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The effect of omental pedicle flap with platelets-rich fibrin on reconstruction of induced-urinary bladder defect in dogs

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Abstract

The research was planned to evaluate the ability of an omental pedicle flap with platelets-rich fibrin (PRF) to augment bladder defects in dogs. Eighteen adult- male dogs were used. After general anesthesia, a partial cystectomy with a circular shape with a diameter of 3cm. was performed on the dorsal surface of the bladder. Then, the animals (N=9) were divided into two equal groups. In the first group, the bladder defect was closed with a double layer of omentum. In contrast, in the second group, the defect was also reconstructed by a double layer of omentum with topical application of platelets-rich fibrin between both layers. The results were evaluated by studying the gross, cystography, and histopathological changes on 7, 15, and 30 days postoperatively. Monitoring of clinical signs with statistical histopathological scoring analysis was also studied. The clinical signs during the first-week post-operation were represented by loss of appetite, straining during urination, and hematuria. These signs disappeared after the first week of surgical repair, and all animals ultimately returned to their normal body status. The complete closure of the bladder defect represented the gross changes. The histopathological results represented the formation of granulation tissue, angiogenesis, and infiltration of polymorphonuclear and mononuclear inflammatory cells in both groups. The proliferation and maturation of granulation tissue were more in the second group, in addition to lower degree of inflammatory cell infiltration. The results of cystography revealed no sign of urine leakage. In conclusion, adding PRF to omentum can improve the healing of bladder defects in dogs.

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Introduction

The urinary bladder is a hollow, muscular organ that stores and empties urine. It located within the pelvic cavity and is different in size, position, and shape according to the storage amount of urine. The bladder may suffer from different problems or pathological conditions affecting biomechanical activity. These pathological conditions include; congenital cases such as bladder hypoplasia, neoplasia, inflammatory disease, and rupture. Also, some complications may occur after surgical intervention (1). The tearing or rupture of the bladder may cause loss of bladder tissues and urine leakage into the abdominal cavity, especially in some lesions of the bladder, such as severe trauma, urinary tract obstruction, cancers, and rough catheterization (2,3). Therefore, bladder augmentation is indicated to reconstruct the bladder defect (4,5). The pathological cases that need to remove some of the bladder tissues may cause a reduction in the average capacity of the bladder. Therefore, correcting complex conditions remains a significant challenge in urology (6,7). Several techniques were used to reconstruct the bladder defect to preserve the capacity of the urinary bladder, such as gastrocystoplasty, enterocystoplasty, and colocystoplasty (6-8). Additionally, different materials can be used to treat the bladder defect, such as autogenous fascia and pericardium. The ideal bladder

augmentation material has not yet been identified (9-11). Several complications, such as hematuria, dysuria, and chronic infection, may develop after surgical grafting or correction of bladder defect due to recurrent relapses (12,13). Therefore, tissue engineering is indicated (6-10). Tissue engineering is used either as a cellular or cellular. Natural or synthetic scaffolds were used to close the bladder tissue defect in the acellular technique. The donor cells were seeded into the scaffold or used alone in the cellular method. The seeded cells in the scaffold are either autologous, allogeneic, or xenogeneic sources (14-17). Revascularizing properties characterize the omentum, accelerating the healing process (18,19). It is easy to apply and sutured over the hollow organs such as the bladder. Also, it can adhere to the defect site and prevent leakage of contents of hollow organs (20). Platelet-rich fibrin (PRF) is an autologous blood product utilized to treat different conditions because of its ability to improve and enhance different stages of tissue healing (21-26).

Therefore, this study aims to know the ability of the omental pedicle flap with platelet-rich fibrin to reconstruct bladder defects in dogs.

Materials and methods

Ethical approve

The research was approved by the ethics of the Institutional Animal Care and Use Committee for the College of Veterinary Medicine, University of Mosul UMVET.2023.038.

Experimental animals

Eighteen adult, local-breed, male dogs were used in this study. The average weight of animals is 25 kg±1.3, aged 2-3 years. The total number of animals was divided into two groups, each composed of 9 animals after induced bladder defect using partial cystectomy.

Anesthesia

Atropine sulfate was injected as a pre-anesthetic agent at a dose of 0.05 mg/kg. subcutaneously before induction of general anesthesia. Then, the general anesthesia protocol used in this work to inject the animals with a mixture of 10 % ketamine HCL at a dose of 10 mg/kg and 2% xylazine at a dose of 2 mg/kg intramuscularly (27).

Surgical operation

After induction of general anesthesia, urethral catheterization was done. The surgical procedure is accomplished by making a ventral mid-line incision, which extends from the umbilical to the pubis region caudally. The incision was continued through the lina alba and peritoneum with a reflection of the penis laterally. The urinary bladder is isolated with sterile moistened sponges and two stay sutures used to facilitate the catching of the bladder. Then, a 3 cm. circular excision was created on the dorsal surface of the urinary bladder wall (Figure 1). The machine of sucker was used before repairing the site of the defect to remove any urine or blood from the bladder cavity (Figure 2). The closure of the induced defect was performed in the first group though the application of an omental pedicle flap pulled from the nearest area to the bladder and sutured with the internal edges of the bladder at the site of the defect by a 3/0 vicryl suture material using a simple continuous suture technique. Then, a second layer of omental pedicle flap was applied and sutured with the external edges of the bladder by using the same suture and technique (Figure 3). Before the closure of the abdominal incision, a considerable amount of normal saline was pushed through the catheter to ensure no leaks at the site of bladder repair. Then, the surgical incision of the abdominal cavity was sutured with routine techniques. The catheter introduced before the surgical operation was fixed externally by a few stiches with the skin. In the second group, the same technique was used to apply PRF between the layers of the omental pedicle flap. The PRF was applied before suturing the second layer of the omental pedicle flap with the bladder.

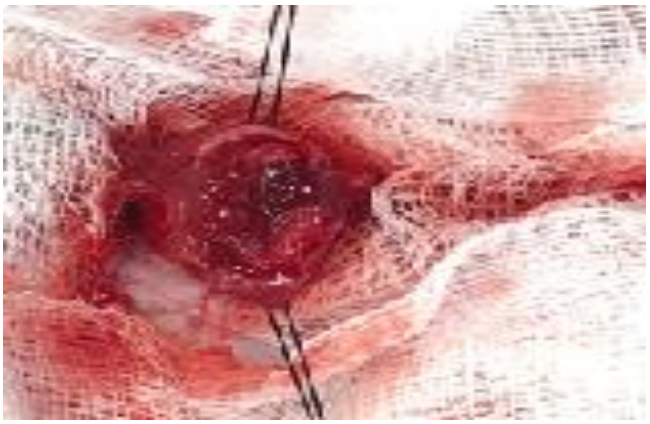

Figure 1: Shows exposure of bladder with induction of defect.

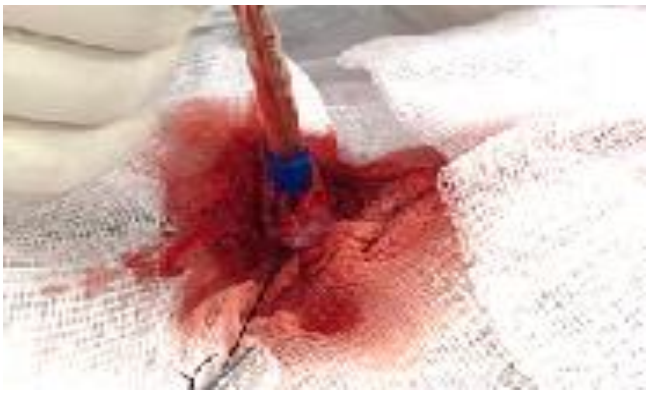

Figure 2: Shows using of a sucker.

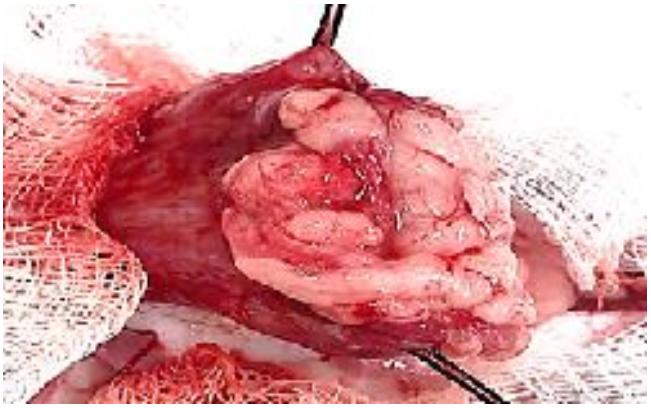

Figure 3: Shows closure of the bladder defect with a double layer of omental pedicle flap.

Post-operative care

The experimental animals were injected with systemic antibiotics and analgesics postoperatively using penicillin-streptomycin (1 ml/10 kg and metalgen 1 ml/day, respectively) for seven days after the surgical operation. Also, the operation site was dressed daily with oxytetracycline spray until the skin wound healed completely. The folly catheter was monitored daily until it was removed seven days postoperatively.

Assessment of healing

The parameters used to assess the efficiency of each technique to repair urinary bladder defects include; Study the clinical signs postoperatively for each animal. Study the gross, and histopathological observations on 7,15, and 30 days postoperatively. Study the statistical analysis of the histopathological scoring. The data of histopathological descriptive scores of the granulation tissue, angiogenesis, and severity of the inflammatory response of the dog urinary bladder were done by a pathologist and analyzed statistically by Kruskal-Wallis test and used Pairwise Multiple Comparison Procedures (Tukey Test) at $P \leq 0.05$. The Sigma Plot (version 12.5) software program analyzed the data for statistical analysis. The histological sections were scored according to the following criteria [1] Intensity of granulation tissue, [2] Intensity of angiogenesis, [3] Intensity of inflammatory reaction, and [4] Positive contrast cystography on 7,15, and 30 days postoperatively (Table 1) (28,29).

Table 1: Showing the histopathological scoring

Criteria	0	1	2	3
Intensity of granulation tissue	Absent	Discrete	Moderate	Intense
Intensity of angiogenesis	Absent	Discrete	Moderate	Intense
Intensity of inflammatory reaction.	Severe	Moderate	Few	Absent

Preparation of platelets rich fibrin (PRF)

PRF was prepared immediately before performing the surgical correction. About 10ml of blood was obtained from the jugular vein after anesthetizing the animal, and then the blood sample was put in the plane sterile glass tube that did not contain anticoagulant. Then, the tube was placed in the machine centrifuge for about 10 minutes at 3000 rpm. After centrifugation, three layers appeared: upper, middle, and lower. The lower layer contains blood cells, the upper layer comprises poor platelets, and the middle layer comprises fibrin-rich platelets. The middle layer, which appears as a gel structure, was gently separated from the tube with toothless forceps (Figure 4) (24,30).

Contrast radiography of the urinary bladder (Cystography)

The contrast radiography of the urinary bladder was applied to observe urine leakage and abnormal tissue growth inside the bladder. The contrast radiography was done on 7-, 15-, and 30-days postsurgical repair through an injected contrast medium called Omnipaque (G.E. Health Care, United States) via the Foley catheter. Before pushing the contrast media, the urinary bladder should be emptied of urine, and the contrast media should be diluted with normal saline at a percentage of 50 ml of contrast with 250 ml saline.

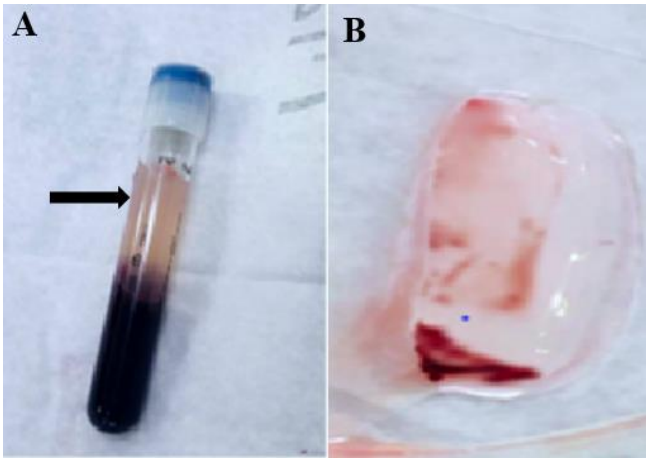

Figure 4: Shows steps of PRF preparation. (A) Three layers appeared in the sterile glass tube after centrifugation. (B) The separated middle fibrin-rich platelets layer.

Results

Clinical signs

The animals, after surgical operation suffered from varying degrees of anorexia, a state of restlessness or discomfort, and straining during urination. In addition, discoloration of urine with small amounts of blood was appeared. However, these signs disappeared after the first week postoperatively, and the animals returned to normal body conditions without any complications.

Gross changes

In all experimental animals, the grafting site at 7-, and 15-days post-operation suffered from varying degrees of adhesion with surrounding tissues. The adhesion was reduced at the end of the study. However, good binding was observed between the implanted piece of omental pedicle flap and urinary bladder defect in all animals during all periods of the study, with slight thickening in the wall of the bladder at the site of grafting; in addition, no sign of urine leakage and tissue dehiscence was observed (Figures 5-8).

Figure 5: Gross image of the urinary defect in the first group at seven days post-operation.

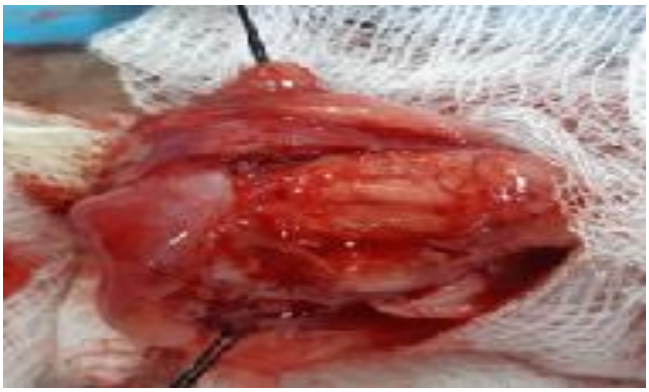

Figure 6: Gross image of the urinary defect in the second group at seven days post-operation.

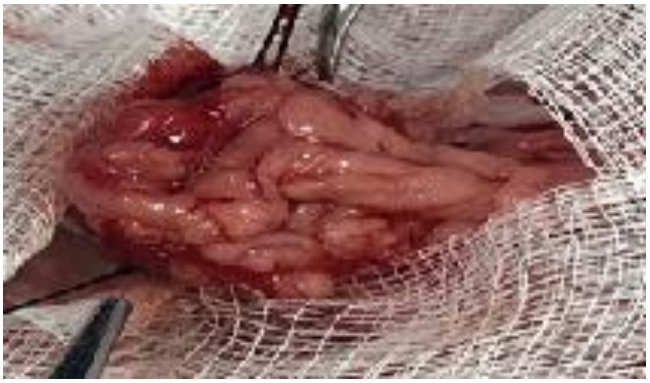

Figure 7: Gross image of the urinary defect in the first group at 30 days post-operation.

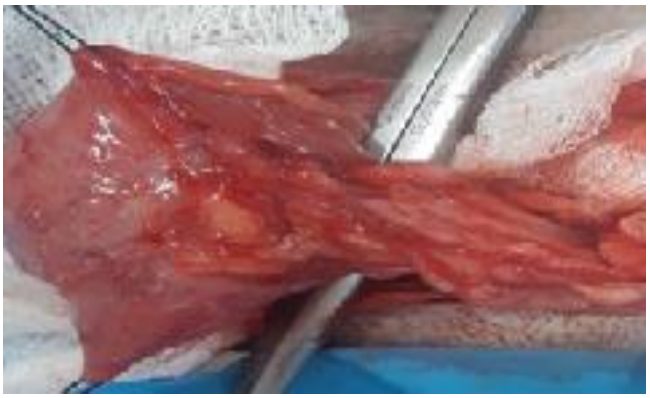

Figure 8: Gross image of the urinary defect in the first group at 30 days post-operation.

Histopathological changes

In the first group (omentum), the histopathological features at the site of bladder augmentation seven days postoperatively were represented by the formation of granulation tissue between the implanted omental pedicle flap and urinary bladder, development of new blood vessels, high infiltration of polymorphonuclear and few mononuclear inflammatory cells, with deposition of fibrin (Figures 9 and 10). On 15 days post-operation, the histopathological sections showed more binding between the implanted omental pedicle flap and bladder by mature granulation tissue. The angiogenesis, and fibrin deposition with high infiltration of polymorphonuclear and few mononuclear inflammatory cells were also observed (Figures 11 and 12). On 30 days post-operation, more maturation of granulation tissue was shown with an increase in angiogenesis. Little infiltration of polymorphonuclear inflammatory cells was shown with an increase in the infiltration of mononuclear inflammatory cells (Figures 13 and 14).

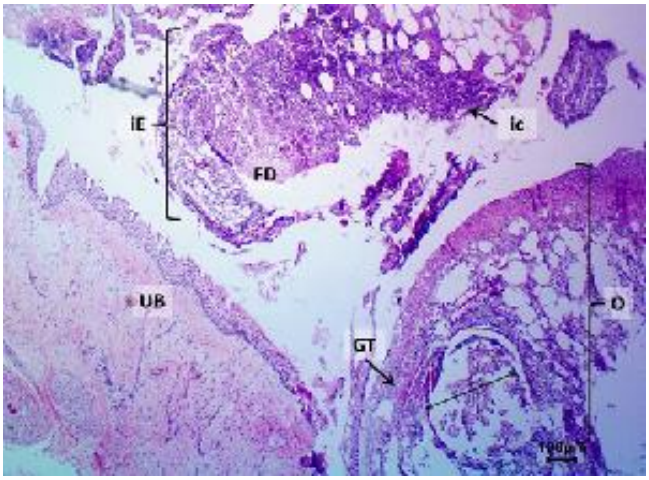

Figure 9: Histopathological section from the first group at 7th P.O. days shows the site of defect with the omentum graft (O), stitch knot (↔), severe inflammatory exudate (iE) composed of fibrin deposition (FD), and high infiltration of inflammatory cells (ic) and granulation tissue (GT). H&E stain,40X.

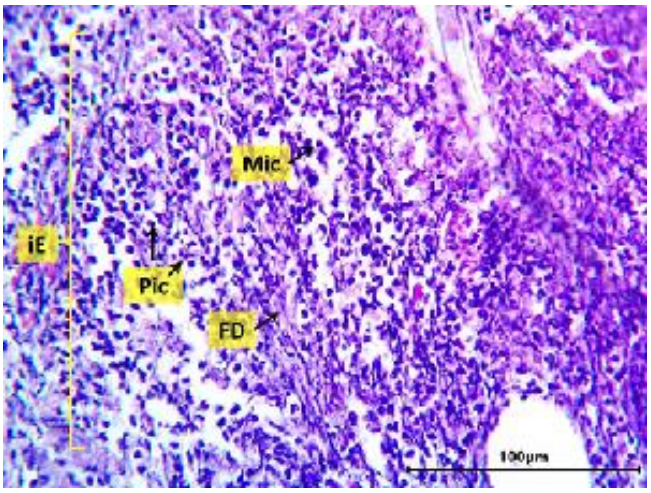

Figure 10: Histopathological section from the first group at 7th P.O. days shows the site of defect with severe inflammatory exudate (iE) composed of fibrin deposition (FD), and infiltration of highly polymorph-nuclear (Pic) and few mono-nuclear inflammatory cells (Mic). H&E stain, 400X.

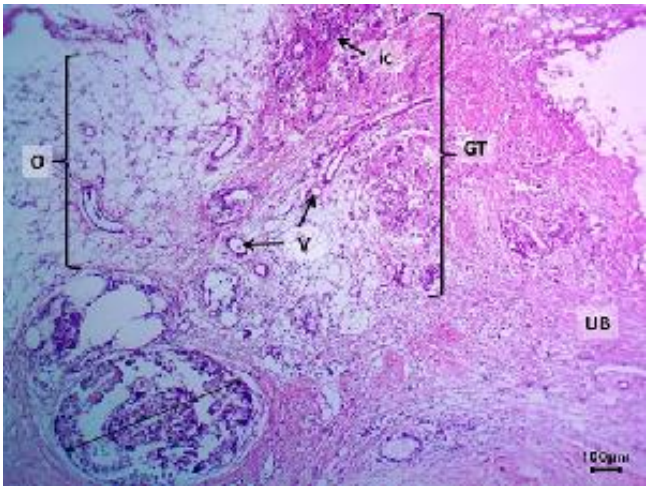

Figure 11: Histopathological section from the first group at 15th P.O. days shows the site of defect with the omentum graft (O), stitch knot (↔), granulation tissue (GT), infiltration of inflammatory cells (ic), and new blood vessels (V). H&E stain, 40X.

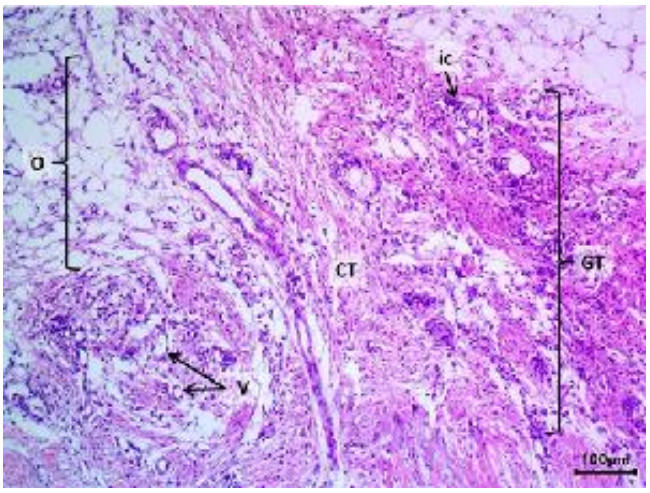

Figure 12: Histopathological section from the first group at 15th P.O. days shows the site of defect with the omentum graft (O), granulation tissue (GT), composed of connective tissue (CT), infiltration of inflammatory cells (ic), new blood vessels (V). H&E stain, 100X.

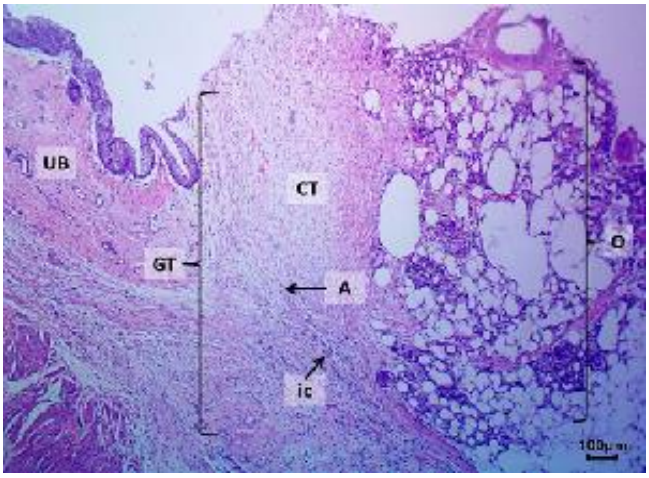

Figure 13: Histopathological section from the first group at 30th P.O. days shows the site of defect with the omentum graft (O), granulation tissue (GT), composed of connective tissue (CT), expectable infiltration of inflammatory cells (ic), high angiogenesis (A) .H&E stain, 40X.

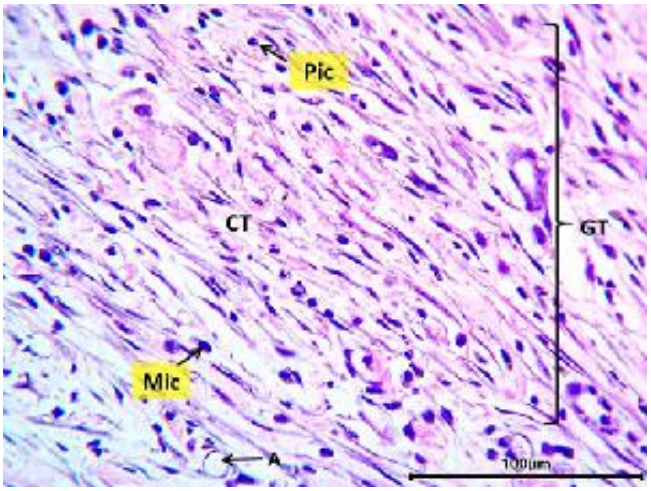

Figure 14: Histopathological section from the first group at 30th P.O. days shows the site of defect with granulation tissue (GT), composed of connective tissue (CT), few polymorph-nuclear (Pic), and high mono-nuclear inflammatory cells (Mic), and angiogenesis (A). H&E stain, 400X.

In the second group (omentum with PRF), the histopathological changes at the site of bladder augmentation seven days postoperatively were represented by good binding between the implanted pedicle flap and bladder through more granulation tissue formation. In addition, fibrin deposition, more angiogenesis, and high infiltration of polymorphonuclear and few mono-nuclear inflammatory cells were observed (Figure 15 and 16). On 15 days post-operation, the maturation of granulation tissue was increased with more angiogenesis and fibrin deposition. The infiltration of polymorphonuclear inflammatory cells decreased with high mononuclear inflammatory cell infiltration (Figures 17 and 18). On a 30 days post-operation, the absence of inflammatory cells and granulation tissue showed more maturation of connective tissue and more angiogenesis (Figures 19 and 20).

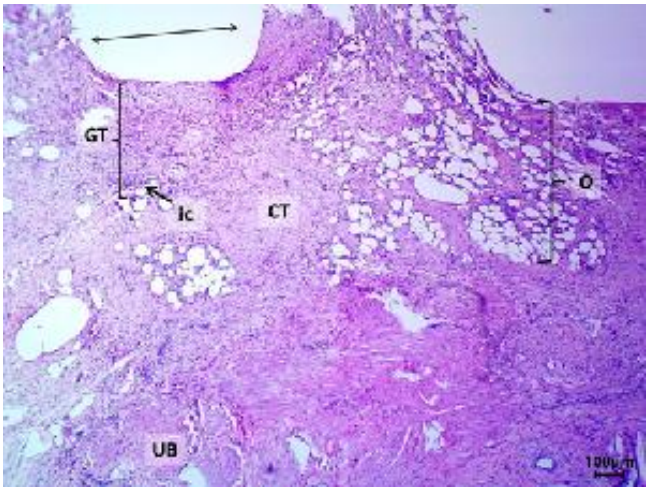

Figure 15: Histopathological section from the second group at 7th P.O. days shows the site of defect with the omentum graft (O), stitch knot (↔), granulation tissue (GT), with connective tissue (CT), infiltration of inflammatory cells (ic). H&E stain, 40X.

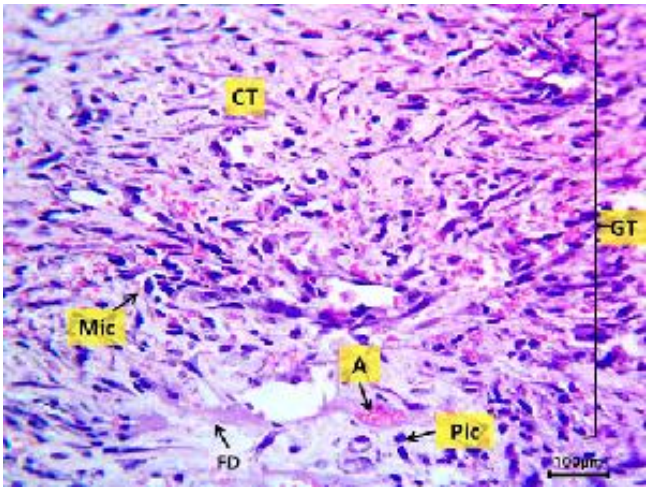

Figure 16: Histopathological section from the second group at 7th P.O. days shows the site of defect with the granulation tissue (GT), composed of connective tissue (CT), fibrin deposition (FD), infiltration of polymorph-nuclear (Pic) more than mono-nuclear inflammatory cells (Mic) and high angiogenesis (A). H&E stain, 400X.

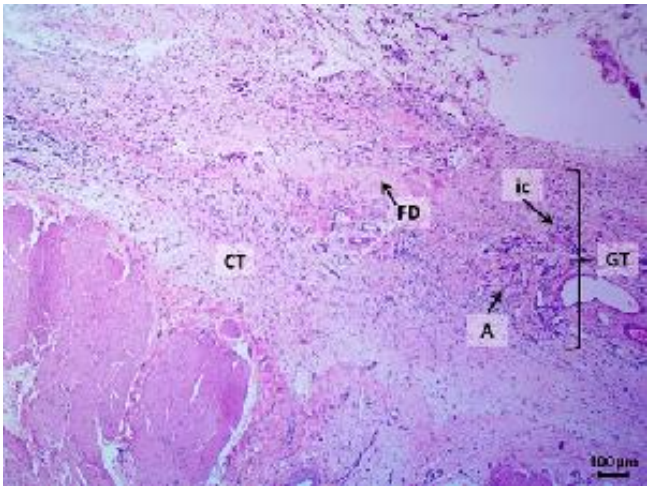

Figure 17: Histopathological section from the second group at 15th P.O. days shows the site of defect with granulation tissue (GT), composed of connective tissue (CT), few polymorph-nuclear (Pic), and high mono-nuclear inflammatory cells (Mic), and angiogenesis or blood vessels (A). H&E stain, 40X.

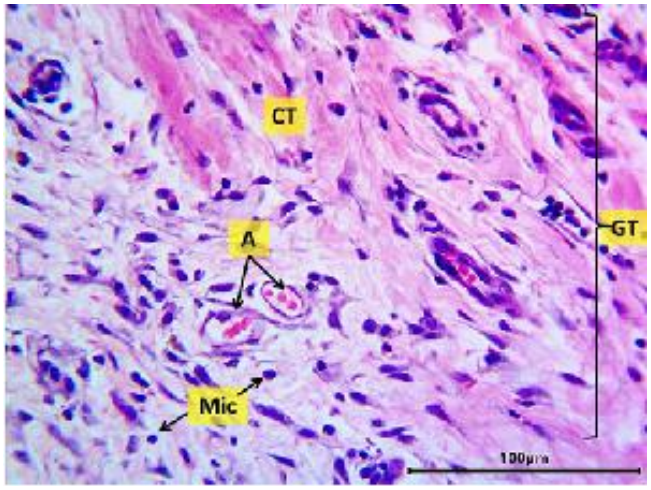

Figure 18: Histopathological section from the second group at 15th P.O. days shows the site of defect with granulation tissue (GT), with connective tissue (CT), redominant mono-nuclear inflammatory cells (Mic), and high angiogenesis (A). H&E stain, 400X.

Histopathological scoring

The statistical analysis of granulation tissue scoring showed a significant difference at P≤0.05 for the second group during all periods of the study, where the formation of granulation tissue in the second group developed more than in the first group on seven days postoperatively and disappeared on the 30-day post-operation (Table 2). The statistical analysis of angiogenesis scoring showed a significant difference at P≤0.05 for the second group during all study periods, where the angiogenesis in the second group developed more than in the first and on seven days postoperatively and reduced on the 30-day post-operation (Table 3). The statistical analysis of inflammation scoring showed a significant difference at P≤0.05 for the second group during all study periods, where the inflammation in the second group developed less than in the first group seven days postoperatively and disappeared on the 30-day post-operation (Table 4).

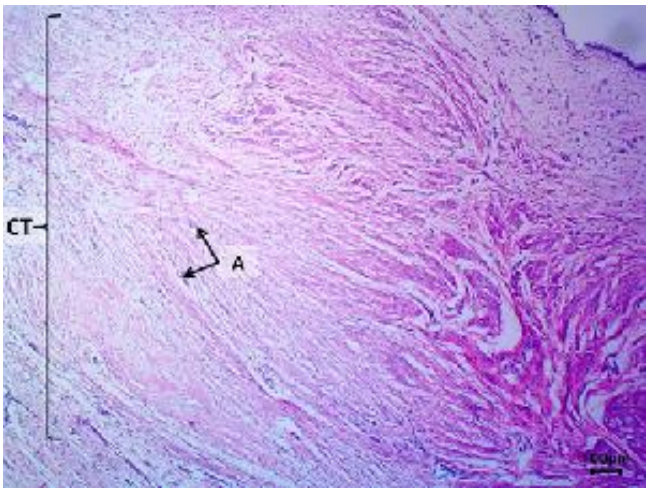

Figure 19: Histopathological section from the second group at 30th P.O. days shows the defect site with mature connective tissue (CT), and angiogenesis (A) without inflammatory cells. H&E stain, 40X.

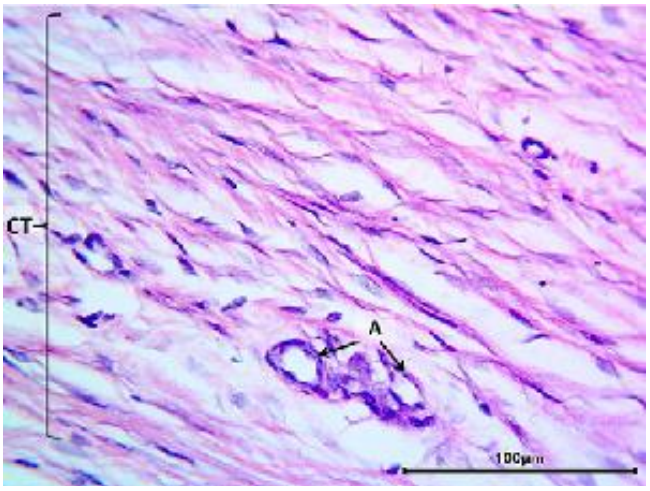

Figure 20: Histopathological section from the second group at 30th P.O. days shows the defect site with mature connective tissue (CT), and angiogenesis (A) without inflammatory cells or granulation tissue. H&E stain,400X.

Table 2: Histopathological Scores of the of granulation tissue

Groups	Post operation days (n=3) median		
	7th	15th	30th
G1	1 (1) Bb	3 (3) Ba	3 (2) Aab
G2	3 (2) Aa	1 (1) Aab	0 (1) Bb

The difference in capital letters means there are significant differences between groups at P≤0.05. The difference in small letters means there are significant differences between periods at P≤0.05.

Table 3: Histopathological scores of the angiogenesis

Groups	Post operation days (n=3) median		
	7th	15th	30th
G1	1 (0) Bb	2 (1) Aab	3 (3) Aa
G2	3 (2) Aa	3 (3) Aa	2 (1) Aa

The difference in capital letters means there are significant differences between groups at P≤0.05. The difference in small letters means there are significant differences between periods at P≤0.05.

Table 4: Histopathological scores of the inflammation

Groups	Post operation days (n=3) median		
	7th	15th	30th
G1	3 (3) Ba	2 (2) Aa	2 (1) Aa
G2	1 (1) Aa	1 (0) Aa	0 (0) Aa

The difference in capital letters means there are significant differences between groups at P≤0.05. The difference in small letters means there are significant differences between periods at P≤0.05.

Radiographic results

The site of bladder defect after the surgical correction appeared on seven days in both groups as a circular and radiolucent shape without any radiographic sign of urine leakage (Figures 21 and 22). At 15 days, no urine leakage or tissue overgrowth within the bladder cavity appeared at the site of urinary. The urinary defects at 30 days postoperatively in both groups disappeared, and the bladder defect ultimately appeared with a radiopaque appearance. Also, no urine leakage or tissue overgrowth was shown. During all study periods, the contrast media filled the cavity of the urinary bladder relatively (Figures 23 and 24).

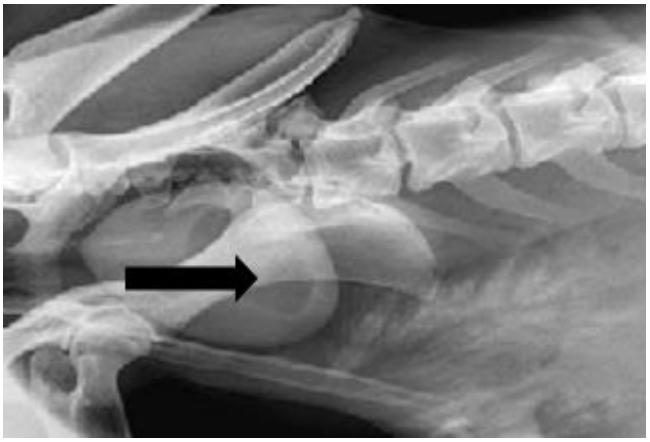

Figure 21: Radiographic image shows urinary defect at seven days post operation in the first group.

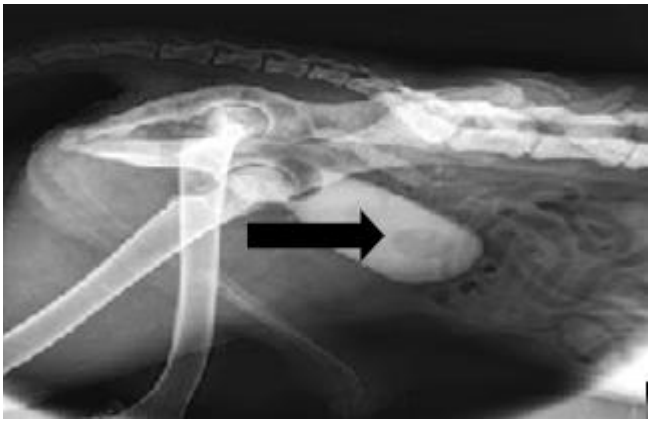

Figure 22: Radiographic image shows urinary defect at seven days post operation in the second group.

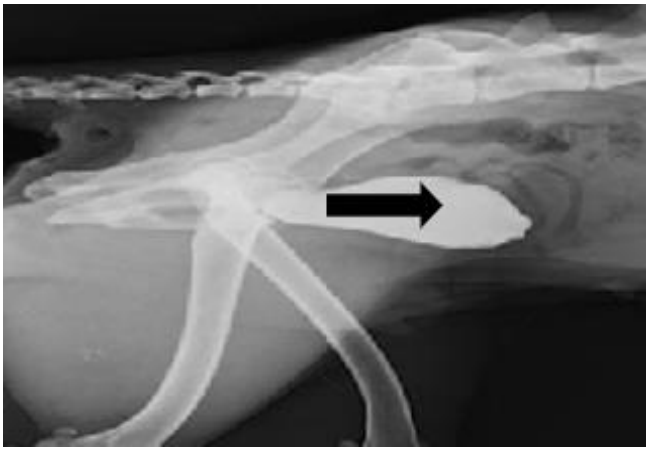

Figure 23: Radiographic image shows urinary defect at 30 days post operation in the first group.

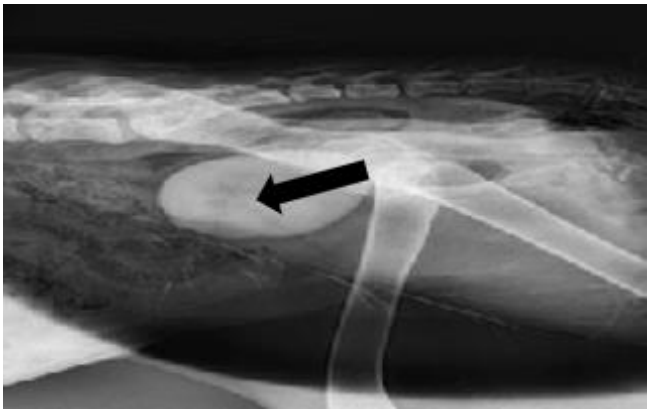

Figure 24: Radiographic image shows urinary defect at 30 days post operation in the second group.

Discussion

In this research, most animals suffered from varying degrees of appetite loss, straining during urination, and hematuria postoperatively. These results occurred due to injury of the bladder tissues. The authors Kamal *et al.* (31), Lenka *et al.* (32), mentioned that the main clinical signs that appear due to severe bladder trauma include; straining during urination and the presence of blood with excreted urine. The process of urethral catheterization may also cause animal restlessness due to pain or tissue damage to the mucosa of the lower urinary tract (33,34). However, these signs were finished, and the animals returned to normal body status after the first week of bladder augmentation. These obtained results indicate the successful closure of bladder defect, in addition to the progress of the healing process of the bladder typically, where the authors Hastings *et al.* (35), Degner and Walshaw (36) noticed the healing process of the bladder with the mucosal defect might occur within one week, and 2 - 3 weeks for full-thickness defects. Additionally, many events happen after cystectomy, which helps improve the healing process, including spasms of detrusor muscle around the incision site to maintain a watertight seal at the suture line and proliferation of smooth muscles.

In both groups, the omental pedicle flap closed, and the induced bladder defect was complete without any surgical complications, especially tissue dehiscence. This excellent result indicated the ability of the omentum to repair the injured tissues where the authors Findlay and DeFreitas (37), Agrama *et al.* (38) noticed the omentum can adhere around the site of injured and inflamed tissues. The omentum plays a vital role in the defense mechanism through adherence to the inflamed area (39-41). The ability of the mentum to adhere belongs to its ability to produce fibrin, and this agreement with Konturek *et al.* (42) concluded that the omentum can be adhered to an injured area due to its capacity for fibrin formation.

The thickening in the bladder wall is a typical sequel to tissue injury. The authors Adams and Senior, Brearley and Cooper (43,44) noticed that the bladder tissue responds to inflammation by hyperplasia and hypertrophy, and this inflammatory response leads to the thickening of the bladder wall. Also, Kropp *et al.* (45), Cornell (46), Desch and Wagner (47) showed that the bladder can be enlarged after partial cystectomy due to epithelial regeneration, proliferation, and smooth muscle hypertrophy with synthesis and scar tissue remodeling.

Varying degrees of adhesion formation appeared between the site of bladder correction and surrounding tissues of the abdominal cavity at 7-, and 15-days post-operation. Then, adhesion subsides relatively at the end of the study. This result was mentioned by Weibel and Majno, Diamond and Freeman, Menzies and Ellis (48-50), who said the adhesion was developed post-abdominal surgery and is regarded as one of the most common complications due to tissue trauma regardless of the type or location of surgical operation in the body.

Many complications may appear following bladder reconstruction, such as chronic infection, and urine leakage (51-54). In both groups, the bladder defect was closed entirely, and no urine leak showed during positive contrast radiography. These results indicate the ability of the omental pedicle flap to heal the bladder defect. The authors Ackermann *et al.* (55), Cartier *et al.* (56), Mokhort and Makarov (57) mentioned that the omentum has a high level of vascularization. Therefore, it can reinnervate and revascularize the affected bladder. The omentum is essential in wound healing acceleration through neovascularizing the devitalized structures (58,59).

In the first group, the histopathological results were characterized by new connective tissue and blood vessel formation with high infiltration of polymorphonuclear inflammatory cells and less infiltration of mononuclear inflammatory cells. These observations resemble those Van *et al.* (60), Zhu *et al.* (61), Dux (62) who said the omentum could improve the healing process of injured or inflamed tissue. In addition, the omentum has a suitable defense mechanism because it contains a high aggregation of leukocytes, macrophages, and lymphocytes, and permits the migration of neutrophils from the circulation. The addition of PRF to the implanted omental pedicle flap improved the healing process of bladder defect by increasing the proliferation of granulation tissue formation and angiogenesis with less degree of infiltration of inflammatory cells, especially the polymorphonuclear cells. These findings are in agreement with Cakir *et al.* (63), Varela *et al.* (64), Saluja *et al.* (65), Naik *et al.* (66), Dohan *et al.* (67), Choukroun *et al.* (68), who noticed the PRF can promote wound healing and regeneration of tissues because it contains many growth factors (G.Fs.) such as essential fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1),

transforming growth factor-1 (TGF-1) and platelet-derived growth factor-BB (PDGF-BB) and cytokines that play a central role in the migration, proliferation, and differentiation of cells, in addition to the process of angiogenesis.

Conclusion

The findings concluded that the omental pedicle flap with PRF could successfully close the bladder defect in dogs.

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Conflict of interest

The authors declare that there is no conflict of interest.

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تأثير سدلة طية الثرب مع الليفين الغني بالصفيحات الدموية على إصلاح أذى المثانة البولية المستحدث في الكلاب

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الخلاصة

صمم البحث لتقييم قابلية سدلة طية الثرب مع الليفين الغني بالصفيحات الدموية على إصلاح أذى المثانة البولية في الكلاب. تم استخدام ثمانية عشر كلبا ناضجا ذكرا في هذا البحث. بعد إجراء التخدير العام تم عمل إزالة جزئية للمثانة البولية على شكل دائري وبقطر ٣ سم. وبعده قسمت الحيوانات الى مجموعتين متساويتين حيث ضمت كل مجموعة على تسع حيوانات. في المجموعة الأولى تم غلق الأذى بواسطة طبقتين من سدلة طية الثرب أما في المجموعة الثانية تم غلق الأذى أيضا بطبقتين من سدلة طية الثرب مع الاستخدام الموضعي لليفين الغني بالصفيحات الدموية بين طبقتي الثرب. تم تقييم نتائج الدراسة من خلال دراسة التغيرات العيانية والشعاعية للمثانة والنسجية المرضية كل ٧، ١٥ و ٣٠ يوم بعد العملية. بالإضافة الى دراسة العلامات السريرية والتحليل الإحصائي للدرجات النسجية المرضية بعد التصحيح الجراحي للأذى. تميزت العلامات السريرية خلال الأسبوع الأول بعد العملية للحيوانات بفقدان الشهية مع عدم الراحة والتعصر أثناء عملية التبول وظهور البيلة الدموية. اختفت هذه العلامات بعد الأسبوع الأول من إجراء العملية الجراحية وعادت الحيوانات الى وضعها الطبيعي بشكل كامل تقريبا. أما التغيرات العيانية فقد تمثلت بانسداد الأذى للمثانة البولية بشكل كامل. تمثلت النتائج النسجية المرضية بتكوين نسيج حبيبي وأوعية دموية جديدة بالإضافة الى ارتشاح الخلايا وحيدة ومتعددة الأشكال الالتهابية في كلا المجموعتين. حيث كانت نسبة تكاثر ونضوج النسيج الحبيبي بشكل أكثر في المجموعة الثانية. في حين أظهرت النتائج الشعاعية الى عدم وجود حالة تسرب للبول. بالاستنتاج إن إضافة الليفين الغني بالصفيحات الدموية للثرب عمل على تحسين عملية التئام أذى المثانة في الكلاب.