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Comparison between the regenerative potential of bone marrow aspirate (BMA) and platelet-rich plasma (PRP) on healing of canine meniscal tear

Ahmed A. Sadek[1*], Sary Kh. Abdel-ghaffar[2,3], Mahmoud Rushdi[4], Mohamed Semieka[1], Samia Moustafa[1]

[1]*Department of Surgery, Anesthesiology, and Radiology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.*
[2]*Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.*
[3]*School of Veterinary Medicine, Badr University in Assiut, Assiut, Egypt.*
[4]*Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.*

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***Correspondence:**

Corresponding author: Ahmed A. Sadek
E-mail address: ahmedsadek90@aun.edu.eg

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ABSTRACT

Repair of meniscal tears in the avascular zone represents an obstacle for orthopedic surgeons. Several therapeutic methods have been suggested to mange these tears including meniscectomy and meniscal allografts; however, their clinical application was restricted due to their disadvantages. These limitations inspired the necessity to develop products that possess the ability to initiate healing in such avascular tears. Hence, the goal of the current study is to assess and compare the regenerative capability of bone marrow aspirate (BMA) and platelet-rich plasma (PRP) to enhance repair of avascular meniscal tears. After preparation of BMA and PRP, meniscal tear was conducted in the inner avascular zones in dogs and left untreated as control or treated with either BMA or PRP. Clinical observation of weight bearing, lameness, pain on manipulation, gait, and functional disability were investigated after 3, 6, 9, and 12 weeks of surgery. In addition, gross and histological evaluations were performed at weeks 4, 8, and 12 after surgery. Both materials demonstrated a positive improvement in clinical observations compared to the control group. Furthermore, repair of meniscal tears was stimulated in tears treated with either BMA or PRP with better gross and histological outcomes in PRP-treated group than BMA-treated group. To conclude, our findings showed that BMA and PRP possess the potential to enhance the healing process of meniscal tears in the inner avascular region with the superiority of PRP.

Introduction

The menisci, intra-articular C-shaped tissues interposed between the condyles of the femur and the tibial plateau, are a key criterion structures to the integrity of stifle joint since they contribute significantly in mechanical support, force distribution, shock resistance, and joint lubrication (Franklin *et al*., 2010). Thus, lesions of the meniscus usually associated with lameness, dysfunction of the stifle joint, and higher strain on the articular cartilage, which increases the risk of articular cartilage destruction and osteoarthritis progression (Kawanishi *et al*., 2014; Koch *et al*., 2019).

Among these lesions, meniscal tears are one of the leading contributors to canine lameness. Meniscal tears are mostly related to cranial cruciate ligament rupture due to instability of the joint with excessive stress on the meniscus (Franklin *et al*., 2010; Hayes *et al*., 2010; McCready and Ness, 2016; Jeong *et al*., 2021). Unfortunately, the meniscal intrinsic healing tendency is impaired particularly in the avascular area owing to the limited blood supply. Therefore, the regeneration of meniscal tears in the inner avascular zone is regarded a major challenge in orthopedic surgery and a substantial health concern with economic consequences (Ballard *et al*., 2014; Kawanishi *et al*., 2014; Koch *et al*., 2019; Xiao *et al*., 2021).

Meniscectomy and meniscal allografting are the commonly used strategies for treatment of meniscal tears. However, their use associated with various drawbacks, whereas meniscectomy is usually associated with occurrence of osteoarthritis, while meniscal allografting are subjected to limited sources of allografts, selection of suitable graft size, failure of grafting, transmission of diseases, and arthrofibrosis (Kawanishi *et al*., 2014; Yu *et al*., 2015; McCready and Ness, 2016; Koch *et al*., 2019; Stocco *et al*., 2022). Therefore, tissue engineering has gained attraction as an innovative strategy to induce meniscal healing in the avascular area based on a combination of growth factors, cells, and scaffolds (Zhang *et al*., 2009; Sadek *et al*., 2023).

Different biological materials have been emerged in recent years for tissue engineering (Lombardo *et al*., 2021). Bone marrow is one of these biological materials that contains hematopoietic stem cells, mesenchymal stem cells (MSCs), growth factors, cytokines, and chemokines (Soltan *et al*., 2009). Bone marrow MSCs possess the potential to differentiate into the various precursors of orthopedic tissues including bone, cartilage, tendon, and meniscus (Abdel-Hamid *et al*., 2005; Huang *et al*., 2008 ; Gianakos *et al*., 2017). In addition, bone marrow contains a variety of the growth factors that initiates and support the process of orthopedic tissues repair such as transforming growth factor-β (TGF-β), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF) (Sugaya *et al*., 2018). Bone marrow was applied in the repair of wounds (Rodriguez-Menocal *et al*., 2015; Chittoria *et al*., 2016; Gupta *et al*., 2017; Mohammed *et al*., 2023), cartilage lesions (Fortier *et al*., 2010; Neubauer *et al*., 2018), tendon rupture (Imam *et al*., 2017), bone defects (Pelegrine *et al*., 2014; U *et al*., 2019), and meniscal tears (Abdel-Hamid *et al*., 2005; Duygulu *et al*., 2012; Koch *et al*., 2019; Xiao *et al*., 2021).

Platelet rich plasma (PRP) is another biological material that has increasingly attained significant attention in recent years and composed of highly concentrated platelets suspended in small amounts of plasma. PRP are considered a enriched concentrated source of the key growth factors that regulate tissue regeneration processes including insulin–like growth factor (IGF), transforming growth factor-alpha (TGF-α), TGF-β, FGF, VEGF, and PDGF (Sugaya *et al*., 2018; Xiao *et al*., 2021). These growth factors

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act synergistically to enhance the cascade of tissue repair including chemotaxis of inflammatory cells to the area of tissue injury, angiogenesis, extracellular matrix (ECM) deposition, fibroplasia, recruitment of MSCs to the site of injury, and differentiation and proliferation of cells (Demidova-Rice *et al*., 2012; Zarei and Soleimaninejad, 2018). Consequently, PRP has been reported to stimulate regeneration of different orthopedic tissues as bones (Kim *et al*., 2014; Zhao *et al*., 2021), tendons (Jiang *et al*., 2020; Teng *et al*., 2016), cartilage (Mifune *et al*., 2013; Gao *et al*., 2019), and meniscus (Ishida *et al*., 2007; Lee *et al*., 2016; Xiao *et al*., 2021).

Thus, the objective of the present study is to investigate the regenerative capability of BMA and PRP to induce meniscal healing in the inner avascular region. Moreover, it aimed to compare the potency of BMA and PRP in repair avascular meniscal tears.

Materials and methods

Ethical approval

The Institutional Animal Care and Use Committee of Research Facilities at the Faculty of Veterinary Medicine, Assiut University, Egypt approved the study's design according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) regulations.

Animals and Study Design

A total of twenty-seven mature mongrel dogs, 11 males and 16 non-pregnant females that aging 2-3 years old and weighing 15 to 20 kg, were selected to be used for this experiment. Animals were subjected to thorough clinical and radiographic examination to ensure good health status and normal musculoskeletal configuration, and then they were housed in an individual boxes in a well-ventilated room at the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Assiut University, Egypt. Dogs were maintained on a standard commercial diet and access to water was ad libitum throughout the study. All animals were acclimatized to their cages for 2 weeks prior to surgical procedure. Animals were randomly placed into three groups (n = 9 for each group); the control group, the BMA-treated group, and the PRP-treated group.

Acquisition of autologous BMA

After induction of general anesthesia using a combination of xylazine HCl 2% (1 mg/kg, Xyla-ject 20mg: Adwia Co., Egypt) and ketamine HCl 5% (10 mg/kg, Ketamine 50mg: Sigma-Tec, Egypt), a 15G Rosenthal bone marrow needle was locked in the iliac crest to aspirate 2ml of bone marrow into a syringe containing heparin (10 IU/mL of bone marrow). The collected BMA was placed at room temperature till injected into tear site as autologous BMA.

Preparation of autologous PRP

PRP was prepared in this study in two steps: preparation of autologous thrombin and preparation of autologous PRP. Autologous thrombin was prepared from the blood as reported previously (Seung-gul *et al*., 2007). In brief, in a plain vacutainer tube about 5mL of autologous blood was collected under strict aseptic conditions. The collected blood was allowed to stand for 30 min at room temperature. Then, blood was centrifugated at 3600 rpm for 12 min and the serum was picked up as autologous thrombin. Subsequently, the collected autologous thrombin was mixed with calcium chloride 10% ($CaCl_2$, Sigma, Egypt) at the ratio 3:1 (300 µL autologous thrombin and 100 µL $CaCl_2$ 10%).

The autologous PRP was prepared according to Yamada *et al*. (2004). Briefly, 10 mL of whole blood was received into sodium citrate 3.8% vacutainers and centrifugated for 5 min at 1100 rpm. Then, the yellow plasma containing the buffy coat was taken up into a plain tube and subjected to a second centrifugation at 2500 rpm for 5 min. Afterwards, the plasma supernatant was removed, and the created pellet was resuspended in the residual plasma (800 µL) to produce PRP. The prepared autologous PRP was stored at room temperature until use.

At last, the PRP gel was formed immediately before intraarticular application through the addition of the thrombin into the PRP (800 µL of PRP and 400 µL of thrombin).

Establishment of avascular meniscal tear model and management

Animals were prepared for surgery through food starvation for 12 hours prior to the operations. All surgical operations were performed under complete aseptic conditions. General anesthesia was used in the experiment that was achieved through IV injection of a mixture of xylazine HCl 2% and ketamine HCl 5%. Then, the right hindlimb was aseptically prepared and draped.

A full-thickness longitudinal tear was performed in the avascular zone of medial meniscus as described by Xiao *et al*. (2021) Fig. 1. Typically, a medial approach to the stifle joint was carried out through a longitudinal incision medial to the patella and parallel to the midline of the stifle joint. The arthrotomy incision passes through the skin, subcutaneous tissue, and joint capsule. Then, the patella was dislocated laterally followed by full joint flexion with a distal displacement of the fat pad for better exposure of the meniscus. By using a scalpel blade, a standard longitudinal avascular full-thickness tear (10 mm in length) was created on the anterior horn of medial meniscus parallel to longitudinal meniscal axis. In the PRP treated group, the PRP gel was applied at the tear site followed by suturing of joint capsule. In control and BMA treated groups, the joint capsule was sutured and then either left untreated or injected with 2ml of BMA, respectively. Finally, the subcutaneous tissue and skin were closed as usual.

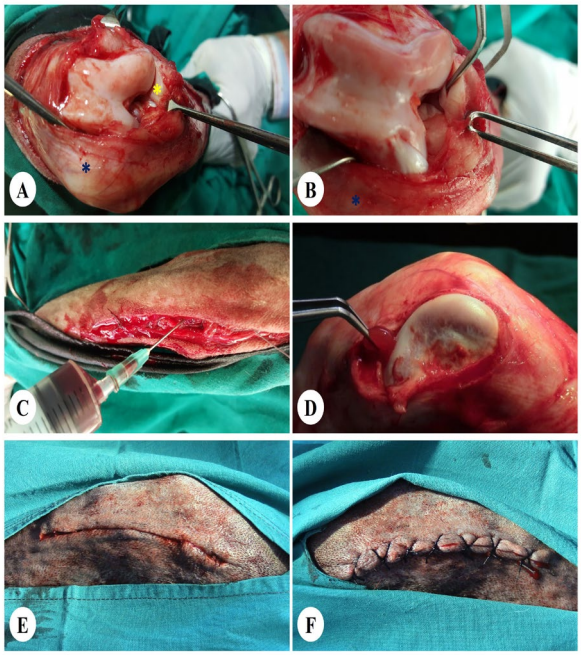

Fig. 1. Establishment of meniscal tears. (A) exposure of the medial meniscus (yellow asterisk) after lateral displacement of patella (blue asterisk). (B) Creation of longitudinal full thickness tear. (C) Application of BMA. (D) Application of PRP. (E) and (F) closure of s\c tissue and skin, respectively.

During the consecutive post-surgical 5 days, each animal was given an intramuscular injection of Cefepime (4.5 mg/kg, Maxipim: Smith-Kline Beecham Co., Egypt). In addition, the operated hindlimbs were placed for 14 days in a cast using a splint bandage. At 30, 60, and 90 days after operation, repair of the meniscal tear was evaluated in different experimental groups.

Clinical investigation

During the postoperative period, animals were subjected to careful inspection of complications such as infection, wound dehiscence, health condition of animals, activity level, gait, and weight bearing on the operated limb. Furthermore, lameness during walking, range of motion, pain on manipulation, and functional disability were assessed (Table 1) and scored at 3, 6, 9, and 12 weeks after surgery (Black *et al*., 2007).

Table 1. Clinical observation lameness scoring system.

Parameter	Description	Score
Lameness during walking	Un-detectable lameness	1
	Intermittent lameness	2
	Persistent lameness	3
Pain on manipulation	No pain	1
	Mild pain	2
	Severe pain	3
Range of motion	No limitation	1
	Pain only at full extension/ flexion	2
	Pain at less than extension/ flexion	3
	Pain at any attempt	4
Functional disability	Normal activity	1
	Slightly stiff gait	2
	Stiff gait	3
	Very stiff gait	4
	Unwilling to walk	5

Gross evaluation of the meniscal tear

After 4, 8, and 12 weeks of induction of meniscal tear, animal were sedated with xylazine HCl (1 mg/kg, Xyla-ject: ADWIA Co., Egypt) followed by euthanasia using an overdose of thiopental sodium (85 mg/kg, Anapental: Sigma-Tec, Egypt). Afterwards, the medial meniscus was removed for gross evaluation including the tear site filling, color, and surface.

Histological assessment

Menisci were collected at different evaluation times (n = 3 from each time at each group) and then fixed in neutral buffered formalin (10%,). The formalin-fixed meniscal samples were routinely dehydrated in ethanol, cleared in methyl benzoate, embedded in paraffin, and sectioned (5 µm thickness). Then, the serial sections were stained with Hematoxylin and Eosin (H&E). Afterward, slides were observed using a microscope (Olympus CX31, Japan) and photographed using a digital camera (Olympus, Camedia C-5060, Japan). Histological interpretation was performed on coded samples blindly.

Histochemical staining of collagen

Crossmon's trichrome staining was conducted for further examination of collagen deposition and cartilage plaques formation within the regenerated area. The paraffin-embedded sections were deparaffinized in xylene, and rehydrated in a graded series of ethanol, stained with Crossmon's trichrome stain, dehydrated in graded alcohol, made transparent with xylene, and mounted. The stained sections were examined and photographed in a blind manner.

Statistical analysis

The obtained data were analyzed with a statistical software (IBM SPSS version 21) and presented as a mean ± standard deviation (SD) at a significant level of p <0.05. The results of lameness scores (n=3 for each time point in each group) were analyzed by two-way ANOVA, followed by Tukey's test.

Results

Clinical investigation

In the present study, animals in different groups withstand the operation and postoperative period without notable complications. In the first 48 hours after surgery, all animals could stand up and walk freely. They returned to the routine life activities such as eating, drinking, and grooming after 72-96 hours of surgery. In addition, dogs suffered from partial weight bearing on the operated limb for 7 days after surgery in the PRP and BMA treated groups compared to 14 days for the control one.

In the control group, dogs showed persistent severe lameness on walking at week 3 after surgery which diminished in a time-dependent manner to moderate lameness at week 6 and continued to week 12 postoperatively. In addition, they displayed very stiff gait and severe pain on manipulation of the operated hind limb with painful reaction at less than extension/flexion on week 3 and 6 after surgery. On 9 and 12 weeks postoperatively, stiff gait and mild pain associated with handling and full extension/flexion of the operated limb were reported.

In the BMA-treated group, mild painful reaction was detected on manipulation and full extension/flexion of the affected hind limb revealed mild pain on week 3 after surgery. This painful reaction on manipulation of the affected limb reduced overtime till disappeared with no motion limitation at weeks 9 and 12 postoperatively. In addition, dogs in BMA-treated group after 3 weeks of surgery demonstrated a moderate lameness during walking with stiff gait that returned to normal gait without lameness at weeks 9 and 12 after surgery.

In the PRP-treated group, animals displayed stiff gait with moderate degree of lameness during walking on week 3 after surgery. Furthermore, handling as well as full extension/flexion of the affected hind limb revealed mild pain. On week 6, 9, and 12 postoperatively, animals showed normal walking and gait without pain on limb manipulation nor limitation of movement.

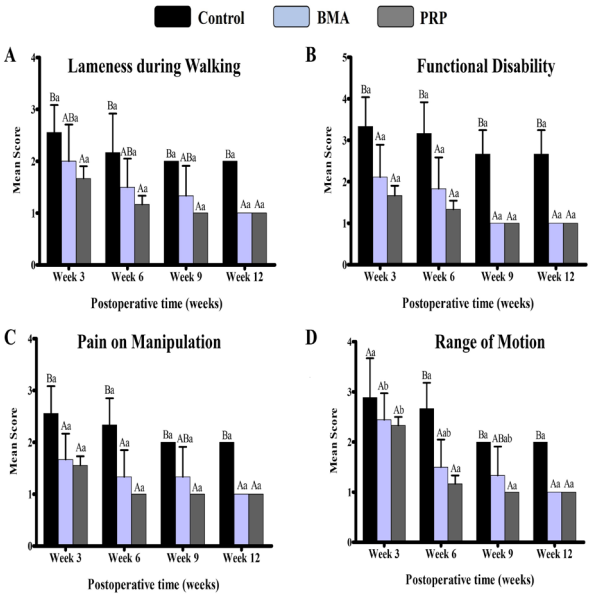

Fig. 2. Lameness score parameters. The percentages of lameness during walking (A), functional disability (B), pain on manipulation (C), and range of motion (D) in different study groups at weeks 3, 6, 9, and 12 after surgical creation of meniscal tears. Error bars ± SD; n = 3 for each group and time point. Bars with the same letter represent values that are not significantly different (two-way ANOVA followed by Tukey's HSD post hoc test). A and B: significance between groups; a and b: significance between time points within the same group.

As demonstrated in Fig. 2, the score of lameness during walking in

the PRP-treated group was significantly lower than the control group during the entire study period. Furthermore, BMA-treated group displayed a lower significance compared to the control group at week 12 after surgery. Regarding the functional disability score, both PRP-treated and BMA-treated groups showed a lower significant difference compared with the control group at different times of evaluation. The pain on manipulation scoring revealed a significance between both PRP-treated and BMA-treated groups and the control group at 3, 6, and 12 weeks after surgery. Additionally, the PRP-treated group showed a significant difference with the control group at week 9 postoperatively. The range of motion score showed a non-significant difference between different experimental groups on week 3 after surgery. However, the PRP-treated and BMA-treated groups were significantly lower than the control group at weeks 6 and 12 after surgery. On week 9 after surgery, the score of range of motion was lower in the PRP-treated group than in control group. Moreover, the range of motion was significantly higher at week 3 after surgery compared to weeks 6, 9, and 12 in the PRP-treated group, while it displayed a significance between week 3 and week 12 in the BMA-treated group. Furthermore, the PRP-treated and BMA-treated groups demonstrated no significant differences between each other throughout the experiment for scores of lameness during walking, functional disability, pain on manipulation, and range of motion.

Gross evaluation of the meniscal tear

As observed in Fig. 3, the site of tears in the control group were well-recognized with a demarcated margins of the tears at 4, 8, and 12 postoperatively.

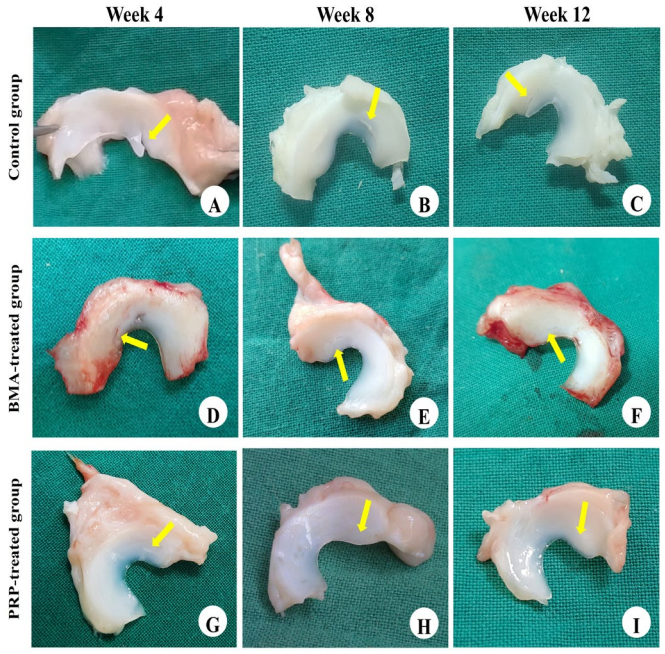

Fig. 3. Gross evaluation of meniscal tear sites (yellow arrow) of control (A-C), BMA-treated (D-F), and PRP-treated (G-I) groups at different evaluation times.

On week 4 after surgery, a distinguishable tear site was observed in the BMA-treated group with a fairly recognized bridging healing tissue connecting the tear margins. However, the PRP-treated group showed a less distinguishable tear site and a recognized tissue between the tear margins. In addition, the tear sites appeared yellow in color with rough surface in BMA-treated group, while the PRP-treated group displayed smooth surface and white color of the tear sites.

On week 8 after surgery, the BMA-treated group showed a less distinguishable rough white tear site with a recognized tissue conjugating the rims of tear with each other. However, the tear sites in the PRP-treated group were indistinguishable with a smooth surface and a color similar to the surrounding tissue.

On week 12 after surgery, the tear sites in the BMA-treated and PRP-treated groups were undistinguished with a color resembling the surrounding normal tissue and smooth surface, indicating complete healing of the tears with a bridging tissue.

Histological assessment

Histological examination was conducted to observe the regenerative potential of BMA and PRP on stimulation of meniscal tissue repair.

On week 4 after surgery, the control group showed an empty tear site gap lined with a single layer of fibroblast cells (Fig. 4A). The tear site in BMA-treated group revealed a large number of epithelial-like cells lining the gap in addition to the presence of neovascularization, collagen fibers deposition, and lymphoid cells aggregations in the healing area (Figs. 4D and 5Aa). However, the PRP-treated tear sites were filled with collagen fibers, inflammatory cells, newly formed blood vessels, and increased fibroblast population (Figs. 4G and 5 Ad). Furthermore, the tear sites in PRP-treated group showed less microscopic gaps than BMA-treated group.

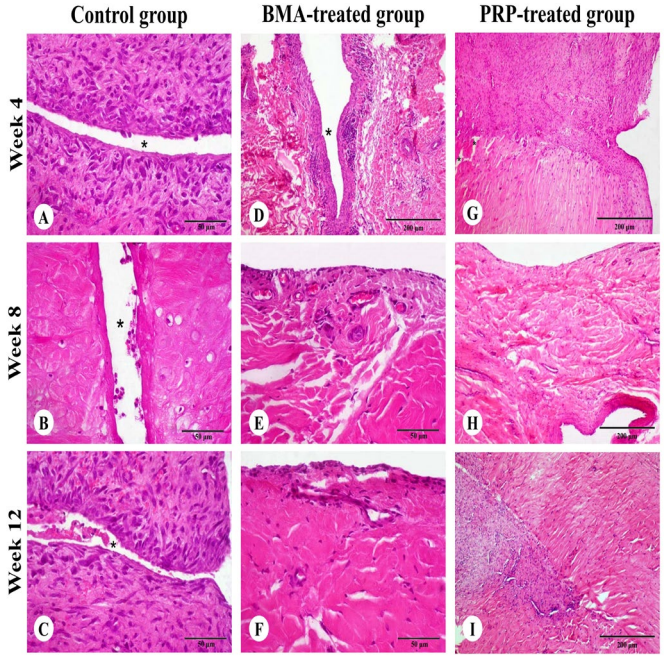

Fig. 4. Histological evaluation of meniscal tear sites. The repair site of the meniscal tear at week 4 (A, D, G), 8 (B, E, H), and 12 (C, F, I) after surgery in control, BMA treated, and PRP treated groups was stained with H&E. Black asterisks: tear gap. The scale bars in panels A-C, E, and F= 50 µm (×400), panels D, G, H, and I = 200 µm (×100).

On week 8 after surgery, empty gaps were still observed in the control group with the presence of few inflammatory cells at tear site (Fig. 4B). However, more regenerated tissue was seen in the PRP-treated tear sites than BMA-treated group. The regenerated tissue is characterized by the presence of neovascularization, inflammatory cell reaction, collagen fibers, and fibroblasts (Figs. 4E, H and 5Ab, Ae).

On week 12 after surgery, the control group remained showing unrepaired empty gaps with no evidence of healing (Fig. 4C). However, the treated groups revealed the presence of repaired tear sites that filled with collagen fibers, newvascularization, and fibroblasts aggregations (Figs. 4F, I and 5Ac). Additionally, fibrochondrocyte plaques were observed at the healed tissue in the PRP-treated group (Figs. 4I and 5Af).

As shown in Fig. 5B, the Crossmon's trichrome stained sections at week 4 revealed deposition of irregular collagen fibers in BMA-treated and PRP-treated groups. However, regular collagen was found at weeks 8 and 12 after surgery in the treated group. Additionally, proliferation of fibrochondrocytes on week 12 was more in the PRP-treated tear sites than BMA-treated sites.

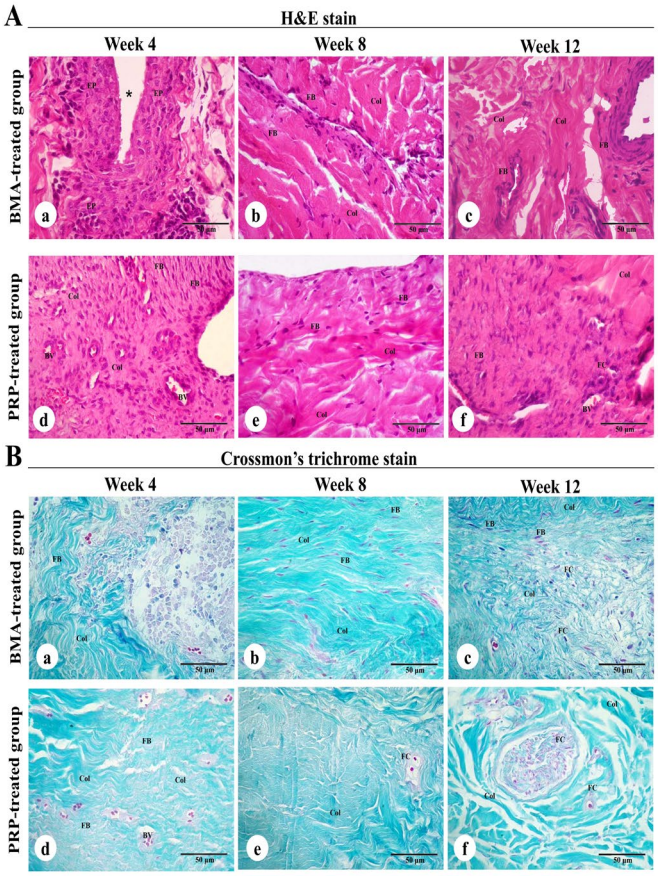

Fig. 5. Histological and Histochemical evaluations of formed tissue at meniscal tear sites. The repair tissue at site of the tears at weeks 4, 8, and 12 after surgery in BMA treated (a-c), and PRP treated (d-f) groups was stained with H&E (A) and Crossmon's trichrome (B) stains. BV: blood vessels, EP: epithelial-like cells, FB: fibroblast cell, FC: fibrochondrocyte, Col: collagen fibers, Black asterisks: tear gap. The scale bars in H&E and Crossmon's trichrome stain panels = 50 µm.

Discussion

Non-repaired avascular meniscal injuries represent a challenging orthopedic obstacle in dogs due to lack of spontaneous healing of these tears without further interference (McCready and Ness, 2016). Various biological materials have emerged in last decades as a potential solution to improve healing potential of the meniscal lesions in the avascular region, but the best material with ideal results has not been reported yet (Abdel-Hamid *et al*., 2005; Ishida *et al*., 2007; Zhang *et al*., 2009; Kawanishi *et al*., 2014; Zellner *et al*., 2014). Thus, our study evaluates the ability of BMA and PRP to enhance avascular full-thickness meniscal tears repair.

Herein, a full thickness longitudinal meniscal tear in the inner avascular zone was performed to evaluate the ability of both BMA and PRP for stimulation of meniscal tissue healing (Xiao *et al*., 2021).

In the current study, animals in all groups showed partial weight bearing during the first 7 days. This may be related to the pain response arises from the inflammation associated with the operation of arthrotomy (Tomas *et al*., 2015). However, animals in the BMA-treated and PRP-treated groups attained full weight bearing at day 7 after surgery in comparison to day 14 in the control group. Additionally, the results of lameness score demonstrated a lower score in BMA-treated and PRP-treated groups than in control group. These observations in BMA-treated groups explained to be related the growth factors contents of BMA that have anti-inflammatory properties (Chahla *et al*., 2016), while in the PRP-treated group might be may be attributed to the ability of PRP to reduced inflammatory response and hasten the process of healing (Abdul Ameer *et al*., 2018).

Meniscal regeneration is a complex mechanism of cellular and molecular interactions that follow extrinsic and\or intrinsic patterns. The extrinsic repair pattern based on neovascularization, undifferentiated MSCs, and granulation tissue formation; however, the intrinsic pattern depends on the self-repair potential of meniscal fibrochondrocytes (Ishida *et al*., 2007; de Albornoz and Forriol, 2012; Vishwakarma *et al*., 2016). The repair process of meniscal tissue passes through inflammatory, proliferative, and maturation stages. Additionally, neovascularization, deposition of collagen matrix, and proliferation and differentiation of cells are evidence of tissue regeneration (Tarafder *et al*., 2020; Nakagawa *et al*., 2021; Yan *et al*., 2021). Moreover, growth factors and bone marrow MSCs have been reported to possess an integral role in meniscal repair. MSCs were recruited at tear site, proliferated, and differentiated into meniscal cells, whereas growth factors involving PDGF, VEGF, IGF-1, TGF-β, and FGF have an integral function in hemostasis, angiogenesis, ECM matrix synthesis, and meniscal fibrochondrocyte cells metabolism (de Albornoz and Forriol, 2012; Twomey-Kozak and Jayasuriya, 2020).

Our findings showed the formation of reparative tissue within the tear sites in BMA-treated and PRP-treated groups compared to empty sites in control group. The newly formed tissue consists of neovascularization, regular collagen fibers deposition, and fibrochondrocyte. However, the new tissue formation was higher in the PRP-treated group than in the BMA group. The power of the BMA for avascular meniscal tear healing suggested to be related to the bone marrow contents of MSCs and growth factors (Abdel-Hamid *et al*., 2005; Seung-gul *et al*., 2007), while the power of PRP attributed the growth factors contents released from activated platelets (Abdul Ameer *et al*., 2018; Sugaya *et al*., 2018; Xiao *et al*., 2021).

In the current study, the main restrictions include inability to use neither arthroscopy for induction of meniscal tears nor diagnostic imaging tools as contrast computed tomography and magnetic resonance imaging for assessment of meniscal tears. In addition, the relatively small animal numbers, so future studies with more animals are recommended.

Conclusion

This study showed a robust anabolic efficacy of BMA and PRP in improvement of lameness and healing of the tear sites grossly and histologically. Thus, the present study suggested that BMA and PRP were promising material for induction of the healing process of avascular meniscal tears in dogs with the superiority of PRP.

Conflict of interest

The authors declare no competing interests.

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