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ORIGINAL ARTICLE

Basic Study

Novel isolated cecal pouch model for endoscopic observation in rats

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Abstract

AIM: To create a new rat model for drug administration, cell transplantation, and endoscopic examination for the treatment of intestinal diseases.

METHODS: F344/NJc l-rnu/rnu rats (10-wk-old males, 350-400 g) were used in this study. The rats were anesthetized *via* 2% isoflurane inhalation. The rat's cecum was isolated from the intestines, and a pouch was created. The remainder of the intestines was rejoined to create an anastomosis. The "side-to-side" anastomosis (SSA) technique initially involves the creation of a 2-cm longitudinal incision into each intestinal wall. To create an anastomosis along the ileal and colonic walls, both intestines were cut, and a continuous suture procedure was performed that included all layers of both intestines. The serous membrane was sutured along the edge and on the anterior wall of the anastomosis. The "end-to-end" anastomosis (EEA) technique was compared with the SSA technique. In the EEA technique, the frontal surfaces of both cut intestinal lumens were joined together by continuous sutures. Additional sutures were made at the serosa. After the anastomotic intestine was successfully constructed, the two intestinal lumens that were cut at the isolated cecum were managed. In addition, one luminal side of the pouch remained open to create an artificial anus on the dorsum as a passage for the residual substances in the pouch. Finally, small animal endoscopy was used to observe the inside of the pouch.

RESULTS: In this animal model, mucus and feces are excreted through the reconstructed passage. Accordingly, the cecal pouch mucosa was not obstructed or contaminated by feces, thus facilitating observations of the luminal surface of the intestine. The endoscopic observation of the cecal pouch provided clear visualization given the absence of feces. The membrane surface of the cecum was clearly observed. Two methods of creating an anastomotic intestine, the "SSA" and "EEA" techniques, were compared with regard to animal survival rate, complication rate, and operation time. The SSA technique resulted in a significantly increased survival rate and a lower incidence of complications in rat models compared with the EEA technique. The complications of stenosis and leakage resulted in death in the EEA technique. Thus, the EEA technique exhibited a lower survival rate compared with the SSA technique. However, the SSA technique required a significantly longer operation time compared with the EEA technique.

CONCLUSION: Our new rat model is potentially useful for the development of a novel treatment for intestinal diseases.

Key words: Animal model; Anastomosis; Endoscopy; Cecal pouch; Microsurgery

Core tip: The most innovative feature of this study involved the creation of a cecal pouch that was isolated from the intestines and rejoined to create an anastomosis and an artificial anus on the rat's dorsum that consisted of one side of a cut that remained open on the lumen of the cecal pouch. Feces were excreted *via* the anastomosis. Thus, the pouch mucosa was not contaminated by feces, and the luminal surface remained clean. This feature enables endoscopic observation *via* the artificial anus. Furthermore, drug administration or cell transplantation into the pouch can be easily and repeatedly performed through the artificial anus, and temporal changes can be observed *via* endoscopy.

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INTRODUCTION

Animal models allow researchers to better understand various processes related to human diseases, development, and physiology. In addition, these models provide valuable information leading to the development of human therapies. Therefore, well-developed animal models are crucial to ensure successful experiments[1-7]. In gastroenterological research, trinitrobenzenesulfonic acid (TNBS)-induced colitis is a conventional experimental colitis model that is widely used to understand the pathophysiology of inflammatory bowel disease (IBD)[8]. This animal model was also recently used in stem cell transplantation studies that involve the transplantation of *in vitro* cultured stem cells into an ulcerative lesion in animal intestines[9]. Typically, drugs and cells are delivered *via* enema in such studies. However, the use of this technique to deliver drugs and cells to target sites of the luminal surface is difficult and inefficient due to limited visualization and obstruction from the animals' waste products. Even if drug and cell delivery is achieved, routine excretion is still unavoidable and often hampers drug absorption and cell engraftment. Therefore, it is necessary to develop a novel animal model that can facilitate efficient drug and cell administration in an experimental animal study.

Unlike human surgeries, operations in rat models are typically performed by a single operator under a microscope[10]. Therefore, a clear and stable field of operation, particularly while suturing intestinal tissues, is indispensable to the operator in terms of maintaining a meticulous surgical technique[11]. In the present, study we developed a new rat intestinal model wherein a cecal-isolated intestinal pouch was created to provide an experimental site for the gastrointestinal study. A separate excretory passage was simultaneously created using the rejoined intestine. The use of surgical procedures to rejoin the intestines were proposed and compared.

MATERIALS AND METHODS

Animal care and use statement

The animal protocol was designed to minimize pain or discomfort to the animals. The rats were housed and cared for in individual cages. The room was maintained at 22-24 ℃ and approximately 45% humidity on a 12 h light and dark cycle. Rats were provided with ad libitum access to food and water. The rats were anesthetized with 2% isoflurane inhalation before the procedures.

Animal preparations

Animal care was performed according to the protocol approved by the Tokyo Women's Medical University Animal Experimentation Committee (IACUC protocol number: 14-69). A total of 33 F344/NJc l-rnu/rnu rats (10-wk-old males, 350-400 g) purchased from Charles River Laboratories Japan (Tokyo, Japan) were used for this study. Prior to anesthesia, the animals were injected with a normal saline solution containing 10% penicillin/streptomycin (Invitrogen™, Life Technologies, 1600 Faraday Avenue Carlsbad, CA 92008 United States) through the superficial dorsal vein of the penis to avoid infection. Subsequently, the rats were anesthetized *via* 2% isoflurane inhalation. The animals

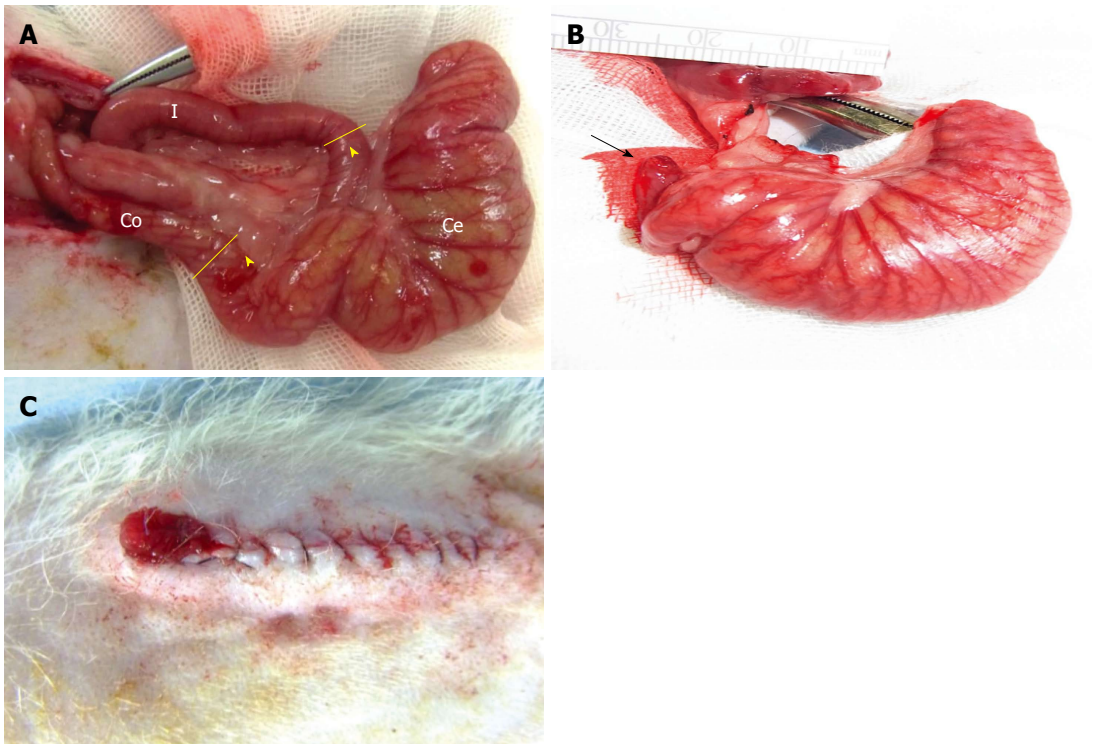

Figure 1 Procedures for intestinal isolation and the creation of an artificial anus on the rat's dorsum. A: Anatomical findings reveal the ileum (I), cecum (Ce), and colon (Co). Two cutting points were denoted (dash line) 2-cm apart from ileocecal and cecocolonic junctions (arrowhead); B: The arrow indicates the colonic lumen that remains open; C: The intestinal membrane of the open colonic lumen was sutured together with the rat dorsal skin to create an artificial anus.

were restrained in the supine position on a micro warm plate (Kitazato Corporation, 81 Nakajima, Fuji, Shizuoka, 416-0907 Japan) set at 37 ℃. Their dorsal hair was shaved, and the surgical area was sterilized with 70% ethanol.

Surgical procedures

A 2.5-cm incision was created on the right side of the rat dorsum. Due to the increased anatomical size of the rats (compared with humans), the cecum and small intestine were easily recognizable after passing through the retroperitoneal layer.

Two incision points were marked 2 cm away from the ileocecal and cecocolonic junctions (Figure 1A). To stabilize and secure an operative field, the posterior wall of the ileum and colon were placed in parallel. Under a surgical microscope, both intestinal walls were then fixed together with five interrupted 7-0 PDS*II (polydioxanone) sutures (Ethicon®, Johnson and Johnson, Medical PTY. Ltd., NSW, United States). The incisions (which were made as previously described) were located to isolate the cecum. Subsequently, the ileum and colon were rejoined to create an anastomotic intestine using each of the two newly proposed techniques, specifically, the "side-to-side" and "end-to-end" anastomosis techniques (see the "Results" section for the comparison of these two methods in detail). After the anastomotic intestine was successfully constructed, the 2 intestinal lumens that were cut at the isolated cecum were managed. The ileal lumen was closed with 7-0 PDS*II sutures, whereas the colonic lumen remained open to create an artificial anus as a passage for residual substances in the pouch. The cecal pouch was subsequently created. Next, the incised retroperitoneum was closed. Finally, an artificial anus was created on the rat dorsum by suturing the intestinal membrane of the colonic lumen, which remained open, to the dorsal skin, which subsequently reversed the mucosa (the inside of the mucosa now faces outwards) (Figure 1B and C).

Cell preparations

Cell preparation procedures were performed as previously described[12-17]. The small intestinal epithelial cells were obtained from the C57BL/6-Tg (CAG-EGFP) mice purchased from Charles River Laboratories Japan (Tokyo, Japan). Primary cells were seeded on temperature-responsive culture dishes (35 mm in diameter, UpCell®, CellSeed Inc., Tokyo, Japan). These cells were co-cultured with mouse embryonic fibroblasts (MEF) serving as feeder cells (ReproCELL Inc., Tokyo, Japan) in Advanced Dulbecco's Modified Eagle Medium/ Ham's F-12 (Life Technologies™) supplemented with N-2 Supplement (Life Technologies™), B-27® Supplement (Life Technologies™), N-acetylcysteine (Sigma-Aldrich®), murine recombinant epidermal growth factor (Life Technologies™), murine recombinant Noggin (PeproTech Inc.), human recombinant R-spondin1 (RD Systems),

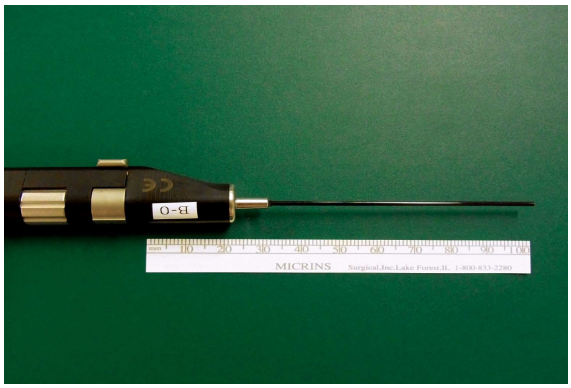

Figure 2 Endoscopic tool used in this animal study.

and Rho-associated protein kinase (ROCK) inhibitor (Y-27632, Sigma-Aldrich®).

Internal observation of the cecal pouch

To observe the inside of the cecum, small animal endoscopy was used (AE-F16070, AVS Co., Ltd., Japan) (Figure 2).

Statistical analysis

The data are presented as the mean ± SD and frequency. A normality test was performed to evaluate sample distribution. The independent sample-t test and Fisher's exact test were used to compare the groups. A *P*-value of less than 0.05 ($P < 0.05$) was considered significant. The statistical methods of this study were reviewed by Dr. Satoshi Iimuro from Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University (TWIns).

RESULTS

Endoscopic observation of cecal pouch membrane

A new rat intestinal model was developed wherein the cecal pouch was created to isolate the cecum from an excretory passage. In this animal model, mucus and feces are excreted through the reconstructed passage. Accordingly, the cecal pouch mucosa was not obstructed or contaminated by feces, thereby facilitating observations of the luminal surface of the intestine (Figure 3A). The cecal pouch was clearly visualized *via* endoscopy given the absence of feces. The membrane surface of the cecum was clearly observed (Figure 3B).

Creation of an artificial ulcerative lesion on the cecal pouch membrane

At 1 wk, during post-operative surgical re-entry of the cecal pouch, we confirmed no fecal contamination. Furthermore, we successively created an ulcerative lesion on the cecal pouch membrane (Figure 4A and B). Figure 4C depicts the administration of cultured small intestinal epithelial cells to an artificial ulcerative lesion.

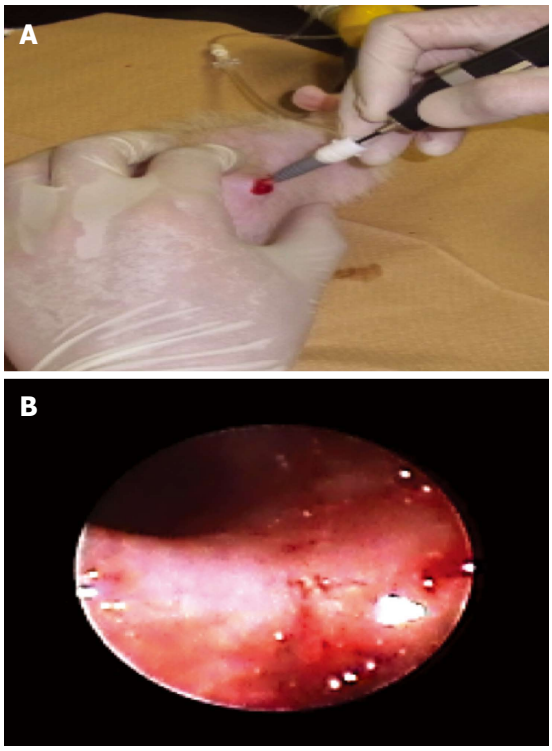

Figure 3 Internal observation of the cecal pouch using a small animal endoscope *via* an artificial anus. A: The endoscope was used to observe the internal cecal pouch *via* an artificial anus; B: An image of the cecal pouch membrane obtained from the endoscopic examination.

Comparison of the "side-to-side" anastomosis with "end-to-end" anastomosis technique

To create an anastomotic intestine, we used and compared two distinct surgical techniques: "side-to-side" and "end-to-end" anastomosis techniques.

Technically, the "side-to-side" anastomosis (SSA) technique involves the creation of a 2-cm longitudinal incision into each intestinal wall. To create an anastomosis along the ileal and colonic walls, both intestines were cut, and a continuous 7-0 PDS*II suture procedure was performed that included all layers of both intestines. The serous membrane was sutured along the edge and on the anterior wall of the anastomosis (Figure 5A and B).

The EEA technique was performed and compared with the SSA technique. In the EEA technique, the frontal surfaces of both cut intestinal lumens were joined together by continuous 7-0 PDS*II sutures (Figure 5C). Additional sutures were placed at the serosa (Figure 5D).

The two methods used to create an anastomotic intestine, the SSA *vs* EEA techniques, were compared with regard to the animal survival rate, complication rate, and operation time. The follow-up period was 7-10 d after the operation. The SSA technique resulted in a significantly increased survival rate (SSA *vs* EEA; 75% *vs* 23%, respectively) (Figure 6A) and exhibited a lower incidence of complications compared with

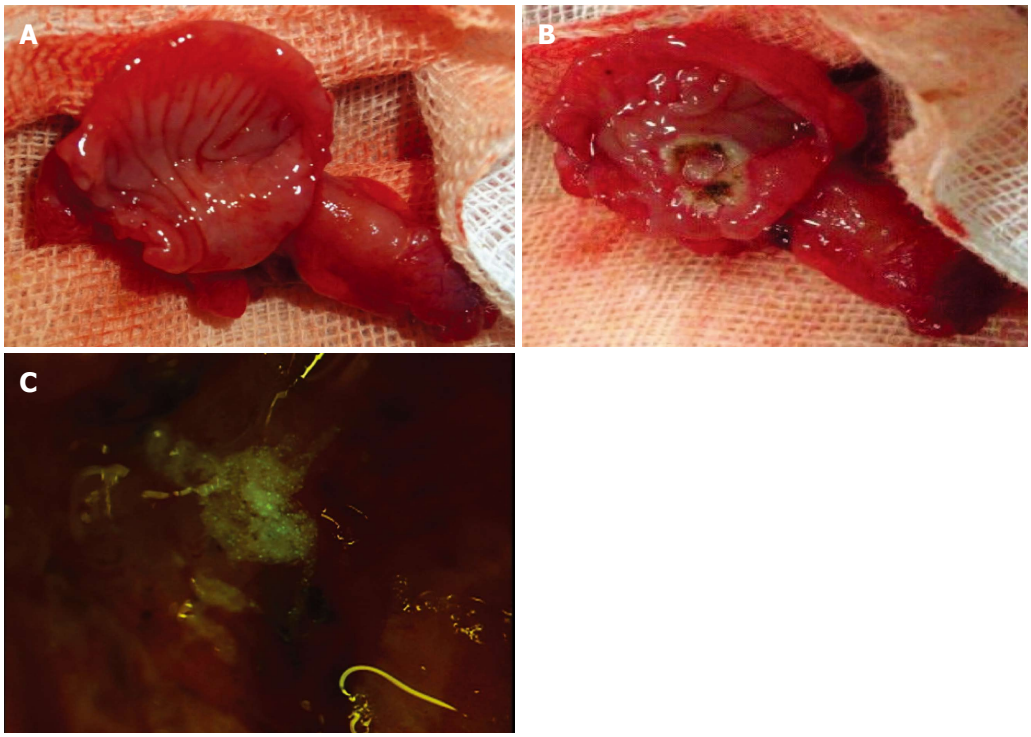

Figure 4 Applications of the newly created isolated cecal pouch model. A: One-week post-operative surgical re-entry confirmed no fecal contamination of the cecal pouch membrane surface; B: An artificial ulcerative lesion was created on the surface of the pouch membrane; C: Microscopic photograph indicating that cultured small intestinal epithelial cells could be applied to an ulcerative lesion on the cecal pouch membrane.

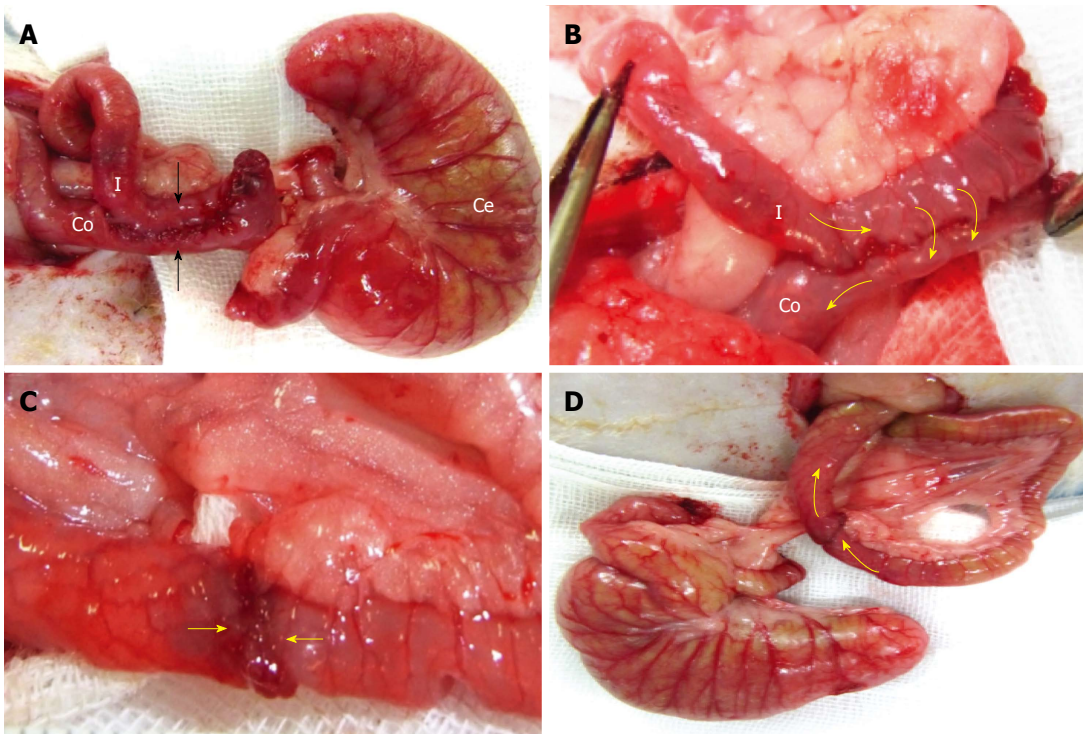

Figure 5 Comparison of two proposed surgical techniques for creating an anastomotic intestine: "side-to-side" anastomosis *vs* "end-to-end" anastomosis technique. A: The ileum and colon were anastomosed together using the SSA technique (arrow). An isolated cecal pouch was created; B: The direction of fecal passage from the ileum to the colon *via* the SSA-anastomotic intestine; C: EEA technique; the frontal surfaces of both cut intestinal lumens were sutured together; D: The direction of fecal passage from the ileum to the colon *via* the EEA-anastomotic intestine. SSA: "Side-to-side" anastomosis; EEA: "End-to-end" anastomosis.

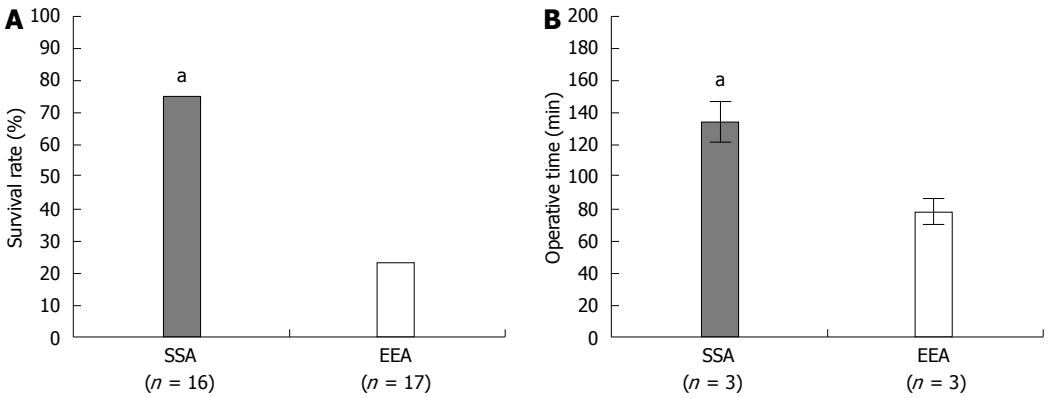

Figure 6 Comparison of the survival rate and operative time between the "side-to-side" anastomosis and end-to-end" anastomosis techniques. A: Survival rate of animals after creating an anastomotic intestine using the "side-to-side" anastomosis (SSA) or "end-to-end" anastomosis (EEA) technique ($^aP < 0.05$ *vs* EEA method, Fisher's exact test); B: Operative time used in the SSA method compared with the EEA method ($^aP < 0.05$ *vs* EEA method, independent sample-*t* test).

Table 1 Incidence of complications in dead animals *n* (%)

Complication	SSA	EEA
Stenosis	0 (0)	7/13 (41)
Leakage	2/4 (12.5)	6/13 (36)
Local infection	2/4 (12.5)	0 (0)
Total	4/16 (25)	13/17 (77)

SSA: "Side-to-side" anastomosis; EEA: "End-to-end" anastomosis.

the EEA technique (Table 1). Stenosis and leakage complications resulted in death in the EEA technique. Thus, the EEA technique exhibited a lower survival rate compared with the SSA technique. However, the SSA technique required a significantly longer operation time compared with the EEA technique (Figure 6B).

DISCUSSION

The possibility of contamination and obstruction of the intestinal tract with animal waste products cannot be eliminated; thus, it would be difficult to interpret the obtained results regarding drug administration and cell transplantation into the intestinal lumen in a conventional bowel disease model. To overcome these problems, in the present study, we developed a new rat intestinal model by creating a cecal pouch that is not exposed to fecal contamination. Furthermore, an excretory orifice was artificially created on the rat dorsum for the excretion of waste product from the cecal pouch.

By preventing fecal contamination inside of the pouch, it is easy to perform intestinal observations and various experimental tasks. Therefore, we attempted to create an ulcerative lesion on the surface of the pouch membrane and transfer cultured cells to a lesion site; these experiments were performed with ease. Our results suggested that this novel isolated cecal pouch model is applicable to several types of animal experiments related to gastrointestinal studies.

In several animal studies, experimental animals must be sacrificed in an attempt to observe temporal changes[1,7,9,18-21]. Because the rat excretory passage is separated from the cecal pouch, the present animal model provided good visualization internally and of the luminal surfaces of the cecal pouch *via* endoscopy (Figure 3B). Using a small animal endoscope, the luminal surfaces of the pouch are easily observed over time *via* a non-invasive procedure, and repeated examinations can be performed as often as necessary in the living animal.

A stable and secured field of operation is a critical factor for a precise operation. However, in small animal operations, a microscope is often used[3], and the operation is routinely performed by a single operator. In the present study, we introduced a primary suture at the posterior wall of the each intestine to maintain the position of the operative site. This additional suturing eased intestinal anastomosis.

Compared with the EEA technique, the SSA technique offered enhanced stabilization and a wider diameter of the anastomotic intestine. This difference likely accounts for the increased survival rate and reduced morbidity observed in the SSA group. When performing the EEA method, the tissues moved easily, which complicated the suturing procedure. Consequently, the unstable suture technique caused stenosis and leakage at the anastomosed area that resulted in mortality, as indicated by the low survival numbers. Therefore, in our model, the SSA technique successfully extended animal. Although the SSA technique was more time-consuming than the EEA technique due to a complex suturing procedure, the increased time did not reduce the survival rates in the SSA group, which was most likely due to the stress-reducing protocol used during surgery. A micro warm plate and bipolar electrocoagulation were used, resulting in reduced stress and a faster operation. One week after the operation, the animals had recovered; they were stabilized and ready for use in further experiments.

In conclusion, researchers can use our new in-

testinal rat model with various approaches. The manipulation of drugs or cells into the isolated cecal pouch was performed *via* an artificial anus without any fecal contamination. Additionally, an endoscope can be employed to conveniently observe the intestinal membrane and administer drugs or cells.

This animal model enables reproducibility with regard to observing the pathological changes and enabling the time-dependent effects of the interventions on the intestinal tract. This reproducibility will be useful in understanding the morbid state of bowel disease and will help researchers to perform successful future studies of gastrointestinal diseases.

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COMMENTS

Background

The models of inflammatory bowel diseases (IBD) have been established in the animal intestines. Trinitrobenzenesulfonic acid-induced colitis is a conventional experimental colitis model that is widely used to understand the pathophysiology of them. These models were also recently used in stem cell transplantation studies that involve the transplantation of *in vitro* cultured stem cells into an ulcerative lesion in animal intestines.

Research frontiers

In these researches, the method of delivery system is done by means of injecting drug or cell per anal. However, a limited visualization and obstruction inside the luminal surface caused by animals' feces hamper an effectiveness of conventional drug or cell delivery system. Therefore reliable models are required for experimental colitis research.

Innovations and breakthroughs

In this study, a new rat model was created. The rats' cecum were isolated to create the cecal pouch. The intestines were reconstructed in order to avoid a contamination by feces. These newly proposed methods allowed a fecal-free internal surface of a cecal pouch, thus, feces are not interfered with drug absorption and cell engraftment. At the same time, a newly created anastomotic intestines and artificial anus enabled the defecation of animals' waste product.

Applications

The present rats' cecal pouch model allowed the researchers to apply transplanted cells or drugs to an intestinal target site directly and easily. As one side of the lumen of this pouch was used as an artificial anus located at the rats' dorsum, this facilitated an application of the small animal's endoscope in observing a luminal surface of a cecal pouch *via* an artificial anus.

Terminology

The IBD is caused by an abnormality of the bowel immune system. The failure of restoration of gastrointestinal mucosa and bowel barrier system is caused by chronic inflammation. Ulcerative colitis and Crohn's disease are representative of the IBDs.

Peer-review

In this paper, a new suturing technique of the animal operation was proposed. This technique helped reducing complications that associated with an anastomosis. A creation of this novel isolated intestinal pouch used for a transplantation site is of which our original idea. This animal model could be applied for the study related to the bowel disease models and stem cell transplantation.

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