

Effects of Polyethylene Glycol (SprayGel™) on Dogs Undergoing Conventional Cystotomy

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Summary

The effectiveness of SprayGel was determine in this study as a barrier to reduce the frequency for the development of abdomino-pelvic adhesions following cystotomy in dogs. Eight dogs were divided into two equal groups; control (A) and treatment (B). A routine cystotomy was experimentally produced in all dogs by conventional mid-ventral laparotomy. The cystotomy wound was closed in both groups by a single layer continuous Cushing pattern. In the treatment group, the closed cystotomy site was sprayed by Spray Gel™, while in the control one, the wound was irrigated with physiologic saline solution. Two dogs from both groups were subsequently euthanized at 14th and 28th days post operatively and were examined for gross intraperitoneal adhesions formation. Macroscopically, a vast difference was detected in the level of adhesion formation between the two groups and statistically the scores for the adhesions was significantly different ($P<0.05$) only at the 14th post-cystotomy day. This concludes that Spray Gel™ acts as an effective barrier against and actively reduced adhesions formation following cystotomy.

تأثر البولي أثلين كلاكول (سبراي جيل) في الكلاب التي خضعت لعملية بضع المثانة البولية
التقليدي

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**مديرية بيطرة السليمانية/ السليمانية- إقليم كردستان العراق

***وزير التجارة في حكومة العراق/ جمهورية العراق

الخلاصة

في هذه الدراسة تم التحقق من اثر مادة السبراي جيل (رذاذ هلامي) في الحد من انتشار الالتصاق في الجوف
البطني- الحوضي الناجمة عن عملية فتح المثانة التجريبي في الكلاب. تم استخدام ثمانية كلاب في التجربة وقسمت
بالتساوي إلى مجموعتين؛ سيطرة (أ) وعلاج (ب). وتم الوصول إلى المثانة البولية بالطرق الجراحية الروتينية وتحت
ظروف معقمة بعد عملية بضع البطن التقليدية من الخط الوسطي لجدار البطن. وبعد استخراج المثانة، تم إجراء شق
تام في جدارها البطني بطول 3-5 سم. وبعد الكشف عن ما بداخل المثانة تم غلقها وفي كلتا المجموعتين بخياطتها
بطبقة واحدة وبنمط غرزكشن المستمر باستخدام خيط بولي كلاكتين 910 حجم 0-3. وبعد إتمام خياطة شق المثانة،
تم رش سطح الجدار و الجرح لمجموعة العلاج (ب) بطبقة من مادة السبراي جيل، وبمحلول الملح الفسلجي العقيم في

مجموعة السيطرة تم انتخاب كلبين عشوائيا من كل مجموعة لقتلهم بطريقة رحيمة وإعادة فتح البطن فيها بعد مرور 14 و28 يوما وعلى التوالي من تاريخ إجراء العملية، وذلك للكشف العيني على مدى تكون الالتصاق في جوف البطن - الحوضي فيها. وبينت نتائج فحص الجثة العيني وجود تباين كبير في مستوى تولد الالتصاق بين المجموعتين. وأظهرت النتائج الإحصائية لإحراز الالتصاق وجود فرق معنوي محسوس ($p < 0.05$) بين المجموعتين فقط في اليوم 14 من بعد فتح المثانة وخياطتها. واستنتج من كل ذلك بان السبر اي جيل قد عمل بفاعلية للتقليل من تكوين الالتصاق الناجم عن فتح المثانة.

Introduction

Adhesion formation following celiotomy and pelvic surgery often causes unavoidable tissue injury that can lead to the formation of post-surgical adhesions and remains one of the leading causes of infertility, small bowel obstruction, and re-operation abdominal or pelvic pain (1). Such injuries include mechanical trauma from retractors and tissue handling, ischemia at suture sites and after electrocautery use, foreign bodies, tissue desiccation and infection (2). Peritoneal adhesions are fibrous bands of tissue that join intra-abdominal organs to each other or to the abdominal wall. These adhesions are a major complication in healing following surgery or infection and can lead to conditions such as intestinal obstruction, infertility, and chronic pain (3). When peritoneal tissue has been traumatized, fibrin deposition is evident within 12 hours, evidence of the cascade that results in adhesion formation. New mesothelium begins to develop between 2 and 3 days after the injury, and re-epithelialization is normally complete within 7 to 9 days (4). Therefore, an ideal adhesion barrier should be nonreactive, but protect tissue at risk during critical wound healing period before being resorbed and cleared; it should remain adherent to target tissue; and it should be easily applicable during conventional or laparoscopic procedures performed on adhesiogenic organs such as ovaries and adnexa. The SprayGelTM adhesion barrier system (Confluent Surgical Inc., Waltham MA) consists of two polyethylene glycol (PEG)-based liquids (clear and blue precursors) that when mixed together rapidly cross-link to form a biocompatible absorbable hydrogel in situ. The hydrogel formation occurs within seconds, with no evolution of heat and no requirement for external energy sources (5, 6). An air-assisted sprayer is usually used to spray the two SprayGelTM liquids onto tissues in both endoscopic and conventional laparotomy procedures. Both liquids are mixed in air to form on target tissue a flexible hydrogel barrier that adheres to patient's tissue. The adherent barrier remains intact for about 5 to 7 days, following application to the site during the critical period when fibrin deposition and fibrinolysis are believed to occur, and then degrades into water-soluble PEG components that undergo renal clearance (5, 6). The effect of medical barriers to utilize anti-adhesive properties had been studied on a varieties of animal models such as in rats (7), mice (8), and rabbits (9, 10). In all models a variety of surgical injuries were produced to mimic adhesions, such as cesarean incisions (10), abrasion or electrocautery applied to a range of organs including uterine horns (11), the caecum, ovaries and the pelvic sidewall to create a nidus for adhesion formation (9). The aim of this study was to create and to determine the extent of abdomeno-pelvic adhesions following cystotomy in dogs, and to evaluate the efficacy of SprayGelTM as an anti-adhesion barrier following cystotomy.

Materials and Methods

1. Animals: Eight stray dogs from both sexes were used in this experiment. They were accommodated for indoor living two weeks prior to surgery. Their body weight and age were; 8.5-14 (10.4 ± 1.8) kg, and 8-12 (10.7 ± 3.6) months, respectively. All dogs were healthy and free of congenital or acquired diseases and were vaccinated against rabies and canine distemper and treated for internal and external parasites.

2. Experimental Design and animal grouping: Conventional cystotomy was performed to all experimental dogs. The cystotomy wounds was sutured by one-layer Cushing mattress using no. 3/0 polyglactin-910. Before the routine closures of the abdominal wall the dogs were divided randomly into two groups:

Group A (control): the sutured cystotomy wounds were irrigated with 0.09 sterile saline solution.

Group B (treatment): the sutured cystotomy wounds were sprayed by thin layer of SprayGelTM.

At the 14th and 28th post-operative days, two dogs from each group (A & B), were subjected to gross postmortem (P.M.) examination and the peritoneal cavity was opened, and the viscera examined by a blinded reviewer for adverse adhesion changes between the urinary bladder and sidewall.

3. Anesthesia: The dogs were fasted 24 hours prior to surgery. During this period, the ventral abdominal wall was aseptically prepared for the laparotomy. Preanesthetic medication with atropine sulphate at a dose of 0.02 mg/kg body weight was given intramuscularly. Thirty minutes later, a mixture of xylazine 2% (1 mg/kg) and ketamine 5% (10 mg/kg) was given by intramuscular route. Incremental doses from the same mixture were given to the animals as needed when the plan of surgical stage of anesthesia was extended.

4. Laparotomy and Cystotomy procedures: The animals were positioned and fixed to the surgical table on dorsal recumbence. Evacuation of urine from the bladder were performed routinely in male dogs by urethral catheterization, while in female dogs, were performed by direct aspiration of urine with a sterile 50 ml syringe from the bladder after opening of abdominal cavity and exteriorization of the urinary bladder. Laparotomy performed by routine midline ventral celiotomy from umbilicus to pubis. Following abdominal exploration, the U. B. was identified, explored and isolated from the abdominal viscera with moistened sterile laparotomy towels. Two retention sutures were placed on the cranial and caudal ends of the bladder which was then completely evacuated using a 50 ml syringe supplied with a 20-gauge needle. A 3-5 cm stab incision was made by a scalpel on the ventral bladder wall, that was extended by a scissor cranially and caudally to explore the bladder cavity for the presence of bladder lesions or calculi and finally flushed with warm saline before its closure.

5. Suturing of urinary bladder wall: In all dogs suturing of U.B. wall was performed by one-full thickness layer of continuous Cushing pattern with 3/0 Polyglactin-910 (Fig. 1). The closed cystotomy wounds were treated in group A only by irrigation with 2 ml sterile 0.09 saline solution. While in group B they were Sprayed with a thin layer of SprayGelTM (Fig. 2). Postoperative concern included monitoring of animals physical status, daily intramuscular injection of systemic antibiotic (penicillin (10.000 IU\ kg) and streptomycin (20 mg\ kg) for 3 days. The skin stitches were removed 8 days post laparotomy.

- 6. Gross postmortem examination for adhesion scoring study:** Two dogs from both groups (A and B) were randomly chosen and euthanized at 14th and 28th post-cystotomy days, respectively. Post-mortem was performed by opening the carcass mid-ventrally from xyphoid to pubis, and laterally from the umbilicus to dorsum. Prospective gross physical examinations were carried by a blinded reviewer to the abdomino-pelvic cavity and viscera for the presence of adhesions related to the previously performed cystotomy. The scores of the developed adhesions were evaluated and recorded according to the criteria of scoring system represented in Table 1 (13).
- 7. Statistical analysis:** One-way analysis of variance (ANOVA) was used to analyze the results of adhesions scores. Post hoc comparison of parameters was applied by selection of Duncan procedure. A (*p*) value of ≤ 0.05 was considered statistically significant. Data are shown as the mean \pm SE.

Table (1) Adhesion scoring system proposed by diZerega *et al.*, (12) used for evaluation of the adhesion grades seen on the type of study dogs at 14th and 28th P.O. cystotomy days

Type	Score
None	0
Filmy, transparent, a vascular	1
Opaque, translucent, a vascular	2
Opaque, capillaries present	3
Opaque, large vessels present	4



Fig. (1) The urinary bladder wall was closed following cystotomy with one layer full-thickness continuous Cushing by 3/0 Polyglactin-910 (Vicryl)



Fig. (2) The cystotomy wound, following suturing was sprayed with a thin layer of SprayGel™ in group B dogs

Results

1. The gross P.M. findings of abdomino-pelvis cavity on the experimental dogs (group A and B), at 14th & 28th days post cystotomy days:

Group A (control): Irrigation of the cystotomy wounds following suturing with 2 ml physiologic saline solution

At 14th P.O. day: Dog No. (1) showed formation of opaque, translucent, avascular bands of adhesion on the urinary bladder at the site of cystotomy between the ventral bladder surface and the omentum at the site of the cystotomy. The grade of adhesion was scored 2. Otherwise no adhesions were found on the rest of the U.B. or the other visceral organs (Fig. 3). Dog no. 2, underwent incisional umbilical herniation 8 days P.O. Its gross P.M. findings revealed multiple thick, opaque vascular bands of adhesions extending from the cystotomy site towards the visceral organs and abdominal wall which scored as grade 3, (Fig. 4).

At 28th P.O. day: Both dogs no. 3 and 4, showed opaque, translucent, a vascular bands of adhesions joining the U.B. to the omentum and other bands of adhesions extending between the abdominal viscera and/or omentum. The adhesions were scored as grade 2 and 3 for dogs no. (3) and (4), respectively (Fig. 5).

Group B (treatment): Coating of the cystotomy wounds following suturing with SprayGel™

At 14th P.O. day: Dog no. 5, two days post-cystotomy, opened its own celiotomy sutures causing the omentum to fall out. It was aseptically and routinely repaired. At P.M. examination a thick band of omento-abdominal adhesion, but no gross adhesions were found on bladder due to cystotomy (Fig. 6). Dog no. 6 showed fine transparent bands of adhesions seen extending from the suture site towards the omentum and scored as grade 1 (Fig. 7).

At 28th P.O. day: In dog no. (7), thin vascular bands of adhesions were seen extending from the cystotomy site on the U.B. toward the wall of pelvic cavity, that were graded as score 2 adhesions (Fig. 8). While, in dog no. (8), score 1 adhesion bands of thin translucent, a vascular adhesions were seen extending from the cystotomized U.B. to the pelvic wall (Fig. 9).

2. Statistical Results for Adhesion Scores: The average mean values (Mean \pm SE) for the scores of adhesions in SprayGel™ treated animals (group B) as seen in Table 2, was lower than that seen in control group A. The difference was significant ($p \leq 0.05$) between the two groups at the 14th post-cystotomy day. While, not between control and treatment groups at the 28th, although lower scores of adhesions between both groups at the was graded.

Table (2) Statistical results for the adhesions scoring between group A and B.

Groups	Numbers	14 th day N=2	28 th day N=2
A	4	2.5 \pm 0.7 B	2.0 \pm 0.0 AB
B	4	0.5 \pm 0.7 A	1.5 \pm 0.7 AB

Values are mean \pm SE; Different letters (A, and B) in the columns indicates significant differences between control and treatment groups ($P < 0.05$).

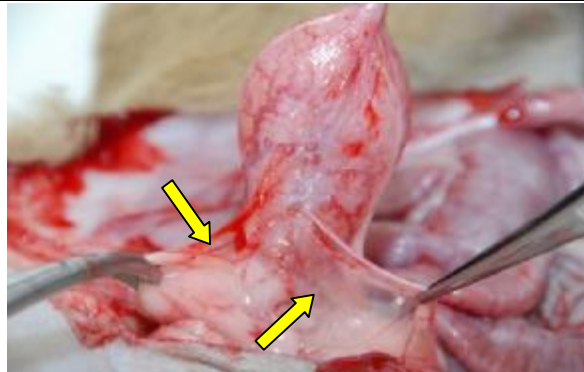


Fig. (3) Dog no. (1), group A. Opaque, translucent, avascular thick bands of adhesions seen joining the ventral aspect of the U.B. from the cystotomy site with the omentum at the site of the cystotomy and graded as score 2

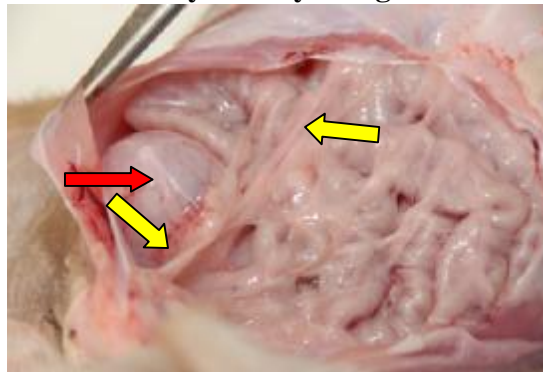


Fig. (4) Dog no. (2), group A. Multiple thick layers of opaque vascular bands of adhesions seen extended from the urinary bladder (red arrow) towards the visceral organs and abdominal wall and were graded at score 3

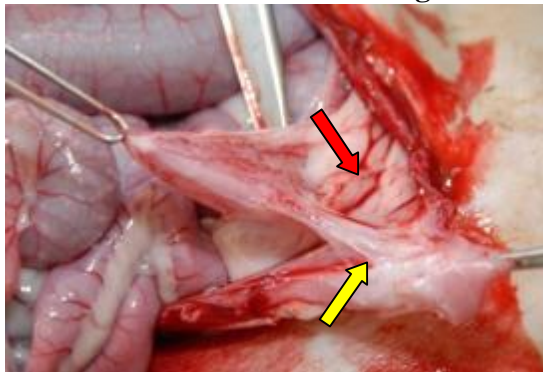


Fig. (5) Dog no. (4) from control group on day 28 P.O. Thick opaque translucent avascular bands of adhesions seen covering the U.B. and extending towards the omentum and between the gut and peritoneum (red arrow), while others were joining the U.B. to the visceral aspect of the abdominal wall. These were graded at score 3



Fig. (6) Dog no. (5), group B. On the 14th P.O. day, the gross P.M. showed absence of intra-peritoneal adhesions (score 0)

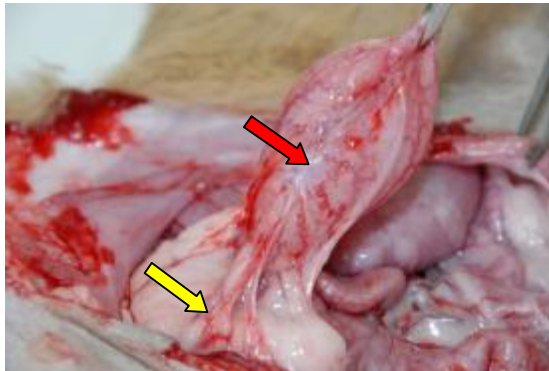


Fig. (7) Dog no. (6), group B. at 14th P.O. day. Thin transparent bands of adhesions seen extending from the ventral aspect of the U.B. from the line of cystotomy wound (red arrow) toward the pelvic sidewall (yellow arrow) that graded at score 1

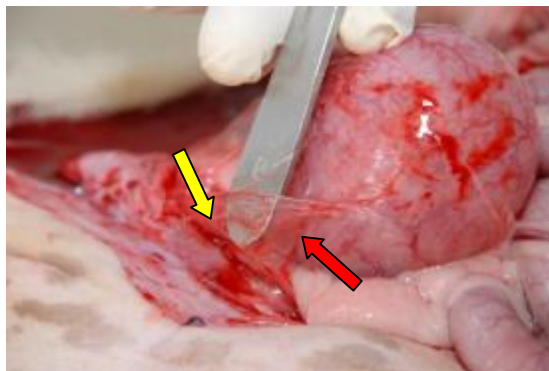


Fig. (8) Dog no.(7) from group B, at 28th P.O. day. Thin vascular bands of adhesions seen extending from the from the ventral aspect of the U.B. from the line of cystotomy wound (red arrow) toward the pelvic sidewall, and graded at score 2

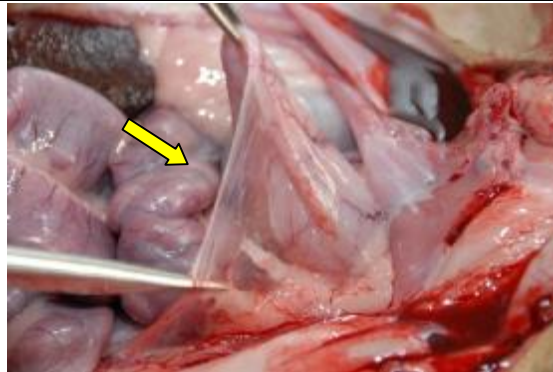


Fig. (9) Dog no. (8), from group B at the 28th P.O. day. Translucent, a vascular bands of adhesions seen extending from the cystotomy site toward the abdomino-pelvic wall and graded at score 1

Discussion

Adhesions need to be recognized as the most frequent complication of abdominal surgery and evidence is increasing to support the efficacy of adhesion-reduction agents to complement good surgical practice, including agents that are relatively inexpensive and simple to use. In the current study, the developed abdomino-pelvic adhesions in the dogs following cystotomy under conventional laparotomy, have shown that even with advances in surgical practice, adhesions continue to represent a significant burden for patients and surgeons. Coating of the cystotomy wound by the blue colored adhesion barrier “SprayGel™” on the cystotomy site significantly reduced adhesion incidence, extent and severity compared to surgical control. While, in the control dogs opened at the 14th P.O. day, the gross P.M. examinations revealed high scores of adhesions. The adhesions bands were existing as opaque, translucent, avascular adhesion that were extending mostly from the cystotomy site toward the abdomino-pelvic wall and visceral organs. On the other hand, in the adhesion barrier treated dogs, during the same critical adhesion formation period (the 14th P.O. day), the observed adhesions were filmy, transparent and avascular in nature and of lower scores. The difference was referred mainly to the mechanical protective barrier provided to the cystotomy wounds by the coated thin film of SprayGel™ which protected the traumatized serosal and other injured U.B. wall layers from friction and further irritation with the neighboring pelvic sidewalls and viscera. Adhesions are generally to develop from seven to ten days postoperatively, making this time period crucial for preventing adhesion formation (13). The use of physical barriers between injured tissues has been proved as an active method to prevent post-surgical adhesion formation (8, 10). From within the strategies in the approaches for prevention of adhesion formation following conventional or laparoscopic celiotomy, is to place a biocompatible absorbable or non-absorbable material on the injured site that serves as a physical barrier limiting tissue apposition during the post-operative period following surgery when adhesion forms. The thin film coat of SprayGel™ applied on the cystotomy site in this approach succeeded in preventing the post-cystotomy abdomino-pelvis adhesions by placing this biocompatible absorbable material in the abdomen that served as a physical barrier during the period at which postoperative adhesion forms by limiting tissue apposition during the time following surgery when adhesions usually form. The obtained results coincide with the strategy against postsurgical adhesion formation, in the use of physical barriers in order to separate adjacent injured areas within the peritoneal cavity (14, 15, 16). In conclusion, SprayGel™ was found safe, well tolerated, and demonstrated efficacy in patients known to be at risk for adhesion formation.

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