open a couple of centimeters at a time.

2.5 | Needle loading

The needle, IPD, and MIST methods all require needle loading as the initial step. Using a pair of tweezers, the intestinal segment lumen was lifted open, and a needle was inserted through the lumen, filling the length of the segment. The needles used were aluminum double-point knitting needles (7 inches long, diameter size 2-size 5, Yarnology, MN, USA), and they were placed in 0.9% saline to allow easy loading. The lumen size of the intestinal segments decreases distally from the stomach to the anus. Hence, a variety of needle diameters were used depending on the diameter of the intestinal segment. For SI-1, we used needles with diameters of 3.75 mm (size 5) or 3.50 mm (size 4). For SI-2 and SI-3, needle diameters of 3.50 mm (size 4) or 3.25 mm (size 3) were used. For the colon, the needle diameters used were 3.25 mm (size 3) or 2.75 mm (size 2).

2.6 | IPD method

Four loaded needles were placed in the designated half-circle wells in the IPD base. The lid was placed on top and clamped to the base, thereby securing the needles in place horizontally and vertically. Four metal slanted cutting guides were inserted into their designated slots in the lid. One operator hand was used to press the cutting guide against the needle to secure the tissue. With the other hand, a scalpel was used to longitudinally cut the length of each segment. The device was disassembled by carefully removing the cutting guides, unclamping, and removing the lid. The needles containing the cut intestinal segments were transferred to the working surface, and tissues were gently removed from the needles and spread open on the working surface.

2.7 | Needle method

A loaded needle was placed directly on the work surface. With one hand, the tissue was securely pressed against the work surface and needle. A scalpel was used to make a longitudinal cut along the needle length. Extreme caution was exerted to avoid cutting fingers. The intestinal segment was gently removed from the needle and spread open on the work surface.

2.8 | MIST method

A loaded needle was placed onto the work surface, and the MIST was placed on top. Pressure was evenly applied onto the tissue in all areas

from the force of the operator hand pressing the MIST down. Along the MIST's built-in cutting guide, a scalpel was used to longitudinally cut open the intestine. The MIST was removed, and the intestine was gently removed from the needle and spread onto the work surface.

2.9 | Intestinal segment and cutting edge measurements

Measuring the cutting edge neatness in comparison with the segment's actual length was achieved using ImageJ (National Institutes of Health, Bethesda, MD, USA). First, the prepped intestines were photographed with a reference ruler. In ImageJ, a scale of 1 cm was set on the basis of the reference ruler in each photograph. Using the segmented line tool, the bottom cut edge of SI-1 was traced and measured. Next, using the same segmented line tool, the middle length of SI-1 was measured.

2.10 | Swiss-roll preparation and histology for colons prepared by the scissors and MIST methods

Colons were prepared and spread onto the work surface. The handle end of a sterile cotton swab was placed across the proximal end of the tissue and used as an anchor to roll the tissue around itself. Once fully rolled, the tissue roll was gently pushed off the end of the handle of the cotton swab using forceps into a single-chamber cassette. Rolls were fixed in Histochoice Tissue Fixative (VWR, Radnor, PA, USA) for 24 h. After fixation, samples were paraffin embedded in which 5 μm sections were cut, mounted on glass slides, and stained with hematoxylin and eosin. Slides were scanned into electronic files using an Aperio AT2 slide scanner (Leica Biosystems, Wetzlar, Germany) at 20 \times magnification for histological evaluation.

3 | RESULTS

3.1 | Optimizing design of the IPD

To improve upon the widely used scissors method (Figure 2A,B), we initially used the IPD (Figures 1A,B and 2C-F), our 3D-printable version of the "gut cutting" device by Yoneda et al.¹² Utilizing 3D printing simplified the construction of the IPD in comparison with the gut cutting device, which was machined out of a duralumin block.¹² In addition, our use of double-pointed knitting needles facilitated insertion into the intestinal lumen, with a lower risk for puncturing the tissue. However, the IPD had some drawbacks, such as cut tissue falling off the needle upon transfer to the work surface (Figure 2F) or needles bending in the device. We next evaluated a "needle method" (Figure 2G,H). An advantage of this method is that it does not require specialized devices or transfer of tissues. The major drawbacks of this approach were the pronounced lack of a safety guard to shield the operator's fingers, the lack of a cutting guide, and poor visualization

of the tissue. Taking the aforementioned needs for improvement into consideration, we engineered the MIST, a small 3D-printable tool. The MIST requires only one hand to apply pressure to secure a needle loaded with intestinal tissue on a dissection tray (Figures 1D and 2I,J). Both ends of the device contain bars to prevent the needle from sliding out during the cut (Figure 1C). A built-in slanted cutting guide permits safe cutting with a scalpel. The instrument also does not contain excess crevices, allowing for easy cleaning and disinfection.

3.2 | The MIST preparation method consistently requires less time

For mouse necropsies that involve analysis of both the small intestine (analyzed in 3 segments) and colon, the total amount of harvest time per mouse can quickly add up for large experimental groups. Hence, we performed objective measurements of cut time required to longitudinally prepare the small and large intestines per mouse to compare the performance of the MIST with that of the IPD, needle, and scissors dissection techniques (Figure 3). We found that the MIST, IPD, and needle methods were all significantly quicker than the benchmark scissors method, which took an average time per mouse of 12.2 min. The IPD method decreased the average time per mouse to 7.7 min. The needle and MIST method further decreased the preparation time by roughly 50% with averages of 6.1 and 6.2 min, respectively. In addition to the significant improvement in timing, the MIST method yielded the smallest range of preparation times, indicating good consistency between samples.

3.3 | The MIST provides increased quality of intestine preparation

The intestinal preparation quality across the various methods is visually evident (Figure 4A,D). We observed that the needle (Figure 4C) and the MIST methods (Figure 4D) have smoother, straighter cut edges, while the scissors (Figure 4A) and IPD methods (Figure 4B) yield many curves and lumps along the cut edge. To objectively quantify these observations, we determined the ratio between the total segment length (measured along the middle of the tissue), and the length of the bottom cut edge (Figure 4E). A ratio of 1 represents a "perfect cut," meaning the cut edge length is equal to the actual length of the segment; a greater ratio indicates a suboptimal cut. Both the needle and MIST methods yielded ratios closer to 1 and were significantly lower than the benchmark scissors method. Similar to the timing data, the MIST method had a low variation in experimental measurements.

3.4 | The MIST device allows for high-quality Swiss-roll preparation for histology

The dimensions of the small and large intestine make it difficult to preserve in its native form; therefore, the Swiss-roll technique was

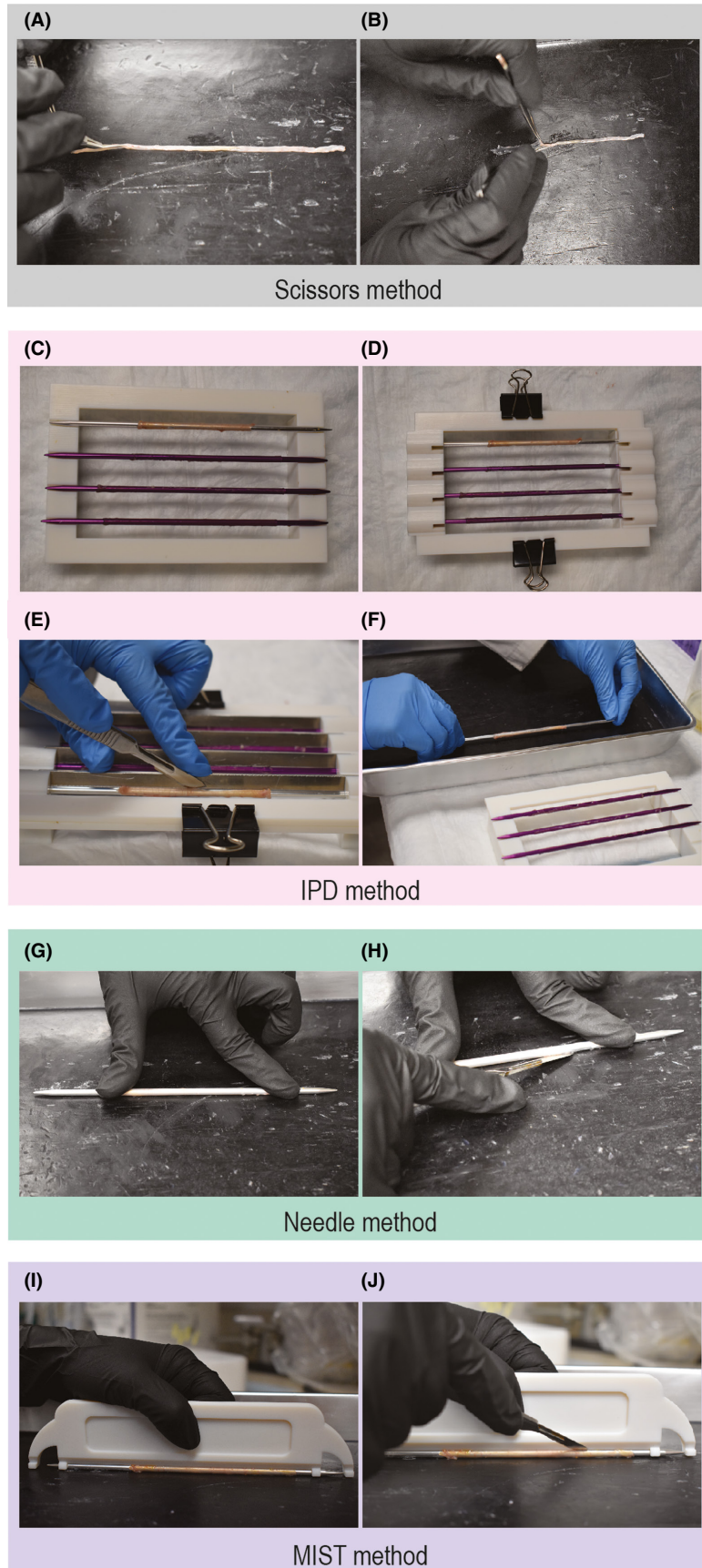


FIGURE 2 Overview of intestinal preparation methods. (A,B) Scissors method: the intestinal segment was cut open longitudinally (A), and the lumen was spread open using tweezers (B). (C–F) IPD method: loaded needles were placed into the IPD base (C) and secured in place by the lid (D). Metal cutting guides were inserted into the lid the tissue was cut with a scalpel (E). The IPD lid was removed, and the needles with cut intestines were transferred to the working surface (F). (G,H) Needle method: tissue was secured against the working surface by 2 fingers (G) and cut with a scalpel (H). (I,J) MIST method: tissue was secured against the working surface by uniform downward pressure from the MIST (I) and cut with a scalpel along the built-in slanted cutting guide (J)

created as a method to preserve the integrity of large lengths of intestinal tissue for histological analysis.¹⁴ The Swiss-roll technique is a straightforward method in which a longitudinally opened section of intestinal tissue is rolled in upon itself around a stick-like implement (toothpick or pin) prior to fixation. The resulting sample, once

embedded, gives an uninterrupted, lateral view of the entire length of embedded tissue (Figure 5A,C). Proper alignment of the tissue edges is important for creating a neatly rolled tissue sample, and aids in optimal orientation of tissue structure for histological analysis. When compared with colonic Swiss-roll samples cut using the scissors method (Figure 5A,B), MIST-method-prepared Swiss rolls were not only easier to roll, but also resulted in better crypt orientation (Figure 5C,D). The even edge created with the MIST method decreased instances of uneven and ruffled sample edges, allowing for more consistent sample orientation without the need to cut deeply into the paraffin block.

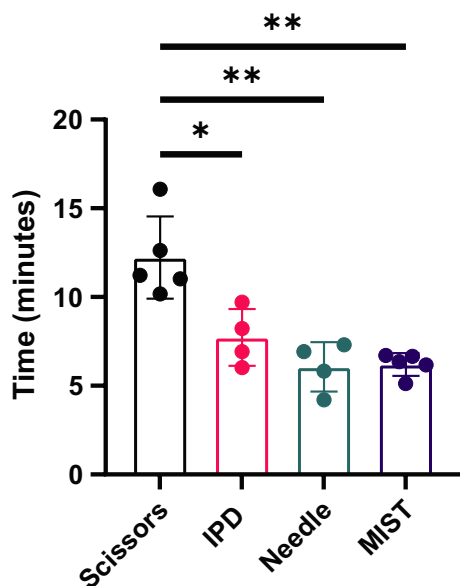


FIGURE 3 Comparison of preparation times across different methods. The preparation time for 4 intestinal segments per mouse was compared with the scissors method. Use of the IPD ($*p = 0.0111$), needle ($**p = 0.0020$), and MIST ($**p = 0.0032$) methods significantly improved prep times. Statistical analysis was performed using Brown-Forsythe and Welch ANOVA tests uncorrected for multiple comparisons. $N = 4$ –5 per group

4 | DISCUSSION

Uniform practices and reproducible analyses of mouse small- and large-intestinal lumen are crucial for animal models of intestinal studies. Here, we engineered the 3D-printed MIST, which we propose as a tool for providing simple, straightforward, and reproducible longitudinally cut mouse intestines.

The amount of time to prepare a mouse's 4 intestinal segments was significantly shorter for all tested methods compared with the conventional scissors method. The needle and MIST methods reduced the time in half to dissect intestinal tissue in comparison with the scissors method (Figure 3). This difference presents a substantial advantage for experiments involving a large number of animals. For every 10 mice that require intestinal preparations, using the MIST will save an average of 1 h.

We noticed an appreciable qualitative difference between the intestine preparations obtained from the 4 methods. The needle and MIST methods resulted in smooth cut edges in contrast to the rough

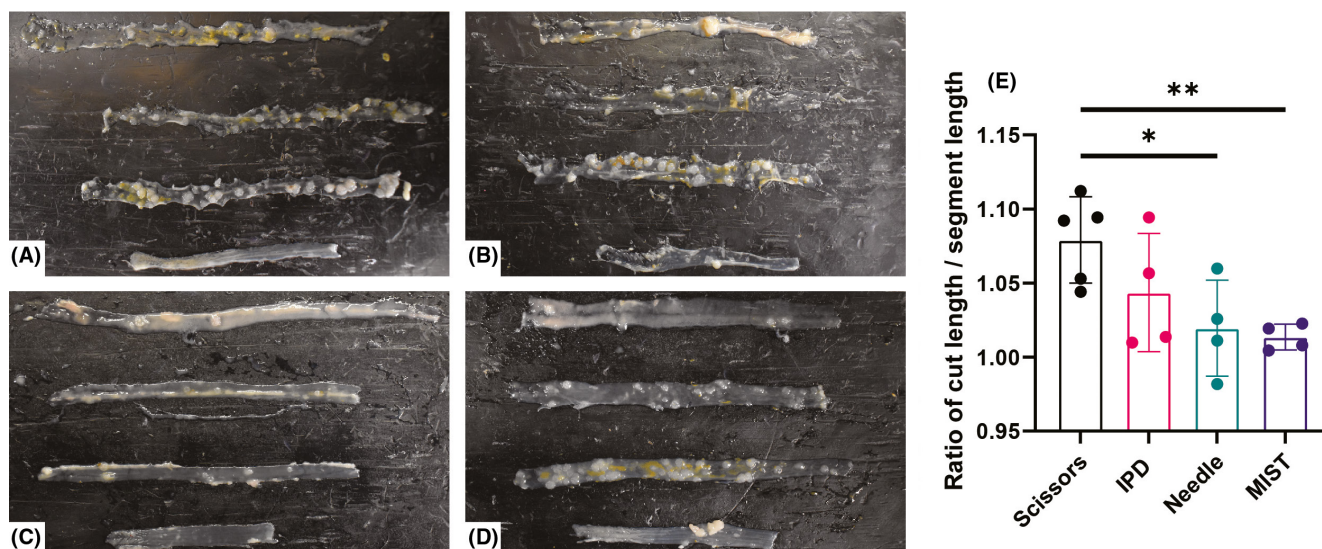


FIGURE 4 The MIST method reproducibly yields the neatest cutting edges. (A–D) Representative photos of longitudinal intestine preparation using the scissors method (A), IPD method (B), needle method (C), and MIST method (D). (E) The neatness of the cutting edge was quantified by the ratio of the edge length to the total segment length, with a ratio of 1 representing a “perfect cut.” Compared with the scissors method, the cutting edge quality was significantly improved for the MIST ($**p = 0.0054$) and needle ($*p = 0.0277$). Statistical analysis was performed using Brown-Forsythe and Welch ANOVA tests uncorrected for multiple comparisons. $N = 4$ –5 per group

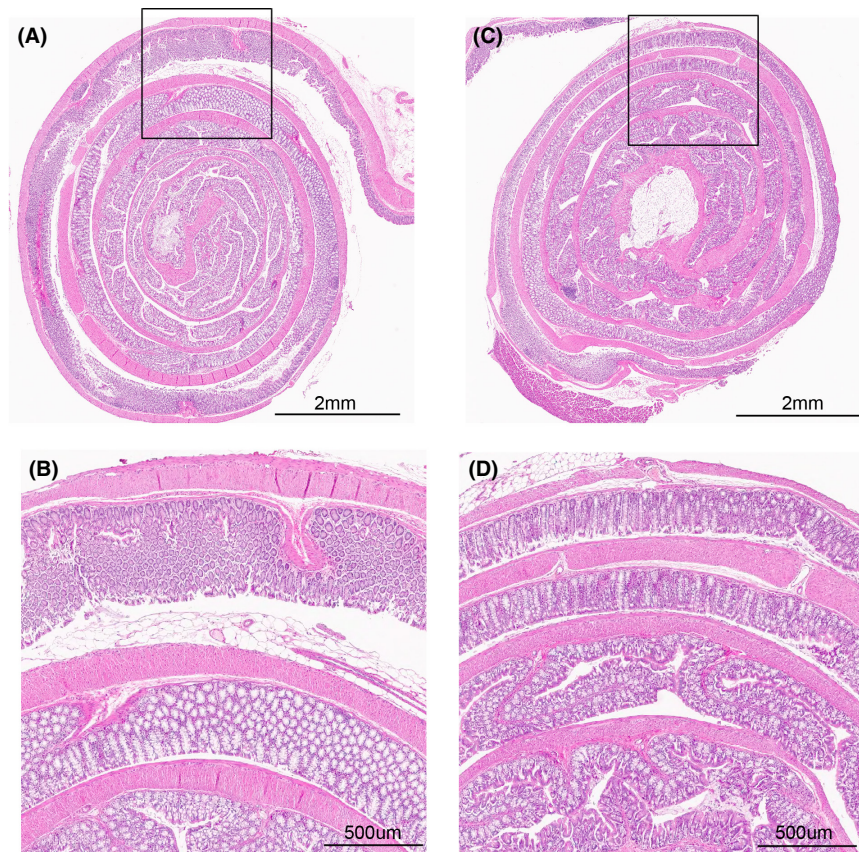


FIGURE 5 The MIST device allows for higher-quality Swiss-roll histology. (A–D) Representative hematoxylin and eosin (H&E)-stained colonic Swiss-roll preparations using the scissors method (A,B) and MIST method (C,D). The insets (B and D) give a higher-magnification view. The even edge obtained by using the MIST (D) results in better and more consistent orientation of the tissue and visualization of the colonic crypts when compared with use of the scissors (B) for paraffin blocks that are sectioned at the same depth

edges obtained with the scissors and IPD methods (Figure 4A–D). For the scissors method, this was likely due to the lack of any cutting guide. The rougher edges obtained with the IPD method are possibly due to the absence of a working surface directly underneath the loaded needles, as well as the transfer process from the device to the working surface. Although both the needle and MIST methods resulted in clean, smooth cut edges, occasionally the needle method resulted in a thin layer of intestine being cut off, as seen on the SI-2 segment (Figure 4C). This was due to the lack of a cutting guide, poor visualization of the tissue, and the need to repeatedly run the scalpel down the length of the needle. To corroborate our visual observations with objective measures, we quantified the ratios between the cut edge to the actual segment length. This confirmed that the needle and MIST methods resulted in significantly straighter cut edges compared with the scissors method (Figure 4E). While the needle and MIST techniques performed similarly in terms of timing and prep quality, using the MIST is preferred because of consistency and safety.

To test the applicability of the high-quality intestine preparation provided by the MIST, we formed Swiss rolls from colons cut open using scissors or the MIST and tissue histology evaluated after staining with hematoxylin and eosin (Figure 5). The even edge obtained by using the MIST resulted in better and more consistent tissue orientation when the blocks were sectioned at the same depth. This will improve histological sample integrity for easier comparisons, as well as reduce waste of experimental samples. The high-quality preparations resulting from the use of the MIST can also be a powerful tool

in making accurate gross histological observations. In the field of cancer research, the accuracy of the enumeration and measurement of adenomas can be improved using MIST. Additionally, researchers interested in inflammatory changes in the intestine can benefit from use of the MIST to visualize gross morphological changes indicative of inflammatory bowel disease.

In summary, we showed that the MIST provides a more time-efficient means of longitudinally preparing mouse intestines, and it is a strong candidate to become the standard technique for this purpose. In sharing the printing plans for the MIST, we aspire to facilitate data collection in preclinical models of gastrointestinal diseases, such as intestinal cancer or inflammatory bowel diseases.

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CONFLICT OF INTEREST

J.C. is a scientific advisor for Seed Health, Inc. Other authors do not have competing interests.

AUTHOR CONTRIBUTIONS

Conceptualization and design: Beckey DeLucia, Sergey Samorezov, Jan Claesen; Investigation: Beckey DeLucia, Sergey Samorezov, Megan T. Zangara, Rachel L. Markley, Lucas J. Osborn, Karlee B. Schultz, Christine McDonald, Jan Claesen; Figures: Beckey DeLucia, Sergey Samorezov, Megan T. Zangara, Christine McDonald, Jan Claesen; Writing of the original draft: Beckey DeLucia Writing, review and interpretation: Beckey DeLucia, Sergey Samorezov, Megan T. Zangara, Rachel L. Markley, Lucas J. Osborn, Karlee B. Schultz, Christine McDonald, Jan Claesen.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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