

Impact of a Hydrophilic Polyethylene Glycol-Based Adhesion Barrier After Experimental Celiotomy in Rabbits

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Abstract

A hydrophilic polyethylene glycol-based adhesion barrier (SprayGel™) was tested on intra-abdominal adhesion in conventional celiotomy in rabbits. Twenty healthy male rabbits of the local breed were randomly divided into two equal groups: control and treatment. All the experimental rabbits were first subjected to mid-line conventional celiotomy under general anesthesia. The ascending colon was tracked out of the laparotomy opening, and a sterile soft toothbrush was used mechanically to abrade its serosal and subserosal layers. Before closing the celiotomy opening, 2 ml of sterile saline solution was applied intraperitoneally into the abdominal cavities of the control group. SprayGel™ was applied to the test rabbits' abraded colons before their closures. Postmortem examinations were performed on the 14th and 21st postoperative days to assess intraperitoneal gross pathological changes, and biopsies were collected for histopathological examinations. The macromorphological and micromorphological examinations showed mild postoperative intra-abdominal adhesion changes in the treatment compared to the control. This was confirmed by significantly lowered gross intra-abdominal adhesion scores with the slightest micromorphological changes in the SprayGel™-treated rabbits. Higher scores of gross transperitoneal adhesions were observed in the control rabbits. In addition, histopathological changes were seen as serosal scars, consisting of raised areas of fibrous tissue replacing the muscularis layer of the mechanically abraded ascending colon, often to the submucosa level. SprayGel™ was an effective barrier substance to reduce intra-abdominal adhesions following celiotomy and experimentally induced mechanical serosal and subserosal abrasions to the ascending colon in rabbits.

Keywords: Postoperative adhesion, celiotomy, intra-abdominal adhesion, hydrophilic polyethylene, anesthesia.

Introduction

Adhesions are fibrinous or fibrous bands that form abnormal unions between two or more surfaces, generally lined with a serosal layer. They may appear as thin layers or thick fibrous tissues (1). Intra-abdominal adhesions are expected consequences of serosal repair in 93% to 100% of patients undergoing laparotomy and may cause severe complications like infertility, intestinal obstruction, and pelvic pain (2). Bowel adhesion typically begins within the first few days postoperation and may form fibrous bands around part of the intestine. As scar tissue restricts the motion of small intestines, the bowel may become blocked. Symptoms may include pain, spasms, vomiting, difficulty passing gas, bowel movements, and abdominal swelling (3).

Adhesions develop as a result of the body trying to repair itself. If a healing organ comes into contact with another healing part, scar tissue may connect the surfaces, causing adhesion. Inflammatory mediators are released from the circulation, and capillaries dilate, allowing leukocytes, erythrocytes, and platelets to concentrate in a fibrinous exudate bundle at the injury site. The exudates transform into an adhesion where collagen fibers deposit and vascularization occurs (4).

Applying liquids and membranes (e.g., phospholipids) prevents adhesion formation by inhibiting adhesiogenesis without restraining wound healing (5). Preventing surface contact using a barrier between raw tissue surfaces is one way to prevent

adhesion by mechanically separating the surgical surfaces and keeping them apart. SprayGel™ is a barrier substance used to prevent postsurgical adhesions. It is a smooth, water-based coating material, also known as hydrogel. SprayGel™ is a polyethylene glycol-based product that has become available to reduce postoperative adhesions' incidence, extent, and severity. The material is sprayed in solution form using a special applicator designed for minimally invasive surgical instrumentation, and it can also be applied during laparotomy. A two-component liquid is applied to the tissue using an applicator. The gel sticks to and coats the surgical trauma's surfaces (6). However, the product's efficacy has not been tested in small animals. Accordingly, the current study was designed to investigate the adhesion-preventive activity of SprayGel™ in an experimentally established transperitoneal adhesion by mechanical abrasion of the ascending colon's serosal and sub-serosal layers in rabbits. In addition, the study tested the effectiveness of the mechanical gel barrier on the ascending colon's abraded parts for preventing or reducing intra-abdominal adhesion after celiotomy in rabbits.

Materials and Methods

Animals and grouping: Twenty local breed adult rabbits (1.5–2.0 kg) were used in this experimental study. The rabbits were reared at room temperatures of 22–25°C and fed barley and vegetables, given twice daily, with free access to water. The twenty experimental rabbits were divided randomly

into equal control and treatment groups. Each rabbit in both groups received the same prophylactic daily dose of the systemic antibiotic enrofloxacin (5 mg/kg) subcutaneously from the day before the celiotomy was conducted and continued for three consecutive days postoperatively. The Animal Care and Use Committee (ACUC) at the College of Veterinary Medicine/University of Sulaimani permitted this study through the approval number AUP-2021-4.

Procedures for celiotomy and adhesions

induction: The preoperative preparations for the experimental rabbits subjected to conventional midline laparotomy included withholding food for 12 hours before the laparotomy. The laparotomy was done under general anesthesia with a mixture of ketamine HCl (50 mg/kg) and xylazine HCl (5 mg/kg) given via the intramuscular route to induce anesthesia. The anesthetized rabbits were restrained on dorsal recumbency, and their ventral abdominal aspects were aseptically prepared, from xiphoid to pubic, all together with the right and left flank regions. Afterward, the surgical field was disinfected with tincture of iodine (2.5%), and sterile drapes were used to cover the rabbit's body.

Celiotomy was performed through a midline ventral abdominal incision from the umbilicus to the pubis. After exploring the abdominal cavity, the ascending colon was identified and gently tracked out of the celiotomy opening. Mechanical abrasion to a circumscribed field of the serosal and subserosal layers (2×2 cm²) was performed

using a sterile toothbrush (7).

Following abrasion induction, the celiotomy openings' abdominal muscles in all experimental rabbits were closed by simple continuous mattress pattern suture without suturing the peritoneum using Vicryl NO 3/0 suture material. Before applying the last abdominal wall stitch, sterile saline solution (2 ml) was injected into the abdominal cavities of the control group rabbits. Finally, the muscles, subcutaneous tissue, and skin were closed routinely using number 3/0 silk. The same operation was done to the treatment group, and the SprayGel™ was applied to the abdominal cavities of the rabbits. The SprayGel™ polymer kit contains components that, when mixed, form an absorbable adhesion barrier. The reconstituted polymer kit syringe assembly is attached to a system applicator that allows the polymer to be applied to tissues (8). The sprayed material (SprayGel™) is a blue-colored hydrogel film formed when the two water-based polyethylene glycol solutions were mixed. Upon application, a quick cross-linked biocompatible, flexible, and absorbable hydrogel was formed instantly on the sprayed portion of the mechanically abraded portion of the ascending colon tissue. This procedure was practiced through a prototype sprayer made of stainless steel with a 5mm quadruple lumen.

Postoperative care: The closed surgical wound was gently washed with sterile saline solution, swabbed with povidone polyvinyl iodine (1%), and sprayed with oxytetracycline wound spray (4%, Intervet, China) to prevent bacterial contamination.

Enrofloxacin (Baytril[®], Germany) was administered subcutaneously daily for three consecutive days at a dosage of 5 mg/kg, with the local wound care and application of the wound spray oxytetracycline (10%), once daily for seven successive days. The skin stitches were removed on the seventh postoperative day.

Gross intra-abdominal macromorphological examination for adhesion scoring and biopsies collection:

In both experimental groups, macromorphological changes in the celiac organs were observed in the rabbits. On the 14th and 21st days, five rabbits from each group were sacrificed with anesthesia using a combination of xylazine and ketamine, followed by exsanguination. A U-shaped incision was made in the anterior abdominal wall, and the skin was retracted backward for maximum exposure. Two independent surgeons examined adhesions macroscopically, and the degree of adhesion formation was graded according to a scale suggested by (9) (Table 1). The gross intra-abdominal pathological study for transperitoneal adhesion formation included macroscopic features of the abdominal organs and the degree of adhesion formation within the viscera or adjacent organs. Tissue

samples were taken from each rabbit of both groups without tissue distortion or damage.

Micromorphological processing and examinations: Biopsy samples collected from the different sites of the abraded region of the ascending colon, adhered organs, and abdominal wall were resectioned and preserved instantly in a sufficient amount of 10% buffered formalin solution. The slides were examined under a light microscope and evaluated blindly by the same pathologist to determine the general structure, fibroblastic activity, and fibrosis following a scaling system described previously (10) (Table 2). The formalin-fixed samples (10%) were processed, sectioned, and stained. The procedure was performed in the Laboratory of Histopathology, Department of Pathology, Ali Kamal Hospital, Sulaimani. The histological sectioned films (5–6 mm) were stained with hematoxylin and eosin (11).

Statistical analysis: Statistical Package for Social Science (SPSS) version 24.0 was used for the statistical analyses. An independent samples T-test was used to determine differences between two independent populations. A probability value of < 0.05 was regarded as significant, and data were presented as mean ± standard error of the mean (S.E.M.)

Table 1. Macromorphological grading of adhesion formation.

Grade	Description
0	No occurrence of adhesion
1	Presence of an adhesion band from the viscera to the abdominal wall or between the visceral organs
2	Occurrence of two adhesion bands from the viscera to the abdominal wall or between the visceral organs
3	Observation of more than two adhesion bands from the viscera to the abdominal wall between the visceral organs
4	Several dense visceral adhesion bands are attached directly to the abdominal wall.

Adapted from (12).

Table 2. Micromorphological scale system used for histopathological assessment of the degree of fibrosis in the adhered tissues.

Degree	Histopathological observation
0	No fibrosis is observed.
1	Occurrence of thin cellular fibrosis clusters
2	Broad fibrosis areas with decreased vascularization
3	Fibrosis areas comprising a thick cluster of collagen fibers

Adapted from (12).

Results

Results of gross inspections of adhesion formation: On the 14th day post-surgery, grade 1 adhesion was observed in two rabbits in the control group, and three had grade 2 adhesions. The adhesion was represented as filmy, transparent, white, thin, fibrous adhesions between the visceral abdominal wall and ascending colon parts. A white coat of fibrinous layer at the abrasion site on the serosal layer of the ascending colon was observed, and the coat was extended to bridge the adjacent parts of the viscera (Figure 1. A) On day 21 post operation, variable degrees of adhesion were found and graded as grade 2 in four rabbits and grade 3, characterized by a thick fibrous adhesion, in one control rabbit (Table 3, Figure 1. B).

The results of the adhesion scoring system (only extension) during the 14th and 21st postoperative days are shown in Table 1. The average values of adhesions in the

Lower adhesion scores were recorded in the treatment group rabbits, where the induced mechanical abraded ascending colon was sprayed with the SprayGel™. On the 14th postoperative day, the rabbits showed adhesion grades ranging between 0 in one rabbit and 1 in the remaining four rabbits. A fibrinous-like coat was on the ascending colon serosa at the abrasion site (Figure 1. C). On the 21st postoperative day, grade 0 was seen in one rabbit, and grade 1 adhesion was found between the loops of the intestine in three rabbits. In one of the five experimental rabbits, a grade 2 adhesion band was found bridging the abdominal wall and urinary bladder (Figure 1.D).

treatment group rabbits were significantly lower than in the control group. The average adhesion grade was 1.6 in the control group on the 14th postoperative day. While on day

21, the average value increased to 2.2. These results indicate that the adhesion extension in the control group of rabbits increased with time. However, the average adhesion grades in all rabbits in the treatment group were 0.8 and 1.0 after 14 and 21 days of operation, respectively. This result means that the adhesion extension in the treatment group was significantly lower ($p < 0.05$).

Histopathological evaluation of postoperative adhesions: On the 14th postoperative day, microscopic sections of the serosal layer of the ascending colon of the control group showed a loss of serosal surface integrity. Fibroblast proliferation invaded the fibrin matrix and produced an extracellular matrix. New blood vessel formation (angiogenesis) was seen (Figure 2. A), accompanied by infiltration of lymphocytes and macrophages, showing loss of the serosal surface integrity, congestion, and infiltration of lymphocytes

and macrophages (Figure 2. B).

On the 21st postoperative day, microscopic sections of the serosal and muscular layers of the ascending colon of the control group rabbits showed loss of the serosal surface integrity, fibrosis, heavy infiltration of lymphocytes and macrophages, and remnants of adhesion bridges (Figure 2. C). On the 14th postoperative day, the histopathological sections from the treatment group rabbits showed mild fibroblast proliferation and moderate to high infiltration of lymphocytes and macrophages within the serosal layer without invasion of the fibrin matrix (Figure 3. A).

On the 21st day post-surgery, healing of the ascending colon's mechanically abraded serosal and muscular layers was noticed, accompanied by slight fibrosis and infiltration of lymphocytes and macrophages within the serosal layer (Figure 3. B).

Table 3. Mean postoperative adhesion scores in the control and treatment groups

Group	Grade of adhesion		
	14 days post operation	21 days post operation	Probability*
Control	1.60 ± 0.25	2.20 ± 0.20	0.09
Treatment	0.80 ± 0.20	1.00 ± 0.32	0.61
Probability*	0.04	0.01	

Values represent the means of five rabbits/group ± S.E.M. *: probability values ≤ 0.05 indicate a statistically significant difference.

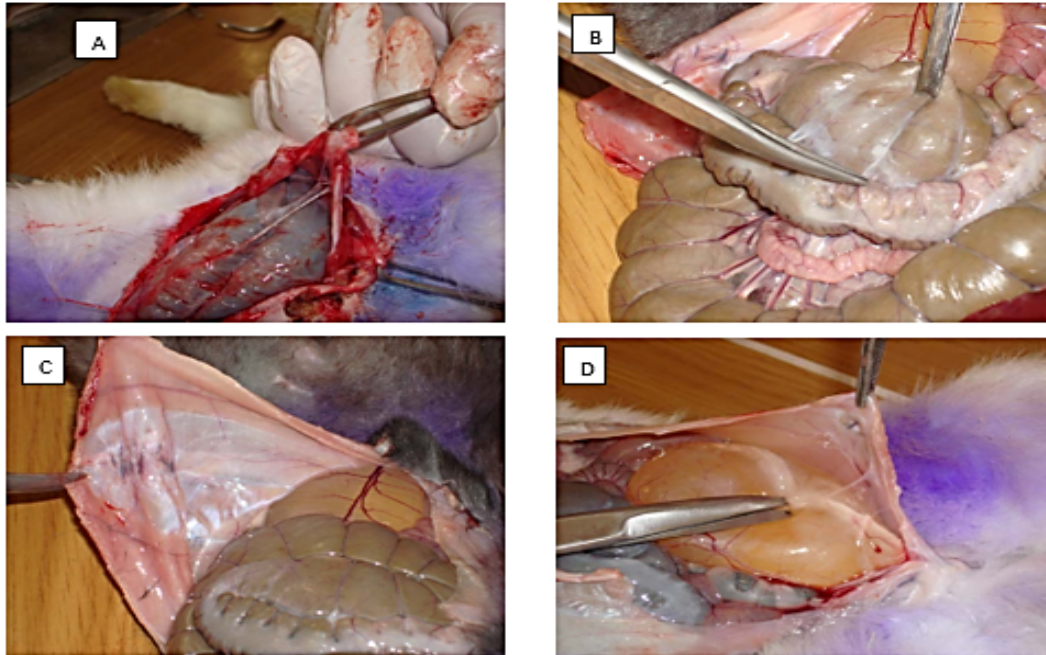


Figure 1. A: The abdominal cavity of a control group rabbit 14 days after the operation. Bands of transparent white thin fibrous adhesions developed between the visceral abdominal wall and ascending colon (grade 2). B: The abdominal cavity in a control group rabbit after 21 days of celiotomy. A moderate fibrinous coat was found covering the serosa of the ascending colon adjacent to the abraded site (grade 3). C: The abdominal cavity in a treatment group rabbit on the 14th postoperative day. Adhesion was found between intestinal loops (grade 1). D: The abdominal cavity in a treatment group rabbit after 21 days of celiotomy. A moderate adhesion band bridges the abdominal wall and the urinary bladder (Grade 2).

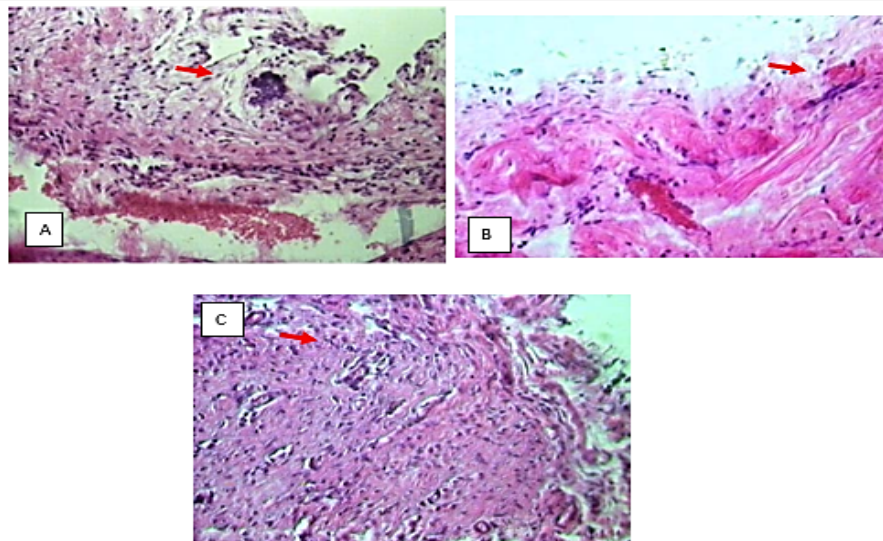


Figure 2. A: Micro photo section of the ascending colon serosal layer in a control group rabbit after 14 days of celiotomy. The section shows loss of serosal surface integrity, blood vessel congestion, and infiltration of lymphocytes and macrophages (red arrow) $\times 200$. B: Microscopic section of the serosa and muscularis of the ascending colon in a control rabbit on the 14th postoperative day, showing loss of the serosal surface integrity, new blood vessels formation (angiogenesis, red arrow), congestion, and infiltration of lymphocytes and macrophages ($\times 200$). C: Serosal and muscular layers of the ascending colon of a control rabbit on the 21st day post operation. Angiogenesis, loss of the serosal surface integrity, and heavy infiltration of lymphocytes and macrophages were seen (red arrow) ($\times 200$).

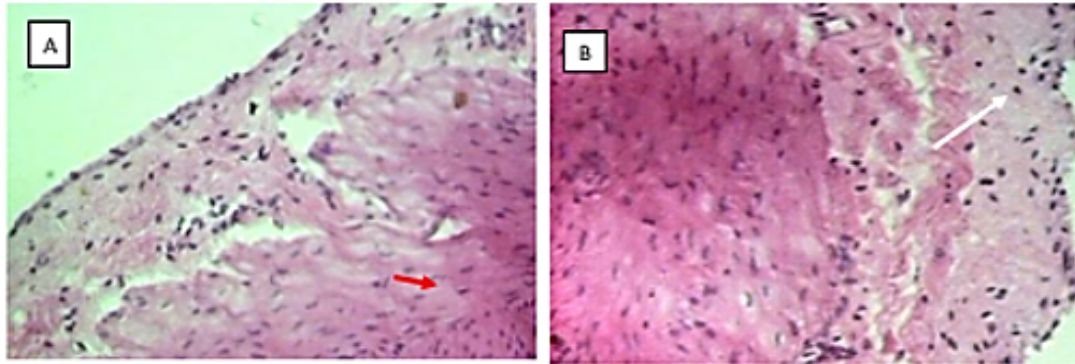


Figure 3. A: Microscopic section of the serosa in a treatment group rabbit after 14 days of celiotomy showing moderate to high infiltration of lymphocytes and macrophages and few fibroblasts (red arrow) without signs of extracellular matrix production ($\times 200$). B: The serosal and muscular layers of the ascending colon (white arrow) of a rabbit of the treatment group show slight fibrosis and infiltration of lymphocytes and macrophages within the serosal layer (H and E, $\times 200$).

Discussion

Adhesions are unfortunate consequences of surgeries. Adhesion formation is a complicated process that involves many steps and substances. The proteinaceous exudates secreted due to the trauma to the intestine are not completely reabsorbed from the peritoneal cavity, and a fibrin matrix is laid down. If this matrix remains, it will become organized, resulting in adhesions (13). Although its pathophysiology is widely elucidated, a definite solution to adhesion is not available yet. Adhesions occur following peritoneal injury, tissue ischemia, or by the effect of exogenous materials. Fibrinogenesis and fibrinolysis occur when two injury areas are in contact, and the balance between these processes will be altered, leading to adhesion establishment (14).

Numerous advances, like careful laparoscopic or microsurgical techniques or various adhesion barriers, were suggested to prevent adhesion. However, no method was utterly effective in randomized controlled tests. Various commercially available substances have been implemented to reduce postoperative intra-abdominal adhesions during the past two decades. With different compositions and characteristics, these materials have limitations and advantages when used in clinical settings. Barriers have been used widely to prevent adhesion. Typically, a barrier should be nonadhesive, biocompatible, resorbable, adherent to the injured surface, efficacious on an oozing surface, applicable through a laparoscope, and cheap. Unfortunately, such a perfect barrier is unavailable (15). In contrast,

SprayGel™ was effective in reducing adhesion formation, according to the current study results.

Many experimental models are proposed to minimize peritoneal adhesions. Scraping is an efficient way to induce peritoneal adhesions since it comprises two stages. The stages are direct mechanical intestinal wall damage due to gauze scraping and vascular ischemia from clamping. We used this model in our study since it mimics abdominal surgery. The peritoneum's postoperative intra-abdominal adhesions usually develop within 7–10 postoperative days and persist after two weeks. Hence, it is crucial to prevent adhesion formation at this stage. We chose to perform a re-laparotomy after 14 days since it was previously reported that adhesion scores peak after that time (16).

Adhesions are associated with five major factors: tissue ischemia, trauma to the serosa, inflammation, infection, and the presence of foreign material (17). Inflamed peritoneal tissue reduces abdominal fibrinolytic activity (18); hence, the intra-abdominal fibrin persists with subsequent adhesion formation (19). In the present study, the procedure and tool used for induction of mechanical abrasion to the ascending colon's serosal and subserosal layers of the rabbits enhanced immediate fibrinous activity associated with the formation of transperitoneal adhesion. Additionally, the conventional celiotomy technique performed on the experimental rabbits in both groups in the present study necessitated exposure of the rabbit's

abdominal viscera to various mechanical and environmental injuries that may produce further serosal damage, which could induce noticeable adhesions.

Postmortem examinations for adhesion scoring and biopsy collections in the treatment group rabbits on the 14th and 21st postoperative days revealed lowered rates in adhesion scores. They encouraged the production of a promising strategy against postsurgical adhesion formation by the locally applied SprayGel™ barrier. The barrier applied on the surgically traumatized ascending colon surfaces effectively protected the exposed subserosal layer and allowed mesothelial regeneration, thus preventing partial adherence of the injured tissue with the adjacent structures and reducing adhesion formation. The polyethylene glycol-based product SprayGel™ has become available to reduce postoperative adhesions' incidence, extent, and severity (20). It is primarily a tissue-friendly, water-based compound that fulfills the need for an efficacious, safe, easy-to-use method to prevent postoperative adhesion successfully. In the present study, SprayGel™ significantly reduced adhesion formation ($p < 0.05$) and had no higher scores of adhesion formation in the treatment group rabbits.

Most adhesions in the treatment group were developed in the non-SprayGel™ covered areas within the abdomen cavity. Application of the physical barrier via the air-assisted sprayer provided the polymer of two synthetic modified polyethylene glycol substances that polymerized together

quickly when mixed at the application site to prevent postoperative intra-abdominal adhesions by limiting tissue apposition during the time following surgery when adhesion usually forms (21).

Adhesions in the rabbits in both groups (control and treatment groups) were evaluated on the extent of adhesion establishment based on a scale of 0 to 4. This allowed a distinct identification of the formation and extent of adhesion between the experimental rabbits. Most adhesion grading methods are based on adhesions: firm or weak and fibrinous or fibrous. Other ways include evaluating the percentage of the traumatized area-covering adhesions (22), measurement of surface area involved, grading of adhesion, replacement of portions of the muscularis layer of the organ with fibrous tissue of variable vascularity, and fibrous tissue projections from the peritoneal surface and fibrous adhesions (23, 24).

The gross appearance of transparent bands of thin white fibrous adhesions was noted between the visceral abdominal wall and ascending colon's parts (Figure 1. A and 1. B) in the control group of rabbits after 14 postoperative days. The white-colored thick fibrinous layers at the abraded surface of the serosal layer of the ascending colon were extended to other abdominal viscera parts in the control group of rabbits. On the 21st postoperative day, a moderate band of adhesion (grade 2) was seen bridging between the abdominal wall and the parietal surface, joined to the visceral aspect of the abdominal wall. In contrast, thick adhesion bands were not observed during postmortem

exploration of the treatment group rabbits at both sessions. This outcome indicated the effectiveness of the barrier agent SprayGel™ on abrasions, which reduced the transperitoneal adhesion resulting from factors rather than the local mechanical trauma to the ascending colon, such as the conventional celiotomy.

Based on the histological evaluation, the creation of adhesions by serosal scarification in this experiment appears efficacious for two reasons: The severity of physical trauma's serosal and muscular damage, and the deposition of fragments of non-degradable foreign material at the experimental site, causing a persistent, multifocal inflammatory reaction (25). The microscopic section of the serosal layer of the ascending colon of a rabbit of the control group on the 14th day showed a loss of the serosal surface integrity. On the 21st day, loss of the serosal surface integrity, fibrosis, blood vessel congestion, heavy infiltration of lymphocytes and macrophages, remnants of adhesion bridges, and loss of the serosal surface integrity were found. On the other hand, the gross appearance of adhesions in rabbits of the treatment group on the 14th postoperative day at the abrasion site was fibrinous coat-like on the serosal surfaces of the abraded gut. On the 21st postoperative day, the adhesion bands were joining between the abdominal wall and the visceral aspect of the abdominal wall.

SprayGel™ acts as a coat on the traumatized tissue surface that prevents tissue surfaces from sticking with others and adhering to them with no signs of bacterial infection on

gross and histological evaluation (26).

Conclusion

Experimentally induced adhesion can be performed successfully by mechanical abrasion of the ascending colon's serosal and subserosal layers. Applying the SprayGel™ barrier appeared to reduce adhesion during conventional celiotomy effectively. However, the effectiveness of SprayGel™ in comparison with other bioabsorbable anti-adhesive barriers needs to be elucidated in future studies.

Conflict of Interest

The authors declare that they have no conflict of interest.

Financial Disclosure

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Animal Rights Statement

The Animal Care and Use Committee (ACUC) at the College of Veterinary Medicine, University of Sulaimani, approved this study through the approval number AUP-2021-4.

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تأثير حاجز التصاق البولي إيثيلين غليكول المحب للماء على تكوين التصاق ما بعد بضع الظهارة في الأرانب

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الخلاصة

تم اختبار تأثير حاجز التصاق البولي إيثيلين جليكول المحب للماء (SprayGel™) على تكوين الالتصاق داخل البطن بعد البضع التقليدي للبطن في الأرانب. تم إجراء الدراسة على عشرين ذكر من السلالة المحلية ، قسمت عشوائياً وبالتساوي إلى مجموعتي ضابطة ومعالجة. تم إخضاع جميع الأرانب التجريبية لأول مرة لبضع البطن التقليدي في الخط الوسطاني تحت التخدير العام. تم تعقب القولون الصاعد من فتحة شق البطن ، واستخدمت فرشاة أسنان ناعمة معقمة ميكانيكياً لمحو طبقاته المخاطية وتحت المخاطية. قبل إغلاق فتحة بضع البطن، تم وضع 2 مل من المحلول الملحي المعقم داخل الصفاق في تجاويف البطن لأرانب التحكم. تم تطبيق SprayGel™ على القولون المتأكل لأرانب الاختبار قبل إغلاقها. تم إجراء فحوصات ما بعد الهلاك في اليومين الرابع عشر والحادي والعشرين بعد الجراحة لتقييم التغيرات المورفولوجية الإجمالية داخل الصفاق، وتم جمع الخزعات لإجراء فحوصات مجهرية. أظهرت الفحوصات الشكلية العيانية والدقيقة تغيرات الالتصاق داخل البطن بعد العملية الجراحية في العلاج مقارنة بمجموعة السيطرة. تم تأكيد ذلك من خلال انخفاض درجات الالتصاق داخل البطن بشكل ملحوظ مع أدنى تغيرات دقيقة في الأرانب المعالجة بـ SprayGel™ لوحظت درجات أعلى من التصاقات عبر الصفاق على أرانب بمجموعة السيطرة ، بالإضافة إلى التغيرات النسيجية المرضية التي ينظر إليها على أنها ندوب مخاطية، تتكون من مناطق مرتفعة من الأنسجة اللبينة لتحل محل الطبقة العضلية للقولون الصاعد المتأكل ميكانيكياً ، غالباً إلى مستوى تحت المخاطية. كان SprayGel™ مادة حاجزة فعالة لتقليل التصاقات داخل البطن بعد بضع البطن والتآكل الميكانيكي المخاطي وتحت المخاط المستحث تجريبياً إلى القولون الصاعد في الأرانب.

لكلمات المفتاحية: التصاق ما بعد الجراحة، بضع البطن، التصاق داخل البطن، بولي إيثيلين المحبة للماء، تخدير.