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(RESEARCH ARTICLE)

Establishment of a postoperative bowel adhesion model in Rats

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Abstract

Postoperative adhesive bowel obstruction is a frequent cause of hospital admission in a surgical department. Emergency surgery is needed in a majority of patients with bowel ischemia or peritonitis; most adhesive bowel obstruction can be managed non-operatively. Many studies have investigated benefits of using oral water-soluble contrast to manage adhesive bowel obstruction. Treatment recommendations are still controversial. Intestinal adhesions commonly occur in patients after abdominal surgery, often between intestines, between intestines and the abdominal wall, or within the peritoneum. Adhesions can lead to symptoms such as abdominal pain, constipation, and bloating, and may result in serious complications such as intestinal obstruction, bowel necrosis, acute peritonitis, and sepsis, which can be life-threatening. Therefore, reducing the occurrence of intestinal adhesions is crucial for abdominal surgery. To minimize adhesions, endoscopic techniques can be employed to reduce the abdominal opening area and thus decrease the probability of adhesion formation. However, this approach requires specialized equipment and surgical skills, making it somewhat challenging. Alternatively, anti-adhesion products can be used to lower the risk of adhesion formation. In this study, a murine cecal abrasion adhesion model was established and will be simulated common adhesion scenarios observed in patients after abdominal surgery. The efficacy of anti-adhesion gel materials will be then evaluated to assess their anti-adhesion properties. The record of gross adhesion severity assessment was performed. The adhesion status between the cecum and abdominal wall was observed on postoperative days 3 and 14. The results was shown that three days post-surgery operation, the group exhibited more severe adhesions upon gross examination. By day 14 post-surgery operation (at animal sacrifice), the histopathological analysis revealed that the group exhibited thicker fibrous tissue thickness, increased neovascularization, and higher collagen accumulation in the peritoneum. Therefore, based on the results of this experiment, a postoperative bowel adhesion rat is reliable and can be safely used to test the efficacy of novel anti-adhesive formulations to prevent intra-abdominal adhesions by using this model.

Keywords: Cecal abrasion adhesion; Postoperative bowel adhesion model; Rat model; Surgery induction

1 Introduction

The more formal term for intestinal adhesions is adhesive small bowel obstruction (ASBO). ASBO occurs when the small intestine is partially or completely blocked due to peritoneal adhesions, thereby affecting the normal flow of intestinal contents. Postoperative adhesions are the most common cause of ASBO, accounting for over 60% of all cases [1-3].

The formation of adhesions can result from various causes, including the natural healing process after surgery, abdominal trauma, infection, radiation therapy, or drug reactions. Abdominal surgery, particularly laparotomy, is the

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most common cause because these procedures directly impact the peritoneum and surrounding tissues. When the peritoneum is injured, the body initiates a pathological healing response, leading to the proliferation of fibroblasts and the formation of adhesions. The formation of adhesions is part of the body's recovery mechanism. When the peritoneum is affected by surgery, trauma, or infection, even if it appears undamaged on the surface, internal bleeding or inflammation in the affected organs prompts the proliferation of fibroblasts responsible for tissue repair, resulting in adhesion formation. These adhesions disrupt the natural separation between the intestines and other organs, causing them to abnormally connect and affecting intestinal function and the normal operation of other organs. Symptoms of intestinal adhesions may include abdominal pain, vomiting, bloating, and constipation. The severity of these symptoms can range from mild discomfort to severe pain, depending on the extent and location of the obstruction. Diagnosing intestinal adhesions usually requires a comprehensive evaluation of medical history, physical examination, and imaging studies such as X-rays, CT (computed tomography) scans, or MRI (magnetic resonance imaging), which help doctors determine the exact location and cause of the obstruction [2-6].

The treatment options for ASBO are varied, ranging from non-surgical to surgical methods, all aiming to restore bowel function and minimize the risk of future adhesions and obstructions. Non-surgical treatment is the preferred approach, especially in patients without emergent conditions such as peritonitis, bowel strangulation, or bowel ischemia. This treatment includes as (1) Fasting: Allowing the intestines to rest by reducing the contents of the intestines, thereby lowering intestinal pressure. (2) Nasogastric decompression: Using a nasogastric tube to drain the contents of the stomach and small intestine, helping to reduce bloating and abdominal pain. (3) Fluid and electrolyte correction: Administering fluids and electrolytes intravenously to maintain the patient's hydration and electrolyte balance. (4) Nutritional support: Providing nutrition through intravenous or other means if fasting is prolonged. (5) Prevention of aspiration pneumonia: Implementing appropriate care measures to prevent aspiration pneumonia [5-8].

These treatments are effective in most cases, allowing the majority of patients to recover without surgery. However, if there is no improvement within 72 hours, or if symptoms worsen, surgical intervention may be necessary. The purpose of surgery is to physically remove or separate the adhesions to relieve the obstruction. However, surgery itself can also cause new adhesions to form, so it must be considered carefully. Both laparoscopic and open surgeries are options to consider. While there are currently no medications that directly treat adhesions, some drugs can be used to reduce postoperative inflammation, which may indirectly lower the risk of adhesion formation [6-7].

To prevent adhesions and their complications, the main strategies are to minimize surgical trauma and use adjunctive materials to reduce the likelihood of adhesion formation. Laparoscopic surgery, which is performed through small incisions, can indeed reduce trauma and the risk of adhesion formation compared to traditional open surgery. The choice of different energy surgical systems can also impact adhesion formation. For instance, bipolar electrosurgery and ultrasonic devices cause less damage to the peritoneum compared to monopolar electrosurgery [6-10].

The formal name for anti-adhesion measures is adhesion barriers. These barriers can be in the form of solid membranes, gels, or liquids, and their primary function is to act as a physical barrier that separates injured peritoneal surfaces, allowing them to heal without forming fibrous attachments that lead to adhesions. Common adhesion barriers include as (1) Hyaluronate carboxymethylcellulose: A solid barrier most suitable for open surgery but can also be placed laparoscopically. Studies in general and gynecologic surgeries have shown that it reduces adhesion formation and the risk of repeat surgeries due to adhesive small bowel obstruction. (2) Oxidized regenerated cellulose: A solid barrier suitable for open surgery. Research in gynecologic surgeries has shown it reduces the incidence of adhesion formation. (3) Icodextrin: A liquid barrier that is easy to apply in both open and laparoscopic surgeries. (4) Polyethylene glycol: A gel barrier that is easy to apply in both open and laparoscopic surgeries [7-10].

In preventing postoperative adhesions, lifestyle adjustments in terms of exercise and diet are also important. Patients should avoid prolonged bed rest after surgery and be encouraged to engage in moderate exercise to promote gastrointestinal motility and appetite, thereby accelerating recovery. Additionally, postoperative dietary habits should focus on eating small, frequent meals to avoid constipation and reduce the burden on the intestines [4-6].

Overall, postoperative bowel adhesions are a challenge many patients who have undergone abdominal surgery may face, significantly impacting their quality of life and health. Treating postoperative bowel adhesions requires close collaboration from a multidisciplinary team, including surgeons, radiologists, nutritionists, and physical therapists. Through appropriate preventive measures, careful treatment decisions, and comprehensive patient care, most adhesion-related issues can be effectively managed [7-10].

Postoperative adhesive bowel obstruction is a frequent cause of hospital admission in a surgical department. Emergency surgery is needed in a majority of patients with bowel ischemia or peritonitis; most adhesive bowel obstruction can be

managed non-operatively. Many studies have investigated benefits of using oral water-soluble contrast to manage adhesive bowel obstruction. Treatment recommendations are still controversial. Intestinal adhesions commonly occur in patients after abdominal surgery, often between intestines, between intestines and the abdominal wall, or within the peritoneum. Adhesions can lead to symptoms such as abdominal pain, constipation, and bloating, and may result in serious complications such as intestinal obstruction, bowel necrosis, acute peritonitis, and sepsis, which can be life-threatening. Therefore, reducing the occurrence of intestinal adhesions is crucial for abdominal surgery. To minimize adhesions, endoscopic techniques can be employed to reduce the abdominal opening area and thus decrease the probability of adhesion formation. However, this approach requires specialized equipment and surgical skills, making it somewhat challenging. Alternatively, anti-adhesion products can be used to lower the risk of adhesion formation [8-10]. In this study, a murine cecal abrasion adhesion model will be established and simulated common adhesion scenarios observed in patients after abdominal surgery. The efficacy of anti-adhesion gel materials will be then evaluated to assess their anti-adhesion properties by using this model.

2 Material and methods

2.1 Experimental Animals

Adult male Sprague Dawley (SD) rats (n = 3, 6-10 weeks old) were procured from BioLASCO Taiwan Co., Ltd. (Yilan, Taiwan) under specific pathogen-free conditions. These rats were maintained on a standard laboratory diet (No. 5001, LabDiet®; PMI Nutrition International, St. Louis, MO, USA) and provided with distilled water ad libitum throughout the experimental period. The animals were housed in an environment with a room temperature of 24-27°C, humidity ranging from 60% to 70%, and a photoperiod consisting of 12 hours of light and 12 hours of darkness. Following a one-week acclimation period, the study commenced. All animal experiments were conducted in accordance with the guidelines outlined in protocol IACUC-112040, which was approved by the Institutional Animal Care and Use Committee (IACUC) of the Agricultural Technology Research Institute.

2.2 Surgery

All SD rats were deeply anesthetized using 4-5% isoflurane. The surgical site was shaved clean, followed by at least three rounds of disinfection using povidone-iodine and 75% alcohol to ensure surgical site cleanliness. A 4 cm incision was made at the surgical site, and the cecum was exposed. Dry sterile gauze was used to rub the lateral and ventral sides of the cecum in SD rats until visible bleeding points appeared. A 1 cm × 2 cm wound was created on the inner wall of the abdominal cavity adjacent to the cecum in situ using a No. 10 surgical blade. Depending on the control group, the corresponding substance (sterile distilled water) was applied to the cecum and the inner wall of the abdominal cavity.

After relocating the cecum, suturing was performed. Abdominal surgery was conducted separately on postoperative days 3 and 14 to observe the adhesion between the cecum and the inner wall of the abdominal cavity. The abdominal cavity was opened, and adhesion between the cecum and the abdominal cavity was observed. During the process, efforts were made to minimize stretching and displacement between the two to prevent artificial adhesion separation. Adhesion severity was photographed and assessed by a senior veterinarian. After the 14th-day adhesion observation, the contact area between the cecum and the abdominal cavity was rinsed with sterile 1× phosphate-buffered saline at 4°C. The rinse solution was collected and refrigerated before being sent to the designated facility.

2.3 Sample Collections and Treatments

Post-surgery, all rats were housed individually in separate cages. All rats were monitored postoperatively 8-hourly for the first 48 h to observe their behavior, physical well-being and appearance. On the 14th day, rats were euthanized under anesthesia by cardiac blood collection. After death, adhesions between the cecum and its serosa, adhesions between the cecum serosa and the adjacent intestinal serosa, and adhesions between the cecum serosa and the peritoneum of the abdominal wall were collected. These tissues were fixed in 10% neutral buffered formalin, followed by paraffin embedding for tissue sectioning. The sections were stained with H&E and Masson's trichrome for histopathological analysis.

2.4 Histopathological Examination

The peritoneum and cecum of rats from different groups were fixed in 10% neutral buffered formalin for at least 24 hours. Subsequently, tissues were trimmed longitudinally and placed into embedding cassettes. Following dehydration, infiltration with paraffin wax, and embedding procedures, paraffin tissue blocks were prepared. Tissue sections of 4 μm thickness were then cut using a rotary microtome (RM 2145, Leica). These sections were stained with Hematoxylin & Eosin (H&E) and observed under an optical microscope (BX51, Olympus) to evaluate the severity of adhesion (fibrous

tissue thickness, degree of neovascularization, and Masson's trichrome staining) in the aforementioned organs [8-10]. The criteria for tissue pathology assessment were as follows:

- Fibrous tissue thickness: 0 = absence of fibrous tissue; 1 = thin fibrous tissue; 2 = moderate thickness of fibrous tissue; 3 = thick fibrous tissue.
- Degree of neovascularization score: 0 = no vascular proliferation; 1 = mild vascular proliferation; 2 = moderate vascular proliferation; 3 = extensive vascular proliferation.
- Masson's trichrome staining: 0 = absence of blue-purple fibers; 1 = sparse blue-purple fibers; 2 = moderate amount of blue-purple fibers; 3 = dense blue-purple fibers.

2.5 Statistical Analysis

All values are presented as mean ± SD (standard deviation). One-way analysis of variance was conducted using Graphpad Prism 5 statistical software to analyze. A significance level of $p < 0.05$ was considered statistically significant.

3 Results

3.1 Scoring of Adhesion Formation

The gross adhesion severity assessment was recorded. The adhesion status between the cecum and abdominal wall was observed on postoperative days 3 and 14. Outcome measures were performed on postoperative day 14. All rats were humanely killed on postoperative day 14 and scored based on the presence and severity of adhesions using a previously validated adhesion scoring system Table 1. This scoring system takes into account the number, strength and distribution of adhesions formed. The score of the day 3 post-surgery operation was significantly higher than that on the day 14 ($p < 0.05$) (Figure 1 and Table 2).

Table 1 Scoring criteria for adhesion formation between the cecum and abdominal wall

Score	Description
0	No adhesions
1	Thin filmy adhesions
2	More than one thin adhesion
3	Thick adhesion with focal point
4	Thick adhesion with planar attachment
5	Very thick vascularized adhesions or more than one planar adhesion

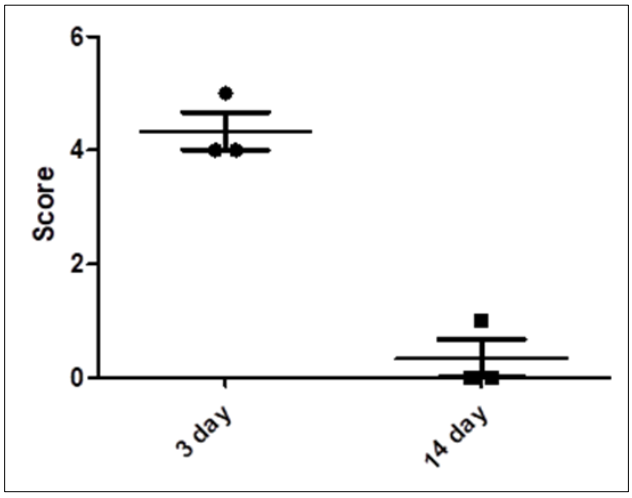

Figure 1 Adhesion formation score in all rats on postoperative days 3 and 14

Table 2 Scoring of adhesion formation in all rats was observed on postoperative days 3 and 14

Surgery induction	No. of rats		
	C1	C2	C3
Day 3 post-surgery induction	5 score	4 score	4 score
Day 14 post-surgery induction	1 score	0 score	0 score

3.2 Histopathological Examination

On postoperative day 14, rats were euthanized under anesthesia, and cardiac blood was also collected for the further analysis. The caecum, and adhesions between the cecal adventitia and adherent, adjacent intestinal serosal surfaces, and between the adventitial aspect of the caecum and the parietal peritoneum of the abdominal wall, were collected and immersion-fixed in 10% neutral buffered formalin. These tissues were then paraffin-embedded, cut at 4 µm, and stained with hematoxylin and eosin (H&E) (Figures 2A-B). Duplicate sections were also stained by the Masson's trichrome technique to demonstrate collagen deposition in fibrous adhesions (Figures 3A-B). Severity scores were assigned by a senior pathologist for fibrous tissue thickness, degree of neovascularization, and Masson's trichrome staining in the peritoneum and cecum of rats.

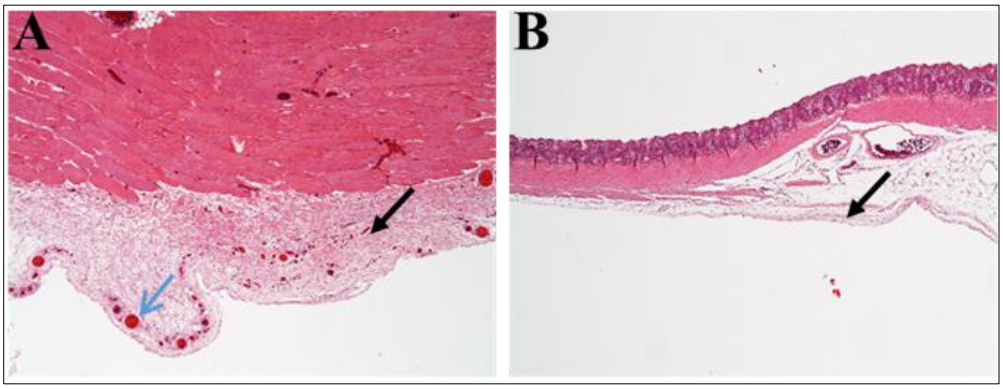

Figure 2 Histological changes in peritoneal and cecal tissues. Black arrows indicated fibrous tissue and a blue arrow indicated neovascularization. A: Peritoneal tissue of the group, B: Cecal tissue of the group. H&E stain. The magnifications were at 40× and 100×

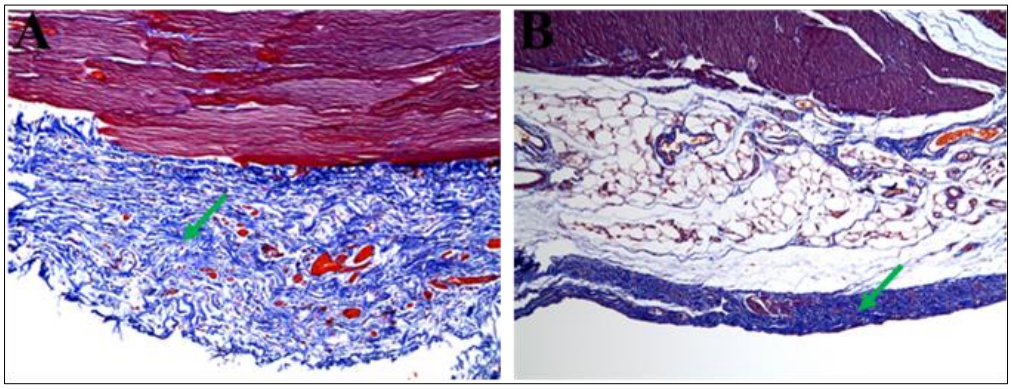

Figure 3 Histological changes in peritoneal and cecal tissues. Green arrows indicated fibrous tissue. A: Peritoneal tissue. B: Cecal tissue. Masson's trichrome stain. The magnifications were at 40× and 100×

Regarding the evaluation of fibrous tissue thickness, the severity scores for the peritoneum and cecum of the group rats were assessed. The total scores for three rats were 4, 3, and 2, respectively (Table 3). Regarding the evaluation of neovascularization, the severity scores for the peritoneum and cecum of the control group rats were assessed. The total scores for three rats were 3, 2, and 2, respectively (Table 3). Regarding the evaluation of Masson's trichrome staining, the severity scores for the peritoneum and cecum of the control group rats were assessed. The total scores for three rats were 4, 3, and 3, respectively (Table 3). For the comprehensive assessment of the severity of the three evaluation

indicators for the peritoneum and cecum of the rats, the results showed that the comprehensive scores for the severity were 11, 8, and 7, respectively (Table 3).

Table 3 Assessment criteria scores for peritoneum and cecal tissues in rats

Assessment criteria	No. of rats		
	C1	C2	C3
Peritoneum			
Fibrous tissue Thickness	3	2	2
Degree of neovascularization	3	2	2
Masson's trichrome stain	3	2	2
Cecum			
Fibrous tissue thickness	1	1	0
Degree of neovascularization	0	0	0
Masson's trichrome stain	1	1	1
Comprehensive score	11	8	7

4 Discussion

Adhesive small bowel obstruction (ASBO) is defined as an obstruction of the small intestine resulting from adhesions that develop following previous abdominal or pelvic surgeries. Adhesions are bands of scar tissue that form between two organs, loops of the intestine, or abdominal walls that are not normally connected. Individuals who undergo open abdominal surgery may develop adhesions postoperatively, which are a common cause of small bowel obstruction [8-10, 1-3].

Postoperative adhesive small bowel obstruction (SBO) is a frequent cause of hospital admission, accounting for 16-20% of hospitalizations due to acute abdominal pain in surgical departments. The Bologna Guidelines for Diagnosis and Management of Adhesive Small Bowel Obstruction, revised in 2018, recommend an initial medical treatment for 72 hours before considering surgical intervention, except in cases where there are signs of severe complications such as strangulation, peritonitis, or intestinal ischemia. This therapeutic strategy for adhesive SBO remains controversial due to a documented 30-40% risk of failure with non-operative management. Many studies have explored the benefits of using oral water-soluble contrast agents to manage adhesive SBO and predict the need for surgery, but the optimal timing for administration remains debated [11-13].

Adhesion formation following abdominal surgery is widely recognized as nearly inevitable and a significant cause of morbidity. Although novel treatments have been proposed, the pre-clinical evaluation of these treatments is hampered by the lack of suitable small animal models, primarily due to inconsistency in adhesion formation in positive control animals [14-16]. In this study, we propose the establishment of a rat model of abdominal adhesions to evaluate the anti-adhesive properties of new agents. In this study, three days post-surgery, the group exhibited fewer intra-abdominal adhesion upon gross observation. By day 14 post-surgery, no apparent adhesions were observed. It is hypothesized that during the observation of intra-abdominal adhesions on day 3 post-surgery, the manipulation of the cecum (removal and repositioning into the abdominal cavity) might have displaced the adhered areas, leading to less noticeable adhesion formation by day 14. Post-sacrifice sampling revealed that the group had thicker fibrous tissue, increased neovascularization, and higher collagen accumulation in the peritoneum.

Combining all the results, it was shown that three days post-surgery, the group exhibited more severe adhesions upon gross examination. By day 14 post-surgery, histopathological analysis revealed thicker fibrous tissue, increased neovascularization, and higher collagen accumulation in the peritoneum. Therefore, a postoperative bowel adhesion rat model was successfully established and is reliable. This model can be safely used to test the efficacy of novel anti-adhesive formulations to prevent intra-abdominal adhesions.

5 Conclusion

Three days post-surgery operation, the exhibited more severe adhesions upon gross examination was found. By day 14 post-surgery operation (animal sacrifice time), the histopathological examination revealed that exhibited thicker fibrous tissue thickness, increased neovascularization, and higher collagen accumulation in the peritoneum of the experimental rats. Therefore, based on the results of this experiment, a postoperative bowel adhesion model in rats has been successfully established.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC 112040 approved by the IACUC ethics committee.

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