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# The impact of application acellular bovine pericardium supported with autogenous platelet-rich fibrin for cystoplasty in dogs: Histopathological assessments

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**Abstract**

The experimental work was planned to determine the efficacy of an acellular bovine pericardium patch with topical application of PRF to enhance the defect of the urinary bladder in dogs. Eighteen male adult local breed dogs were utilized in this study. Animals undergo the protocol of general anesthesia. The experiment was divided randomly into two equal groups, nine for each group. In G1, acellular bovine pericardium (ABP), a circular defect about 3cm in diameter will be created on the dorsal part of the urinary bladder then the site of operation wrapped completely with 4 cm in diameter of acellular bovine pericardium, whereas in G2, acellular bovine pericardium with platelet-rich fibrin (ABP+PRF) group the site of cystoplasty was reinforced with platelet-rich fibrin. Clinically, all animals were mentioned daily for 45 days, and the histopathological and serological assessments were performed at 0, 3, 7, 15, 30, 45 days post-surgery. The results of the decellularization of the bovine pericardium (BP) patch showed the absence of cells and nuclear components. Clinically, the animals seemed healthy and survived until the completion of the study. Some signs occurred in the first 2 days, such as hematuria, pain, arching of the back and urine incontinence, which subsided after 48-72 hours post-surgery. The biochemical and serological evaluation showed an increase in the concentration of urea at day 7, 15 post-surgery and creatinine at day 7 post-surgery in G1 superior to G2. The analysis of CRP concentration showed an increase in serum CRP levels at day 3, 15 post-surgeries in G1 superior to G2. The histopathological changes were represented by good healing in both groups without inflammatory reaction at 45 days post-surgery. The healing process of UB defect was superior and earlier in G2 than in G1, represented by the formation of fibrous tissue bridges between the graft and original tissue with the neovascularization. Both groups had high vimentin expression at 45 days post-surgery, whereas there was high VEGF expression in G2 at 15, 30 days, and there was a very weak reaction in G1. In conclusion, the experimental study presented that applying PRF to the implanted ABP patch improved the healing process of bladder defects in dogs, which is important to the PRF.

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## Introduction

Many disorders affect the construction of the bladder, which results in inadequate or limited bladder emptying (1). Inflammatory diseases like infections of the urinary tract, nerve injury, and congenital illnesses are among the other genital and urinary system disorders that cause bladder damage. Medical therapies are restricted, so surgical

treatment, especially cystectomy, is commonly needed (2). Cystectomy is indicated for urinary bladder diseases, involving correct patent urachus, treatment of cystic carcinoma, accidental damage, rupture of bladder and persistent interstitial cystitis (3). Urinary bladder reconstruction is typically done to alleviate persistent irritation and congenital abnormalities (4). Many complications were recorded as uroabdomen, postoperative infection of the urinary tract, urolith, development of cystic stones, and obstruction of the lower urinary tract that can accompanied by cystoplasty (5). BP represents one of the most bioactive materials used with varying degrees in different defects to improve regenerative processes in the surgery field (6). ABP has been successfully used in the restoration of the defects of the abdominal wall in dogs, tenorrhaphy, patch and valve transplants in heart operations performed on humans, and healing of rabbits' cutaneous wounds (7,8) and used as strengthening of suturing for the gastrointestinal anastomotic (9) and to substitute numerous types of tissues like dura mater (10), with good outcomes and computability with recipient tissue without immunological reaction. BP may be an excellent choice for restoring the urinary bladder (UB) due to its obtainability and low cost (11). So, the bioactive material should be biocompatible and perform as an implant to reconstruct bladder tissues (12). The decellularized materials are widely utilized as naturally derived urological materials, augmented as xenogenic, allogenic and autologous tissue is used to harvest them. The matrix is decellularized by chemical or mechanical processing, eliminating all cellular constituents and exposing a naturally occurring substrate for tissue growth (13). PRF is a membrane of single fibrin containing immunological and platelet concentrates that gathers all blood elements required for immunity, healing, and reducing the inflammatory process following surgery (14). PRF matrix plays an essential role in angiogenesis as it consists of six main angiogenesis soluble factors like platelet-derived growth factor (PDGF), fibroblast growth factor basic (FGFb), vascular endothelial growth factor (VEGF) and angiopoïetin (15) as well as PRF is easy applicable with low cost and high concentrations of growth factors which play a role in healing of soft and hard tissue which widely used in veterinary medicine like reconstitution of the mandibular defect (16) and intestinal anastomosis (17). In the biochemical examination, changes in the physical and chemical elements of blood deliver a better understanding of the infection developments and are helpful in differential diagnosis, healing and prognosis (18).

This study planned to evaluate the application of ABP supported by topical application of autogenous PRF to improve the healing process of defective urinary bladder in dogs.

## Materials and methods

### Ethical approval

The Ethics Committee of Mosul University's Faculty of the College of Veterinary Medicine approved the experimental design NO.UM.VET.2023.069.

### Experimental design

This study planned on eighteen adult male stray dogs. Their age and weight were (2±2.2years and 25±1.1kg) respectively. The experiment was divided randomly into two equal groups. Nine of each. cystoplasty of dogs using (ABP) G1(n=9), cystoplasty of dogs using (ABP+PRF) G2 (n=9).

### Preparation of ABP

The pericardium patches were taken from the nearby slaughterhouse and preserved right away in an ice-cold liquid of sterilized phosphate-buffered saline (PBS, pH 7.4) supplemented with a protease inhibitor (0.02% EDTA) and an antibiotic with broad spectrum (Amikacin-1 mg/ml). Next, all the adherence waste and blood and lipids were removed inside the cleaning room and thoroughly cleaned with PBS and sterilized gauze. A modified approach was used to decellularize the pericardium of cows. The pericardium membranes were submerged in 2% sodium deoxycholate SDS solution for nine hours while continuously exciting at 220 rotations per minute in a horizontal orbital shaker. This allowed for the best possible interaction between the compounds and the membranes. Hematoxylin and Eosin (H&E) staining was accompanied by a microscopic examination to ascertain the cellularity of the treated pericardium specimens (Figure 1). After that, the collected ABP was cleaned six times over two hours using a sterilized solution of PBS to get rid of any leftover materials (19) and lastly kept at 4°C in solution of PBS having 0.1% amikacin (8).

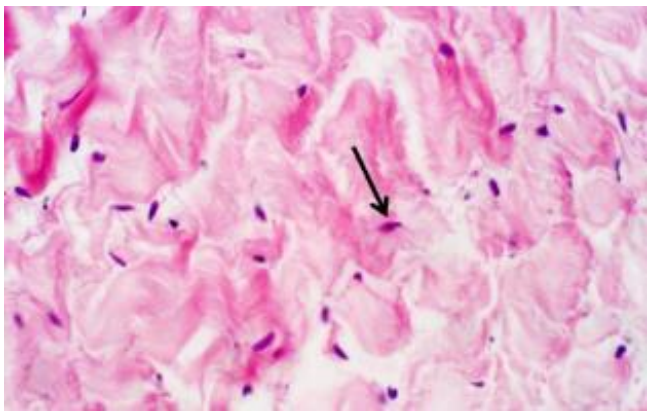

Figure 1: A micrograph of the normal bovine pericardium exhibited the presence of the nucleus and cells before decellularization (black arrow), and H&E staining was 400 x.

**Preparation of PRF**

10 ml of blood aseptically is drawn from the jugular vein and put into test tubes without an anticoagulant. The blood is then placed in testing tubes without an anticoagulant agent and centrifuged for 15 minutes at 2500 rpm. The final product has three layers that are as follows: for achieving a homogenous material, place the tube in the refrigerator after layer one, which is composed of cellular plasma, PRF mass in the central, RBC and WBC at the lowest (16).

**Surgical operation**

A protocol of anesthesia included administering atropine sulphate at a dose of (0.04 mg/kg B.W.) subcutaneously. Ten minutes later, Ketamine 5% and Xylazine 2% at a dose of (15,5 mg/ kg B.W.) intramuscularly were used (20). After anesthesia, the dog was positioned in dorsal recumbency and under a septic condition. The partial cystectomy was performed before the operation, the urine was evacuated by intraurethral catheterization at about 8-10 diameter, depending on the size of the animal (21). A mid-line laparotomy incision was made from the umbilicus hole to the pubis. After interring to the abdominal cavity, the UB was placed, exteriorized out of the abdomen and isolated from the abdominal cavity. The UB was evacuated from urine by needle and syringe, then encircled with moist cotton. Three tension stay sutures were fixed on the apex and two sides of the bladder wall using 3-0 polyglactin 910 (Vicryl) suture material to aid in stabilizing the UB on the operation site. An incision was made at the dorsal aspect of the bladder. In the first group, a circular defect about 3cm in diameter will be created on the dorsal aspect with a full thickness of the urinary bladder. The site of operation will closed with 4cm of acellular bovine pericardium, then sutured with 3-0 polyglactin910 (Vicryl) suture material (Yangzhou Super Union- China) with two rows by continuous inverting as Lembert and Cushing suture technique respectively (Figure 2) while in the second group is a same manner of first group will be considered except the site of operation closed completely with 4 cm of acellular bovine pericardium reinforced with a suitable amount with PRF as a circular shape along the edge of the graft and secured with 3-0 polyglactin910 (Vicryl) suture material with simple interrupted suture technique (Figure 3). Then the laparotomy incision was closed with routine manner.

For 45 days, the animals were observed daily in order to assess the biochemical and serological findings. Using an anticoagulant medication, five millilitres of blood were extracted from the jugular vein at various times to measure urea and creatinine levels in the blood serum at days 0, 3, 7, 15, and 16 before and after surgery. The urea kit was manufactured by the Spanish company Biosystem. In contrast, the French company Biolabo manufactured the creatinine kit (22,23) for serological investigations, including the C-reactive protein level in blood serum on days 0,3,7,15, pre-and post-surgery. The enzyme-linked immunosorbent assay technique (ELISA) was used to evaluate the serum sample (CRP Cat: ELk9107) (ELK Biotechnology, USA). The biopsies were collected to evaluate histopathological and immunohistochemical investigation from 3 animals for each group at 15, 30, and 45 days post-surgery to determine the healing process in the site of grafting (24-26) (Table 1).

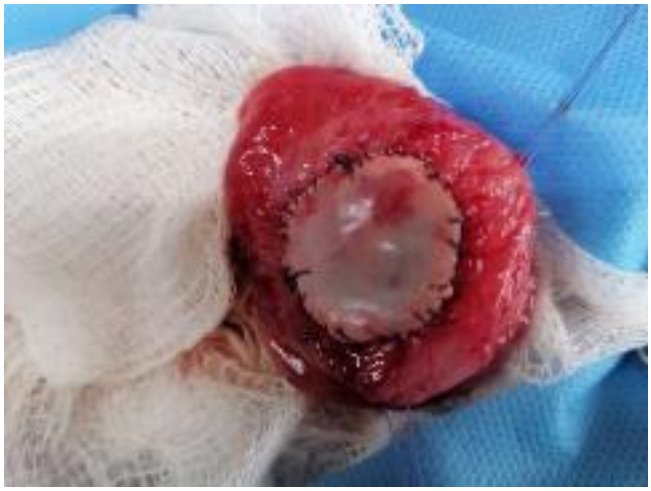

Figure 2: The photographic image shows cystoplasty with an ABP implant diameter of 4cm in G1.

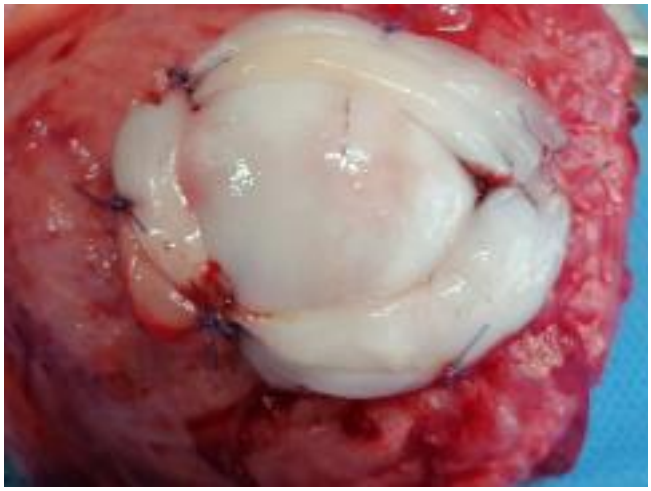

Figure 3: The photographic image shows cystoplasty with an ABP implant with a diameter of 4 cm reinforced with PRF in G2.

**Statistical analysis**

Statistical analysis performed with the Data was presented as M±SE error, and the comparison among periods was done by one-way ANOVA test. At the same time, the T-test achieved the comparison between the two groups, and the significant level was set as $P \leq 0.01$.

Table 1: Shows the immunohistochemical scoring system of Vimentin (VIM) and VEGF

| Standards | 4+ | 3+ | 2+ | 1+ | 0+ |
|---|---|---|---|---|---|
| Vimentin | Positive +++ More than 25 cells/field | Positive ++ 11 - 25 cell/field | Positive + 4 - 10 cell/field | Weak± 1-3 cell/field | Negative 0 cell/field |
| VEGF | Positive+++ More than 25 cells/field | Positive++ 11-25 cell/field | Positive + 4 - 10 cell/field | Weak± 1-3 cell/field | Negative 0 cell/field |

(-) no (±) very weak (+) low (++) moderate (+++) high positive immunoreactivity.

**Results**

The decellularization process of a bovine pericardium graft exhibited the absence of cells and nuclear components and was preserved without collagen degradation (Figure 4). In the present study, the clinical signs exhibited that all dogs in G1 and G2 survived a long study period with good general health conditions after surgery. All the clinical signs in both groups were slight and simple except the presence of hematuria, which was continued for 10-12 hours post-surgery, as well as signs of pain, arching of back and urine incontinence during urination, which continued for 24-48 hours post-surgery then all the clinical signs were subsided gradually after 48-72 hours and return of normal urination. There is no stitch abscess, graft rejection, uroabdomen, urine extravasation or stone formation during the studied period. The urea concentration results revealed a significant difference among periods within G1 at P≤0.01, except between 3 and 7day there was no significant difference. The same result appeared within periods of G2, except between zero and 7 days. There was no significant difference. The results showed the concentration of urea in G1 were 84.9± 3.9 mg/dl and 60.4±3.6 mg/dl higher than that in G2 and were 62.7±11.8 mg/dl and 28.3±4.7 mg/dl with a significant difference at P≤0.01 among the periods of 7,15 day (Table 2). The results of creatinine concentration indicated no significant difference among periods within G1 at P≤0.01, except that 7 days showed a significant difference with other periods within this group. The same result appeared within periods of G2 except between zero and 15 days and between 3 and 7 days; there was no significant difference. The results showed the concentration of creatinine in G1 was 2.37± 0.26 mg/dl higher than that in G2 and was 1.73±0.3 mg/dl with a significant difference at P≤0.01 among the periods of 7 days (Table 3). The results of Dog CRP concentration revealed no significant difference among periods within G1 and periods within G2 at P≤0.01, except that 7 days showed a significant difference with other periods within this group. The results showed the concentrations of dog CRP in G1 were 0.29±0.02 mg/dl, 0.30±0.02 mg/dl respectively higher than that in G2 and were 0.23±0.01 mg/dl, 0.21±0.014 mg/dl respectively with significant difference at P≤0.01 among the periods of 3 day as well as between period of 15 day (Table 4).

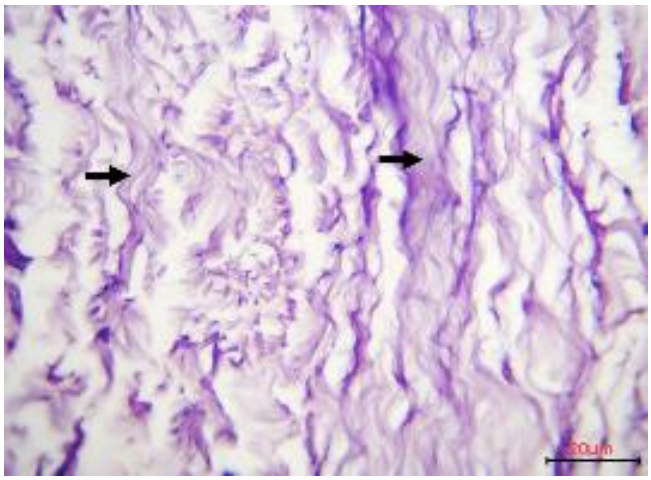

Figure 4: The histological section of the acellular bovine pericardium patch exhibited no cellular contain and deposition of collagen fiber (black arrow). (20µm H&E).

Table 2: Shows the mean values of urea concentration in serum mg/dl through the study in both groups

| Days | G1 | G2 |
|---|---|---|
| zero day | 29.0±3.4 [a] | 25.8±2.0 [a] |
| 3day | 100.8±11.2 [b] | 95.03±4.2 [b] |
| 7day | 84.9±3.9 [b] | 62.7±11.8 [c*] |
| 15day | 60.4±3.6 [c] | 28.3±4.7 [a*] |

The different letters in each column mean significant differences at P≤0.01 among periods. Star: Meaning significant difference at P≤0.01 among G1 and G2 groups.

Table 3: The mean values of creatinine concentration in serum through the study in both groups

| Days | G1 | G2 |
|---|---|---|
| zero day | 1.30±0.03 [a] | 1.06±0.03 [a] |
| 3day | 1.73±0.12 [a] | 1.50±0.05 [b] |
| 7day | 2.37±0.26 [b] | 1.73±0.30 [b*] |
| 15day | 1.43±0.16 [a] | 1.30±0.08 [a] |

The different letters in each column mean significant differences at P≤0.01among periods. Star: Meaning significant difference at P≤0.01 between G1 and G2 groups.

Table 4: Shows the mean values of C reactive protein concentration in serum mg/dl through the study in both groups

| Days | G1 | G2 |
|---|---|---|
| zero day | 0.23±0.018 [a] | 0.21±0.03 [a] |
| 3day | 0.29±0.02 [a] | 0.23±0.01 [a*] |
| 7day | 0.20±0.016 [a] | 0.20±0.02 [a] |
| 15day | 0.30±0.02 [a] | 0.21±0.014 [a*] |

The different letters in each column mean significant differences at P≤0.01 among periods. Star: Meaning significant difference at P≤0.01 among G1 and G2.

The histopathological findings at 15-day post-surgery in G1 was indicated existence of focal infiltration of mononuclear cells around and inside the graft (Figure 5) whereas in G2 revealed existence of fibrous tissue with a number of fibrocytes, the neovascularization started to appear inside the tissue, with dense fibrin clot and deposition of fibrin network (Figure 6). Moreover at 30 days post-surgery in the G1 presented heavy focal infiltration of mononuclear cells, formation of blood capillaries (angiogenesis), presence of fibrous tissue with a number of fibrocytes (Figure 7) whereas in G2 revealed an infiltration of inflammatory cells in the tissue and around the suture materials, clear fibrin clot and deposition of fibrin network with formation of fibrous tissue bridges between the graft and original tissue (Figure 8). At 45 days post-surgery in G1was exhibited a few inflammatory cells were found adhering to the surface of the implant and around the suture materials, small blood vessels among the two tissues representing neovascularization, formation of immature connective tissue between the two tissues (Figure 9) while in in G2 revealed a fibrous connective tissue and collagen bundles between the urinary bladder and the area of ABP implant, with new blood vessels and multiple spindle shape fibroblasts (Figure 10).

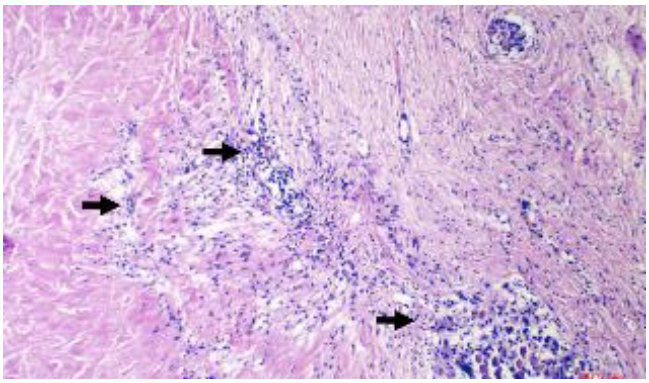

Figure 5: The histological section of G1 is on day 15 after grafting. Focal infiltration of mononuclear cells (lymphocytes, macrophages, giant cells) around and inside the graft (black arrows) (50µm H&E).

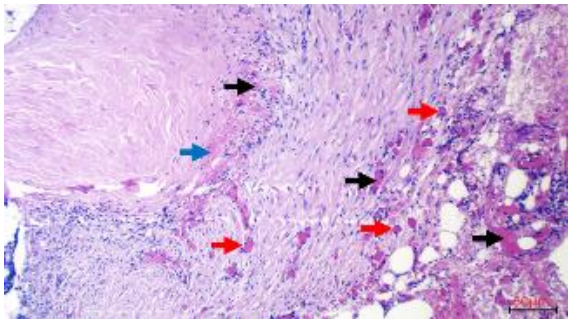

Figure 6: The histological section of G2 is on day 15 after grafting. The BP is closely intertwined in the urinary bladder with the presence of fibrous tissue with several fibrocytes (blue arrow); the neovascularization started to appear inside the tissue (red arrows), with dense fibrin clot and fibrin network deposition (black arrows). (50µm H&E).

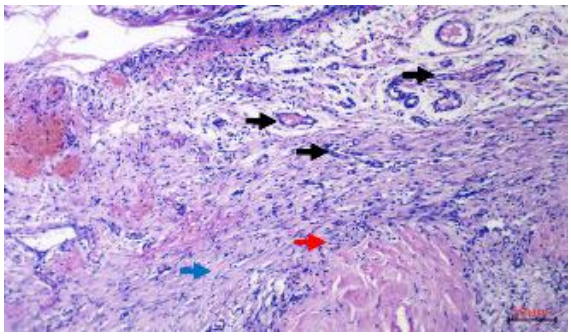

Figure 7: Histological section of G1 at day 30 after grafting. The BP implant is thoroughly intertwined in the urinary bladder, with heavy focal infiltration of mononuclear cells (red arrows), formation of blood capillaries (angiogenesis) (black arrows), and presence of fibrous tissue with several fibrocytes (blue arrows). (50µm H&E).

The results of immunohistochemistry staining for vimentin of G1 at day 15 after implantation in a dog urinary bladder showed a moderate positive immunoreactivity (++) vimentin expression (VIME), which represented as a nuclear brown spot in the fibroblast cells within the tissue (Figure 11) whereas in G2 was exhibited as same as the G1 (Figure 12). Moreover at 30 days, post-surgery of G1 presented high positive immunoreactivity (+++) vimentin expression which appearance as nuclear brown spot in the fibroblast cells within the tissue (Figure 13) though in G2 was exhibited a moderate positive immunoreactivity (++) vimentin expression which represented as nuclear brown spot in the fibroblast cells within the tissue (Figure 14). At 45 days post-surgery of G1 showed high positive immunoreactivity (+++) vimentin expression which represented as nuclear brown spot in the fibroblast cells inside the tissue (Figure 15) though in the in G2 was exhibited as same as the G1 (Figure 16).

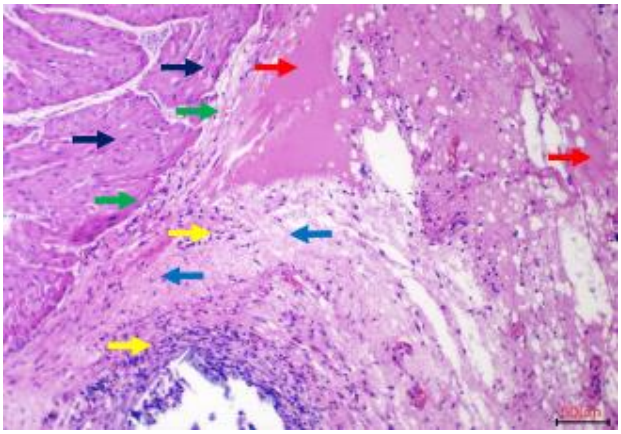

Figure 8: The histological section of G2 at day 30 after grafting shows the connection among the urinary bladder (black arrows) with the area of ABP (blue arrow), with infiltration of inflammatory cells in the tissue and around the suture materials (yellow arrows), apparent fibrin clot and deposition of fibrin network (red arrows), with formation of fibrous tissue bridges among the implant and original tissue (green arrows). (50µm H&E).

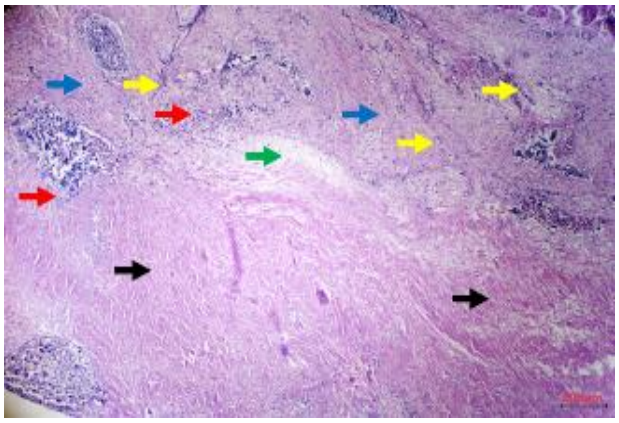

Figure 9: Histological section of G1 at day 45 after grafting. Cross section showing the contact between the muscular layer of the urinary bladder (blue arrow) and the area of ABP (black arrows), a few inflammatory cells were found adhering to the surface of the implant and around the suture materials (red arrows), small blood vessels among the two tissues demonstrating neovascularization (yellow arrows), formation of immature connective tissue between the two tissues (green arrow). (200µm H&E).

The results of immunohistochemistry staining for vascular endothelium growth factor of G1 at day 15 after transplanting in a dog urinary bladder showed very weak positive immunoreactivity (±), which represented a nuclear brown spot in the angioblasts cells inside the tissue (Figure 17) while in G2 was showed high positive reactivity (+++) which show as a nuclear brown spot in the angioblasts cells (Figure 18). Moreover at 30 days post-surgery of G1 showed low positive reactivity (+) which represented as nuclear brown spot in the angioblast's cells (Figure 19) while in the G2 was exhibited showed high positive reactivity (+++) which show as nuclear brown spot in the angioblast's cells (Figure 20). At 45 days post-surgery of G1 showed high positive reactivity (+++) which represented as nuclear brown spot in the angioblast's cells (Figure 21) though in G2 was exhibited as same as the G1 (Figure 22).

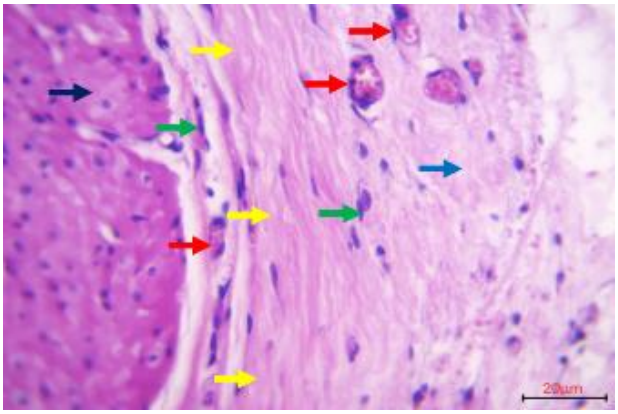

Figure 10: Histological section of G2 at day 45 after implantation. The section shows well-organized fibrous connective tissue and collagen bundles (yellow arrows) among the urinary bladder (black arrows) and the area of ABP (blue arrows), with neovascularization (red arrows) and multiple spindle-shaped fibroblasts (green arrows). (20µm H&E).

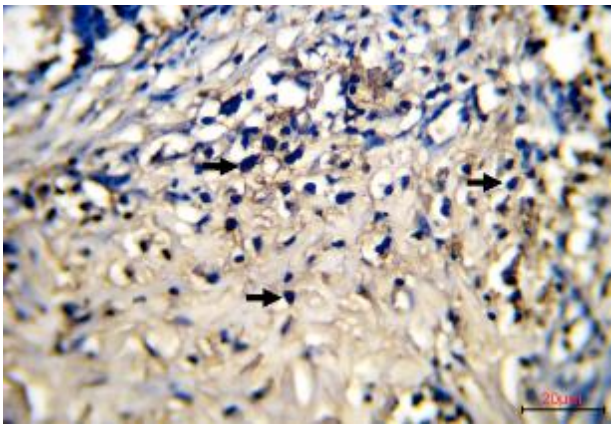

Figure 11: Immunoreactivity staining for vimentin in G1 at day 15 after implantation. Presented (++) VIME shows a nuclear brown spot in the fibroblast cells inside the tissue (arrows). IHC. 20µm.

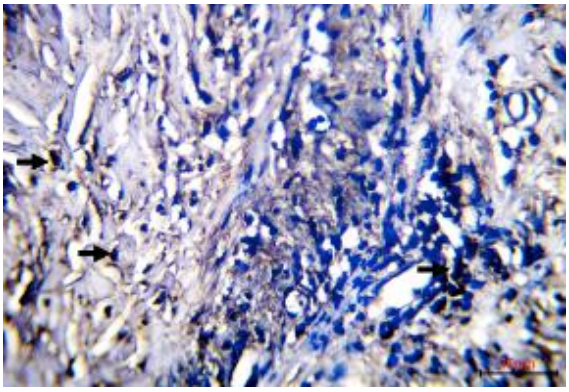

Figure 12: Immunoreactivity stain for Vimentin in G2 at day 15 after transplanting. Presented (++) VIME shows a nuclear brown spot in the fibroblast cells inside the tissue (arrows). IHC. 20µm.

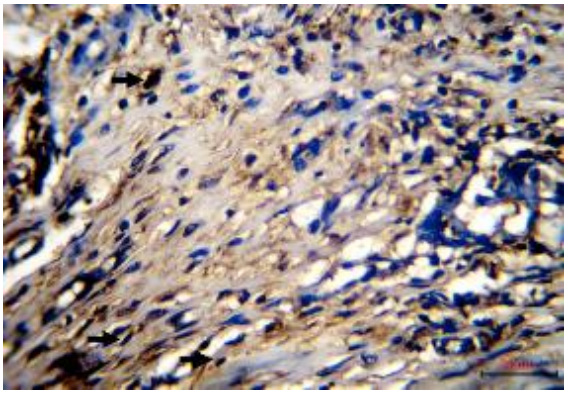

Figure 15: Immunoreactivity stain for Vimentin in G1 at day 45 after transplanting. Presented (+++) VIME shows a nuclear brown spot in the fibroblast cells inside the tissue (arrows). IHC. 20µm.

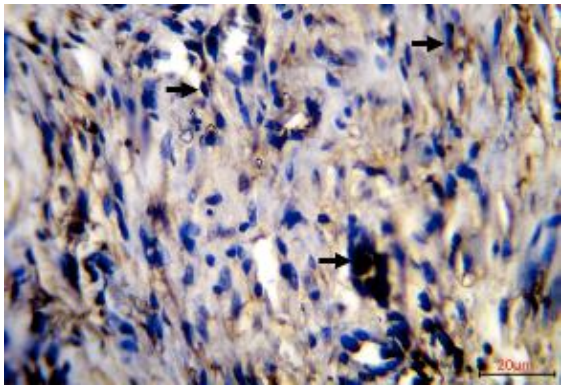

Figure 13: Immunoreactivity stain for Vimentin in G1 at day 30 after transplanting. Presented (+++) VIME shows a nuclear brown spot in the fibroblast cells inside the tissue (arrows). IHC. 20µm.

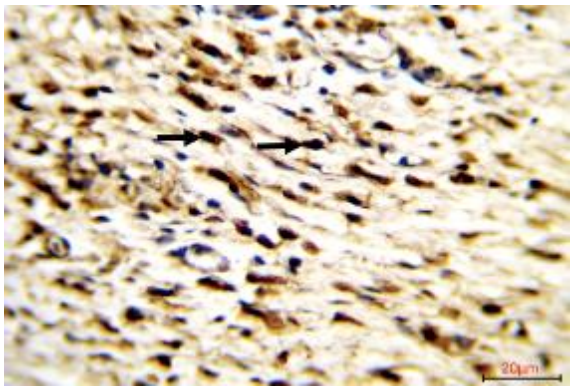

Figure 16: Immunoreactivity stain for vimentin in G2 at day 45 after transplanting. Presented (+++) VIME shows a nuclear brown spot in the fibroblast cells inside the tissue (arrows). IHC. 20µm.

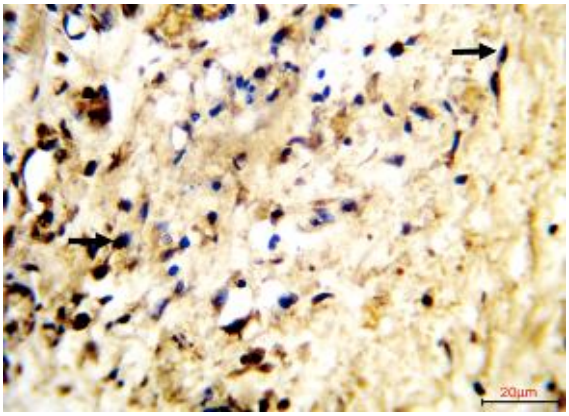

Figure 14: Immunoreactivity stain for Vimentin in G2 at day 30 after transplanting. Presented (++) VIME shows a nuclear brown spot in the fibroblast cells inside the tissue (arrows). IHC. 20µm.

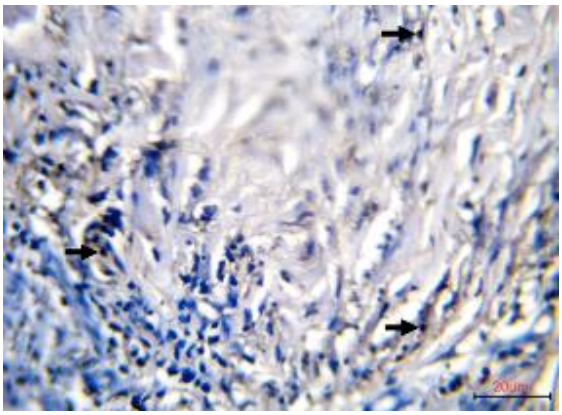

Figure 17: Immunoreactivity stain for VGEF in G1 at day 15 after transplanting. Presented (±) VEGFE shows as a nuclear brown spot in the angioblasts cells inside the tissue (arrows). IHC. 20µm.

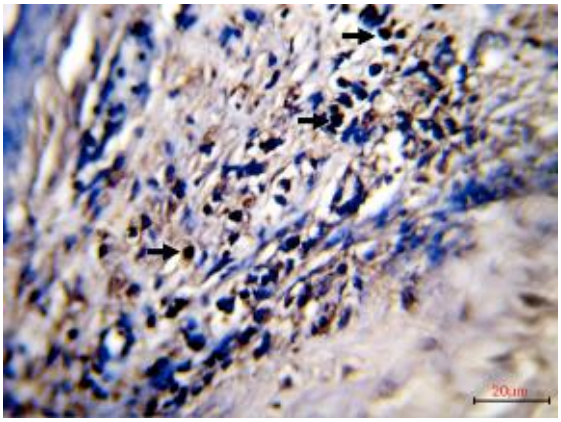

Figure 18: Immunoreactivity stain for VGEF in G2 at day 15 after transplanting. Presented (+++) VEGFE is shown as a nuclear brown spot in the angioblasts cells inside the tissue (arrows). IHC. 20µm.

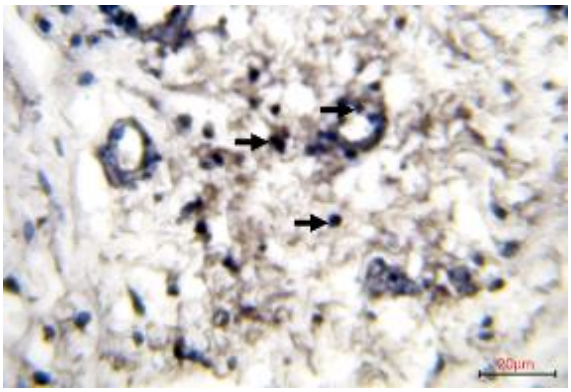

Figure 19: Immunoreactivity stain for VGEF in G1 at day 30 after transplanting. Presented (+) VEGFE shows a nuclear brown spot inside the tissue of the angioblast cells (arrows). IHC. 20µm.

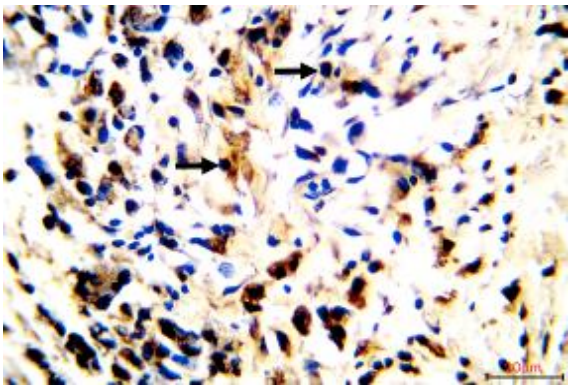

Figure 20: Immunoreactivity stain for VGEF in G2 at day 30 after transplanting. Presented (+++) VEGFE is shown as a nuclear brown spot in the angioblasts cells inside the tissue (arrows). IHC. 20µm.

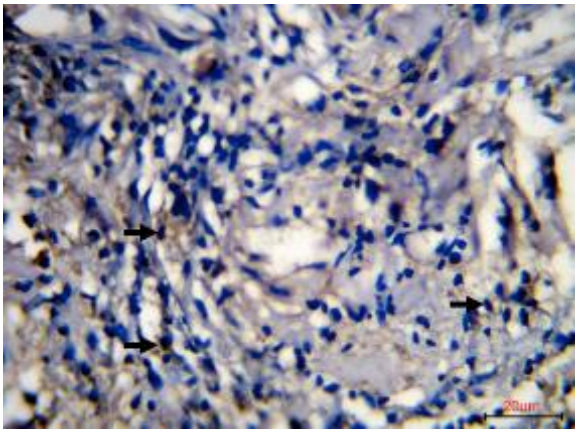

Figure 21: Immunoreactivity stain for VGEF in G1 at day 45 after transplanting. Presented (+++) VEGFE is shown as a nuclear brown spot in the angioblasts cells inside the tissue (arrows). IHC. 20µm.

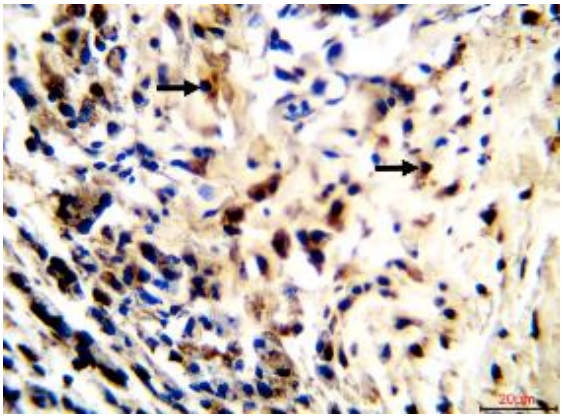

Figure 22: Immunoreactivity stain for VGEF G2 at day 45 after transplanting. Presented (+++) VEGFE is shown as a nuclear brown spot in the angioblasts cells inside the tissue (arrows). IHC. 20µm.

**Statistical analysis of immunohistochemical scoring**

The immunohistochemical results of vimentin expression showed no significant difference among periods of G1 and G2 at P≤0.01. additionally, the results showed no significant difference among the two groups at P≤0.01 for all periods of study in both groups (Table 5). The immunohistochemical results of VEGF expression showed no significant difference between 15 and 30 days in G1. However, there is a significant difference between 15 and 45 days and between 30 and 45 days at P≤0.01, whereas the periods group of G2 presented no significant difference at P≤0.01. The results presented that the expression of VEGF was higher in 15 and 30 days in G2, while the period of 45 days showed no significant difference at P≤0.01 among the two groups (Table 6).

Table 5: Show the scores of the immunohistochemical expression of the vimentin through the study in both groups

| Days | G1 | G2 |
|---|---|---|
| 15 day | 3.25±0.47 [a] | 3.25±0.25 [a] |
| 30 day | 3.50±0.28 [a] | 3.75±0.25 [a] |
| 45 day | 3.75±0.25 [a] | 4.00±0.00 [a] |

Similar letters in columns mean no significant difference among periods at P≤0.01. The star means the significant difference between the two groups at P≤0.01.

Table 6. Show the scores of the immunohistochemical expression of the VEGF in both groups throughout the study

| Days | G1 | G2 |
|---|---|---|
| 15 day | 1.5±0.28 [a] | 3.5±0.28 [a *] |
| 30 day | 2.0±0.40 [a] | 3.75±0.25 [a *] |
| 45 day | 3.5±0.28 [b] | 4.00±0.00 [a] |

Similar letters in columns mean no significant difference among periods at P≤0.01. Star mean significant difference between the two groups at P≤0.01.

**Discussion**

Biodegradable pericardial implants from cows caused the UB to grow. However, the implant gradually reabsorbed over time, and the bladder wall grew to normal, restoring the bladder's proper capacity and physiology (27). Under these circumstances, the present research examined the application and assessment of ABP and ABP implants enhanced with PRF for cystoplasty in dogs, representing clinical conditions. Additionally, this research provides information on applying ABP and ABP implants reinforced with PRF for repairing defective bladders in dog models for the first time. In order to prevent leakage of urine, adhesion, and potential vasculogenesis, the dorsal aspect of the UB is incised in the current investigation (28). Leakage of urine and stone formation were not recorded in this study. Suture type, pattern, and tissue of ABP together may play a role in preventing these complications. These thoughts also agree with many studies which recommended utilizing the running technique with a single layer in bladder augmentation and cystotomy procedures because it is stronger, takes less time, and sutures remain in the tissue of the bladder without remark any leakage (29).

The main clinical signs showed dogs' good general health condition during the first six weeks post-surgery. Hematuria occurred in some animals for both groups post-surgery and continued for 10-12 hours. This probably occurred due to trauma at the surgical site of the operation. There were no unusual alterations in the animal's behaviour or activity, with the exception of minor discomfort and hematuria that lasted for two days before progressively subsiding post-surgery, according to him also (30-32). They recorded the hematuria affected in the experimental animals and then mentioned that hematuria disappeared in the first two days post-surgery in all animals without any treatment. Hematuria was shown in dogs experiencing repair of UB through one week. The signs of urine incontinence and arching of the back during urination, which occur during the first two days post-surgery, may be attributed to the feeling of pain at the surgical site of operation with an abdominal muscle strain (33). Then, all these signs subsided gradually after 48-72 hours, and return to normal urination which were observed shortly after anesthesia recovery and persisted for the first week in the group of inverting, but vanished that day in the dog belonging to the opposing group (31). In this study, urine incontinence was impermanent and stopped naturally without treatment. This may be attributed to the inflammation of UB, the clinical signs like appetite loss, straining during urination, and hematuria postoperatively (34). They said that these results occurred due to injury of the bladder tissues. In the current investigation, the intraurethral tube was left in the site until the day following surgery, to relieve tension on the suturing site and avoid urine buildup (35).

In the present study, the results of the serological assessment exhibited an increase in the concentration of urea and creatinine on days 7,15, respectively, post-surgery, especially in G1, more than in G2. This explained that restoration of UB using ABP led to a weakening of the function of the urinary system as well as the addition of PRF to the implanted ABP graft improved immune response and the healing process of bladder defect by increasing the proliferation of granulation tissue formation and angiogenesis and decrease of inflammatory response post-surgery (14). Contrarily, the G2 recorded slight changes in urine and blood elements after cystoplasty, which was constant with cystoplasty consequences using other implants (36). Similar results were observed an increase in BUN and creatinine levels in the sheep tunica vaginalis xenograft grocomparedared with other groups (33). The analysis of dog CRP concentration showed an increase in serum CRP levels at days 3,15, respectively, post-surgery in G1 more than G2, and the degree progressively dropped to pretreatment values in G2; this outcome can be related to inflammation brought on by surgical trauma post-surgery (37). The AC via the decellularized extracellular matrix group of sheep tunica vaginalis preserved homeostasis of normal blood and was linked to the change of postoperative infective problems (33,38,39).

The site of the defect in both groups was represented by an improvement in the healing process without any inflammatory reaction, especially at 45 days post-surgery. The healing process of UB defect was superior and earlier in the G2 than in G1, respectively. However, the histopathological findings showed progress healing process especially in G2 as compare with G1 at 15,30,45 days post-surgery which was represented by formation of fibrous tissue bridges among the implant and original material with the

neovascularization and presence of collagen bundles and fibrous connective tissue among the UB and the area of a ABP graft and this attributed to tissue regeneration is facilitated by the natural milieu that BP's collagen-rich extracellular matrix creates for host cell proliferation and migration (40). As well as the addition of PRF to the implanted ABP graft that play role in improvement 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 because it contains many growth factors, the BP increases the creation of fibrous tissue, embeds and restores tissue integrity, and has the ability to induce cells regeneration in vivo, all causing a little inflammatory reaction and function of UB storing (41). Also, the treatment groups with PRF result in neovascularization and deposition of collagen fibres, which accelerates the healing process of hernia in addition to the ability of PRF to reduce the incidence of adhesion and inflammation compared to the control group (42), the PRF could promote healing of wound and restoration of cells that play a central role in the differentiation, proliferation and migration of cells, in addition to the process of angiogenesis as well as its releases significantly through super than seven days large amounts of important healing particles as growth factors, chemokines and cytokines and So, it exemplifies one of the most important biomaterials used in regenerative therapy, because it is self- obtained with low cost and provides a high rate of tissue regeneration. In addition, it does not have any contraindications (43,44).

The immunohistochemical results of vimentin expression showed that there is no significant difference at 15, 30, and 45 days post-surgery among periods of G1 and G2, and this revealed the capability of ABP + PRF graft to significantly improvement and accelerate the healing process of the urinary bladder, and this attributed to the ABP graft is rich in collagen primarily type-I as well as glycosaminoglycans (GAGs), glycoproteins, chemokines, cytokines and growth factors and play role in formation of fibrous tissue and collagen fiber in the site of grafting (45). Also agree with previous results which used other scaffolds for the reconstruction of bladder defect (46), the BAM is composed of complex structural and functional proteins involving proteoglycans, glycosaminoglycans, fibronectin, elastin and collagen (type I and III) and in proportions similar to those in the tissue of normal bladder, the expression with vimentin stains revealed weak staining for the frame, control and suture methods at one week, but highly positive stains for all methods after four weeks (47). Based on these results, they concluded that the frame method of rat subcutaneous implantation was successfully and easily carried out and had a less inflammatory reaction. Also, the degradation phenomenon of the natural scaffold was not restricted to SIS; it was also observed in the pericardium following subcutaneous implantation in a rat aorta, which demonstrated tissue deterioration occurs when the scaffold vanishes or is reabsorbed into the tissue (48). The tissue deterioration might arise from host cells replenishing the transplanted cellular tissue (49). It was postulated that the bioactive material broke down quickly and was substituted by host tissue that organized and differentiated like the bladder tissue (50).

The immunohistochemical results of VEGF expression showed that the expression of VEGF was higher at days 15 and 30 in G2, as compared with G1, and this revealed the capability of ABP + PRF graft significantly improvement and acceleration of the healing of the urinary bladder and this attributed to PRF is a direct impact on angiogenesis and is composed of major soluble factors involved in angiogenesis and growth factors including PDGF, VEGF, FGFb and angiopoietin. These factors deliver a suitable environment for the migration, proliferation and growth of UB epithelial and muscle cells, the treatment group resulted in increasing VEFG expression in the epithelial cells of blood vessels, and they found that the angiogenic activity of PRF has a large effect on wound healing and consider (15,42,51) as well as cytokines and interlukines, therefore, the ABP graft is a constant source of VEGF, which significantly contributes to the healing of UB (52).

## Conclusion

The current investigation results demonstrated that cystoplasty was successful and that the urinary system function was restored in the ABP + PRF group and, to some extent, the ABP groups.

## Acknowledgment

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## Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication and/or funding of this manuscript.

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# تأثير تطبيق غشاء التامور البقري اللاخلوي المدعم بالليفين الغني بالصفيحات الدموية الذاتي لترقيع المثانة في الكلاب: التقديرات النسيجية المرضية

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**الخلاصة**

صمم هذا العمل التجريبي لتقدير كفاءة غشاء التامور البقري اللاخلوي مع التطبيق الموضعي لليفين الغني بالصفيحات الدموية لتحسين عيب المثانة البولية في الكلاب. استخدم في هذه الدراسة ثمانية عشر من الكلاب المحلية البالغة. الحيوانات تحت بروتوكول التخدير العام. قسمت التجربة عشوائيا الى مجموعتان متساويتان تسعة لكل مجموعه. في المجموعة الأولى (غشاء التامور البقري اللاخلوي) تم عمل عيب دائري بقطر ٣ سم في الجزء الظهري للمثانة البولية وتم التغليف الكامل لموقع العملية باستخدام غشاء التامور البقري اللاخلوي بقطر ٤ سم بينما في المجموعة الثانية (غشاء التامور البقري اللاخلوي) مع الليفين الغني بالصفيحات الدموية فأن موقع الترقيع المثانة أسندت بالليفين الغني بالصفيحات الدموية. سريريا روقبت جميع حيوانات التجربة يوميا خلال ٤٥ يوم. أنجزت التقديرات النسيجية المرضية والمصلية للأيام ٤٥،٣٠،١٥،٧،٣،٠ بعد العملية الجراحية. أظهرت نتائج الإزالة الخلوية لرقعة غشاء التامور البقري غياب الخلايا والمكونات النووية. سريريا تبدو الحيوانات بصحة جيدة وبقيت على قيد الحياة الى حد تكامل الدراسة وحدثت بعض العلامات في اليومين الأولى مثل التبول الدموي، الألم، تقوس الضهر وسلس البول وبعدها تم اختفائها بعد ٤٨- ٧٢ ساعة بعد العملية. أظهرت التقديرات المصلية والكيموحيوية زيادة تركيز اليوريا في المصل في اليوم ١٥،٧ بعد العملية الجراحية والكرياتينين في اليوم ٧ بعد العملية في المجموعة الأولى أكثر من المجموعة الثانية. أظهرت نتائج تحليل تركيز بروتين سي التفاعلي زيادة في بروتين سي التفاعلي في المصل في اليوم ٣و١٥ بعد العملية في المجموعة الأولى أكثر من المجموعة الثانية. تمثلت التغيرات النسيجية المرضية بعمليات الالتئام الجيدة لكلا المجموعتين من دون أي تفاعلات التهابية في اليوم ٤٥ بعد العملية. أن عمليات الالتئام لعيوب المثانة البولية كانت راقيه وحدثت بشكل مبكر في المجموعة الثانية بالمقارنة مع المجموعة الأولى على التوالي والتي تمثلت بتكوين جسر النسيج اليفي بين الرقعة والنسيج الأصلي مع تكوين أوعية دموية جديدة. يوجد تعبير عالي للفايمنتين في كلا المجموعتين في اليوم ٤٥ بعد العملية بينما يوجد تعبير عالي لعامل نمو بطانة الأوعية الدموية في اليوم ٣٠،١٥ في المجموعة الثانية وتعبير ضعيف جدا في المجموعة الأولى. من النتائج نستنتج، أن معلومات التجربة أظهرت بأن تطبيق الليفين الغني بالصفيحات الدموية الى رقعة غشاء التامور البقري اللاخلوي المغروسة حسنت من عمليات الالتئام لعيب المثانة البولية في الكلاب مع الأهمية لليفين الغني بالصفيحات الدموية.

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