

## A novel rat model of inflammatory bowel disease developed using a device created with a 3D printer

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### Abstract

Objective Inflammatory bowel disease (IBD) is an intractable condition. Existing models of experimental IBD are limited by their inability to create consistent ulcers between animals. The aim of this study was to develop a novel model of experimental colitis with ulcers of reproducible size. Design We used a 3D printer to fabricate a novel device containing a small window (10×10 mm) that could be inserted rectally to facilitate the creation of a localized ulcer in the rat intestinal mucosa. The mucosa within the window of the device was exposed to 2,4,6-trinitrobenzene sulfonic acid (TNBS) to generate ulceration. We evaluated the effects of conventional drug therapies (mesalazine and prednisolone) and local transplantation of allogeneic adipose-derived mesenchymal stem cells (ASCs) on ulcer size (measured from photographic images using image analysis software) and degree of inflammation (assessed histologically). Results The novel method produced localized, circular or elliptical ulcers that were highly reproducible in terms of size and depth. The pathological characteristics of the lesions were similar to those reported previously for conventional models of TNBS-induced colitis that show greater variation in ulcer size. Ulcer area was significantly reduced by the administration of mesalazine or prednisolone as an enema or localized injection of ASCs. Conclusion The new model of TNBS-induced colitis, made with the aid of a device fabricated by 3D printing, generated ulcers that were reproducible in size. We anticipate that our new model of colitis will provide more reliable measures of treatment effects and prove useful in future studies of IBD therapies.

<b>Keywords</b>	3D printer, IBD models, allogeneic adipose-derived mesenchymal stem cells , mesalazine, prednisolone, 2,4,6-trinitrobenzene sulphonic acid,
<b>Corresponding Author</b>	Masayuki Yamato
<b>Corresponding Author's Institution</b>	Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University
<b>Order of Authors</b>	Tomoko Kuriyama, Masayuki Yamato, jun honma, Yusuke Tobe, Katsutoshi Tokushige
<b>Suggested reviewers</b>	Masaaki Ito, Seung-Jae Myung

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October, 7, 2019

Dear Prof. Okano

On behalf of all the authors, I would like to ask you to consider our manuscript entitled, "A novel rat model of inflammatory bowel disease developed using a device created with a 3D printer" for publication in *Regenerative Therapy* as an original research article.

Although various models of experimental colitis are available, some require invasive techniques (such as laparotomy) and most do not produce ulcers of similar size and depth. The present study describes the development and validation of a novel model of experimental colitis that can be used to generate ulcers of reproducible size easily. We utilized a 3D printer to fabricate a novel device with a small window (10×10 mm) that was inserted rectally into the rat, and a localized ulcer was created in the intestinal mucosa by exposure of the region within the small window to 2,4,6-trinitrobenzene sulfonic acid (TNBS). Ulcer size showed minimal variation between animals and was significantly reduced by the administration of mesalazine or prednisolone, which are commonly used to treat inflammatory bowel disease (IBD) in patients. Furthermore, localized injection of adipose-derived mesenchymal stem cells also resulted in a decrease in ulcer size, suggesting that cell-based therapy potentially could be developed into a future treatment for IBD. I can confirm that the study design was approved by an ethics review board.

We anticipate that our new, simple model of experimental colitis will provide more reliable measures of treatment effects due to its high reproducibility and prove useful in future studies of IBD therapies. We feel that findings from this study will be of special interest to readers of *Regenerative Therapy*.

Without wishing to take a liberty, my coauthors and I would like to suggest Dr. Masaaki Ito ([maito@east.ncc.go.jp](mailto:maito@east.ncc.go.jp)) and Dr. Seung-Jae Myung ([sjmyung@amc.seoul.kr](mailto:sjmyung@amc.seoul.kr)) as potential referees.

This manuscript has not been published and is not under consideration for publication elsewhere. All the authors have read the manuscript and approved this submission.

I look forward to hearing from you.

Yours sincerely,

Masayuki Yamato M.D.

Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjyuku-ku,

Tokyo 162-8666, Japan, Phone: +81-3-3353-8112, Fax: +81-3-3359-6046

E-mail: [yamato.masayuki@twmu.ac.jp](mailto:yamato.masayuki@twmu.ac.jp)

## **Title**

**A novel rat model of inflammatory bowel disease developed using a device created with a 3D printer**

## **Authors**

Tomoko Kuriyama<sup>1</sup>, Masayuki Yamato<sup>2</sup>, Jun Homma<sup>2</sup>, Yusuke Tobe<sup>3</sup>, Katsutoshi

Tokushige<sup>1</sup>

## **Author affiliations**

1. Institute of Gastroenterology, Department of Internal Medicine, Tokyo Women's Medical University, Shinjyuku, Tokyo, 162-8666, Japan

2. Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Shinjyuku, Tokyo, 162-8666, Japan

3. Department of Integrative Bioscience and Biomedical Engineering, Graduate School of Advanced Science and Engineering, TWIns, Waseda University, Shinjyuku, Tokyo, 162-8480, Japan

## **Corresponding author**

Masayuki Yamato M.D.

Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjyuku-ku, Tokyo 162-8666, Japan

Phone: +81-3-3353-8112

Fax: +81-3-3359-6046

E-mail: [yamato.masayuki@twmu.ac.jp](mailto:yamato.masayuki@twmu.ac.jp)

## **Abstract**

### **Objective**

Inflammatory bowel disease (IBD) is an intractable condition. Existing models of experimental IBD are limited by their inability to create consistent ulcers between animals.

The aim of this study was to develop a novel model of experimental colitis with ulcers of reproducible size.

### **Design**

We used a 3D printer to fabricate a novel device containing a small window (10×10 mm) that could be inserted rectally to facilitate the creation of a localized ulcer in the rat intestinal mucosa. The mucosa within the window of the device was exposed to 2,4,6-trinitrobenzene sulfonic acid (TNBS) to generate ulceration. We evaluated the effects of conventional drug therapies (mesalazine and prednisolone) and local transplantation of allogeneic adipose-derived mesenchymal stem cells (ASCs) on ulcer size (measured from photographic images using image analysis software) and degree of inflammation (assessed histologically).

### **Results**

The novel method produced localized, circular or elliptical ulcers that were highly reproducible in terms of size and depth. The pathological characteristics of the lesions were similar to those reported previously for conventional models of TNBS-induced

colitis that show greater variation in ulcer size. Ulcer area was significantly reduced by the administration of mesalazine or prednisolone as an enema or localized injection of ASCs.

## **Conclusion**

The new model of TNBS-induced colitis, made with the aid of a device fabricated by 3D printing, generated ulcers that were reproducible in size. We anticipate that our new model of colitis will provide more reliable measures of treatment effects and prove useful in future studies of IBD therapies.

## **Highlights**

**Adipose-derived stem cells (ASCs) are being explored as a new treatment for IBD because they can downregulate inflammation and improve tissue repair.**

**A new model of TNBS-induced colitis was developed using a custom-designed device fabricated by a 3D printer.**

**The novel model of colitis generated ulcers that were highly reproducible in size.**

**The size of the ulcer was reduced by mesalazine or prednisolone (administered as an enema) or by localized injection of ASCs.**

## **1. Introduction**

Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic and relapsing inflammation of the intestinal tract. The incidence and prevalence of IBD are particularly high in Europe and North America[1] [2] , and IBD is becoming increasingly more common in previously low-incidence areas such as Eastern Europe, Asia and most developing countries[3]. The pathogenesis of IBD is not clearly understood but is thought to involve genetic, environmental and immunological factors as well as the intestinal bacterial flora[4]. Despite the availability of a variety of therapeutic agents, many patients have refractory inflammation and develop cancer of the mucosa[5].

Several different models of experimental colitis have been developed to investigate the mechanisms underlying IBD and novel potential treatment strategies, and these have included chemical, immunological and genetic engineering models[6] [7]. The chemical model has the advantages of being low-cost and straightforward, but an important disadvantage is that it can be difficult to create inflammation of the same size and depth in different animals. Therefore, novel animal models of IBD are needed that create ulcers easily and reproducibly. Three-dimensional (3D) printing is potentially a novel technique for generating a reproducible model of IBD. 3D printing technology is developing rapidly and becoming more widely applied in the field of medicine[8]. 3D printing has been used

to create models of diseased organs in patients, such as intracranial and visceral aneurisms, that could be utilized in clinical practice as tools for patient education and preoperative training[9] [10]. 3D printing technology has also been applied in the fabrication of an oral drug delivery device that was safe for use in patients[11].

Therefore, the aim of this study was to utilize 3D printing technology to make a device that would help to create a novel model of experimental colitis with inflammation of consistent size and depth. An additional objective was to use the novel model of colitis to examine the effects of two drug used clinically in the treatment of IBD, namely mesalazine (5-aminosalicylic acid, an anti-inflammatory drug) and prednisolone (a glucocorticoid), as well as cell-based therapy. Regenerative medicine and tissue engineering using stem cells have emerged as new therapeutic techniques with widespread potential applications in the medical field[12]. Mesenchymal stem cells (MSCs) have attracted particular attention because they are capable of self-renewal, exerting immunomodulatory effects and differentiating into various cell lineages[13] [14]. In the field of IBD, two recent reviews of clinical trials concluded that local injection of MSCs was an effective and safe treatment for perianal fistula in Crohn's disease[15] [16]. Furthermore, MSCs have been shown to alleviate colitis in a mouse model[17]. MSCs can be harvested from various sources, such as bone marrow, adipose tissue, umbilical



cord and placenta. Humans are rich in adipose tissue, which can be easily obtained using liposuction. Therefore, the present study selected adipose-derived MSCs (ASCs) for use as a cell-based therapy.

## **2. Methods**

### **2.1. Experimental animals**

Male Sprague Dawley (SD) rats aged 7 weeks (Japan SLC, Inc, Tokyo, Japan) were used for the experimental model of colitis and for isolation of ASCs assayed by flow cytometry. ASCs were also isolated from 3–5-week old male SD rats (SD-Tg[CAG-EGFP]; Japan SLC, Inc, Tokyo, Japan) expressing enhanced green fluorescent protein (EGFP). All animals were housed in a room with acclimatized temperature (22-24 °C and approximately 45% humidity) under a 12/12-hour day/night cycle and with free access to water and food. All experimental protocols were approved by the Animal Welfare Committee of Tokyo Women's Medical University.

### **2.2. Device fabrication**

A novel device was made from ultraviolet-curing resin (MED610; Stratasys Ltd, Eden Prairie, MN, USA) using a 3D printer (Objet Eden350; Stratasys Ltd). The device had an outer diameter of 9 mm and a length of 40 mm so as to fit inside the intestine of the rat,

and its tip was rounded to avoid damage to the intestinal mucosa. In addition, the device contained a 10 mm × 10 mm window along its length so that a localized ulcer could be easily created and evaluated (figure 1).

### **2.3. Induction of colitis with 2,4,6-trinitrobenzene sulfonic acid (TNBS)**

Colitis in each rat was induced using 30 mg TNBS sodium salt dihydrate (Wako Pure Chemical Industries, Osaka, Japan) diluted in 1 mL of 50% ethanol. The device was inserted rectally into the rat with the aid of lubricating jelly. A 5 cm × 5 cm piece of gauze was placed into the device so as to cover the window, and the gauze was then soaked with TNBS before the gauze was inserted into the device. The device and TNBS-soaked gauze were maintained in place for 10 minutes to induce colitis (figure 2A).

### **2.4. Measurement of ulcer size**

A photograph of the ulcer was taken using a digital camera (CASIO EXILIM EX-ZR 1300, Tokyo, Japan), and ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to trace the ulcer margin and calculate the ulcer area.

### **2.5. Administration of drugs**

After the creation of a local ulcer on the colon, mesalazine (50 mg/kg/day; Pentasa enema, Kyorin Pharmaceutical Co. Ltd, Tokyo, Japan) or prednisolone sodium phosphate (0.3 mg/kg/day; Predonema enema, Kyorin Pharmaceutical Co. Ltd) were administered

rectally. The drugs were administered by enema daily for 7 days (figure 2B). The optimal doses were chosen according to a previous study[18].

## **2.6. Isolation of ASCs**

ASCs were isolated from inguinal adipose tissue using a modification of a previously reported method (Figure 2C)[19]. Briefly, isolated adipose tissue was enzymatically digested at 37°C for 1 hour with 0.1% type-2 collagenase (Worthington Biochemical Corporation, Lakewood, NJ, USA). The stromal vascular fraction was collected after centrifugation for 5 minutes at 2000 bpm, and cells in the interstitial vascular fraction were seeded in a 10-cm dish. The cells were cultured in complete culture medium (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) containing 20% fetal bovine serum (Life Technologies, Frederick, MD, USA) and 1% penicillin/streptomycin (Fujifilm Wako Pure Chemical Corporation) at 37°C under a 5% CO<sub>2</sub> atmosphere. The culture medium was changed at 24 hours, and the cells were then passaged every 3–7 days using 0.25% trypsin-EDTA (Fujifilm Wako Pure Chemical Corporation) and plated at a density of  $1-2 \times 10^5$  cells/dish. Cells at passage 3–4 were used in this study.

## **2.7. Cell characterization**

ASCs were characterized by measuring their colony forming, adipogenic and osteogenic abilities. ASCs at passage 3 were plated at a density of 100 cells per 10-cm dish and

cultured in complete medium for 7 days. For the colony forming assay, the cells were stained with 0.5% crystal violet (Kanto Chemical, Tokyo, Japan) in methanol for 5 min and washed twice with distilled water. For the adipogenesis assay, the medium was changed to complete medium supplemented with 0.5 mmol/L dexamethasone (Fuji Pharma, Tokyo, Japan), 0.5 mmol/L isobutyl-1-methyl xanthine (Sigma-Aldrich, St. Louis, MO, USA) and 50 mmol/L indomethacin (Wako Pure Chemical Industries). After 21 days, the cells were fixed with 4% paraformaldehyde (Muto Pure Chemical, Tokyo, Japan) for 1 hour and stained with fresh oil red O solution (Fujifilm Wako Pure Chemical Corporation) for 3 hours. For the osteogenesis assay, the medium was switched to complete medium supplemented with 100 nmol/L dexamethasone (Fuji Pharma, Tokyo, Japan), 10 mmol/L  $\beta$ -glycerophosphate (Sigma-Aldrich) and 50 mmol/L ascorbic acid (Wako Pure Chemical Industries). After 21 days, the cells were stained with 1% alizarin red S solution (Wako Pure Chemical Industries).

## **2.8. Flow cytometry assay**

One million cells were suspended in 100 mL phosphate-buffered saline (PBS) containing 10 mg/mL of each specific antibody. Fluorescein isothiocyanate (FITC)-coupled primary antibodies against CD11b, CD29 (BD Biosciences, Franklin Lakes, NJ, USA, catalog numbers; 555005 and 554982), CD31, CD45 and CD90 (BIORAD, Hercules, CA, USA, catalog

numbers; MCA1334F, MCA43FT, and MCA47FA) were used to detect surface markers. For the isotype controls, fluorescein isothiocyanate-coupled non-specific mouse IgG1 (AbD Serotec), IgG2a and IgA and non-specific hamster IgM (BD Biosciences) were substituted for the primary antibodies. After incubation for 30 min at 4°C, the cells were washed with PBS and then suspended in 1 mL PBS for analysis. Cell fluorescence was determined using a Gallios flow cytometer (Beckman Coulter, Tokyo, Japan), and data were analyzed using Kaluza for Gallios software (Beckman Coulter).

## **2.9. Injection of ASCs or saline into the ulcer**

The ulcer was injected with ASCs or saline on day 1 after its creation (figure 2C). ASCs ( $3 \times 10^6$  cells in 100  $\mu$ L saline) at passage 3 were injected locally into the submucosal layer of the ulcer using a 29G syringe (Terumo Corporation, Tokyo, Japan). The injection of 100  $\mu$ L saline only was used as a control. The tip of the syringe was inverted to inject ASCs into the intestinal wall.

## **2.10. Histological and immunohistochemical analyses**

Rats were anesthetized with isoflurane (Fujifilm Wako Pure Chemical Corporation) and sacrificed on days 1, 7, 10, 14, 21 and 28 after creation of the ulcer (figure 2B). The rectum was excised from each rat, rinsed with saline solution, fixed in 4% paraformaldehyde (Muto Pure Chemical, Tokyo, Japan) and embedded in paraffin. For

histological analysis, 5- $\mu$ m thick sections were stained with hematoxylin-eosin (H&E). For immunohistochemical analysis, the sections were heated, blocked with HistoVT One (Nacalai Tesque, Inc., Kyoto, Japan) and incubated with green fluorescent protein-conjugated primary antibody (Anti-green fluorescent protein, rabbit IgG fraction Polyclonal: Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) at 4°C overnight. The sections were then incubated for an hour at room temperature with secondary antibody for 1 hour at room temperature. (Alexa Fluor 488 goat anti-rabbit IgG [H+L] Antibody; Life Technologies), and 4',6-diamidino-2-phenylindole (Dojindo, Kumamoto, Japan) was used to stain the nucleus. The sections were observed under a fluorescence microscope (U-RFL-T; Olympus, Tokyo, Japan).

### **2.11. Histological score**

A histological score was obtained based on a modification of methods used in previous studies[17] [20]. The following features were graded: the extent of destruction of the mucosal architecture (0, normal; 1, mild damage; 2, moderate damage; and 3, extensive damage), the presence and degree of cellular infiltration (0, normal; 1, mild infiltration; 2, moderate infiltration; and 3, extensive infiltration), and the extent of bowel wall thickening (0, normal; 1, mild thickening; 2, moderate thickening; and 3, extensive thickening). The scores for each feature were summed to provide an overall score with a

maximum possible value of 9.

## **2.12. Statistical analysis**

The analysis was performed using JMP Pro 12.0.0 (SAS, Cary, NC, USA). All values are shown as the mean  $\pm$  standard error of the mean (SEM). The Wilcoxon test was used to compare two groups, and Dunnett's test was applied for comparisons of multiple groups.  $P < 0.05$  was considered significant.

### **3. Results**

#### **3.1. Temporal changes in localized ulcer area and inflammation**

Representative images illustrating the size of the localized ulcer at 1, 7, 10, 14, 21 and 28 days are shown in figure 3A. The area of the ulcer gradually decreased from day 1 to day 28 (figure 3B). Notably, ulcer area on each day was very similar between animals, as evidenced by the small SEM (figure 3B). Representative histological images are shown in figure 3C. The histological score was highest on day 7 but then subsequently improved from day 10 to day 28 (figure 3D). Moderate inflammation was observed on day 28 due to strong infiltration of inflammatory cells around the remaining small ulcer. Thus, ulcers of consistent size could be easily created using the novel device.

#### **3.2. Therapeutic effects of mesalazine and prednisolone**

To determine whether the present model would be suitable for use as an experimental model of IBD, we investigated the effects of mesalazine and prednisolone, which are known therapeutic agents. Because the ulcer was created near the anus, mesalazine and prednisolone were administered daily by enema, which is a method also used in patients. Representative images illustrating ulcer size at day 7 in control, mesalazine-treated and prednisolone-treated animals are shown in figure 4A–C. Ulcer area at day 7 was significantly smaller ( $p < 0.001$ ) in the mesalazine group ( $37.0 \pm 6.6 \text{ mm}^2$ ) and



prednisolone group ( $38.8 \pm 10.7 \text{ mm}^2$ ) than in the control group ( $73.5 \pm 1.81 \text{ mm}^2$ ;  $p < 0.001$ ; figure 4D). Representative histological images at day 7 are shown in figure 4E–G. The histological score at day 7 (figure 4H) was not significantly different between the control group ( $8.6 \pm 0.55$ ), mesalazine group ( $8.2 \pm 0.84$ ,  $p = 1.0$ , compared to control group) and prednisolone group ( $8.6 \pm 0.55$ ,  $p = 0.54$ , compared to control group). There was a tendency for the control group to exhibit greater weight loss than the other two groups (figure 4I), although there were no significant differences between groups. Furthermore, blood in the stool or diarrhea was not observed in any group (data not shown). The improvement in ulcer size in response to the administration of clinically used therapeutic agents indicates that this is a suitable experimental model of IBD.

### **3.3. Characterization of ASCs**

Crystal violet, oil red O and alizarin red S staining were used to evaluate the characteristics of the ASCs. The ASCs formed colonies and stained with oil red O and alizarin red S (figure 5A–C), demonstrating that they had self-renewal, adipogenic and osteogenic abilities. Additionally, flow cytometry was used to characterize the cell surface markers. The ASCs stained positively for CD29 and CD90 but negatively for CD11b, CD34 and CD45 (figure 5D), indicating that the characteristics of the ASCs were compatible with those of MSCs.

### **3.4. Therapeutic effects of ASC injection**

Next, the effects of locally injected ASCs were investigated in our novel model of IBD.

Representative images comparing ulcer size and histological characteristics at day 7

between the ASC-treated and saline-treated (control) groups are shown in figure 6A–D.

Immunofluorescence imaging revealed that the ASCs had been successfully delivered to

the ulcerated region of the colon (figure 6E). Ulcer area at day 7 was significantly smaller

in the ASC group than in the control group ( $52.34 \pm 9.60 \text{ mm}^2$  vs.  $73.13 \pm 8.24 \text{ mm}^2$ ,  $p <$

$0.01$ ; figure 6F). There was no significant difference in histological score between the

ASC group ( $7.8 \pm 0.84$ ) and control group ( $8.8 \pm 0.45$ ; figure 6G).

## 4. Discussion

The current study has described the use of a novel device, fabricated using 3D printing technology, to create localized ulcers in the large intestine with high reproducibility.

Several other methods to generate ulcers have already described, including a gastric ulcer model based on acetic acid injection into the stomach wall[21], a duodenal ulcer model based on acetic acid contact with the duodenal serosa[22], a colitis model in which acetic acid is injected into the colonic serosa[23], and a colitis model in which TNBS is injected into the colonic serosa[24]. Our novel model has advantages over these previous models in that it does not require laparotomy, allows the direct observation of localized ulcers and permits the administration of drugs or other treatments (such as cell-based therapy).

In addition, we consider our new model to be safe from the viewpoint of animal welfare because no animals died during the procedure used to create the ulcer and no animals subsequently exhibited a body weight decrease of 20% or more.

Exposure of the large intestinal mucosa of a rat to TNBS resulted in the formation of a rounded ulcer with a visually distinct boundary that was readily observable with image analysis software. In addition, the standard deviations in ulcer area were small (1.74, 0.81, 0.98, 0.64, 0.15 and 0.17 mm<sup>2</sup> on days 1, 7, 10, 14, 21 and 28, respectively), indicating that this novel technique was able to create ulcers of reproducible size. One limitation of

our technique is that visible ulcers had almost disappeared by day 28, implying that this model may not be suitable for long-term studies of chronic ulceration. Chronic ulcers that better resemble the longitudinal ulcers of Crohn's disease and are suitable for long-term evaluation potentially could be created using a device with a rectangular window that is larger than that in the current device. In addition, it may be possible to create a model of stenosis by repeatedly creating contiguous localized ulcers to produce a ring-shaped ulcer in the intestinal tract. Indeed, another advantage of the method described here is that ulcers of various sizes can be created easily by altering the design of the device before its fabrication with a 3D printer.

Several treatments for IBD are available, including mesalazine, dietary intervention, corticosteroids, immunomodulator drugs such as azathioprine and anti-tumor necrosis factor- $\alpha$  biologics[25]. In the present study, the localized ulcers were treated with a mesalazine or prednisolone enema, allowing the drug to reach the ulcer easily. There was a significant improvement in ulcer size at day 7 in response to both drugs, demonstrating that this novel model of experimental colitis is suitable for use in preclinical studies of IBD.

Conventionally, TNBS-induced colitis is considered to be a good model of Crohn's disease. In this study, the TNBS was dissolved in ethanol and administered rectally to

create an experimental colitis model. It is believed that colitis occurs due to destruction of the intestinal mucosal barrier by ethanol followed by an immune response to TNBS, which is a hapten that binds nonspecifically to various proteins[26]. Pathologically, the ulcerative lesions are deep and exhibit extensive infiltration of inflammatory cells into the submucosa and muscle layers along with crypt loss and distortion, and there is also an overall thickening of the colon wall[27]. In this study, the printed device enabled TNBS to be brought into contact with a localized region of intestinal tract, and this succeeded in creating a reproducible degree of inflammation. Although the administration of mesalazine and prednisolone resulted in a significant decrease in ulcer size, there was no significant improvement in histological score. One possible reason for this apparent discrepancy is that the present study evaluated the histological score only in the central region of inflammation. The central region may not have shown substantial changes in response to therapy because it had the highest degree of inflammation, whereas a reduction in the severity of inflammation might have been detectable in more peripheral regions.

Cell-based therapy has emerged as a new treatment for IBD, and the effects of MSCs have been investigated in animal models of colitis and in patients with perianal fistula[15] [16] [17]. In this study, ASCs were administered directly to the mucosa of the

localized ulcer, and this resulted in a reduction in the size of the ulcer. However, the improvement in ulcer size was slightly smaller than that achieved with mesalazine or prednisolone, two of the main drugs used to treat IBD. A previous study exploring the optimal route of MSC administration reported that resolution of colitis was better for intraperitoneal administration than for intravenous administration or anal injection[28]. Moreover, tissue engineering of cell sheets may provide an even better approach to the transplantation of cells for cell-based therapy[29]. Compared to the injection of a cell suspension, engineered cell sheets have advantages that include the retention of cells at the transplantation site for a longer period of time and local delivery of growth factors and cytokines[30] [31]. A recent investigation has described the endoscopic transplantation of MSC sheets in a rat model of experimental colitis[32]. Therefore, further studies are needed to evaluate whether changing the route of delivery or duration of administration of MSCs might result in therapeutic effects that are comparable to established treatments such as mesalazine and prednisolone.

In conclusion, the present study has described the creation of a new model of TNBS-induced colitis using a novel device fabricated with a 3D printer. Our technique generated ulcers that were reproducible in size between animals, which will facilitate more reliable evaluation of treatment effects than conventional models. We anticipate that our new

model of colitis will prove useful in future studies of IBD therapies.

## **5. Conclusion**

The new model of TNBS-induced colitis, made with the aid of a device fabricated by 3D printing, generated ulcers that were reproducible in size. We anticipate that our new model of colitis will provide more reliable measures of treatment effects and prove useful in future studies of IBD therapies.

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## **Competing interests**

The authors have declared that there is no conflict of interest.

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None.

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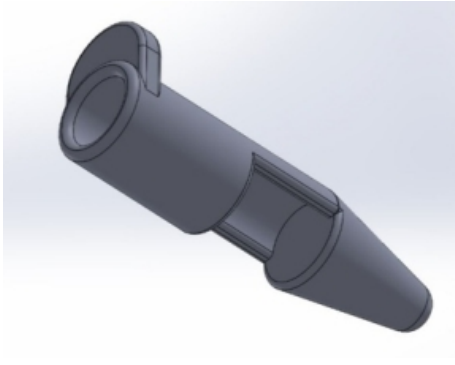
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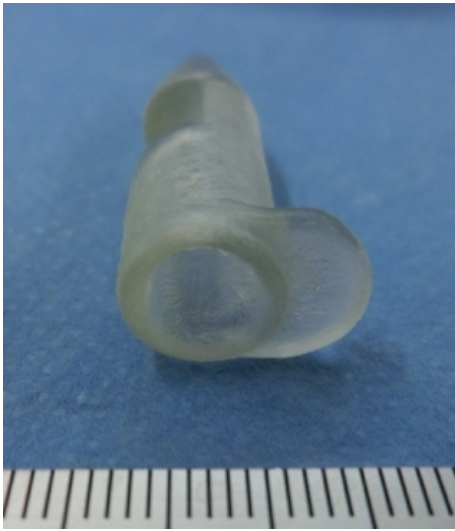
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Figure 1

A



B



C

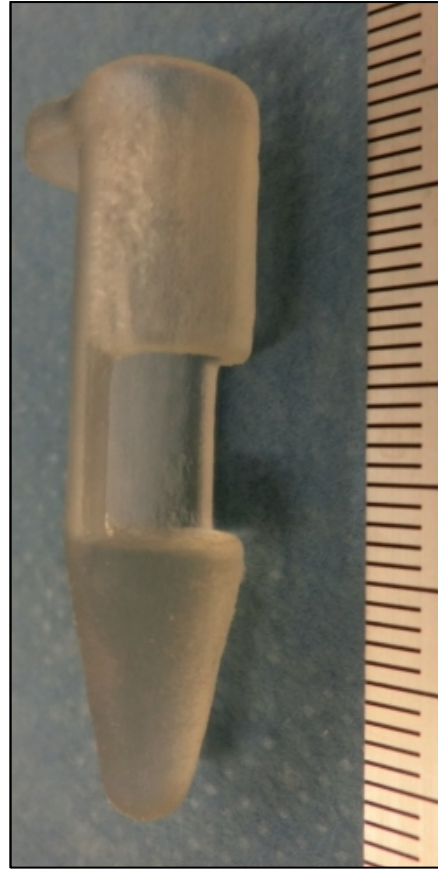


Figure 2

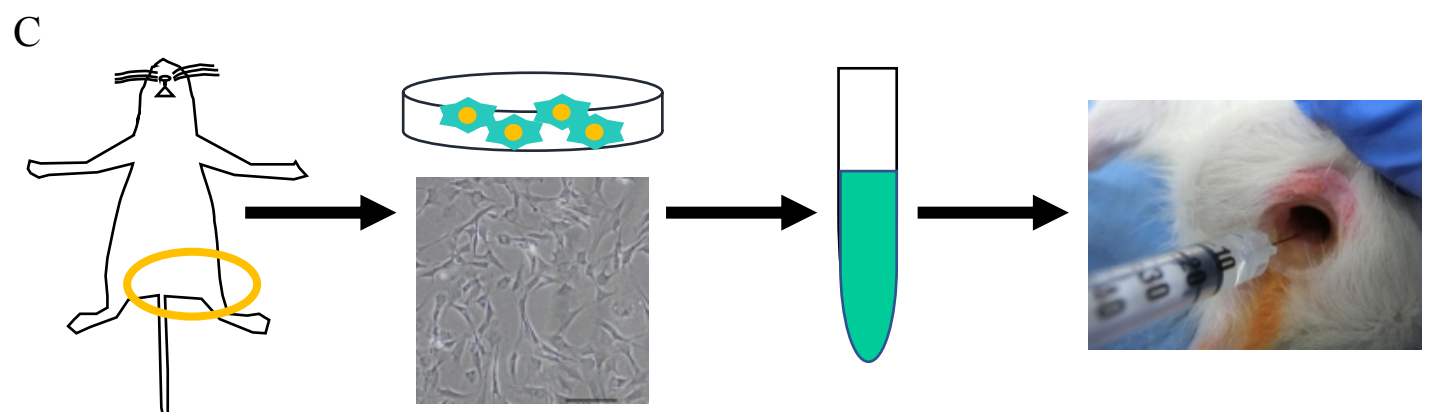
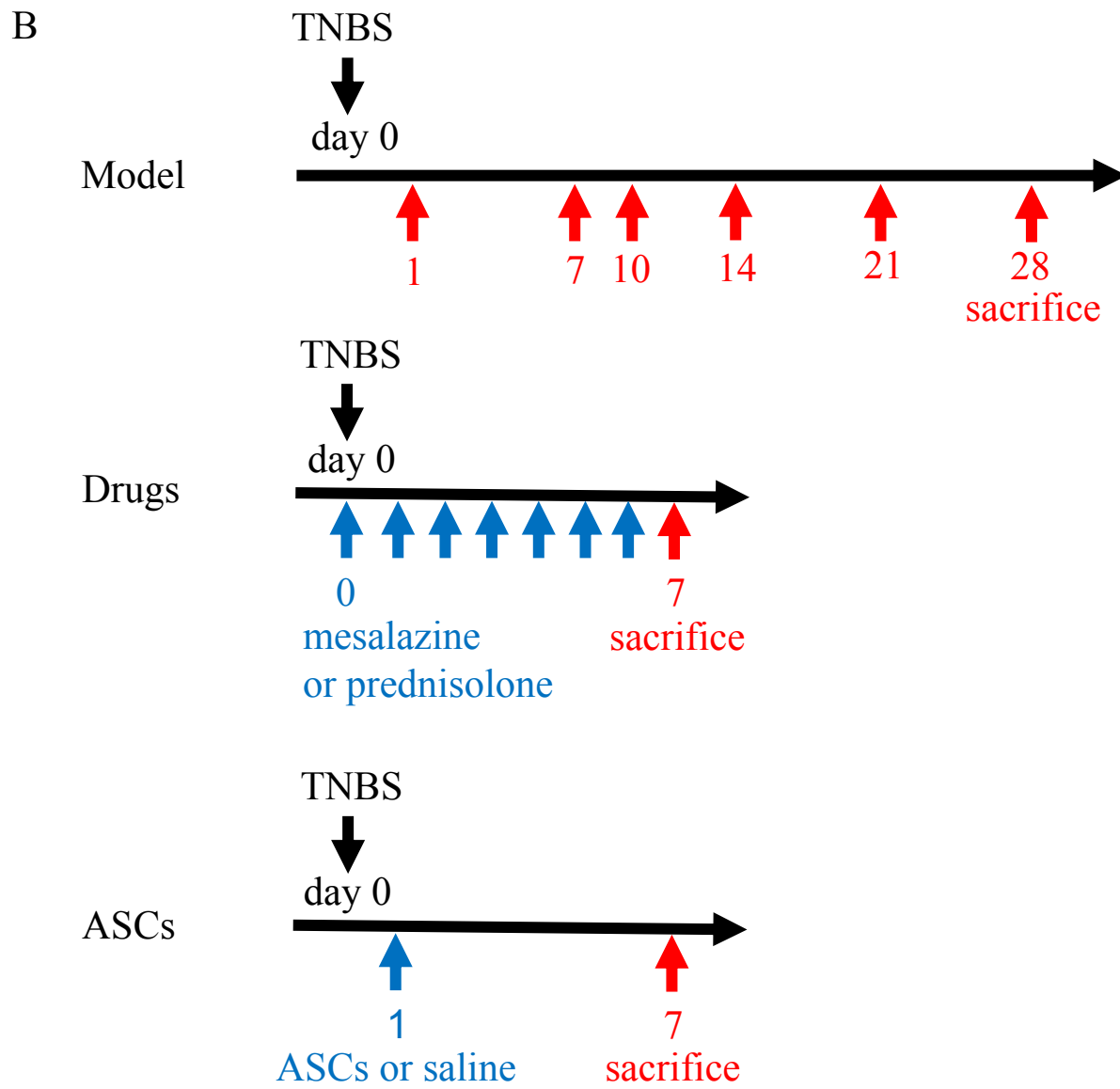
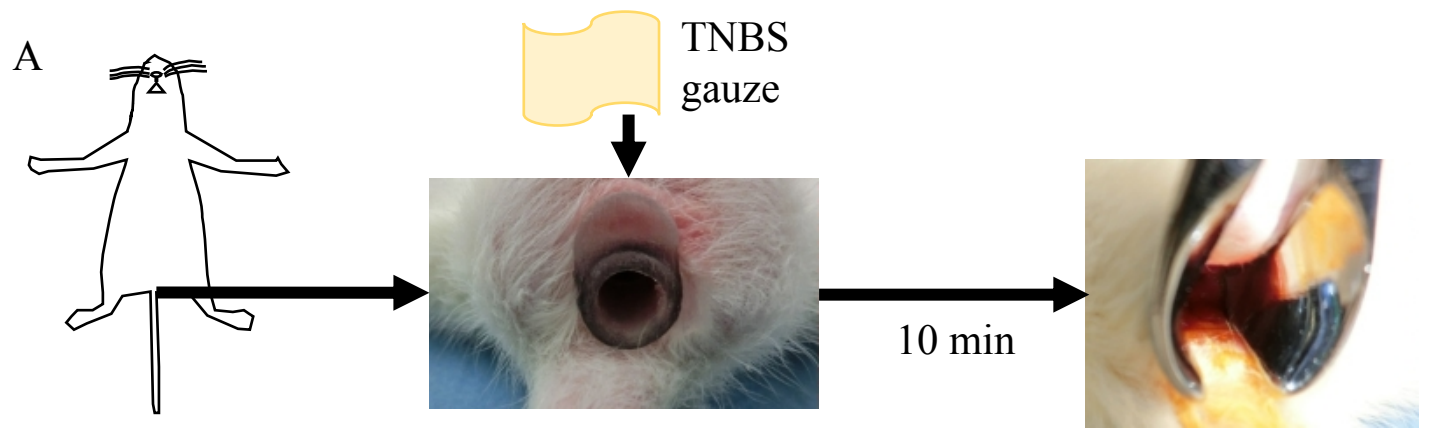
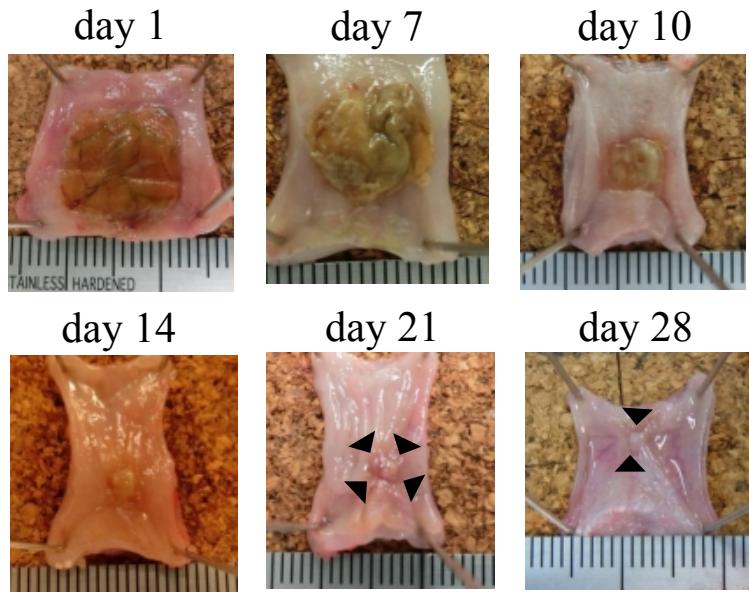
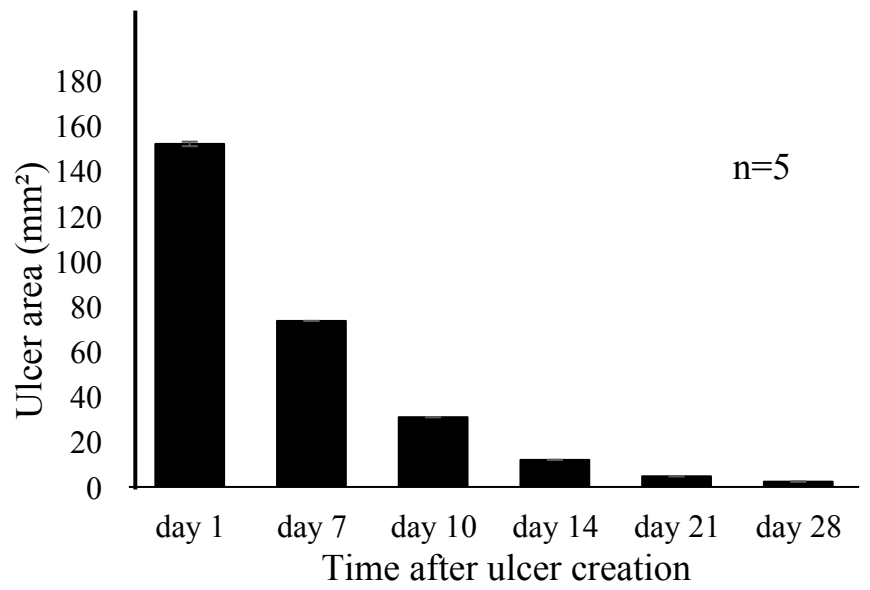


Figure 3

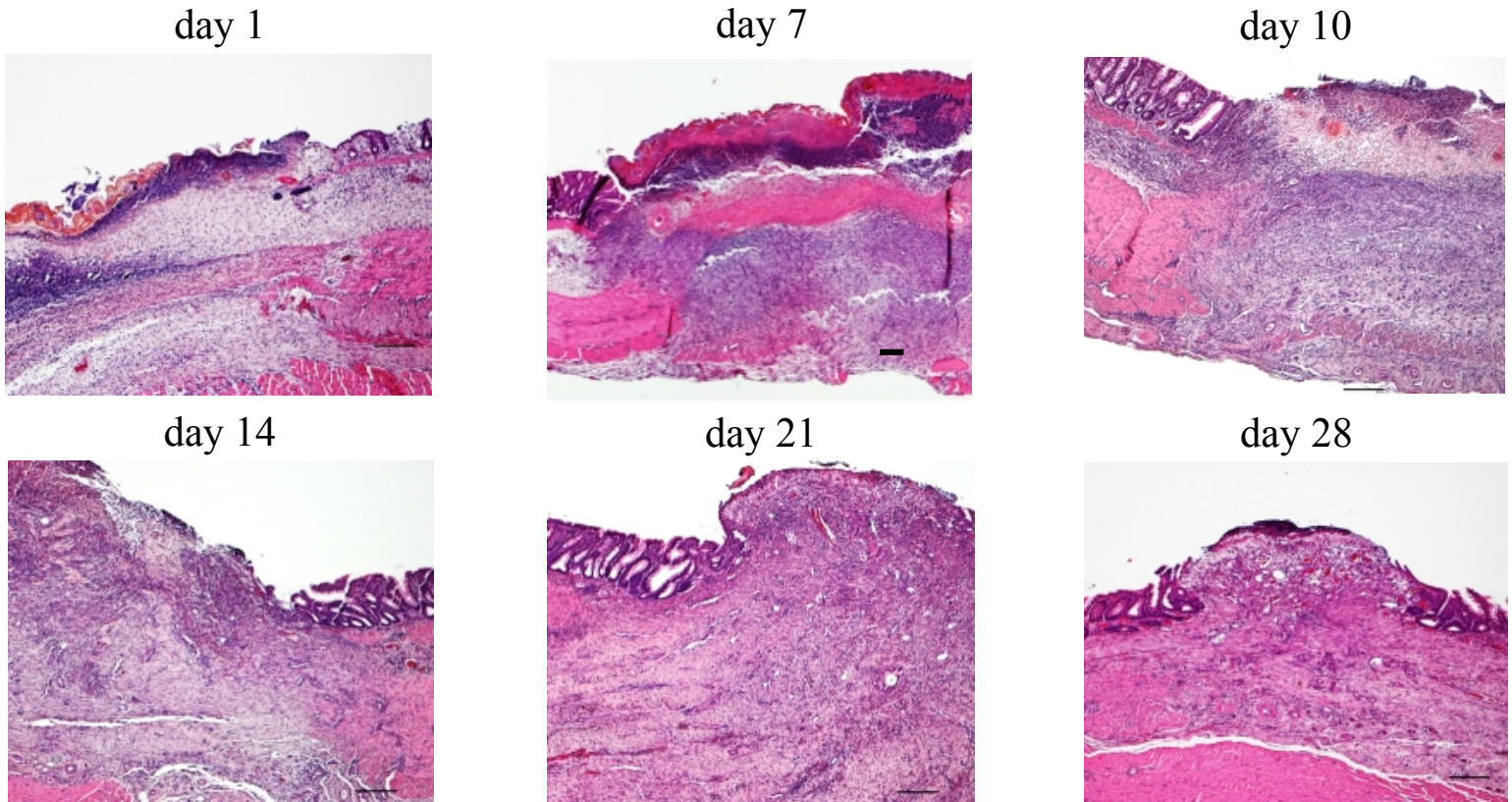
A



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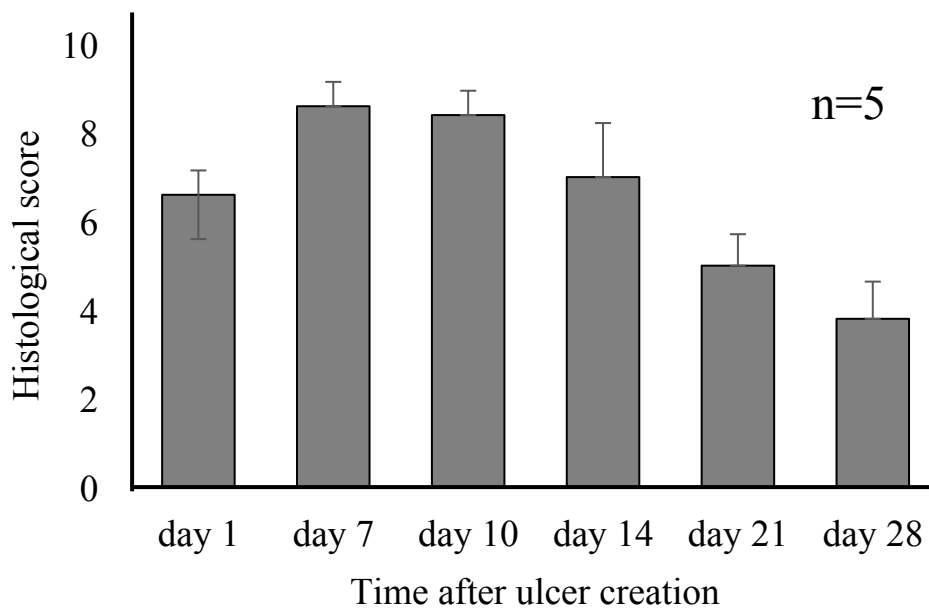


Figure 4

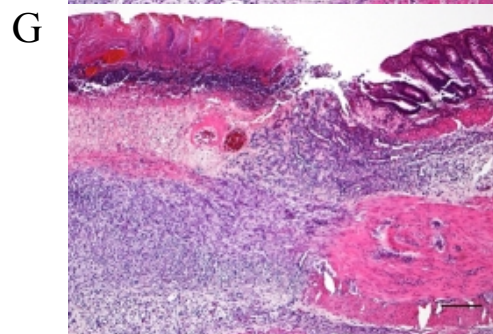
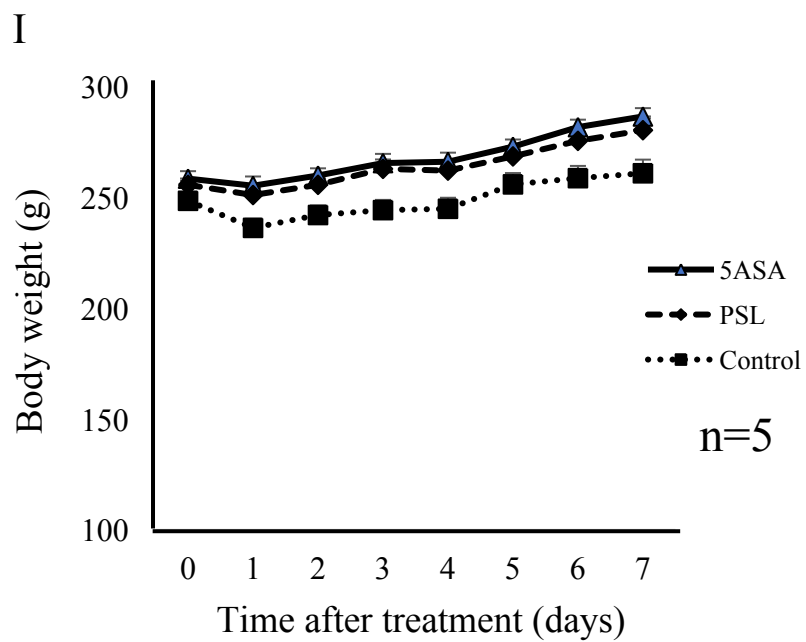
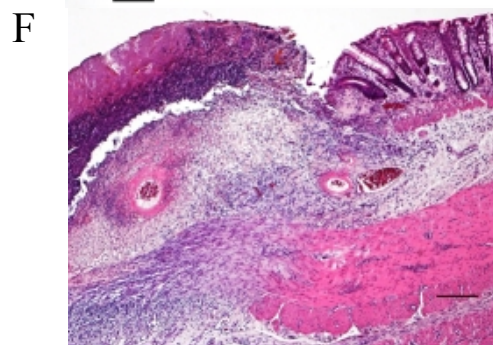
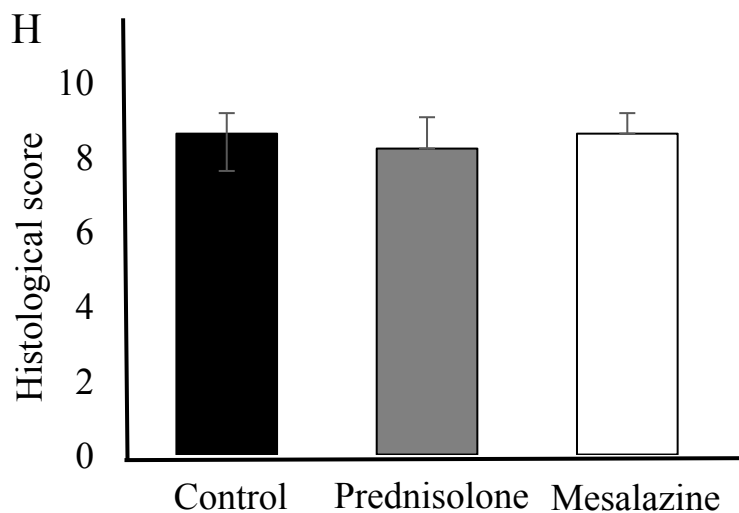
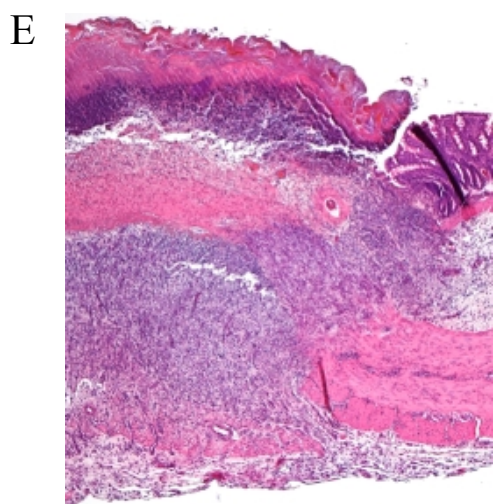
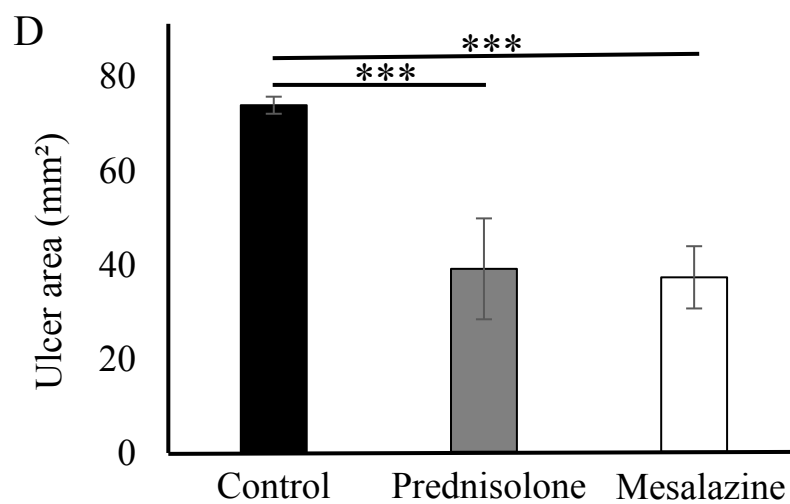
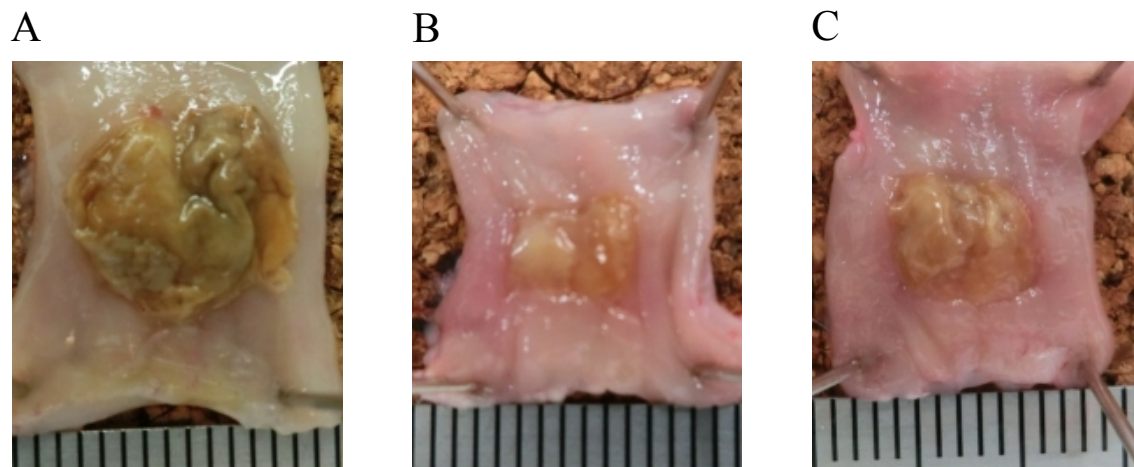


Figure 5

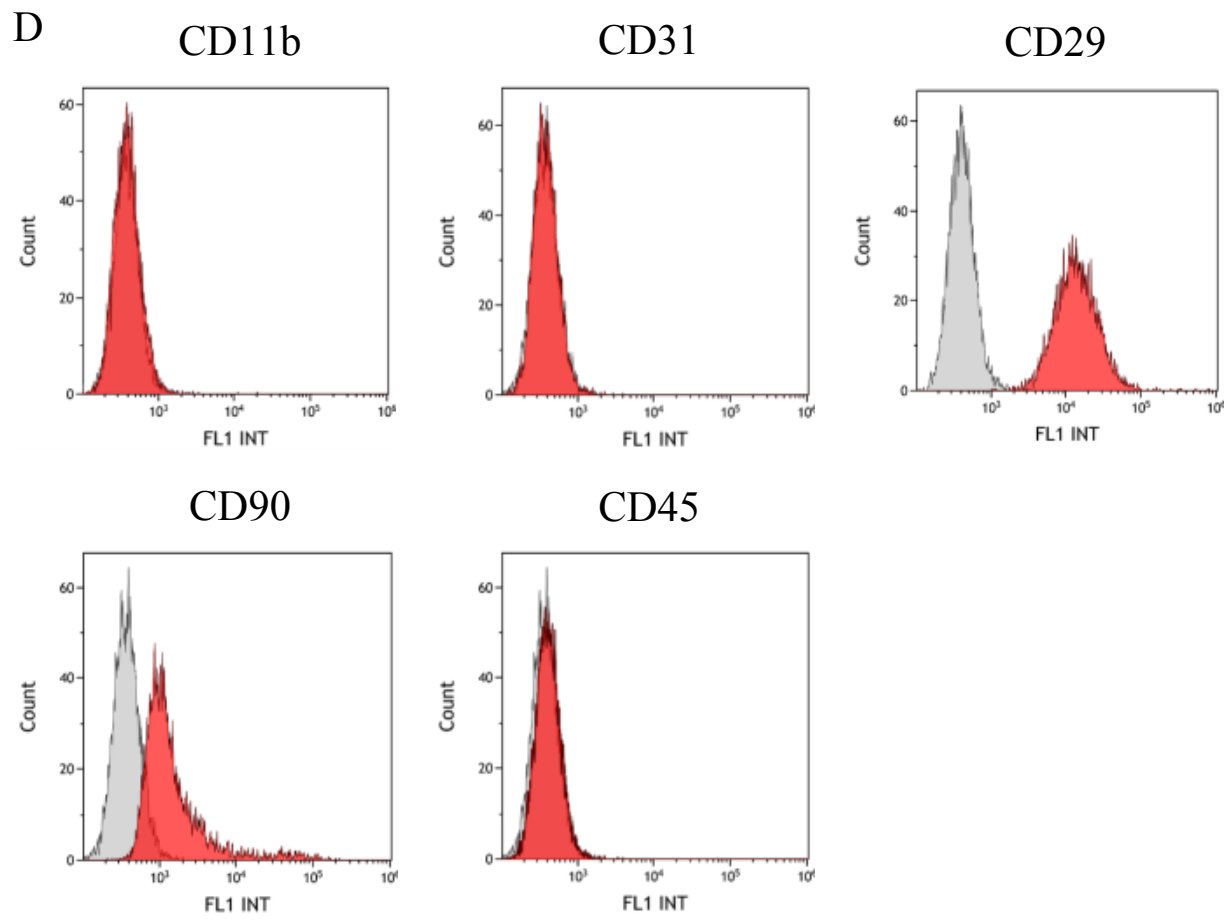
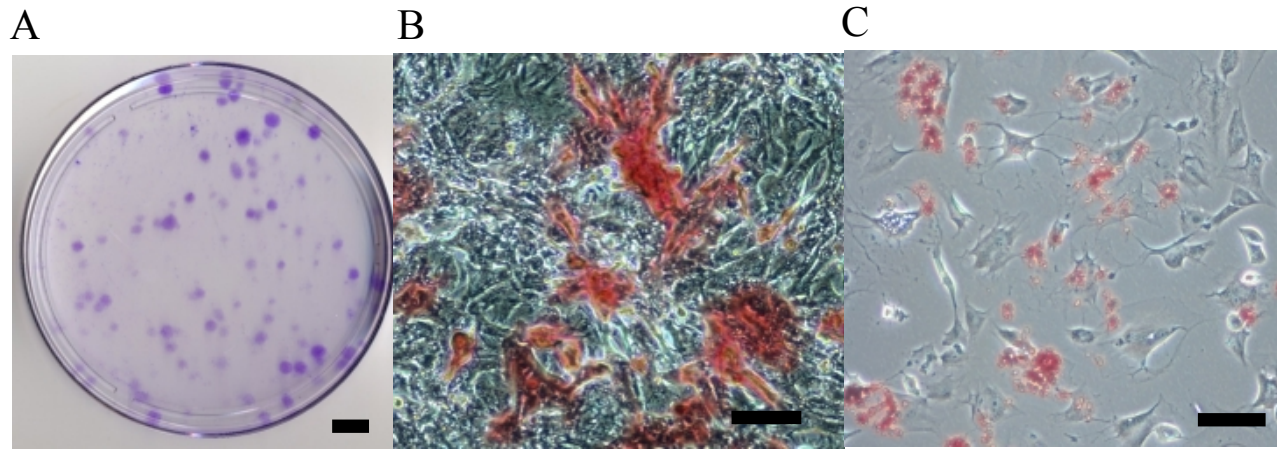
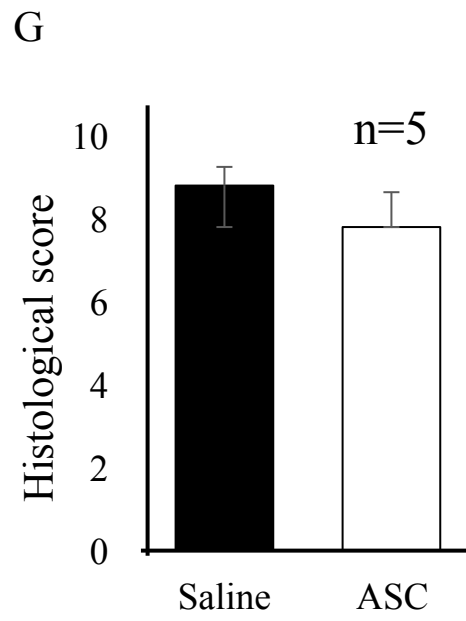
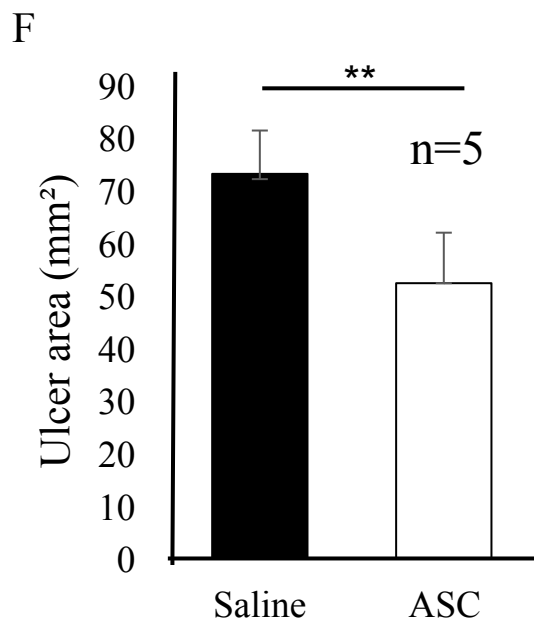
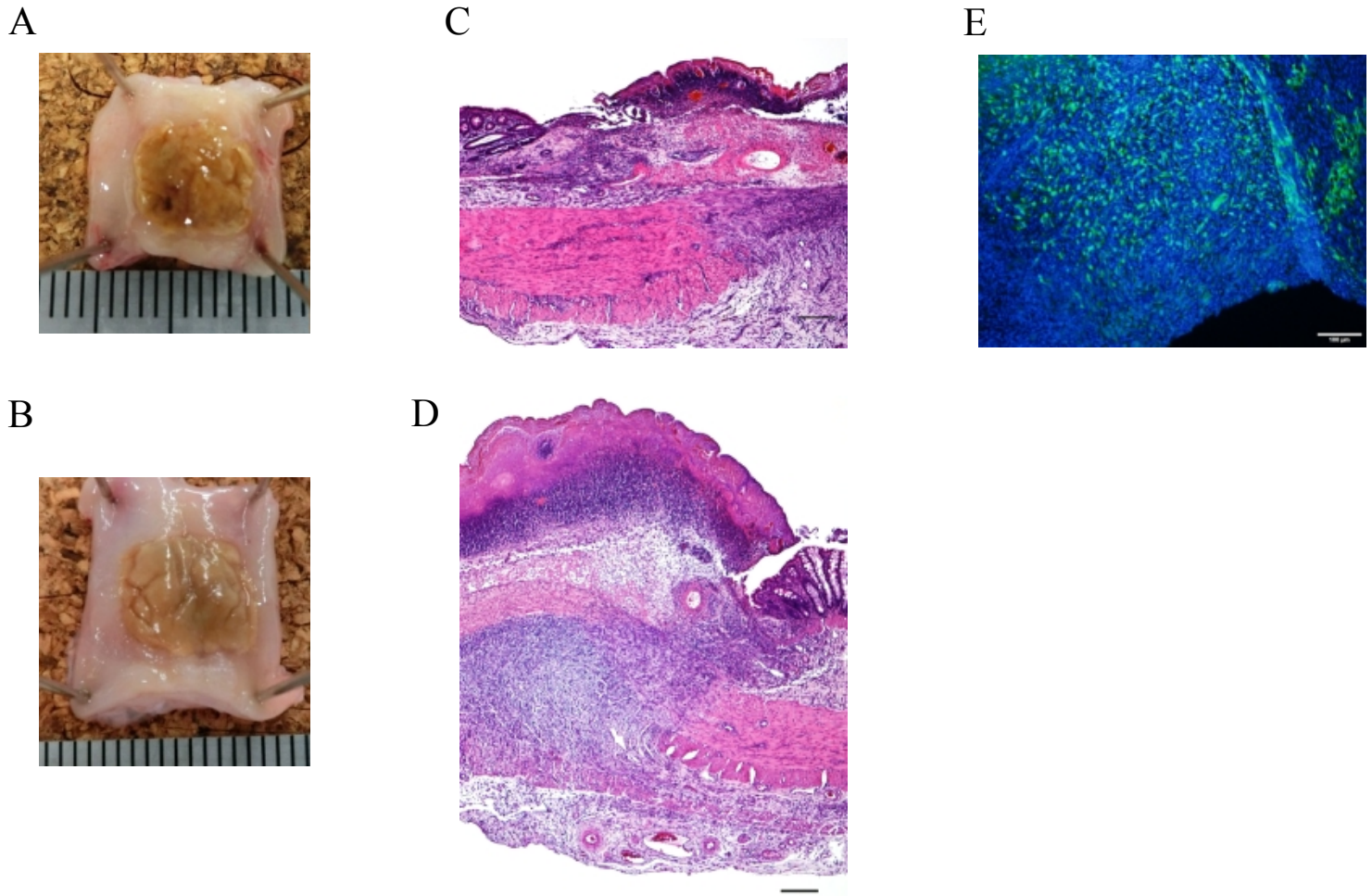


Figure 6





### **Figure 1.**

The novel device created by a three-dimensional (3D) printer. (A) The design of the device shown using 3D computer-aided design software (Solid Works). (B) End-on view of the novel device. (C) Side view of the novel device from a different perspective.

### **Figure 2**

Study methodology. (A) Technique used to create a localized ulcer in the intestinal mucosa. The novel device was inserted rectally into the rat, and a 5 cm × 5 cm piece of gauze soaked with 2,4,6-trinitrobenzene sulfonic acid (TNBS) was placed into the device. After 10 minutes, the device was withdrawn. (B) Experimental timeline. For evaluation of the temporal changes in ulcer characteristics, localized ulcers were created in 30 Sprague Dawley (SD) rats. The rats were randomly divided into 6 equal groups and sacrificed on days 1, 7, 10, 14, 21 or 28 after ulcer creation for further analysis. For assessment of the effects of drugs on ulcer characteristics, 10 SD rats were randomly divided into 2 equal groups and treated daily for 7 days with a mesalazine enema or a prednisolone enema. The rats were sacrificed after 7 days of treatment for further analysis. For investigation of the effects of adipose-derived mesenchymal stem cells (ASCs), 10 SD rats were randomly divided into 2 equal groups, and the ulcer was injected with ASCs

or saline (as a control) on the day following ulcer creation. The rats were then sacrificed on day 7 for further evaluation. (C) Preparation and administration of ASCs. ASCs were isolated from the inguinal adipose tissue of SD-Tg[CAG-EGFP] rats. A suspension of ASCs in saline (100  $\mu$ L) was then injected into the submucosal layer of the ulcer using a syringe and needle. Scale bar: 100  $\mu$ m.

### **Figure 3**

Temporal changes in the size and pathological characteristics of the ulcer. (A) Representative macroscopic images illustrating ulcer size at day 1, 7, 10, 14, 21 and 28 after creation of the localized ulcer. (B) Comparison of ulcer area between different time points after creation of the ulcer. The macroscopic photographs of the localized ulcers were digitized, and the ulcer area at 1, 7, 10, 14, 21 and 28 days was quantified using ImageJ software. The data are shown as the mean  $\pm$  SEM (n = 5). The average local ulcer area at 1, 7, 10, 14, 21 and 28 days was 151.9, 73.5, 30.9, 12.0, 4.7 and 2.3 mm<sup>2</sup>, respectively (n = 5). (C) Representative histological images showing sections of ulcer tissue stained with hematoxylin and eosin at day 1, 7, 10, 14, 21 and 28 after creation of the ulcer. Scale bars: 200  $\mu$ m. (D) Analysis of the histological score at different time points after ulcer creation. The data are shown as the mean  $\pm$  SEM (n = 5). The average

histological score at day 1, 7, 10, 14, 21 and 28 was 6.6, 8.6, 8.4, 7.0, 5.0 and 3.8, respectively.

#### **Figure 4**

Effects of mesalazine and prednisolone (administered by enema) on the size and pathological characteristics of the localized ulcer. (A–C) Representative macroscopic images illustrating ulcer size at day 7 after ulcer creation in the control group (A), prednisolone-treated group (B) and mesalazine-treated group (C). (D) Comparison of ulcer area at day 7 between the control, mesalazine and prednisolone groups. The data are shown as the mean  $\pm$  SEM (n = 5). Average ulcer area was 73.5 mm<sup>2</sup> in the control group, 38.8 mm<sup>2</sup> in the prednisolone group and 37.0 mm<sup>2</sup> in the mesalazine group. \*\*\* P < 0.001. (E–G) Representative histological images showing sections of ulcer tissue stained with hematoxylin and eosin for the control group (E), prednisolone group (F) and mesalazine group (G) at day 7. Scale bars: 200  $\mu$ m. (H) Analysis of the histological score at day 7. The data are shown as the mean  $\pm$  SEM (n = 5). The average histological score was 8.6 in the control group, 8.2 in the prednisolone group and 8.6 in the mesalazine group. (I) Changes in body weight in the control, prednisolone and mesalazine groups during the 7 days after ulcer creation. The data are shown as the mean  $\pm$  SEM (n = 5).

### **Figure 5**

Biopotency of rat adipose-derived mesenchymal stem cells (ASCs). (A) Colonies of ASCs grown in a 100-mm dish for 7 days stained positively with crystal violet. Scale bar: 100 mm. (B) The cells stained positively with oil red O after culture in adipogenic induction medium for 21 days. Scale bar: 100  $\mu$ m. (C) The cells stained positively with alizarin red S after culture in osteogenic induction medium for 21 days. Scale bar: 100  $\mu$ m. (D) Flow cytometry analysis showed that the rat ASCs were positive for CD29 and CD90 but negative for CD11b, CD34 and CD45.

### **Figure 6**

Effects of local injection of rat adipose-derived mesenchymal stem cells (ASCs) on ulcer characteristics. (A) Representative macroscopic image of a localized ulcer at day 7 in the ASC-treated group. (B) Representative macroscopic image of a localized ulcer at day 7 in the saline-treated group (control). (C) Representative histological image from the ASC-treated group showing a section of ulcer tissue at day 7 stained with hematoxylin and eosin. Scale bar: 200  $\mu$ m. (D) Representative histological image from the ASC-treated group showing a section of ulcer tissue at day 7 stained with hematoxylin and eosin. Scale

bar: 200  $\mu\text{m}$ . (E) Representative immunofluorescence image of a specimen of ulcer tissue after the injection of ASCs. ASCs are stained green and nuclei are stained blue. Scale bar: 100  $\mu\text{m}$ . (F) Comparison of ulcer area at day 7 between the ASC and saline groups. The data are shown as the mean  $\pm$  SEM (n = 5). Average ulcer area was 73.1  $\text{mm}^2$  in the saline group and 52.3  $\text{mm}^2$  in the ASC group. \*\* P < 0.01. (G) Analysis of the histological score at day 7. The data are shown as the mean  $\pm$  SEM (n = 5). The average histological score was 8.8 in the saline group and 7.8 in the ASC group.