Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Effect of autologous peritoneum and platelet-rich fibrin graft on healing of intestinal anastomosis in dogs

N.M. Raffea and A.H. Allawi

Department of Surgery and Theriogenology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Article information

Article history:
Received June 14, 2021
Accepted September 3, 2021
Available online February 24, 2022

Keywords:
Peritoneum graft
PRF
Intestinal anastomosis
Dogs

Correspondence:
A.H. Allawi
asmaallaw@uomsul.edu.iq

Abstract

In the present study, the effect of autogenous peritoneum graft (PG) with platelet rich fibrin (PRF) was evaluated on intestinal anastomosis healing in dogs. Eighteen local breed adult dogs were anesthetized then jejunal intestinal resection and anastomosis were created on all animals. Animals were divided into 3 equal groups (6 for each). In first group (control): An end-to-end intestinal anastomosis was performed using simple interrupted suture pattern only. In group PG: The anastomosis site was sutured as in control group and was wrapped with PG. Where as in PRF+PG group: The anastomosis site was sutured also as in first group and was wrapped with PRF and PG. Healing process was studied in all above mentioned groups clinically, grossly, histologically and radiographically at 15 and 30 days post operation. Results were revealed that the utilizing of PG caused a valuable influence on anastomosis site healing. This impact was manifested by faster re-epithelization of mucosa, increased collagen deposition, fibroblast proliferation, reduced adhesions, and decreased stenosis degree comparison with control group. Also there was less adhesions, less stenosis degree, increased in epithelization of the mucosa in the group treated with PRF+PG than the group treated with PG alone. In conclusion the current study has shown that autologous peritoneal graft application has a positive and beneficial effect on intestinal anastomosis healing. Also the results highlighted the promising effects of PRF in conjunction with PG on intestinal anastomosis healing. The application of PRF with PG at the intestinal anastomosis site is successful, safe, and highly efficient with no complications.

DOI: 10.33899/ijvs.2021.130529.1840,

Introduction

In gastrointestinal surgery the bowel anastomosis is a commonly procedure for the development of contact between two historically separate areas of the intestine, it becomes necessary to perform when a diseased segment on intestinal tract is resected for treatment strangulation, intestinal obstruction, neoplasm, irreducible intussusceptions, intestinal torsion, mesenteric parasite and intestinal rupture (1). To perform a good anastomosis a number of aspects must be considered. Several factors have an influence on the healing of anastomosis site for example type of surgery, surgical technique, intraoperative contamination, reperfusion, obstructive jaundice, peritonitis, circulation of intestinal segments being approximated, malnutrition, hypoalbuminemia, vitamin deficiency, previous irradiation, steroids, anemia, tension in suture line, chemotherapy and radiotherapy (2-4). Poor wound healing of anastomotic sites results in severe and life-threatening complications such as ileus, stricture, peritonitis, short bowel syndrome, shock, leak, dehiscence, abscess, fistula, necrosis, perforation, prolonged hospitalization, discomfort, further surgeries, increased health care cost and increased morbidity and mortality rates (1,2,4-6). Despite many developments in surgical techniques, equipment and management after operation, the percentages of complications for intestinal anastomosis have still not been reduced to a negligible level (3,7).

Recently, a number of studies have focused on identifying a plan to prevent or decrease these complications, which represents a failure in the surgical process that can be potentially fatal (5-9). Some of these searches have suggested covering of the anastomosis by a variety of substances in both experimental and clinical studies. Different types of materials have been used, and many of them have succeeded to show convincing results (10). In spite of the promising results of using reinforcement materials, additional studies are needed to improve this strategy. Autografts are regarded as the gold standard to which all other substitutes are measured. In comparison with other natural implantable materials these grafts have biological characteristics and the properties necessary for reconstruction of tissue structure and new tissue regeneration. Moreover, immunological rejection of autologous grafts after transplantation is not noticed (11). A peritoneum is serous membrane lining the abdominal wall. It is covered by mesothelial cells and supported by a connective tissue ground work, or stroma. The connective tissue stroma is composed of collagen, elastin fibers, macrophages, lymphocytes, mast cells, glycosaminoglycan and adipose cell (12,13). This tissue has been shown to induce tissue restoration based on the capacity of cells that it is contain to differentiate into another cells such as fibroblasts, adipocytes, hepatic cells, myoblasts and other cells under special conditions (13,14). Furthermore, peritoneum cells have been shown to secrete many growth factor (GFs) such as fibroblast growth factor (FGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF) which have a critical positive role on tissue healing process (12-14). The protective role of the peritoneal membrane for controlling the escape of intestinal contents from perforations has been well established for many years (15,16). On the other hand, Platelet Rich Fibrin; is totally an autologous blood product has been broadly utilized as a viable fibrin biomaterial in reconstruction surgery to enhance tissue healing. It is contain a large quantity of platelets and leukocyte, stem cells, cytokines and growth factors such as PDGF, TGF-β, IGF, EGF, VEGF, interleukins-1β, 4 and 6 as well as several other adhesion factors. The cytokinesis and growth factors that it is contain are retained in three dimensional tetra molecular network of fibrin to maintain its morphological aspects and properties and can be gradually released up to approximatelly15 days. These factors serve different roles in different phases of the wound-healing process. The PRF was shown to providing the essential elements for wound repairing and healing, it is play important critical role in cell migration, differentiation and proliferation of different cells such as fibroblasts, endothelial cells and osteoprogenitor cells, facilitate tissue healing process and producing negligible minimal immunological reaction (17-19).

Recently, numerous studies have investigated the benefits in various disciplines of surgery solely or in combination with other approaches such as oral surgery, cosmetic surgery, digestive system surgery and orthopedic surgery (19-22). In spite of there is few previous clinical and experimental studies have been used PRF in intestinal healing, no one of them studied the combination use of peritoneum graft with PRF.

Therefore, the goal of this study is to investigate if the usage of the peritoneum alone or in combination with PRF affects the healing process in an experimental intestinal anastomosis model. Moreover, we needed to investigate if these substances reduce the complication of intestinal anastomosis site or not.

Materials and methods

All the procedures carried out and listed in this study were approved by Institutional Animal Care and Use Committee of the Veterinary Medicine Collage, Mosul University (UM.VET.2021.007). Eighteen healthy adult dogs from local breed, average weight 24 kg, aged from 18 - 24 months were used. All animals were housed for 3 weeks at the facility for adaptation and were supplied with healthy food and drink through the period of experiment. All animals underwent surgery were fastened from water for 12 hours, 24 hours from the food. Animals were anesthetized through intramuscular injection a mixture of 5% ketamine hydrochloride (10 mg/kg) and 2% xylazine (3 mg/kg), when needed this mixture was used to maintain the anesthesia.

Preparations of PRF

At the time of operation, 10 ml venous blood sample was drawn from the jugular vein and collected in sterile tube without anticoagulant and was centrifuged at 3000 rpm for 10 min (22). Then, after centrifugation the red blood cells are obtained in the lower part, platelet poor plasma in the upper part and the PRF at the intermediate part (Figure 1).

Preparations of peritoneum graft (PG)

After opening of the abdomen and performing intestinal resection and anastomosis the parietal peritoneum membrane was then isolated and the desired graft size approximately 2×5 cm was harvested. The harvested peritoneal graft was used immediately to wrapping the anastomosis site (14).

Surgical procedure

The abdomen was prepared under aseptic conditions. Animal was positioned in dorsal recumbancy. Ventral midline abdominal incision was made caudal to umbilicus (approximately 7-10 cm in length). The segment of jejunum to be removed was isolated from the remaining viscera and the peritoneal cavity with moistened laparotomy sponges. Approximately 5 cm segment of jejunum was removed. Anastomosis with end-to- end technique was done with

single row of simple interrupted suture using Polygalactin 910 (3/0) (Figure 2), then the defect of mesentery was closed by same technique and suture material used for intestine. The animals were randomly assigned to three groups (6 dogs for each). First group (control group (C)): subjected to end-to- end intestinal anastomosis without grafting. Second group (peritoneum graft group (PG)): the anastomosis site was sutured as in control group and was wrapped with PG which was fixed to intestine by simple interrupted stiches using the same suture used for anastomosis (Figure 3). Third group: PRF and PG groups (PG+PRF): the anastomosis site was sutured as in control group and was wrapped with PRF and then PG which was fixed to intestine as in group two (Figure 4). The intestine was retained to the abdominal cavity. The abdominal incision was closed by routine manner. The animals were given glucose 5% intravenously at a dose 10 ml /Kg B.W. twice a day for two days after operation, after that they were allowed to take soft food. All dogs received penicillin streptomycin 10000 IU and 10 mg/kg respectively for 5 days as a postoperative antibiotic therapy. Clinical examination to animals was performed daily which included the observation of defecation, urination, wound inspection and general condition of the animals, also gross, radiographical and histological examination to anastomosis were made at 15 and 30 days after operation.

For macroscopic examinations the abdominal cavity of three animals for each group at 15 and 30 days post operation was opened and inspected for presence of anastomotic leakage, intraabdominal adhesions, abscess, any kind of free fluid collection and peritonitis. The degree of adhesion formation was scored according to a criteria established according the (23) (Table 1).

Table1: Criteria and the grades used to evaluate the degree of adhesion formation at the anastomosis site.

Grade	Criteria
0	No adhesion around the anastomosis
1	Spontaneously separating Adhesions
2	Separating adhesions by traction
3	Separating adhesions by dissection

To perform the radiographical examination three animals from each group at 15 and 30 days' post-surgery were anesthetized. A segment approximately 15 cm of intestine including the anastomotic site was resected from the animals and kept in physiological saline for other studies. One end of the intestinal piece was closed tightly by forceps and filled with Barium sulfate at concentration 25% and another end afterward was closed too. The intestinal specimens were examined by X-ray machine using 50 kilovolts and 3 MAs in order to calculate the stenosis degree of intestine at the site of anastomosis by using the following formula: degree of stenosis = 100(1-2a/(b+c)), where; a: is the diameter of intestinal anastomosis site. b and c: are the l diameters of intestine 2 cm before and after the site of anastomosis. The results were statistically analyzed using ANOVA and Duncan tests. Statistical differences between groups was considered significance at $P<0.05$. After tacking the radiograph, specimens containing the anastomotic site were collected and fixed in buffered formalin at concentration 10% for10 days and processed routinely according to standard procedures and then was stained by hematoxylin and eosin (H&E).

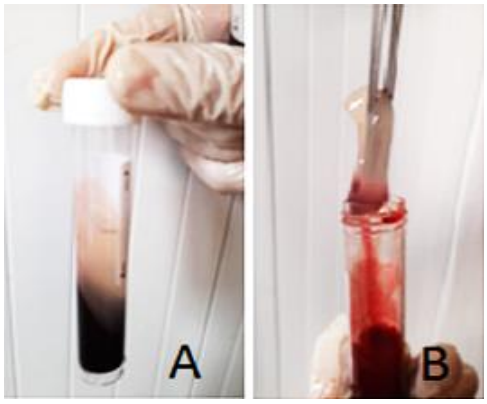

Figure1: Platelet rich fibrin preparation.

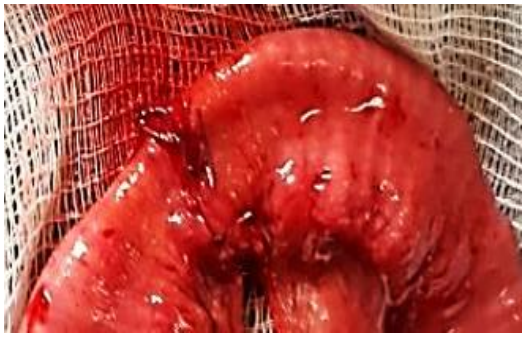

Figure 2: Suturing an anastomosis with a simple interrupted suture pattern.

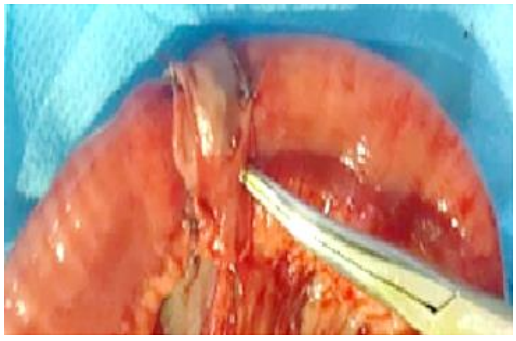

Figure 3: Anastomosis site wrapped by PG.

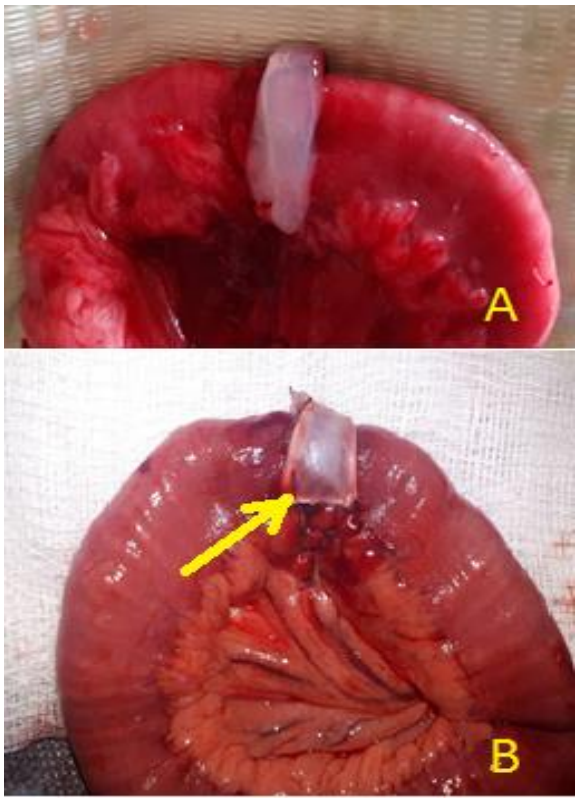

Figure 4: Anastomosis site wrapped by PRF (A) and then by PG in PRF+PG group.

Results

Clinical results showed there is no animal from the all groups died. The defecation and urination were normal. The site of operation healed without any complications. Macroscopic evaluation showed no postoperative complications such as peritonitis, necrosis, abscess, dehiscence, fistula and leak at site of anastomosis in any animal in all groups. Adhesions around the anastomosis site were observed in the three groups at 15 and 30 days' post operations. The adhesions were more severely in control group than the other groups, while the PRF+PG group was showed less severity of adhesions than the other groups (Table 2). In the group C, 33.3% of dogs had a grade 3 adhesion scores, 66.6% of dogs had grade 2 adhesion scores. In the PG group 16.6% of dogs had grade 2 adhesion score, 66.6% had a grade 1 adhesion scores while 16.6% of dogs had no adhesion scores in PRF+PG group, 33,3% of dogs had a grade 1 adhesion score, 66,6% of dogs had no adhesion scores. Adhesions in control group were between the anastomosis site and the adjacent small bowel loops or omentum (Figures 5 and 6), adhesions in group 2 were between the anastomosis and the omentum or mesentery (Figures 7 and 8), while adhesions in group 3 were between the anastomosis and the mesentery only (Figures 9 and 10).

Table 2: Degree of adhesions produced

Group	Degree of adhesions n (%)			
	0	1	2	3
C			4 (66.6)	2 (33.3)
PG	1 (16.6)	4 (66.6)	1 (16.6)	
PRF+PG	4 (66.6)	2 (33.3)		

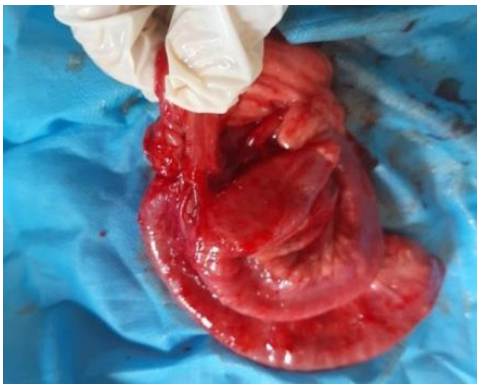

Figure 5: Adhesion the anastomosis area with another intestinal loop and omentum in group C (grade 3).

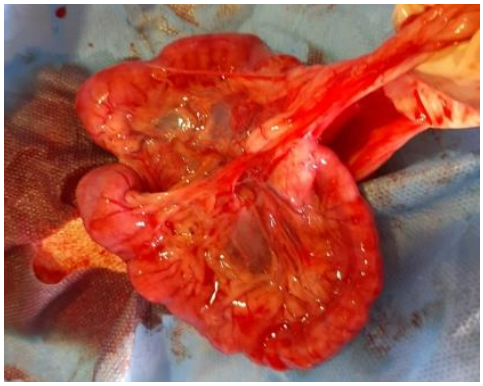

Figure 6: Adhesion the anastomosis area with omentum and mesentery in group C (grade 3).

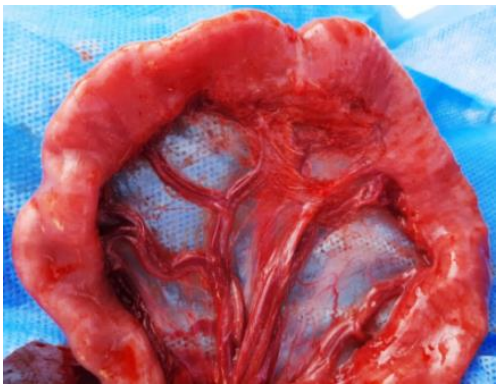

Figure 7: Adhesion the anastomosis area with mesentery in group PG (grade 1).

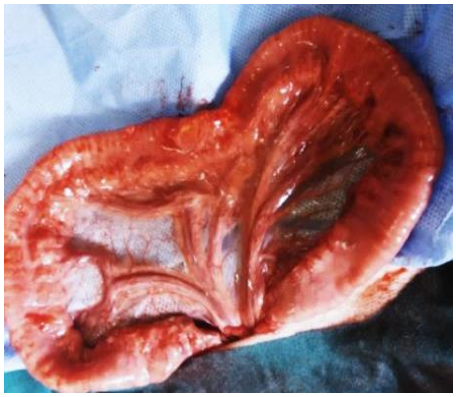

Figure 8: Adhesion the anastomosis area with mesentery in group PG (grade 2).

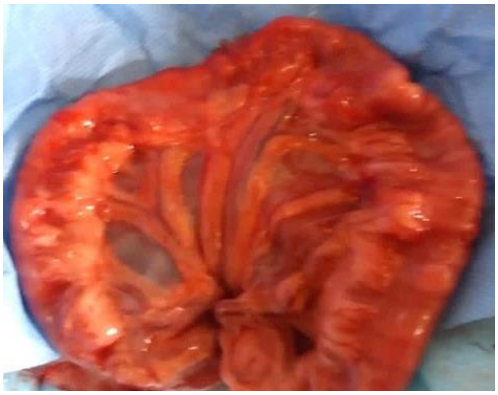

Figure 9: No adhesions at the anastomosis area in group PRF+PG (grade 1).

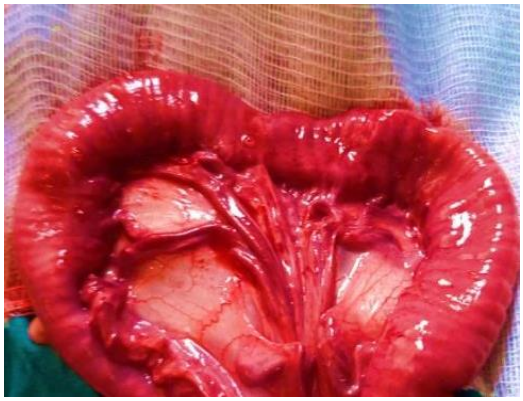

Figure 10: Adhesion the anastomosis area with mesentery in group PRF+PG (grade 1).

Result of contrast radiographic study revealed that there was significant difference in degree of stenosis between all groups at 15 and 30 days' post operation (Table 3). The C group (Figure 11) showed a higher stenosis degree than the PG (Figure 12) and PRF+PG (Figure 13) groups on the 15 and 30 postoperative day. The PRF+PG group showed a lower stenosis degree than the other groups on day 15 and 30 post operation. Also in PG and PRF+PG groups there was significant difference in degree of stenosis at 15 and 30 days' post operation, while the difference in C group was statically not significant. The smallest degree of anastomotic stenosis was seen in PRF+PG at 30 days 12.05%. The largest degree of anastomotic stenosis was seen in C group at 15 days 61.03%.

Table 3: The mean stenosis degree (%) of intestinal of all groups at 15 and 30 days' post operation

Time	C group	PG group	PRF+PG group
15	61.0 ± 0.2^{a}	46.1 ± 1.4^{b}	32.1 ± 1.5^{c}
30	54.1 ± 0.4^{a}	$28.3\pm1.6^{b*}$	$12.0\pm0.2^{c*}$

Litters have significant difference between groups at $P<0.05$. *mean significant difference at time in each group at $P<0.05$.

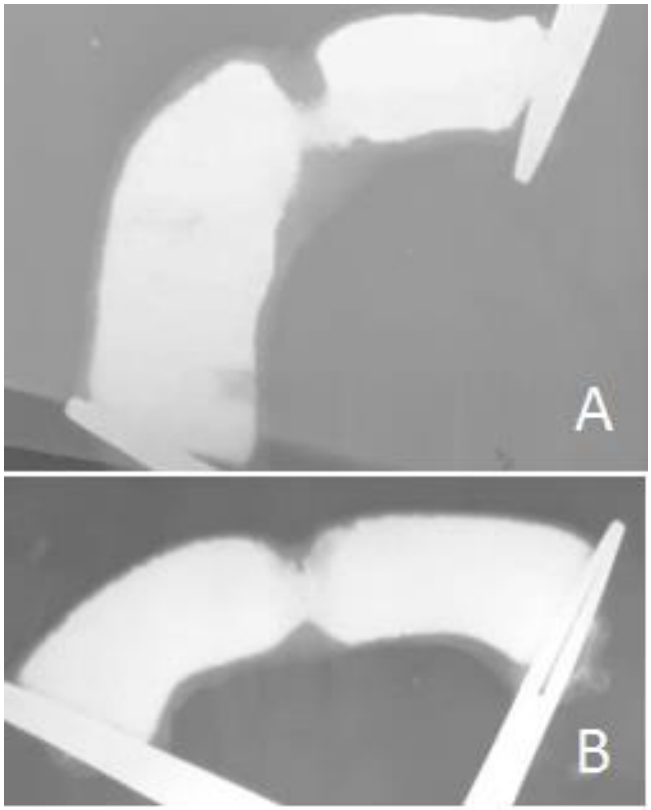

Figure 11: Radiographic image showing stenosis at the anastomosis site (A) 15 days after operation (B) after 30 days after operation in control group.

Histopathology of anastomosis in group C at 15 days' post operation showed formation of granulation tissue, new blood vessels and severe inflammatory reaction characterized by foci of inflammatory cells (Figure 14).

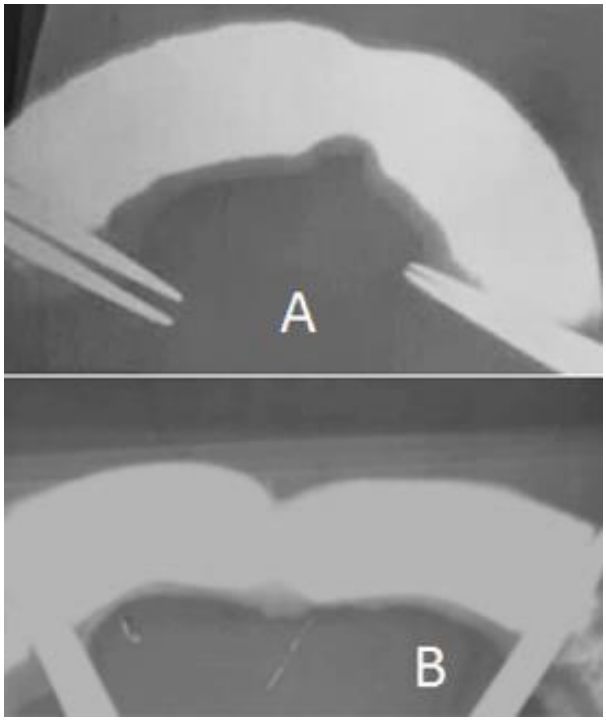

Figure 12: Radiographic image showing stenosis at the anastomosis site (A) 15 days after operation (B) after 30 days after operation in PG group.

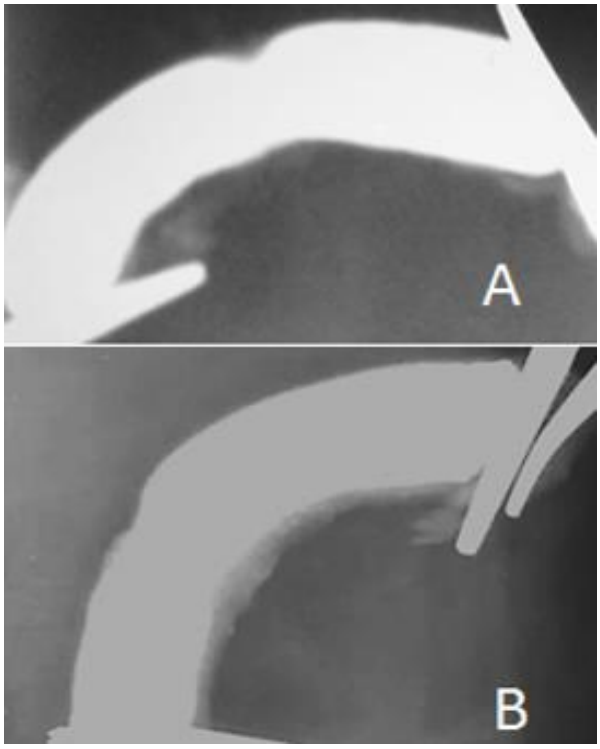

Figure 13: Radiographic image showing stenosis at the anastomosis site (A) 15 days after operation (B) after 30 days after operation in PRF+PG group.

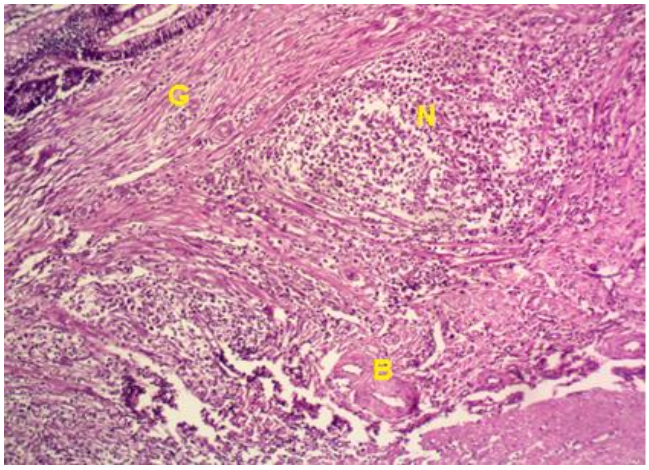

Figure 14: Histological section of intestinal anastomosis site in the C group, 15 days after operation. There is a nodular foci of chronic inflammation (N) surrounded by connective tissue with presence granulation tissue (G) and new blood vessels (B). (H&E X10).

At 30 days' post operation was revealed presence of inflammatory reaction, fibrous tissue and healing of mucosa by re-epithelization (Figure 15). In PG group at 15 days after operation good healing with granulation formation, angiogenesis, immature fibrous tissue and moderate inflammatory response, In addition to that. Beginning of re-epithelization and muscularis mucosa formation were observed. Graft degradation was characterized by loss of a distinct boundary between newly deposited host tissue and the original graft material (Figure 16). While at 30 days' post operation good healing with granulation tissue formation were present characterized by mild - moderate inflammatory reaction, angiogenesis, fibrous connective tissue formation and more re-epithelization than which was seen at 15 days and muscularis mucosa formation (Figure 17).

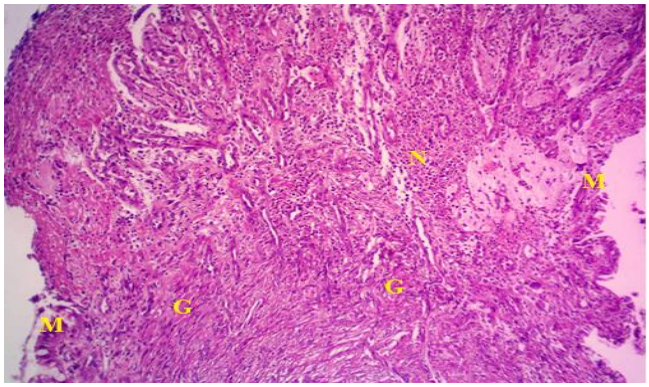

Figure 15: Section of intestinal anastomosis site in the C group, 30 days after operation show healing with granulation (G), re-epithelization and inflammatory reaction (N). (H&E X10).

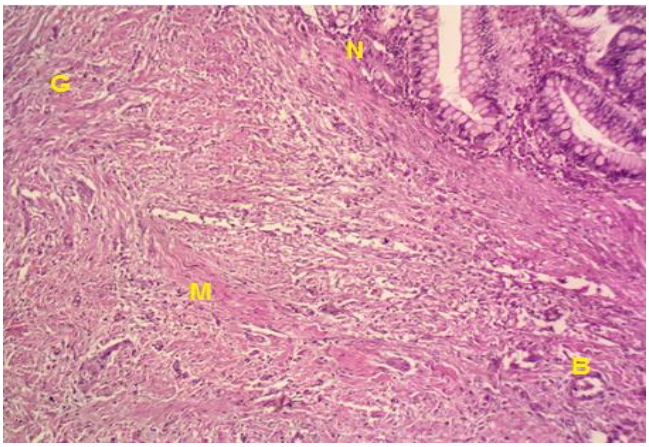

Figure 16: Section of intestinal anastomosis site in the PG group, 15 days after operation show inflammatory reaction, granulation formation (G), new blood vessels with formation of muscularis mucosa (M). (H&E X10).

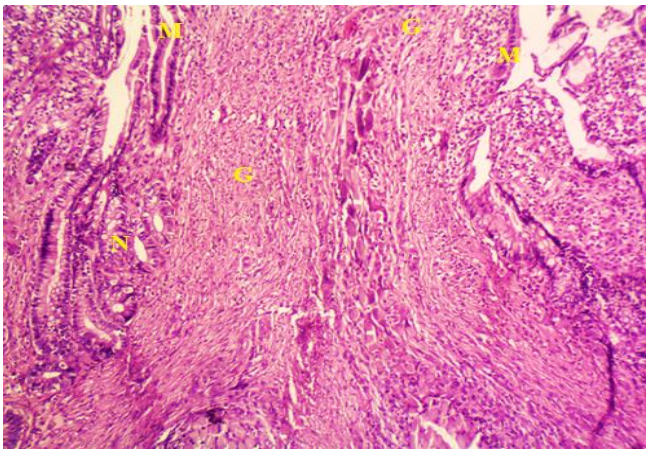

Figure 17: Section of intestinal anastomosis site in the PG group, 30 days after operation show healing with granulation formation (G), re-epithelization, moderate inflammatory reaction (N), angiogenesis, fibrous connective tissue formation and muscularis mucosa formation. (H&E X10).

In the PRF+PG group, histopathological examination at 15 days revealed mild inflammatory response with good healing represented by presence of mature connective tissue and re-epithelization of intestinal mucosa for the site of anastomosis (Figure 18). After 30 days, there was very good healing represented by mature connective tissue at the healing region, organized collagen fibers, angiogenesis, re-epithelization with formation of goblet cells and mild inflammatory response (Figure 19).

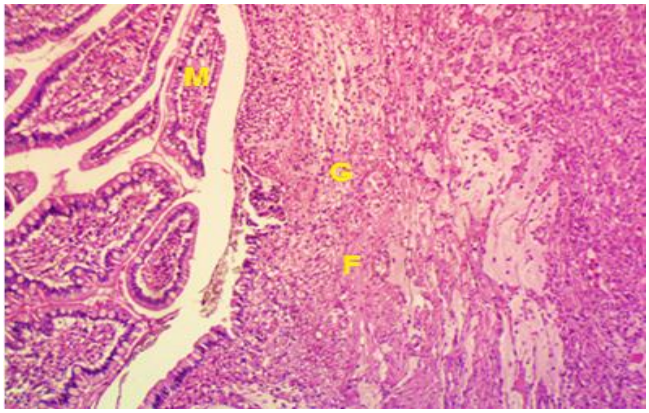

Figure 18: Section of intestinal anastomosis site in the PRF+PG group, 15 days after operation show good healing with granulation tissue formation (G), re-epithelization (M), and fibrous connective tissue formation (F). (H&E X10).

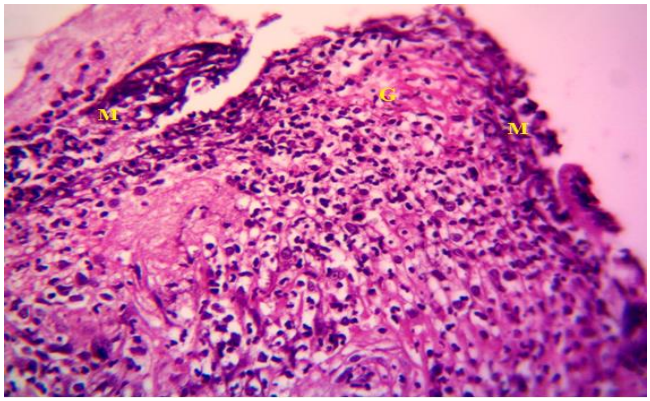

Figure 19: Section of intestinal anastomosis site in the PRF+PG group, 30 days after operation show very good healing, connective tissue formation (G), re-epithelization (M) and mild inflammatory reaction. (H&E X40).

Discussion

According to our knowledge, no study has been published describing the combination use of PRF with autologous PG in this form of utilization, we were the first to application this idea. We designed successfully a study to investigate whether these materials can be used for this purpose. There has been massive number of clinical and experimental researches focusing on application of various types of protection in the intestinal anastomosis site (23-26). Nevertheless, no use of PRF-PG combination has been studied up to now.

Several complications can occur as a result of small intestinal surgery including abdominal adhesions, dehisces, ileus, leakage and postoperative bowel syndrome. One of the most concerns is adhesion because it may contribute to poor functional outcome (1,3,27). It is reported that adhesions following open abdominal surgical procedures

are present in 93% of patients and at a lower rate behind laparoscopic operations. Patients with intra-abdominal adhesions in most cases are asymptomatic. In spite of that, adhesions are considered a common cause for small bowel obstruction, infertility and chronic abdominal pain (27). Since, in the last decades much effort has been put into developing effective adhesion prevention devices.

The main goal of adhesion prevention device is to eliminate or diminish the occurrence, extent, severity, and consequences of adhesion with preserving normal healing process and preventing contamination (28,29). Although many different approaches have been estimated, some have been effectual, but unfortunately others have even been deleterious (23-28). Recently a significant decrease in formation of intra - abdominal adhesions after intestinal surgery with the use of tissue grafts have been found in both human and animal studies (24,25). Tissue grafts are used widely to prevent adhesions formation in different organs, which have promoted the formation of several related structures for different tissue targets (14,16,30).

In our study the PG and PRF autograft were used and the gross examination revealed considerably less adhesion formation in the animals who received PG compared with the group who served as control. Moreover, we found less rate of adhesion in anastomoses treated with combination of peritoneum and PRF compared to samples treated with PG alone or control. The efficacy of PG in preventing postoperative adhesion in different organs has been previously demonstrated by several randomized control studies (31,32). Uema *et al*. (32) described a technique used to prevent adhesion between a synthetic vascular graft and the intestinal loop by putting of bovine peritoneum fold between the intestine and the vascular graft in rabbits (31). Yin, 2005 reported a protective effects of PG in decreasing the degree of adhesion and prevention of stenosis, scar tissue formation, fistulas, abscess and contracture in 30 patients with severe defects in the small intestine, colon and rectum (16). PG has been successfully used for tissue reconstruction in several studies. It is appearing to be easy to obtain, safe, effective and cheap. Harvesting autologous grafts requires additional incision. But in contrast, a suitable size of PG can be obtained rapidly without another procedure. Rapid obtaining of graft is very important in cases when emergency surgery is necessary (14-16,33). It is attracting to note that PG is often used by surgeons other than digestive system surgeons, and more often for organs other than gastrointestinal tract organs. It was used to repair blood vessels (33) urogenital organ lung (34,35) and lung (36).

In fact, in the review of literatures, the use a combination of multiple agents was suggested to be have a synergetic effect and sufficient for adhesion formation prevention than either component alone (37). This result explained why the adhesion was less in group treated with combination PG and PRF. Different platelet concentrates such as leukocyte platelet-rich plasma, PRF, advanced PRF have been utilized in modern regenerative medicine due to their hypothesized positive influence on support tissue regeneration and enhance healing by facilitating angiogenesis, cell recruitment, proliferation, differentiation and another phases during wound healing (17-22). Several studies demonstrated that the use of these product that contain high level of growth factors and cytokines in combination with different grafting materials yielded superior results in term of faster new tissue formation, decrease adhesion and improved healing processes, it indicated that this combination resulted in increased angiogenesis, tissue cell proliferation, improve graft materials properties by providing immune cytokines, growth factors, circulating stem cells and leukocytes with decrease tissue adhesion in comparison to graft alone (14,38,39). Several prevention adhesions strategies have been used, such as inhibition of inflammation (30). We supposed that the anti-inflammatory properties of PRF (22,39) would reduce the formation of adhesions in PRF+PG group compression with other groups. The anti-inflammatory effect of PRF may attribute to PDGF and TGF-β1 that it is contain (40).

The bowel stenosis following intestinal anastomosis is a frequently observed process. It can be influenced by a wide range of factors, such as adhesion, blood flow impairment, a history of radiotherapy, suture failure, mesenteric panniculitis, and diverting stomas (41). Although intestinal anastomosis healing has been broadly studied, the pathology, physiology and contributing factors are yet still not understood completely; briefly, leakage, adhesion, tissue ischemia, method of suturing, chemotherapy and radiotherapy have been shown to be implicated (1,2,6,8). Currently, no methods have been established that capable of completely preventing anastomotic stenosis.

In this study the radiographic examination revealed presence of stenosis in all groups and the major luminal stenosis was observed in group C 61.03% at 15 days postoperative and minor luminal stenosis was observed in PRF+PG group 12.05% at 30 days postoperative. The greater percentage of lumen stenosis in C group may be due to the extensive adhesion that led to compression, and not allow the expansion of lumen at the suture line; this result coincides with other researchers (42). While, minor stenosis in PG group may be due to the graft used which was resulted in low amount of adhesion formation and finally minimum luminal stenosis. This phenomenon coincides with other worker (19,25,27) who said that the using of barrier materials to covering the anastomosis site can reduce the risk of adhesion and later intestinal stenosis and obstruction. However, decreased luminal stenosis in all groups at 30 days as compare with 15 days postoperatively which was not significant statistically in C group while significant in PG and PRF+PG groups may be due to increase metabolism and absorption degree of suture materials at 30 days as compared with 15 days which was led to removal of purse - string effect that induced by this

suture material and finally result in increased luminal diameter of intestine at 30 postoperative days (42). Also we hypothesize that degradation and absorption of graft were faster in PRF+PG group than PG group which was led to decrease the compression on the anastomosis site as a result leading to decrease the degree of stenosis. This result agrees with other stated that a combination of grafts with platelet concentrates added an advantage to these grafts that could yielded regenerative results through the promote expression of various growth factors and inductive proteins, leading to enhance tissue healing and degradation of graft material (14,38,43).

The microscopic findings in groups treated with PG showed migration and proliferation of fibroblasts at the anastomosis site, intestinal layer regeneration, incorporation of PG components with intestinal tissue and improvement of host tissue repairing. The peritoneum graft remodeling process by host tissue revealed complete implant degradation, resolution with deposition of a new intestinal tissue without presence signs of foreign body reaction or fibrous capsule formation around the tissue graft. We hypothesized that this reinforced biomaterial has positive effect on anastomosis healing process, firstly with mechanical action as barrier to prevent adhesion and leakage and definitively with the improvement of anastomosis site healing. The ability of peritoneum to enhance and improve tissues healing has been well established (15,16,32-35).

The peritoneum composed of single layer of mesothelial cells with sub-mesothelial stromal connective tissue. Cells of mesothelium have been shown to promote and induce tissue healing and also have been identified to possess the potential capacity to differentiate into other cells depending on the environmental changes at the site of location and the type and severity of initiation and activation (13,34,35). Some researchers have found precursor pluripotent cells have similar composition to mesenchymal stem cells in the stromal tissue. These cells have been used for repairing and engineering of the internal organs (44). Also peritoneum composed of collagen, glycosaminoglycan and different cells such as mast cells, macrophage, lymphocyte, adipose cells (13,14). The peritoneum cells that's contain released growth factor play a critical role for tissue repair, these factor include: Fibroblast growth factor (FGF), endothelial growth factor (EGF), insulin like growth factor (IGF-I) and transforming growth factor (TGF). These factors are exerting various effects such as cell proliferation, fibroblast stimulation, macrophage recruitment, collagen production, stimulates angiogenesis and enhances tissue healing (13,14, 44). Furthermore, peritoneum graft is considered natural biomaterial (12,13). Atala *et al*. (13) indicated that biomaterials used for tissue engineering provide suitable three-dimensional environment that lets cells to develop new tissues exactly similar to host tissue with appropriate structure and function. These biomaterials are designed usually to replicate the physical and biological function of the native host extra cellular matrix (ECM) found in the body to increase and enhance new tissue formation. Thus, the ideal characteristics of biomaterial are: should be biocompatible, induce tissue cell proliferation and provide structural tissue support and growth without producing severe inflammatory activities that lead to foreign body reactions or fibrous tissue deposits that induce adhesion. Therefore, it is frequently preferable to use a biodegradable graft material because these implanted biomaterials provide a microscopic scaffold upon which host tissue cells growth can occur and possess rapid incorporation properties. So that after time will disappear, leaving behind only the generated tissue (45).

The histopathological examination revealed a sharp difference between the anastomosis sites treated with PG and group has been considered as control. PG resulted in earlier re-epithelization, increase vessel formation and tissue maturation compared with C group. This might be attributed to the previous mentioned biological properties of this material, which acts as a protective shield against adhesions and supports the healing process of the intestine. These results agreed with previous reports on using PG (13-15). Additionally, by comparison between the PRF+PG group and other groups after 15 and 30 days' post operation, it has demonstrated that treatment with combination of PRF and PG graft enhanced the healing process through the decreased of inflammation, increased deposition of collagen fiber, increased proliferation of new blood vessels and enhanced re-epithelization of mucosa and formation of villi. This variation in response in treatment might be attributed to the biological components of the PRF. This result coincides with others said that the PRF has the ability to enhanced hard and soft tissue regeneration without or with minimal inflammatory reaction at the wound site. PRF significantly improved the wound healing quality and can help in homeostasis and improve it. The therapeutic effect of PRF is mainly due to the high variety of platelet-derived protein molecules, which include growth factor, cytokines, and other bioactive peptides in addition to the matrix in which cytokines, growth factors, and platelet cells are retained and can be constantly released (17-22).

Intestinal anastomosis treated with PG and PRF in this present study displayed more re epithelization, angiogenesis with faster collagen formation and organization compared to non-treated group. This result could be explained by the composition of the PG and PRF, which contains GFs and macrophages cell (12,13,17-35,44). This result is agreement with (1) who said that healing of the Intestinal wound is classically included three overlapping phases (lag, proliferative and maturation phases). In these phases the inflammatory cells especially macrophages play special positive role in wound debridement and the production of GFs which is responsible for collagen production, angiogenesis and cell proliferation. Also this result is agreement with previous researches have been assumed that the increase the local growth factor concentrations at the

site of healing accelerating the wound healing process (14,17-22).

In our study the use of PRF in combination with PG graft accelerated intestinal anastomosis healing and increases the rate of graft acceptance. This result coincides with other such as Simonpieri *et al*. (46) which was evaluated the efficacy and advantages of the use of PRF with grafts. They found that the fibrin mesh present in PRF facilitates cellular migration, vascularization and survival of the graft. In addition to that it plays an important role in maintaining and stabilizing the graft material and serves as a biological adhesive for particulate graft material. Also, the physiologic polymerization in PRF allows the cytokines and growth factors to be released gradually as the fibrin matrix is resorbed, thus ensuring bioactive levels for a long time period and creating a continual l repairing process. Lastly, cytokines and leukocytes are the major component in the fibrin matrix, play an important role in inflammatory response regulation within the implanted biomaterial (46). In this study the peritoneum and PRF did not cause local and systemic unfavorable reactions and were absorbed by host tissue before 15 days after application. This result corresponds with others who said that an ideal adhesive barrier should be not toxic, non- allergic biocompatible, easy to utilize, and is dissolved ideally after 7 to 15 days (47).

Conclusion

The present research showed that the combination application of PRF with PG led to decrease in both adhesions and degree of stenosis rates of the anastomosis site. Furthermore, using of this combination affected the histopathological process, and so it can be regarded as a very safe, cheap and easily obtained autograft materials. We recommend that clinical studies for this new application should be performed to clarify this topic, as it may have potentially important effects in surgery.

Acknowledgement

This study is supported by Veterinary Medicine College in University of Mosul. We are very thankful for assistance that it was provided it, which helped to faster and improve the quality of this work.

Conflict of interest

The authors announce that there is no conflict of interest regarding publishing or funding of this article.

References

1. Radlinsky M, Terry W. Fossum TW. Surgery of the Digestive System. 5th ed. Philadelphia: Laura Pardi Duprey; 2019. 331-512 p.
2. Nerstrom M, Krarup PM, Jorgensen LN, Agren MS. Therapeutic improvement of colonic anastomotic healing under complicated conditions: A systematic review. World J Gastrointest Surg. 2016;8(5):389-401. DOI: 10.4240/wjgs.v8.i5.389
3. Farghalya AE, Ammara MS, Algammala AS, Arafab AS. Risk factors for leak in emergent small bowel anastomosis. Menoufia Med J. 2019;32(2):574-580. DOI: 10.4103/mmj.mmj_111_18
4. Awad S, Abd El-Rahman AI, Abbas A, Althobaiti W, Alfaran S, lghamdi S, Alharthi S, Alsubaie K, Ghedan S, Alharthi R, Asiri M, Alzahrani A, Alotaibi N, Shoma A, Abou MS. The assessment of perioperative risk factors of anastomotic leakage after intestinal surgeries; A prospective study. BMC Surg. 2021;21:29. 10.1186/s12893-020-01044-8
5. Dandi PP, Aaudichya AS, Juneja IA, Vaishnani BV and Bhatt JG. A prospective comparative study of intestinal anastomosis, single layer extra mucosal versus double layer. Int J Res Med Sci. 2015;3(8):2099-2104. DOI: 10.18203/2320-6012.ijrms20150333
6. Fang AH, Chao W, Ecker M. Review of colonic anastomotic leakage and prevention methods. J Clin Med. 2020;9:4061:1-17. DOI: 10.3390/jcm9124061
7. McDermott FD, Heeney A, Kelly ME, Steele RJ, Carlson GL, Winter DC. Systematic review of preoperative, intraoperative and postoperative risk factors for colorectal anastomotic leaks. Br J Surg. 2015;102(5):462-479. DOI: 10.1002/bjs.9697
8. Lyra HF, Rodrigues IK, Schiavon L, Acampora AJ. Ghrelin and gastrointestinal wound healing. A new perspective for colorectal surgery. Acta Cir Bras. 2018;33(3):282-294. DOI: 10.1590/s0102-865020180030000010
9. Gorur M, Sozutek A, Oktay IO, Karakaya B. The influence of platelet-rich plasma (PRP) on colonic anastomosis healing impaired by intraperitoneal 5-flourouracil application: An experimental study. Acta Cir Bras. 2020;35(5):e202000504. DOI: 10.1590/s0102-865020200050000004
10. Mooloughi S, Joudi M, Dalili AM, Dalili A. Different types of anastomotic methods: A review of literature. Rev Clin Med. 2015;2(4):178-181. [available at]
11. Horst M, Madduri S, Gobet R, Sulse T, Hall H, Eberli D. Scaffold characteristics for functional hollow organ regeneration. Materials. 2010;3:241-263. DOI: 10.3390/ma3010241
12. Bellenger CR. Textbook of small animal surgery. 3rd ed. Philadelphia: Saunders; 2003. 420 p.
13. Isaza-Restrepo A, Martin-Saavedra JS, Velez -Leal JL, Vargas-Barato F, Riveros-Duenas R. The peritoneum: Beyond the tissue: A review. Front Physiol. 9:738. DOI: 10.3389/fphys.2018.00738
14. Allawi AH, Alkattan LM, Al- Iraqi OM. The effect of autogenous peritoneal graft augmented with platelets- plasma rich protein on the healing of induced achilles tendon rupture, in dogs. Iran J Vet Med. 2020;14(2):111-119. Doi: [available at]
15. Castillo JM, Flores-Plascencia A, Perez-Montiel MD, Garcia S, Vergara N, Perez-Blanco A, Sanchez-Valdivieso EA. Parietal peritoneum graft for duodenum injuries in an animal model. Arq Bras Cir Dig. 2019;32(1):e1418. DOI: 10.1590/0102-672020180001e1418
16. Yin W. Free peritoneal graft for repair of severe seromuscular defect of bowel: From experiment to clinical practice. J Surg Res. 2005;125(1)3-8. DOI: 10.1016/j.jss.2004.11.013
17. Pavlovic V, Ciric M, Jovanovic V, Trandafilovic M, Stojanovic P. Platelet-rich fibrin: Basics of biological actions and protocol modifications. Open Med. 2021;16:446-454. DOI: 10.1515/med-2021-0259
18. Al Deeb M. Role of Platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) in oro-facial tissue regeneration: A narrative review. J Adv Oral Res. 2020;11(1):5-11. DOI: 10.1177/2320206819895836
19. Yu ding Z, TanY, qian Peng Q, jun Zuo J and li N. Novel applications of platelet concentrates in tissue regeneration. Exp Thera Med. 2020;21:226. DOI: 10.3892/etm.2021.9657
20. Zhang S, Cao D, Xie J, Li H, Chen Z, Baob Q. Platelet-rich fibrin as an alternative adjunct to tendon-exposed wound healing: A randomized controlled clinical trial. Urns. 2019;4(5):1152-1172. DOI: 10.1016/j.burns.2019.01.007
21. Ozcay N, Ozdemir H, Besim H. Role of platelet-rich fibrin on intestinal anastomosis wound healing in a rat. Biomed Mater. 2018;17;13(4):045006. DOI: 10.1088/1748-605X/aab8e1

22. Thanoon MG, Eesa MJ, Abed ER. Effects of platelets rich fibrin and bone marrow on the healing of distal radial fracture in local dogs: Comparative study. Iraqi J Vet Sci. 2019;33(2):419-425. DOI: 10.33899/ijvs.2019.163169
23. Uludag M, Citgez B, Ozkaya O. Effects of amniotic membrane on the healing of primary colonic anastomoses in the cecal ligation and puncture model of secondary peritonitis in rats. Int J Colorectal Dis. 2009;24:559-567. DOI: 10.1007/s00384-009-0691-5
24. Kornfeld D. Reinforcement of rectal anastomoses with a collagen-based haemostatic patch: A case series report. J Surg Case Rep. 2021;4:1-3. DOI: 10.1093/jscr/rjaa228
25. Testini1 M, Gurrado A, Portincasa P, Scacco S, Marzullo A, Piccinni G, Lissidini G, Greco L, Antonietta De Salvia M, Bonfrate L, Debellis L, Sardaro N, Staffieri F, Carratu M, Crovace A. Bovine pericardium patch wrapping intestinal anastomosis improves healing process and prevents leakage in a pig model. PLoS ONE. 2014;9(1):e86627. DOI: 10.1371/journal.pone.0086627
26. Reisch S, Wilhelm D, Helmut Friess H, Neumann PA. Innovative approaches for induction of gastrointestinal anastomotic healing:an update on experimental and clinical aspects. Langenbeck's Arch Surg. 2020;12(2):23-30. DOI: 10.1007/s00423-020-01957-1
27. Ntourakis D, Katsimpoulas M, Tanoglidi A, Barbatis C, Karayannacos PE, Sergentanis TN, Kostomitsopoulos N, Machairas A. Adhesions and healing of intestinal anastomoses: The effect of anti-adhesion barriers surgical innovation. Surg Innovat. 2015;23(3):1-11. DOI: 10.1177/1553350615610653
28. Shahzamzni S, Jahandideh A, Abedi G, Akbarzadeh A, Hesaraki S and Parsaei P. Histopathological assessment of nano-acetyle cysteine effect on postoperative adhesion in rats. Iraqi J Vet Med. 2021;35(3):589-597. DOI: 10.33899/ijvs.2020.126857.1400
29. Park H, Baek S, Kang H, Lee D. Biomaterials to prevent post-operative adhesion. Materials. 2020;13:3056. DOI: 10.3390/ma13143056
30. Wu W, Chenga R, Nevesc J, Tanga J, Xiaoa J, Nib Q, Liu X, Pana G, Lia D, Cuia W. and Sarmentoc B. Advances in biomaterials for preventing tissue adhesion. J Cont Rel. 2017;261:318-336. DOI: 10.1016/j.jconrel.2017.06.020
31. Laukka M, Hoppela E, Salo J, Rantakari P, Tove J. Gronroos T, Orte K, Auvinen K, Salmi M, Gerke H, Thol K, Peuhu E, Kauhanen S, Merilahti P, Hartiala P. Preperitoneal fat grafting inhibits the formation of intra-abdominal adhesions in mice. J Gastrointestinal Surg. 2020;24:2838-2848. DOI: 10.1007/s11605-019-04425-4
32. Uema TR, Fazan SVP, Cherri J, Piccinato CE. Bovine peritoneum protection role on intestinal adhesions to a vascular graft: A morphological analysis. Int J Morphol. 2008;26(2):269-274. DOI: 10.4067/S0717-95022008000200004
33. Nicolas Meurisse N, Ansart F, Honore P, De Roover A. Glutaraldehyde-fixed parietal peritoneum graft conduit to replacecompletely the portal vein during pancreaticoduodenectomy: A case report. Inter J Surg Case Rep. 2020;74:296-299. DOI: 10.1016/j.ijscr.2020.07.046
34. Suwajo P, Ratanalert W, Sooksatian K, Uerpairojkit K, Dusitanond N, Chaovanalikit T, Jindarak S, Udomsawaengsup S. Pedicled peritoneal flap vaginoplasty in male-to-female gender affirmation surgery: A case report. Female Pelvic Med Rec Surg. 2020;26:23-26. DOI: 10.1097/SPV.0000000000000888
35. Trenti E, Palermo SM, Elia C, Comploj E, Ladurner C, Huqi D, Tischler T, Mian C, Pycha A. an alternative technique for treating complex ureteral strictures and defects using a peritoneal graft. Biomed J Sci Tech Res. 2017;1(5):1531-1535. DOI: 10.26717/BJSTR.2017.01.000478
36. Andrade CF, Fontena E, Cardoso PG. Pereira R, Gustavo G, Forgiarini LF, Moreira J, Felicetti J. Peritoneal free autologous fat graft for the control of pulmonary air leaks in emphysematous rat lungs. Ann Thorac Surg. 2014;98:271-6. DOI: 10.1016/j.athoracsur.2014.03.027
37. Brian C. Ward BC, Panitch A. Abdominal adhesions: Current and novel therapies. J Surg Res. 2011;165:91-111. DOI: 10.1016/j.jss.2009.09.015
38. Allawi AH, Alkattan LM, Al Iraqi OM. Clinical and ultrasonographic study of using autogenous venous graft and platelet-rich plasma for repairing Achilles tendon rupture in dogs. Iraqi J Vet Sci. 2019;33(2):453-460. DOI: 10.33899/ijvs.2019.163199
39. Barbon S, Stocco E, Macchi V, Contran M, Grandi F. Alessio Borean A, Parnigotto PP, Andrea Porzionato A, De Caro R. Platelet-rich fibrin scaffolds for cartilage and tendon regenerative medicine: From bench to bedside. Int J Mol Sci. 2019;20:1701-1783. DOI: 10.3390/ijms20071701
40. Mudalal Y, Sun X, Li X, Zhou Y. The evaluation of leukocyte-platelet rich fibrin as an anti-inflammatory autologous biological additive: A novel in vitro study. Saudi Med J. 2019;40(7):657-668. DOI: 10.15537/smj.2019.7.24302
41. Hayashi M, Ikeda A, Yokota M, Sako H, Uchida H, Ikeda K, Okusawa S. Early anastomotic stricture occurring after colectomy that responded well to transanal decompression and local steroid therapy: A case report. Int J Surg Case Rep. 2017;37:52-56. DOI: 10.1016/j.ijscr.2017.06.023
42. Hasan NJ. Comparative study of three methods of end to end intestinal anastomosis in dogs [MSc Thesis]. Mosul: Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Mosul; 2000. 62 p.
43. Nair MB, Varma HK, Menon KV, Shenoy SJ, John A. Reconstruction of goat femur segmental defects using triphasic ceramic-coated hydroxyapatite in combination with autologous cells and platelet-rich plasma. Acta Biomater. 2009:5(5):1742-1755. DOI: 10.1016/j.actbio.2009.01.009
44. Chen B, Chen X, Wang W, Shen J, Song Z, Ji H, Zhang F, Wu J, Jie Na J and LiS. Tissue-engineered autologous peritoneal grafts for bladder reconstruction in a porcine model. J Tissue Eng. 2021;12:1-12. DOI: 10.1177/2041731420986796
45. Atala A, Kasper FK, Mikos AG. Engineering complex tissues. Sci Transl Med. 2012;14:4(160):160-172. DOI: 10.1126/scitranslmed.3004890
46. Simonpieri A, Del Corso M, Sammartino G, Dohan, Ehrenfest DM. The relevance of choukroun's platelet rich fibrin and metronidazole during complex maxillary rehabilitations using bone allograft. Part II: Implant Surgery, prosthodontics and survival. Implant Dent. 2009;18:220-9. DOI: 10.1097/ID.0b013e318198cf00
47. Beck DE. The role of *Seprafilm bioresorbable* membrane in adhesion prevention. Eur J Surg Suppl. 1997;577:49-55. DOI: 10.1002/jbm.10040

تأثير الليفين الغني بالصفيحات الدموية ورقع البريتون الذاتية على التئام تفاغر الأمعاء في الكلاب

نبراس محمد رافع و أسماء حسين علاوي

فرع الجراحة وعلم تناسل الحيوان، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تم في هذه الدراسة تقييم تأثير الليفين الغني بالصفيحات الدموية ورقع البريتون الذاتية على التئام تفاغر الأمعاء في الكلاب. تم استعمال ١٨ حيوانا من الكلاب المحلية البالغة إذ تم تخديرها ومن ثم إزالة جزء منها وتفاغرها في جميع الحيوانات. قسمت الحيوانات إلى ثلاثة مجاميع متساوية (٦ لكل منها). مجموعة السـيطرة تم فيها إجراء تفاغر الأمعاء

نهاية -ل- نهاية باستعمال تقنية الخياطة البسيطة المتقطعة فقط. في مجموعة رقع البريتون تم خياطة مكان التفاغر كما في مجموعة السيطرة ومن ثم إحاطته برقع البريتون. بينما في مجموعة الليفين الغني بالصفيحات الدموية ورقع البريتون تم خياطة مكان التفاغر كما في مجموعة السيطرة ومن ثم إحاطته بالليفين الغني بالصفيحات الدموية ورقع البريتون. عملية الالتئام في المجاميع كافة درست سريريا، عيانيا، نسجيا وشعاعيا للمدد ١٥ و٣٠ يوما بعد العملية. أشارت النتائج أن استعمال رقع البريتون له تأثير قيم على مكان التفاغر وهذا التأثير تميز بسرعة تجدد ظهارة المخاطية، زيادة ترسب الكولاجين، تكاثر الاورمات الليفية، قلة الالتصاقات وقلة درجة التضيق مقارنة بمجموعة السيطرة. إضافة لذلك هنالك قلة بالالتصاقات، قلة بدرجة التضيق وزيادة في تظهر المخاطية في مجموعة الليفين الغني بالصفيحات ورقع البريتون مقارنة بمجموعة رقع البريتون لوحدها. نستنتج من هذه الدراسة أن استعمال رقع البريتون الذاتية له تأثير إيجابي ومفيد على تفاغر الأمعاء. أيضا أبرزت النتائج الأثار الواعدة للاستعمال المشترك لليفين الغني بالصفيحات ورقع البريتون على التئام تفاغر الأمعاء. إن استعمال الليفين الغني بالصفيحات مع رقع البريتون على مكان التفاغر ناجح، وآمن وفعال من دون حدوث مضاعفات.