FULL PAPER

Surgery

Enhancement of abdominal wall defect repair using allogenic platelet-rich plasma with commercial polyester/cotton fabric (Damour) in a canine model

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ABSTRACT. Platelet-rich plasma (PRP) has an important role in musculoskeletal surgery; however, it has been underutilized for accelerating the healing of abdominal wall defects in veterinary practice. Therefore, the aim of this study was to evaluate the use of commercial polyester/cotton fabric (Damour) as a new composite mesh for the repair of experimentally induced abdominal wall defects in canine models, and to investigate the possible role of PRP for improving such repair and reducing allied complications. For this purpose, abdominal wall defects were created in 24 healthy mongrel dogs and then repaired with mesh alone (control group) or mesh and allogenic PRP (PRP group). Dogs were euthanized after 2 or 4 months for gross examination of implantation site, detection of adhesion score and hernia recurrence. Moreover, tissue samples were collected for histological and gene expression analyses for neovascularization, collagen formation and tissue incorporation. Hernia recurrence was not recorded in PRP-treated dogs that also displayed significantly more neovascularization and less severe adhesion to the underlings (1.08 ± 0.51) in comparison to control group (2.08 ± 0.99). Histological and molecular evaluation confirmed the gross findings that collagen deposition, new vessel formation, and overexpression of angiogenic and myofibroblastic genes (*COL1α1*, *COL3α1*, *VEGF* and *TGFβ1*) were observed more frequently in the PRP group, at both time points. In conclusion, we found that addition of allogenic PRP to Damour mesh enhanced neovessel formation, and increased tissue deposition and incorporation, with subsequent reduction of peritoneal adhesion and recurrence rate.

KEY WORDS: abdominal wall defect, Damour, dog, platelet-rich plasma

Surgical management of abdominal wall defects in animals and its associated complications remains a subject of debate. Degradable and non-degradable synthetic polymers, used traditionally for hernia repair, are usually accompanied with several complications, such as infection, erosion, adhesion formation, mesh extrusion, contraction, fistula formation and recurrence [12, 33]. The fore mentioned complications, as well as the difficulty in finding a single ideal mesh, have led to growing interest in the search for new materials, like composite meshes and/or enhancements of the tissue responses after implantation using synthetic growth factor/chemokine products.

The premise of new mesh designs is the combination of more than one material, thereby giving rise to the term 'composite mesh'. The main advantage of such meshes is that they can be used in the intraperitoneal space with minimal adhesion formation.

Almost all composite meshes use one or other of three basic materials (Polypropylene, Polyester and ePTFE) either in combination or with a range of additional materials, such as titanium, omega 3, monocryl, and hyaluronate [31].

Commercial polyester/cotton fabric (Damour) is a new composite mesh that has been clinically evaluated for hernioplasty in ruminants, where post-implantation follow-ups revealed high macroscopic success rates (79.82%) with relatively low rates of complications (12.5%) and recurrence (8.3%) [29]. However, direct clinical application did not provide information on tissue incorporation, neovascularization and peritoneal adhesion formation.

Synthetic growth factor/chemokine is a supplementary product for hernia repair that was investigated for its ability to improve tissue responses after mesh implantation. It showed improved mesh incorporation, with significantly decreased mesh bacterial colonization, as well as increased host tissue response and mechanical strength, and diminished incidence of incisional hernia formation [10, 14].

Platelet-rich plasma (PRP) is an available growth factor rich autologous blood product, containing several components of growth factors and chemokines, enhancing soft tissue repair through cellular proliferation and neovascularization [37]. PRP therapies have been studied extensively in human clinical medicine and in experimental animal models for facilitating the healing of bone, tendon and ligament, as well as for intra-articular treatment of osteoarthritis. Likewise, in clinical veterinary medicine, PRP has been used extensively in equine medicine to treat joint disease, ligament and tendon injuries, and lower limb wounds [9, 39].

Despite its promising results in healing the musculoskeletal system, PRP has never been applied to hernia repair, especially in veterinary practice. Thus, the aim of this study was to experimentally evaluate the use of commercial polyester/cotton fabric (Damour) for the repair of abdominal wall defects through gross evaluation, histological techniques and gene expression analysis, and to test the hypothesis that the use of allogenic PRP in such procedures enhances wound healing and diminishes frequently associated complications.

MATERIALS AND METHODS

Animals

The experiment was carried out on twenty-four healthy mongrel dogs with an average age of 1.5–2.0 years and weight of 20–30 kg. All dogs were healthy upon clinical and biochemical examinations, they were kept in closed boxes at the Veterinary Clinic, Mansoura University, and were fed on balanced rations. All animals were kept in similar conditions throughout the experimental duration.

Experimental study

The experimental study was approved by the committee of animal welfare and ethics, Faculty of Veterinary Medicine, Mansoura University. Experimental dogs were allocated randomly to two groups (twelve each). The first group was the control group (Damour alone); the second group was PRP group (Damour + allogenic PRP). Six additional dogs were used as blood donors for PRP isolation.

Animals were kept in a clean, healthy environment until euthanasia was carried out at 2 and 4 months. Six animals from each group were used at each time point. Meanwhile, four animals were kept as substitutes, to replace any animals that suffered from complications necessitating exclusion, which fortunately did not occur.

PRP preparation, quantification and activation

PRP was prepared using a technique described by Okuda *et al.* [32]. Forty m*l* of allogenic blood, withdrawn from the jugular vein of a donor dog 1 hr prior to the experiment, were deposited into two tubes containing 3.8% sodium citrate anti-coagulant. The anti-coagulated blood was centrifuged at 300 ×g for 10 min to separate PRP and platelet-poor plasma (PPP) portions from the red blood cell fraction. The blood was separated into three following parts: red blood cells (at the bottom of the tube), platelet-rich plasma (a discrete grey line in the middle of the tube) and platelet-poor plasma (at the top of the tube). The PRP and PPP portions were again centrifuged at 650 ×g for 15 min to separate PRP from PPP. A 0.5 m*l* sample of the prepared PRP was used for platelet counting using an automatic cell counter. In the present study, 40 m*l* of whole citrated blood was used to prepare 1 m*l* of the PRP.

To evaluate the enhancement of platelet concentration in the PRP, baseline platelet counts were obtained from all blood samples before processing and after PRP preparation. Platelet counts were performed using a hematology analyzer (Sysmex kn21, Sysmex, Norderstedt, Germany). The mean peripheral blood platelet count was $154.3 \pm 9.8 \times 10^9/l$ (range: 148.2 to $168.9 \times 10^9/l$), and mean platelet count of PRP was $1,158 \pm 90 \times 10^9/l$ (range: 1,113 to $1,287 \times 10^9/l$).

The PRP (1 m*l*) was activated just before surgical application with 10% calcium chloride (4.5 mEq/5 m*l*, Biodiagnostic, Co., Cairo, Egypt), 50 μl/m*l* and thromboplastin-D, 200 IU/m*l* (commercially available for prothrombin time test; Biodiagnostic, Co.). The average of platelet number in the final solution of PRP was 6–8 times higher than the platelet count in peripheral blood.

Preoperative preparation and anesthesia

Feed was withheld for 4–6 hr before surgery. Dogs received a preoperative dose of systemic broad-spectrum antibiotic amoxicillin and flucloxacillin (flumox, E.I.P.I.C.O, Cairo, Egypt) before atropine sulphate at a dose of 0.1 mg/kg (1 mg/m*l*, Adwia, Cairo, Egypt) was administered intramuscularly followed by xylazin HCl (Xylaject, Adwia) at a dose of 1 mg/kg by the same route. General anesthesia was induced and maintained by using thiopental sodium (2.5%; Thiopental sodium, E.I.P.I.C.O). The anesthetized animals were positioned in dorsal recumbency. The skin, at the ventral abdominal region, from xiphoid to the pubic
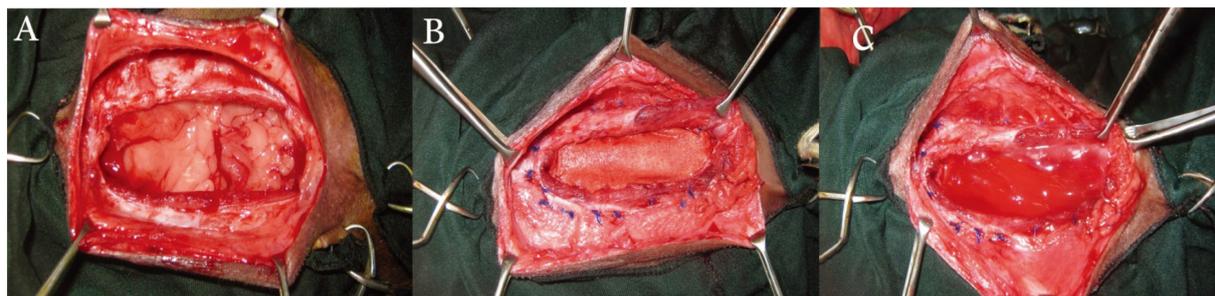
Fig. 1. Surgical technique: a full thickness abdominal wall defect was created (A), mesh was implanted using an underlay technique (B), and allogenic activated platelet-rich plasma (PRP) was applied to the mesh surface prior to skin closure (C).

symphysis at both flank regions, were prepared aseptically.

Surgical technique

Before beginning the operation procedures, hair was clipped at the abdominal region prior to sterilization process of the skin using three scrubs of ethanol (75%; Ethanol, Elgamhoria, Co., Cairo, Egypt) and chlorhexidine (Chlorhexidine, Elgamhoria, Co.). A septic technique was sustained throughout the surgical operation.

In the control group, a rectangular full-thickness skin flap (15 × 10 cm) was incised from three sides, before a full-thickness abdominal wall defect (10 × 6 cm), including muscles and peritoneum, was created at the same site. After control of bleeding, a sterilized piece of commercial polyester/cotton fabric produced by (Misr Spinning and Weaving Co., Mahalla al-Kubra, Egypt) was prepared, in accordance with the size of the hernial ring, to cover the boundaries of the formed defect allowing for 5–8 mm underlay. It was then placed under the visceral peritoneum, after omentopexy (part of omentum was grasped and loosely stitched to the implant). The fabric was secured to the recipient tissue with an interrupted overlapped pattern using No. 1 Polypropylene monofilament suture material (Prolene Ethicon; Johnson& Johnson, Brussels, Belgium). The meshes of PRP-treated dogs were soaked for 10 min in activated PRP before the implantation procedures, and the rest of the PRP was added to the mesh surface after suture fixation and prior to incisional closure, with simple interrupted suture using silk No. 1/0 (Silk, Proadvantage, Co., Cairo, Egypt; Fig.1).

Post-operative care and follow-up

Post operatively, cage rest was prepared for all dogs, and an abdominal bandage was applied to all animals for 5 postoperative days for protection. All dogs received ketoprofin (Amria, Co., Alexandria, Egypt) at a dose of 1 mg/kg at the end of the operation for 3 consecutive postoperative days, at 12-hr intervals. Additionally, preoperative antibiotics were continued for 5 successive postoperative days, at 12-hr intervals. The external wound was dressed twice daily using povidone iodine (Betadine Antiseptic, Pharos, Co., Cairo, Egypt).

The feeding regimen was started in low quantities of soft food, with one third in the first week, and increased gradually over two weeks, until normal feeding was resumed. Animals were routinely monitored during the experimental protocol to avoid postoperative complications, until euthanasia was performed. Animals were euthanized using an overdose of thiopental sodium at a concentration of 5%, prior to tissue harvesting procedures.

Gross evaluation of implantation and tissue sites was carried out at the afore-mentioned time points, for histological and gene expression analyses. The main items for comparison were degree of neovascularization and collagen production, cytokine gene expression, peritoneal adhesion severity and incidence of hernia recurrence.

Gross evaluation and adhesion severity scoring

An examination of the site of implantation was performed before skin removal for complete assessment of the healing process and detection of hernia recurrence. A rectangular area of skin was removed at a width of 6 cm, and examination of the prosthetic implant was conducted for detection of covering connective tissue and implant shrinkage rate. A three borders abdominal wall defect was excised and reflected to observe neovascularization and the grade of adhesion, using Modified Hopkins Adhesion Score performed by Dubcenco et al.; numerical scores ranged from 0 to 4 are weighted according to five different parameters: adhesion formation, frequency, size, density and dissection difficulty (Table 1) [11]. Mean and standard deviation adhesion scores were determined, for each group, at different time points.

Microscopic evaluation

Mesh samples and adjacent tissues were collected at the two time points for histological processing and evaluation. The specimen was preserved in 10% neutral-buffered formalin (Elgamhoria, Co.) for 48 hr prior to paraffin embedding. Paraffin-embedded tissues were sectioned on a microtome at a thickness of 5 μm. To assess microscopic neovessel formation and tissue deposition, sections were routinely stained with hematoxylin, eosin and Masson's trichrome (Sigma Aldrich, St. Louis, MO, U.S.A.).

Table 1. Modified Hopkins Adhesion Score, adapted by Dubcenco *et al*. [11]

Score	Frequency	Size/Width (cm)	Density	Dissection
0	0	No adhesion	No adhesion	No adhesion
1	1	<1	Single thin, filmy adhesion	Minimal blunt dissection, tears easily
2	1–2	1–2	Multiple thin, filmy adhesions	Blunt dissection only
3	3–4	2–3	Dense adhesion(s) with or without filmy adhesions	Sharp dissection or electrocautery, no organ/serosal damage
4	4+	3+	Matted adhesion(s) with or without filmy adhesions	Sharp dissection or electrocautery, with unavoidable organ/serosal damage

Table 2. List of primers involved in the gene expression

Primer	Sequence	Accession number
GAPDH	F: AGTATGATTCTACCCACGGCAAA R: CACAACATACTCAGCACCAGCAT	NM_001003142
Col1α1	F: AAGAGCCTGAGCCAGCAGAT R: AGTCGGAGTGGCACATCTTG	NM_001003090
Col3α1	F: GGCACAGCAGCAAGCTATTG R: GGTTCTGGCTTCCAGACATCTC	XM_845916
VEGF	F: TTGCTGCTCTACCTCCACCAT R: TGTGCTCTCCTCCTGCCATAG	AJ133758
TGFβ1	F: CCTGCTGAGGCTCAAGTTAAAAG R: CTGAGGTAGCGCCAGGAATC	NM_001003309

GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; *Col1α1*, Collagen type I Alpha 1; *Col3α1*, Collagen type III Alpha 1; *VEGF*, vascular endothelial growth factor; *TGFβ1*, Transforming growth factor beta 1.

Gene expression analysis: Mesh implants were separated from the adjacent muscle tissues at each time point, homogenized and lysed using Trizol reagent (Invitrogen, Carlsbad, CA, U.S.A.). RNA concentrations and purities were measured using an Implen spectrophotometer (Implen, Westlake Village, CA, U.S.A.). For each sample, cDNA was synthesized from 1 μg total RNA using a Sensi Fast cDNA synthesis kit (Bioline, Taunton, MA, U.S.A.). The newly formed cDNA was mixed with master mix (TaKaRa, Otsu, Japan) and appropriate target primers to investigate tissue response to the induced wound: collagen type 3 α1 (*COL3α1*) and collagen type 1 α1 (*COL1α1*) to determine collagen deposition, vascular endothelial growth factor A (*VEGF*) to assess angiogenesis, and transform growth factor β1 (*TGFβ1*) to assess wound closure. Reactions were performed on a Pikoreal system (Thermo Fischer Scientific, Waltham, MA, U.S.A.). At each time point, gene expression of the removed implant of Damour with (D w PRP) and without (D w/o PRP) PRP was compared to glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), as a housekeeping gene. Results were adjusted to normal, according to the level of *GAPDH*. Three replicates from every biological sample were used, and results were expressed as mean and standard error. The primers involved in the gene expression are listed in Table 2.

Statistical analysis

Data analyses were performed using SPSS V.20. A two-tailed independent *t*-test using the $2^{-\delta\delta ct}$ method was used for both gene expression analysis and to determine the adhesion score in the two groups at both time points.

RESULTS

Gross examination and adhesion score

All dogs from both groups tolerated the surgical procedure well and survived until the determined date of euthanasia. Wound healing was uncomplicated, except for seroma formation and limited stitch dehiscence. Seroma formation was recorded more frequently in the control group 5/12 (42%) than the PRP group 3/12 (25%). Solitary skin wound stitch dehiscence and areas of infection were grossly observed in two dogs only, both from the control group. Hernia recurrence was detected in one dog from the control group at the 2-month euthanasia time point (Fig. 2A).

At 2 months, gross inspection of the implantation site revealed wrapping of the implanted materials with a thin layer of white fibrous connective tissue and good incorporation with the recipient abdominal wall, with only slight decreases in diameter (4 × 7 cm average). Furthermore, the outer neovascularization could be observed by the naked eye and was larger and more pronounced in the PRP group than in the control group (Fig. 2B and 2C). Thickness of both the covering connective tissue and neovascularization were increased at 4-month euthanasia time point.
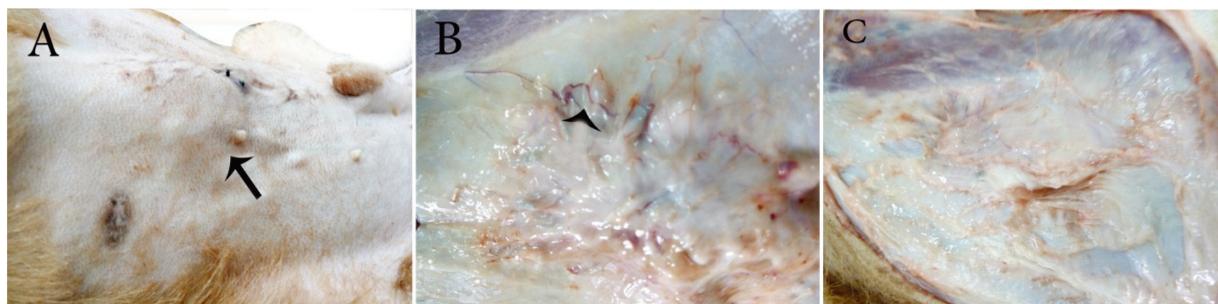
Fig. 2. Hernia recurrence (arrow) was evident at the time of necropsy in one dog from the control group (A). Gross evaluation of the mesh at 2 months revealed a pronounced neovascularization (arrowhead) in the platelet-rich plasma (PRP) group (B) compared to the control group (C).

Table 3. Animal distribution according to the Modified Hopkin Adhesion Score, adapted by Dubcenco *et al*. [11]

Score	Control group		PRP group	
	2 months	4 months	2 months	4 months
0	-	-	-	1
1	2	2	5	4
2	1	3	1	1
3	2	1	-	-
4	1	-	-	-

PRP, platelet-rich plasma.

Fig. 3. Representative images of all intra-abdominal adhesion scores: 0 (A, PRP group); 1 (B, PRP group); 2 (C, PRP group); 3 (D, control group); 4 (E, control group). PRP, platelet-rich plasma.

According to the Modified Hopkins Adhesion Score, adapted by Dubcenco *et al.*, all intra-abdominal adhesion scores were recorded at both euthanasia time points (Table 3). The PRP group shows less severe adhesion to the underlings (1.08 ± 0.51) compared to the control group (2.08 ± 0.99). In PRP treated animals, one dog showed a score of 0 (no adhesion), and no dogs showed scores of 3 and 4. In the control group, all adhesion scores, except 0, were represented, even scores of 4 where matted adhesion between the meshes and both the liver and intestines were observed, leaving unavoidable serosal damage upon dissection (Fig. 3).

Gene expression and histological analysis

Histological examination at 2 months revealed more pronounced parallel collagen fibers in the PRP treated group, compared to the control group (Fig. 4A and 4B), and collagen fibers were more abundant, with formation of muscular islets, in the PRP group at 4 months (Fig. 4C and 4D). Gene expression analysis confirmed this histological finding, where the PRP group over-expressed both COL1α1 and COL3α1 at 4 months compared to the control group ($P \leq 0.05$; Fig. 4E and 4F), however, there was no difference between the two groups at 2 months. Additionally, the PRP group was found to have a larger inter-connecting network of blood vessels, clearly penetrating the implanted Damour at 2 months (Fig. 5A and 5B), that increased in number and size by 4 months compared with the control group (Fig. 5C and 5D). Molecular analysis confirmed this finding as expression of angiogenic factor, VEGF, and revealed a two- and five- fold increase in the PRP group after 2 and 4 months, respectively ($P \leq 0.05$; Fig. 5E). Such angiogenesis improvement in the PRP group was linearly correlated with increased tissue thickness and architecture, compared to
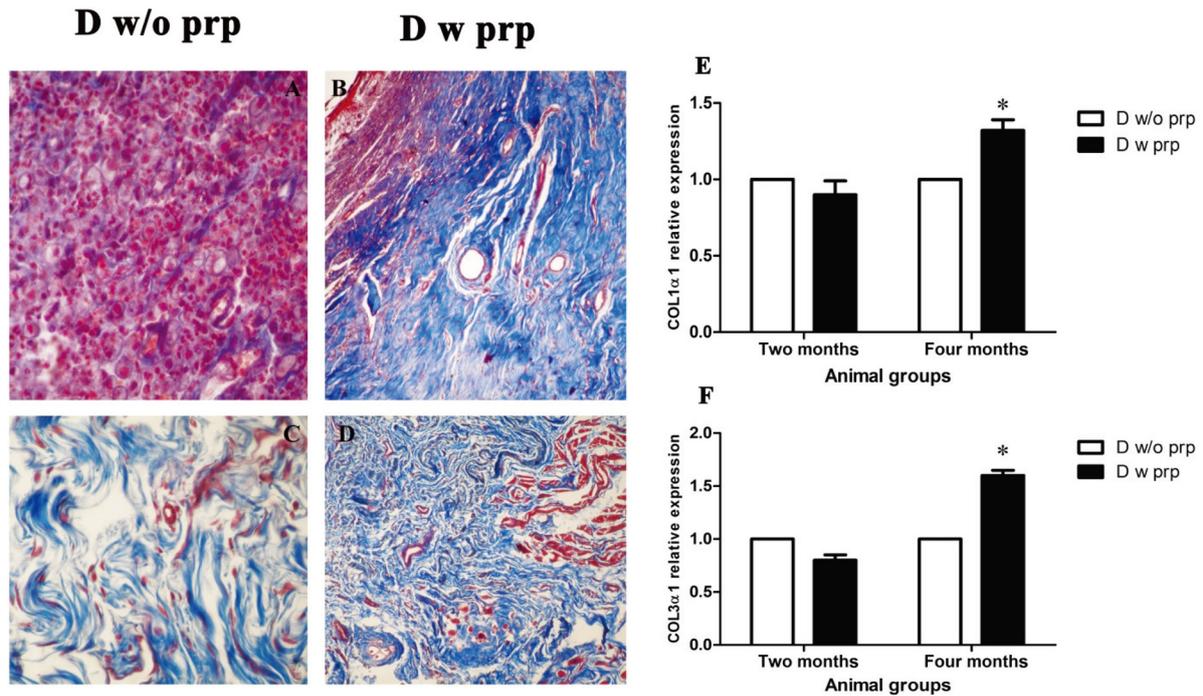
Fig. 4. The control group (Damour without PRP- D w/o PRP) revealed less collagen fiber deposition at 2 months (A) compared to the PRP group (Damour with PRP- D w PRP) (B). At 4 months, collagen fibers in the PRP group had increased in density and displayed restoration of the normal histo-architecture (C, D). Masson trichrome 10×. mRNA expression of *COL1α1* (E) and *COL3α1* (F) indicated a significant increase in gene expression in the PRP group compared to the control group at 4 months. Significant differences were found every month (where bars contain an asterix), indicating significant changes compared to the control group (*P*≤0.05). PRP, platelet-rich plasma; *COL1α1,* Collagen type I Alpha 1; *COL3α1*, Collagen type III Alpha 1.
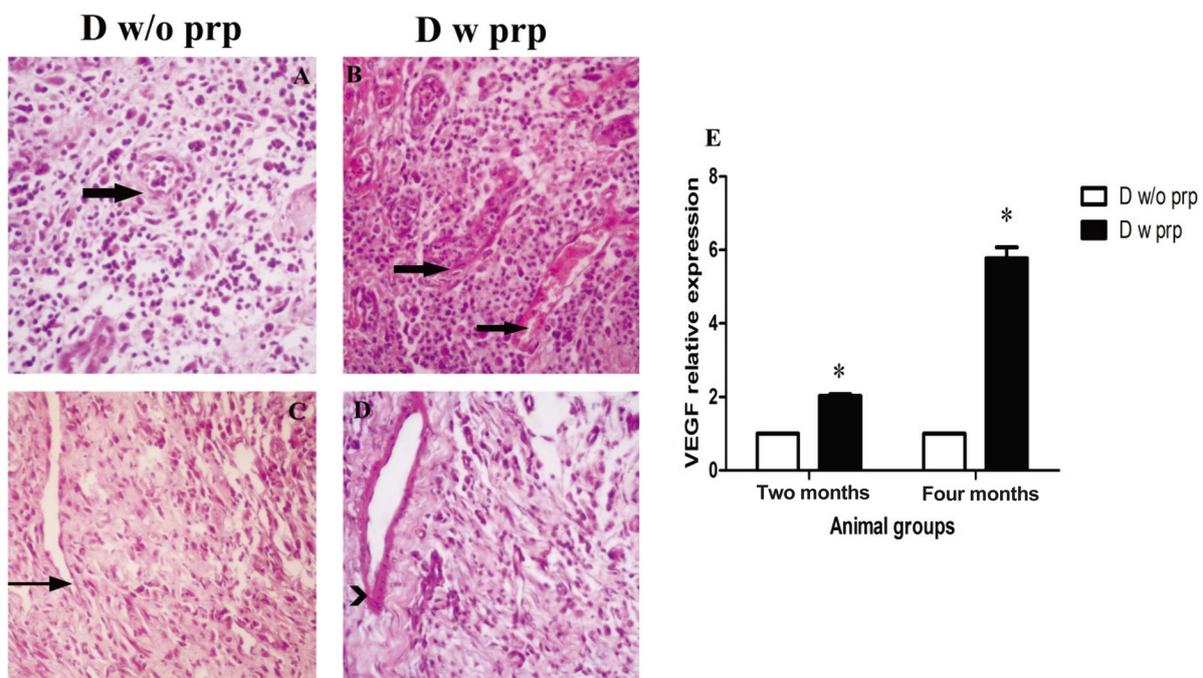

Fig. 5. The control group (Damour without PRP- D w/o PRP) showed fewer poorly-developed capillaries (arrows) by 2 months (A) compared to the PRP group (Damour with PRP- D w PRP) (B). By 4 months, well-developed blood capillaries were observed in both groups with significant increases in size and number in the PRP group (D) than in the control group (C). H&E 40×. There was a significant over-expression of VEGF at 2 and 4 months (by 2- and 5- fold, respectively) in the PRP group compared to the control group. Significant differences were found every month (where bars contain an asterix), indicating significant changes compared to the control group (E; *P*≤0.05). PRP, platelet-rich plasma; *VEGF*, vascular endothelial growth factor; H&E, hematoxylin and eosin.
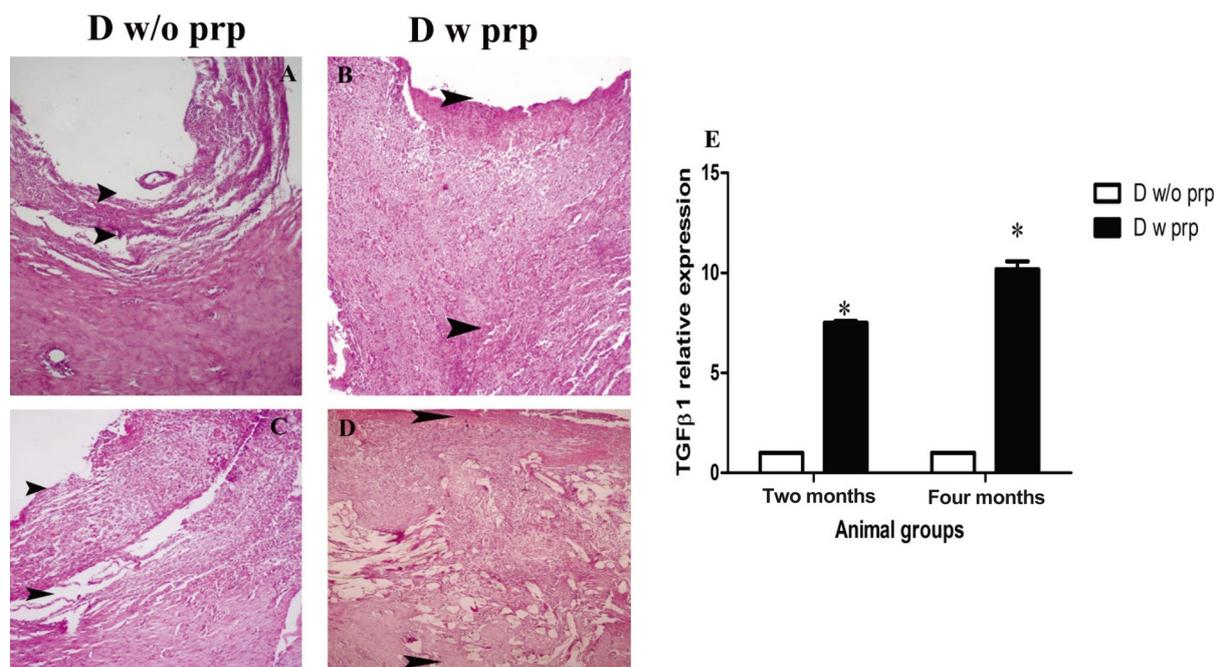
Fig. 6. The PRP group demonstrated more abundant and clearly defined new tissue formation (B, D) than the control group (A, C) at both 2 and 4 months. H&E 10×. There was a significant over-expression of TGFβ1 at 2 and 4 months (by 7- and 10- fold, respectively) in the PRP group compared to the control group. Significance differences were found every month (where bars contain an asterix), indicating significant changes compared to the control group ($P \leq 0.05$). H&E, hematoxylin and eosin, PRP, platelet-rich plasma; *TGFβ1*, transforming growth factor β1.

the control, at 2 months (Fig. 6A and 6B). This enhanced tissue thickness and collagen deposition was further noted at 4 months (Fig. 6C and 6D). Similarly, significant over-expressions of TGFβ1 were recorded at 2 and 4 months in the PRP treated group, by 7- and 10- fold, respectively ($P \leq 0.05$; Fig. 6E).

DISCUSSION

Hernia recurrence continues to be a significant complication after abdominal repair, with substantial economic impacts in both humans and animals [22, 34]. Prosthetic herniorrhaphy was found to decrease recurrence from 50% to less than 25% [21], however, at a high relative cost, especially for animals, in developing countries. Commercial polyester/cotton fabric (Damour) was found by Mosbah and Abouelnasr to be a promising hernioprosthetic material in ruminants, owing to its availability, flexibility, tissue compatibility and cost effectiveness, with associated recurrence and postoperative complication rates of 8 and 12%, respectively [29]. This study showed that the use of PRP with Damour for the repair of abdominal wall defects is associated with increased tissue deposition, incorporation and neovascularization, and subsequent decreased hernia recurrence and other associated complications.

The size of abdominal wall defects (10 × 6 cm) created in the present study is considered large in relation to the animal size. This was done to ensure that they were representative of the large-sized hernias that occur in large animals, necessitating prosthetic herniorrhaphy, as stated by Kumar *et al.* Such cases previously relied on recommendations from the surgical literature for humans, which emphasize the use of prosthetic materials for hernioplasty when the size of the hernial ring exceeds 3 cm in diameter [21, 22, 38].

Implantation of a mesh using an underlay technique and fixation of the mesh with an interrupted suture pattern using polypropylene suturing material, played a crucial role in decreasing the rate of hernia recurrence recorded by Abouelnasr *et al.* [1]. This was due to the even distribution of stress over the mesh and thus reduced tendency of the suturing material to harbor microorganisms, as explained by Kawcak and Stashak [20] and Ladurner *et al.* [23]. PRP is a plasma constituent with a high concentration of platelets. Activated platelets are associated with many growth factors that stimulated cell migration, proliferation, differentiation, angiogenesis, elimination of tissue debris and regeneration of appropriate tissues [9]. In our investigation, for obtaining adequate PRP, we performed double centrifugation at a sufficient rotation force to avoid premature release of the growth factors into the platelets [30]. Furthermore, the addition of thrombin and calcium gluconate to PRP gel spontaneously motivated the alpha granules to release different growth factors that play an effective role in angiogenesis [13]. PRP is easy to prepare, low in cost and does not require a high level of technical skill, rendering it highly accessible and easy-to-use within veterinary medicine. In the present study, the platelet numbers in the prepared PRP were 6–8 times higher than the platelet number in the peripheral blood, concordant with Nixon who reported that increased platelet numbers improve healing by stimulating the increased release of

growth factors [30].

Few animals developed seroma, with no significant differences between the two groups; seroma formation could be attributed to either local circulatory disturbances, resulting from the tight suture or the size of the dead space created between the mesh and the host tissues, as mentioned by Amid [2]. Surprisingly, only one dog from the control group developed hernia recurrence, whilst in the PRP group recurrence was not recorded. This may be due to PRP increasing neovessel formation and subsequently tissue deposition, which were confirmed by histological and gene expression analyses. This result is promising in comparison of other studies that have used synthetic prostheses; recurrence rates of 25% and 70% were recorded with the use of a universally accepted polypropylene mesh [3] and cellular dermal matrices (ADM) for hernia repair in animal models [37], respectively. Additionally, Molloy *et al.* added that inflammatory wound conditions encouraged the release of growth factors and inflammatory cells that initiated the neovascularization and collagen biosynthesis [28].

Histological examination with Mason trichrome stain revealed that the deposition of newly formed tissues increased in thickness because of the formation of organized collagen and muscle fibers. Takamura *et al.* suggested that in rabbits, PRP can initiate a remodeling phase and promote tendon repair through production of collagen fiber bundles in a unidirectional manner [36]. The rapid formation of collagen fiber bundles was attributed to rapid migration of fibroblasts [40]. The current result was further confirmed through a qPCR technique that revealed PRP over-expressed *COL1α1* and *COL3α1* at 4 months. Meanwhile, Jo *et al.* explained that PRP caused cell proliferation of tenocytes and induced significant over-expressions of *COL1α1* and *COL3α1* during PRP treatment within 14 days [17]. In addition, the role of PRP in stimulating collagen biosynthesis might be attributed to the stimulation of collagen production from PRP treated cells rather than existing cells, suggesting a delay in the gene expression of *COL1α1* and *COL3α1* in the first two months [41]. Similarly, Van Eps *et al.* claimed that PRP was capable of inducing *COL1α1* and *COL3α1* expression in a rodent ventral hernia model after 3 months of surgical procedures, however, there is no record of improvement in collagen deposition in less than 3 months from the date of surgical procedure [37].

Histological examination of newly formed blood vessels revealed the presence of an inter-connected mesh of blood vessels that increased in number and size in the 4th month. Platelet rich-plasma consists of abundant angiogenic factors, such as angiopoetin-1, that stimulate the production of endothelial cell growth, differentiation and migration [25]. Furthermore, the high concentration of growth factors in autologous platelet rich plasma stimulates angiogenesis and neovascularization [5]. The up-regulation of VEGF has a beneficial effect by recruiting hematopoietic stem cells to the site of injury that produce capillary plexus and form mature vessels [8]. Therefore, the angiogenesis process was further assessed with gene expression analysis of *VEGF* that showed a significant over-expression after 2 and 4 months, indicating the role of PRP in the stimulation of angiogenesis and process of neovascularization in implanted tissues with Damour. VEGF stimulates the production of integrins in the endothelial lining, which promote endothelial cell migration and enhance neovascularization [35].

PRP is capable of producing several angiogenic factors that stimulate angiogenesis in newly formed tissues [16]. VEGF is considered a critical signal transduction in angiogenesis [18] through angiogenesis regulating and wound healing [24]. The induction of other growth factors by PRP was further extended to TGFβ1, known for its potential role in the regulation of several mammalian tissue wound healing processes, including angiogenesis, cell proliferation and collagen deposition [19]. The increase in the levels of *TGFβ1* by 8- and 10- fold in the PRP group was due to the secretion of TGFβ1 with platelets from alpha granules resulting from PRP therapy [6].

Regarding peritoneal adhesions, our results support the pervious finding of Van Eps *et al.* that PRP can reduce adhesion incidence and severity when used for hernia repair in rat models [37]. Peritoneal adhesions are formed initially after fibrin exudate formation due to trauma; exudates are either absorbed through a fibrinolytic system or transformed to mature tissue adhesion by inflammation or ischemia [7]. In the present study, the role of PRP in reduction of adhesion formation could be attributed to the anti-inflammatory properties of the platelet rich concentrates [4, 26], which could lead to the fibrinolysis of adhesions and reduce mature transformation. This finding is of substantial importance, because a reduction in adhesion formation would reduce complications, such as intestinal obstruction and fistulation, necessitating further surgical intervention [15, 27]. Moreover, our findings were the first study in an advanced animal model that mimicked hernial repair in large animals

In summary, this study demonstrates the effectiveness, in terms of low recurrence rates, of a relatively cheap prosthesis (Damour) for repairing abdominal wall defects with the simple addition of allogenic PRP, which serves to enhance neovessel formation and increase tissue deposition and incorporation, with reduced postoperative complications, such as peritoneal adhesions. Our results are encouraging for the clinical application of PRP Damour for hernia repair in general veterinary surgery.

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