ISSN: 1110-7219; e-ISSN: 2682-2512 (Online) Journal homepage: http://vetj.mans.edu.eg/

Effect of Platlate Riched Fibrin and mononuclear cells on regeneration of osteochondral defect in rabbits

Mohamed Salem, Awad Rizk, Esam Mosbah, Mohamed Hamed, Gamal Karrouf, Adel Zaghloul

To cite this article: Mohamed Salem, Awad Rizk, Esam Mosbah, Mohamed Hamed, Gamal Karrouf, Adel Zaghloul. Effect of Platlate Riched Fibrin and mononuclear cells on regeneration of osteochondral defect in rabbits. Mansoura Veterinary Medical Journal 2020; 21, 2: 1-10.

To link to this article: https://doi.org/10.35943/mvmj.2020.201

Published online: 25 June 2020

Mansoura Veterinary Medical Journal 21:2(2020) 1-10

https://doi.org/10.35943/mvmj.2020.201

Original Article *Surgery*

Effect of Platlate Riched Fibrin and mononuclear cells on regeneration of osteochondral defect in rabbits

Mohamed Salem[1*], Awad Rizk[1], Esam Mosbah, Mohamed Hamed[2], Gamal Karrouf[1*], Adel Zaghloul[1]

[1]Department of surgery, Anesthesiology and Radiology, Faculty of veterinary medicine, Mansoura University
[2]Department of Pathology, Faculty of veterinary medicine, Mansoura University

ARTICLE HISTORY

Received: 14.01.2020

Revised: 18.02.2020

Accepted: 11.03.2020

Address correspondence to M. Salem, G. Karrouf; Tel: +201095959721, +201009016696; E-mail: vetsurgery2018@gmail.com, drgamalkarrouf1966@gmail.com

ABSTRACT

Objective: To assess the effectiveness of Platlate riched fibrin (PRF) and bone marrow derived mononuclear cells (BMNCs) in regeneration of osteochondral defects in rabbits.

Design: A randomized-controlled experimental study.

Animals: Forty-eight adult New Zealand rabbits were allocated randomly into four groups (n=12).

Procedures: An osteochondoral defect of a 4 mm diameter and 5 mm depth was made in the trochlear groove of the left stifle joints. The defect was left for spontaneous healing in group A, filled with Platlate riched fibrin (PRF) in group B, filled with BMNCs in group C and combination of PRF and BMNCs in group D. Healing of the defects was assessed grossly and microscopically at 3, 6 and 12 weeks postoperative.

Results: Grossly, the degree of defect repair, integration to border zone and appearance of defect area were significantly higher in group D than other groups (P≤0.05). Microscopically, surface architecture, tissue morphology, cell distribution and safranin O staining of the matrix were significantly higher in group D than other groups (P≤0.05).

Conclusion and clinical relevance: The results of the present study indicated that, combination of PRF and BMNCs encourages quicker and better healing of osteochondral defects.

Keywords: PRF, BMNCs, Osteochondral defect, Rabbits.

1. INTRODUCTION

The hyaline articular cartilage permits the sliding movement of the joint, distributes loads and creates together with synovial fluid a frictionless surface between the bones [1, 2]. Osteochondoral defects of the stifle joint are a common challenge in orthopedic surgery as failing in its repair leading to osteoarthritis development [3]. Articular cartilage repair teqniques includes osteochondral grafting [4], microfracture [5], platelets riched plasma [6], bone marrow derived mononuclear cells (BMNCs) [7], autologous chondrocyte transplantation [8] and Platlate riched fibrin (PRF) [9].

PRF is a platelet concentrate containing multiple growth factors as tissue growth factor β (TGF- β), vascular endothelial growth factor(VEGFs), platelet-derived growth factors (PDGFs), fibroblast growth factors (FGFs), insulin-like growth factors (IGFs) and epidermal growth factor (EGF) [10]. The growth factors present in PRF are released gradually over a prolonged times, making it an excellent scaffold for the migration of mesenchymal stem cells (MSCs) during cartilage repair [9, 11-13].

BMNCs are a mixed group of bone marrow-derived cells contain a large number of MSCs and some growth factors as TGF-ß and consisting of varying amounts of differentially matured B, T cells, monocytes, MSCs and progenitor cells [7]. Nowadays, BMNCs showed attractive results in treatment of numerous clinical diseases as the ischemic heart diseases [14], peripheral arterial disease [15], articular cartilage regeneration [16] and type 2 diabetes mellitus [17]. This study evaluated the effectiveness of PRF and BMNCs in regeneration of osteochondral defects of the stifle joint in rabbits

2. MATERIALS AND METHODS

2.1. Animals, housing and feeding

This study was performed on 48 adult male New Zealand white rabbits (10-12 month old) and weighing 2.5 to 3 kg. The rabbits were retained under constant conditions and supplied with standard diet and water ad libitum. This investigation was performed at Mansoura Experimental Research Center (MERC), Mansoura University and approved by scientific

research Ethical Committee, Faculty of Veterinary Medicine, Mansoura University (PhD: 15).

2.2. Preparation of PRF

For preparation of PRF, 4 ml of blood were collected from the ear vein of each rabbit into 4ml evacuated tube without anticoagulant. The tube was centrifuged at 3000 rpm for 10 minutes. The PRF layer was detached from the middle layer of the tube using tissue forceps [10].

2.3. Preparation and isolation of BMNCs

Bone marrow was collected from femur and iliac crest using 18 gauge biopsy needle. Five ml of bone marrows were harvested from each rabbit in groups C and D (2.5 ml from each femur) in tube containing 500 IU heparin. Each sample was mixed gently with 10 mL phosphate buffered saline (PBS) using pipette. The sample was poured on the wall of 50 mL falcon tube containing 10 mL ficole (Ficole®, Sigma). The sample was centrifuged at 3000 rpm for 30 minutes and then translucent ring containing mononuclear cells was aspirated. The aspirate was washed twice via mixing with PBS and centrifugation at 2000 rpm for 10 minutes. The cell pellet was resuspended in 60 µL of PBS after discard of the supernatant. The viability of BMNCs was evaluated by trypan blue dye exclusion [16].

2.4. Study design:

The anesthesia was accomplished by intramuscular injection of xylazine HCL (Xylaject, ADWIA, Cairo, Egypt) at dose of 5mg/kg; ketamine HCL (Aneket®, NEON Laboratories Ltd, Mumbai, India) at dose of 35 mg/kg; and buterphanol (Alvegesic, CP. Pharma, Germany) at dose of 0.1 mg/kg [18]. Lumbosacral epidural analgesia was performed using combination of 4mg/kg lidocaine HCL (Debocaine, Arab Company for Gelatin and Pharmaceutical industries, Cairo, Egypt) and 4mg\kg Tramadol (Minpharm, Grünethal, Germany). All animals were given preoperative cefotaxime (Cefotax, Eipico, Cairo, Egypt) at dose of 50 mg/kg.

After arthrotomy of the stifle joint the patella was luxated on the lateral side. An osteochondral defect of 5 mm depth and 4 mm width was made in the middle of the trochlear groove by an electric drill with a drilling bit of 3.5 mm diameter. The patella was immediately returned to its position then the capsule was sutured using 3-0 polyglycolic acid (EGYSORB, Taisier Med, Cairo, Egypt). Subcutaneous tissue and skin were closed by routine manner [16]. Preoperative antibiotic was continued for five days and meloxicam (ADWIA, Cairo, Egypt) at dose of 0.3 mg/kg was administered for three days intramuscularly. These animals were randomly allocated into four groups (n=12). In Group A (control): The osteochondoral defect was kept for spontaneous healing. Group B (PRF group): Osteochondoral defect was filled with PRF, Group C (BMNCs group): 6×10^6 cells suspended in 60 µL PBS was putted at the floor of the defect. Group D (PRF and BMNCs group): 6×10^6 cells suspended in 60 µL PBS was putted at the floor of the defect then the defect was filled with PRF.

2.5. Evaluation of osteochondoral defect regeneration

2.5.1. Gross evaluation

The gross evaluation score of the International Cartilage Repair Society (ICRS) was used for macroscopical evaluation of the repaired tissue [19] at 3, 6 and 12 weeks postoperatively (Table 1).

2.5.2. Microscopical evaluation

The healing defect areas with healthy margins were excised for microscopic examination at 3, 6 and 12 weeks postoperative. Samples handling and processing was performed according to Bancroft and Gamble [20]. Slides were stained with hematoxylin and eosin (H&E), Masson's trichrome (MT) and Safranin O (SO) stains. Histological scoring for the defect was assessed according to ICRS scale [21] (Table 2).

2.6. Statistical analysis

Statistical analyses were carried out using a commercial program (JMP, version 5.1). Homogeneity of groups was evaluated by kruskal Walis test. To study the effect of various interventions and time, a repeated measure ANOVA was performed. The results were presented considering the effect of time, time× treatment interaction. When there is a significant effect, a one-way ANOVA was performed at each time point. Results were considered significant when $P \leq 0.05$.

3. RESULTS

3.1. Gross findings

The degree of defect repair showed an increase of osteochondral filling rate in treated groups compared with control at 3 weeks (50% vs 25), 6 weeks (75 vs 50) and 12 weeks (100 vs 75%). Similarly, the overall repair was higher for treatment groups in comparison with control one at 3 weeks (grade II vs III), 6 weeks (grade I vs III) and 12 weeks (grade I vs grade II) (Table 3). At 3 weeks postoperative, the defect area has several fissures with reddish coloration and moderate congestion in group A (Figure 1 A), but for groups B and C, the defect has small scattered fissures and cracks with whitish opaque appearance (Figure 1 B and C) while, it showed small scattered fissures and cracks with transparent appearance and whitish ring in group D (Figure 1 D).

At 6 weeks postoperative, the osteochondral defects have small scattered fissures, whitish opaque appearance and mild to slight congestion in control group (Figure 1 E). The color of the defect area was transparent appearance with whitish ring in group B (Figure 2 F), transparent appearance with reddish color in group C (Figure 1 G) and transparent appearance in group D (Figure 1 H). At 12 weeks, the osteochondral defects showed fibrillated surfaces and transparent appearance with whitish ring in control group (Figure 1 I). While in groups B, C and D it was at the level of surrounding cartilage with intact smooth surface and transparent appearance (Figure 1 J, K and L).

3.2. Microscopical findings

At 3 and 6 weeks postoperative, tissue morphology, collagen type II staining and safranin O staining showed significant increase in group D than other treated groups. At 12 weeks, although the score was higher in group D, the differences among groups B, C and D was not-significant. Likewise, the overall repair was higher for treatment groups in comparison with control one at 3 weeks (grade II vs III), 6 weeks (grade I vs III) and 12 weeks (grade I vs grade II) postoperative (Table 4). At 3 weeks, the regenerated tissue contains fibrous tissue with irregular and erosive surface in control group (Figure 2 A), fibrocartilage with moderate irregular tissue architecture in group C (Figure 2 C) and mixed hyaline and fibrocartilage with smooth and incontinuous appearance in groups B and D (Figure 2 B and D). The cells in the regenerated tissue were irregular disorganized in control animals (Figure 3 A) and mixed columnar and clusters cell arrangement in groups B, C and D (Figure 3 B, C and D). The amount of fibrous tissue in the regenerated tissue was large in control group (Figure 4 A), moderate in groups B and C (Figure 4 B and C) and little in group D (Figure 4 D). Safranin O staining of the regenerated tissue, revealed absence of proteoglycan in control group (Figure 5 A), slight amount in group C (Figure 5 C) and moderate amount in groups B and D (Figure 5 B, D).

At 6 weeks postoperative**,** fibrous tissue with exuberant proliferation of fibrocartilagenous tissue filling the osteochondral defect with moderately irregular surface in group A (Figure 2 E), hyaline cartilage with moderately irregular surface and cartilage fibrillation in group C (Figure 2 G) and hyaline cartilage with smooth and incontinuous tissue architecture in groups B and D (Figure 2 E and H). The cell arrangement was cluster shaped in control group (Figure 3 E), mixed cluster and columnar in group C (Figure 3 G) and columnar in group B and D (Figure 3 F and H). With Masson trichrome, the healed tissue has moderate amount of bluish-stained fibrous tissue in control group (Figure 4 E) and normal chondroid matrix in groups B, C and D (Figure 4 F, G and H). Safranin O matrix staining showed moderate amount of red stained proteoglycan in control animals (Figure 5 E) and normal red-stained chondroid matrix in groups B, C and D (Figure 5 F, G and H).

At 12 weeks postoperative, the healed tissue in animals of control group contains mixed hyaline and fibrocartilage with smooth and incontinuous surface (Figure 2 I) while the osteochondral defects in animals of groups B, C and D had hyaline cartilage with smooth and continuous surface (Figure 2 J. K and L). The cell arrangement was mixed cluster and columnar cells in groups A and C (Figure 3 I and K) while columnar in groups B and D (Figure 3 J and L). Safranin O matrix staining showed moderate amount of red stained proteoglycan in control group (Figure 5 I) and normal chondroid matrix with high amount of proteoglycan in groups B, C and D (Figure 5 J, K and L).

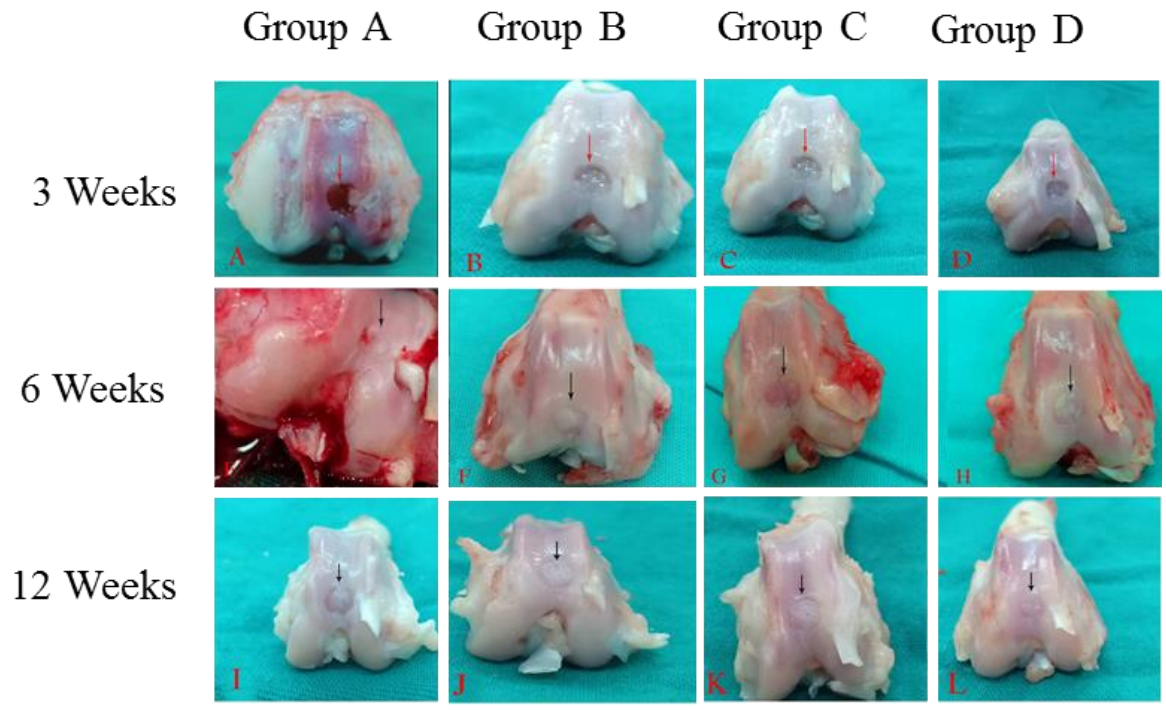

Figure 1. Gross appearance of the osteochondral defects in trochlear groove of left femur in New Zealand White rabbits at 3, 6 and 12 weeks postoperative. Gross appearance showed better healing in group D followed by groups B, C, and A respectively in terms of degree of defect repair; Integration to border zone; Appearance of defect area; coloration of defect area, Defect area congestion; Group A: Control Group, Group B: PRF, Group C: MNCs, Group D: PRF and MNCs.

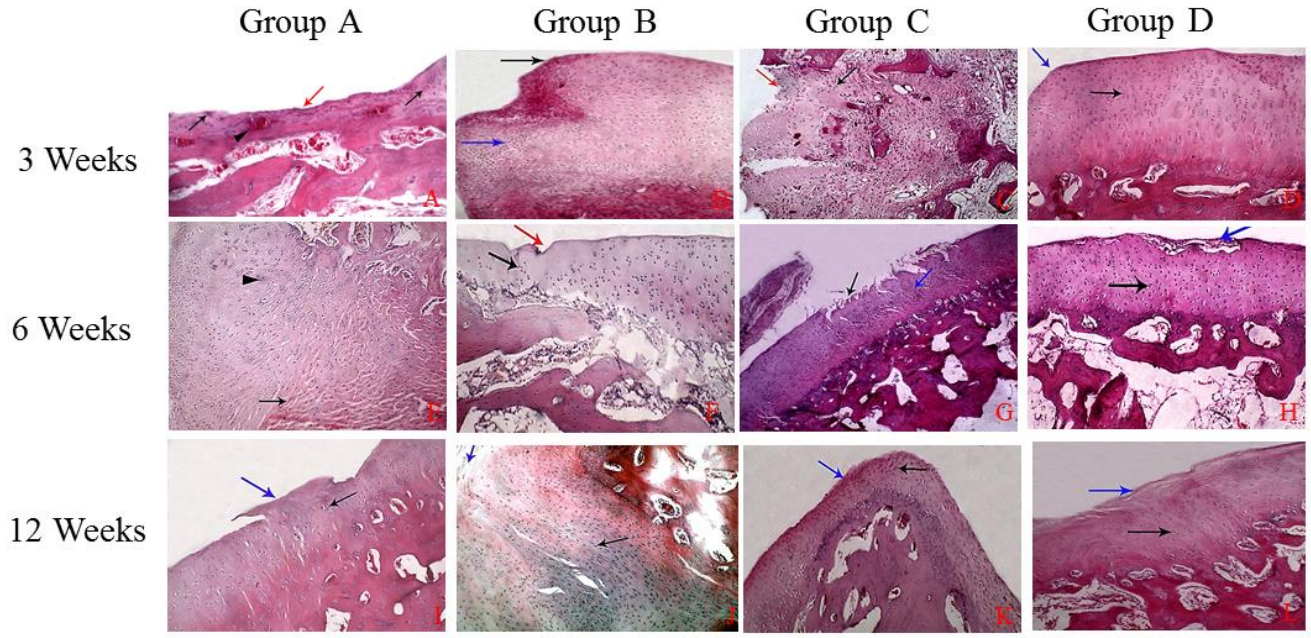

Figure 2. Histopathological view of osteochondral defect at trochlear groove of left stifle joint of white New Zealand rabbit at 3, 6 and 12 weeks postoperative showed better healing in group D followed by groups B, C, and A respectively (HE, 100x).Group A: Control Group, Group B: PRF, Group C: MNCs, Group D: PRF and MNCs

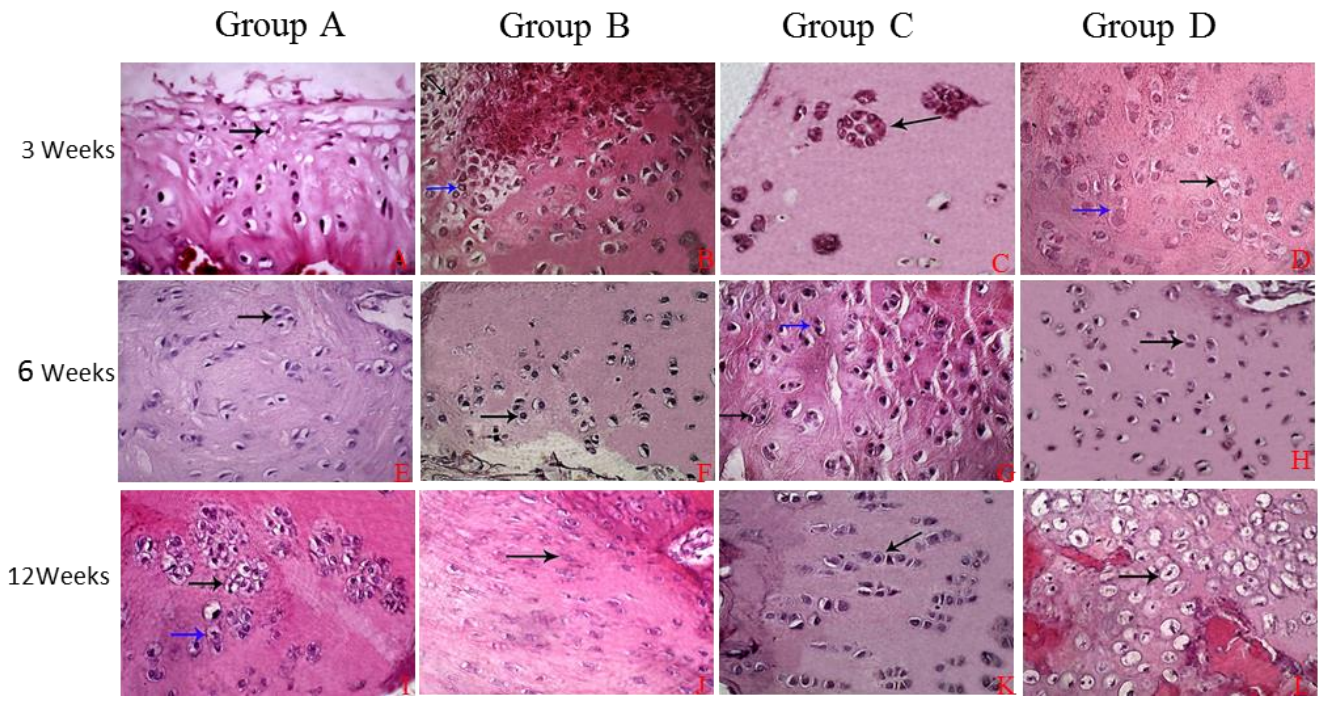

Figure 3. Histopathological view of osteochondral defect at trochlear groove of left stifle joint of white New Zealand rabbit at 3, 6 and 12 weeks postoperative showed chondrocytes shape and arrangement during different time of evaluation (HE, 400 x).

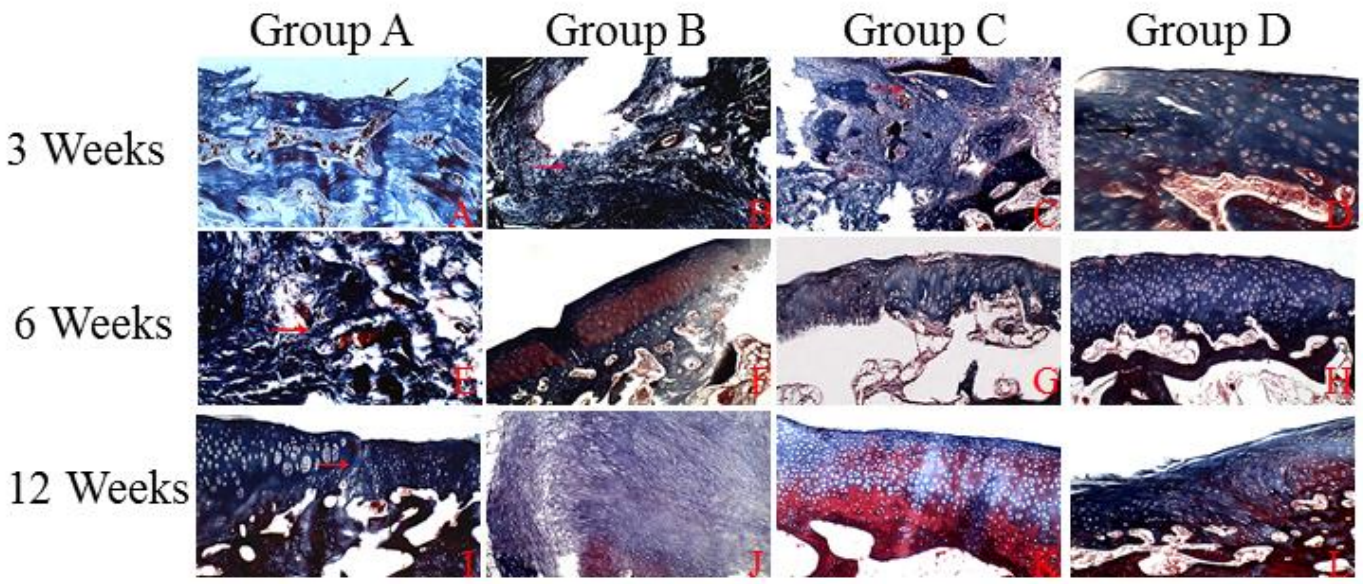

Figure 4. Histopathological view of osteochondral defect at trochlear groove of left stifle joint of white New Zealand rabbits at 3, 6 and 12 weeks postoperative showed amount and distribution of fibrous tissue in different groups at different time of evaluation (Masson trichrome, 100x).Group A: Control Group, Group B: PRF, Group C: BMNCs, Group D: PRF and BMNCs.

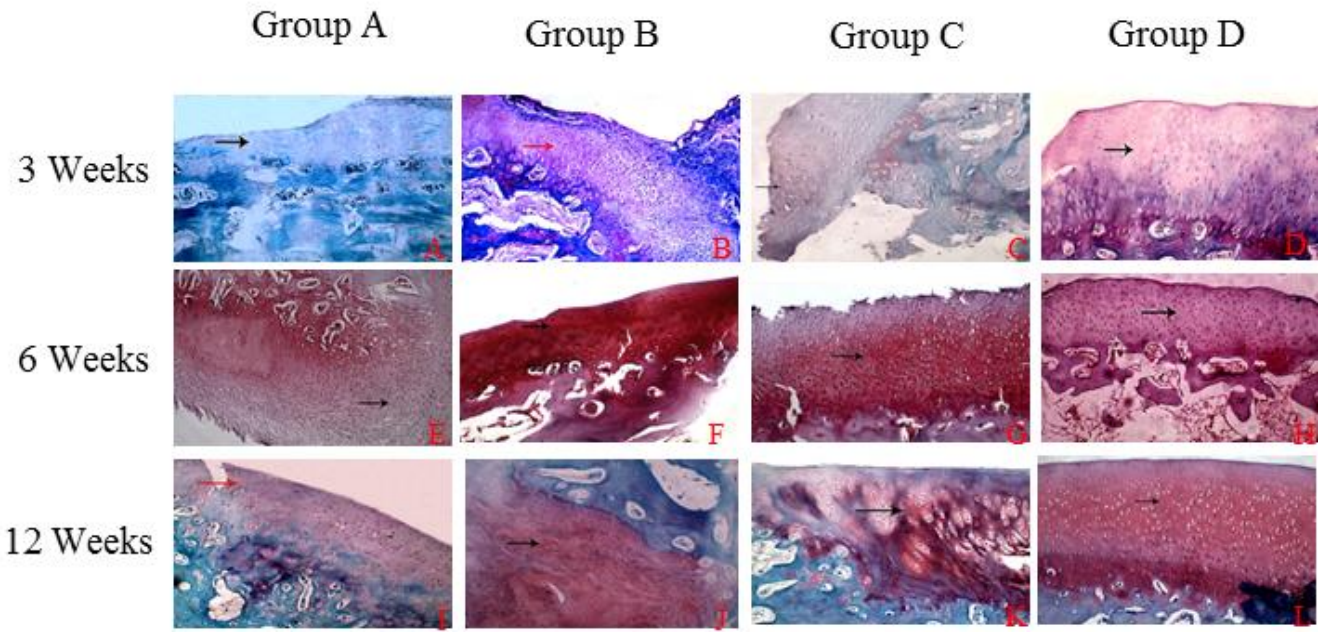

Figure 5. Histopathological view of osteochondral defect at trochlear groove of left stifle joint of white New Zealand rabbits at 3, 6 and 12 weeks postoperative showed normal matrix staining by safranin O (red color) and chondrocytes arrangement in group D followed by groups B, C, and A respectively (safranin O stain, 100x).Group A: Control Group, Group B: PRF, Group C: BMNCs, Group D: PRF and BMNCs.

Table 1. Modified ICRS gross assessment scale for macroscopic evaluation of osteochondoral defect repair in rabbits.

Parameters	Score				
	0	1	2	3	4
Degree of defect repair	0% repair of defect depth	25% repair of defect depth	50% repair of defect depth	75% repair of defect depth	In level with surrounding cartilage
Integration to border zone	¼ of graft integrated with surrounding Cartilage or no contact	½ of graft integrated with surrounding cartilage, ½ with a notable border > 1 mm	¾ of graft integrated, ¼ with a notable border > 1 mm width	Demarcating border < 1 mm 3	Complete integration with surrounding cartilage
Appearance of the defect area	Total degeneration of grafted area	Several, small or few but large fissures	Small, scattered fissures or cracks	Fibrillated surface	Intact smooth surface
Coloration of the defect area	Reddish appearance and whitish ring	Yellowish opaque appearance and whitish ring	Whitish opaque appearance	Transparent appearance with whitish ring	Transparent appearance
Congestion of the defect area	Severe congestion	Moderate congestion	Mild congestion	Slight congestion	No congestion
Overall repair assessment	Grade V: very severely abnormal	Grade IV: severely abnormal	Grade III: abnormal	Grade II: nearly normal	Grade I: normal

Table 2. Modified ICRS histological assessment scale of osteochondoral defect repair in rabbits.

Parameters	Score			
	0	1	2	3
Surface architecture	Severely irregularity	Moderate irregularity	Smooth and in continuous	Smooth and continuous
Tissue morphology	Fibrous tissue	Fibrocartilage	hyaline and fibrocartilage	Hyaline
Cell distribution	Individual cells and disorganized	Clusters	columnar and clusters	Columnar
Cell population viability	<10% viable	50% viable	75% viable	Predominantly viable
Subchondral bone	Detached/fracture/callus at base	Bone necrosis and granulation tissue	Increased remodeling	Normal
Abnormal calcification	Severe	Mild to moderate	Absent	Hyaline cartilage
Type-II collagen staining of the matrix	None	Slight	Moderate	Normal or nearly normal
Safranin-O staining of the matrix	None	Slight	Moderate	Normal or nearly normal
Overall score	Grade IV: severely abnormal	Grade III: abnormal	Grade II: nearly normal	Grade I: Normal

Table 3. Mean ± SD of macroscopic evaluation during osteochondoral defect repair in stifle joint of rabbits. **Group A:** Control Group, **Group B**: PRF, **Group C:** BMNCs, **Group D:** PRF and BMNCs.

		ICRS macroscopic parameters						
Groups	**Weeks**	**DDR**	**IBZ**	**ADR**	**CDA**	**DAC**	**Overall**	**Grade**
A	3	1.5±0.6[a]	1.5±0.6 [a]	0.8±0.5 [a]	0.8±0.5 [a]	1.5±0.6[a]	0.8±0.5[a]	III
B		2.0±0.0[b]	2.5±0.6 [b]	2.0±0.0[b]	2.3±0.0 [b]	3.3±0.8[b]	2.0±0.0[b]	II
C		1.5±0.5 [a]	2.6±0.4 [a]	2.7±0.5 [b]	2.8±0.4 [b]	3.2±0.6[b]	2.0±0.0[b]	II
D		2.0±0.0 [b]	3.0±0.0 [b]	3.0±0.0 [b]	3.0±0.0 [b]	3.5±0.4 [b]	2.0±0.0[b]	II
A	6	2.2±0.5 [a]	2.0±0.0 [a]	1.8±0.9[a]	2.0±0.0[a]	2.2±0.5[a]	1.0±0.0[a]	III
B		4.0±0.0 [b]	3.0±0.0 [b]	4.0±0.0 [b]	3.3±0.5 [b]	3.5±0.6 [b]	2.6±0.5 [b]	I
C		3.5±0.5 [b]	3.3±0.5 [b]	3.4±0.5 [b]	3.14±0.4 [b]	3.4±0.5 [b]	2.5±0.5 [b]	I
D		4.0±0.0 [b]	4.0±0.0 [b]	4.0±0.0 [b]	4.0±0.0 [b]	4.0±0.0 [b]	3.0±0.0 [b]	I
A	12	3.5±0.3 [a]	3.0±0.0 [a]	3.1±0.6 [a]	2.8±0.5 [a]	3.5±0.5 [a]	2.0±0.00 [a]	II
B		4.0±0.0 [b]	4.0±0.0 [b]	4.0±0.0 [b,a]	4.0±0.5 [b]	4.0±0.0 [b]	3.0±0.00 [b]	I
C		3.7±0.5 [b]	3.7±0.5 [b]	3.8±0.5 [b]	3.8±0.5 [b]	4.0±0.0 [b]	3.0±0.00 [b]	I
D		4.0±0.0 [b]	4.0±0.0 [b,a]	4.0±0.0 [b,a]	4.0±0.0 [b,a]	4.0±0.0 [b]	3.0±0.00 [b]	I

Means with different superscript litters for each parameters at each time point are significantly different at P<0.05

DDR= Degree of defect repair; IBZ= Integration to border zone; ADR= Appearance of defect area; CDA= coloration of defect area; DAC= Defect area congestion; overall= Overall macroscopic score; Grade= Grade of macroscopic score

Table 4. Mean ± SD of histopathological evaluation during osteochondoral defect repair in stifle joint of rabbits**. Group A:** Control Group, **Group B**: PRF, **Group C:** BMNCs, **Group D:** PRF and BMNCs.

		ICRS Histological parameters									
Group	weeks	SA	TM	CD	CV	SBA	AC	CMS	SMS	Overall	Grade
A		0.8±0.5^{a}	1.0±0.0^{a}	0.8±0.5^{a}	1.0±0.0^{a}	0.8±0.5^{a}	0.8±0.5^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.8±0.5^{a}	III
B	3	2.3±0.5^{b}	2.5±0.0^{b,c}	2.3±0.5^{b}	1.8±0.5^{b}	1.3±0.5^{a}	1.8±0.5$^{b.a}$	1.8±0.0^{b,c}	1.8±0.5^{b,c}	2.0±0.0^{b}	II
C		1.8±0.5 ^{b}	1.3±0.5 ^{b}	1.8±0.5 ^{b,a}	1.8±0.5^{b}	1.3±0.5^{a}	1.8±0.5$^{b.a}$	1.3±0.0^{b}	1.5±0.5^{b}	1.8±0.5^{b}	II
D		2.3±0.5 ^{b}	2.3±0.5 ^{c}	2.3±0.5 ^{b}	2.3±0.5 ^{b}	1.5±0.4 ^{a}	2.3±0.5^{b}	2.3±0.0^{c}	2.3±0.0^{c}	2.3±0.5^{b}	II
A		1.0±0.0^{a}	1.3±0.5^{a}	1.0±0.0^{a}	0.8±0.5^{a}	1.0±0.0^{a}	1.5±0.5^{a}	0.8±0.5^{a}	0.8±0.5^{a}	1.0±0.0^{a}	III
B	6	2.8±0.5 ^{a,b}	2.8±0.5 ^{b}	2.8±0.5 ^{b}	2.8±0.5 ^{c}	2.5±0.0 ^{b}	2.5±0.5 ^{b}	2.8±0.5 ^{b}	2.8±0.5 ^{b}	2.8±0.5 ^{b,c}	I
C		2.3±0.5 ^{a,b}	2.5±0.6 ^{b}	2.3±0.5 ^{b}	1.75±0.5 ^{b}	2.3±0.5$^{a, b}$	2.3±0.5 ^{b}	2.5±0.56 ^{b}	2.6±0.5 ^{b}	2.5 ±0.5 ^{b}	I
D		2.8±0.5 ^{b}	3.0±0.0 ^{b}	2.8±0.5 ^{b}	3.0±0.0 ^{c}	2.8±0.5 ^{b}	3.0±0.0 ^{c}	3.0±0.0 ^{b}	3.0±0.0 ^{b}	3.0±0.0 ^{c}	I
A		1.8±0.5 ^{a}	2.3±0.5 ^{a}	1.8±0.5 ^{a}	2.0±0.0 ^{a}	1.8±0.5 ^{a}	1.5±0.6^{a}	2.0±0.0 ^{a}	1.8±0.5 ^{a}	2.0±0.0 ^{a}	II
B	12	3.0±0.0 ^{b}	3.0±0.0 ^{b}	3.0±0.0 ^{b}	3.0±0.0 ^{b}	3.0±0.0 ^{b}	3.0±0.0 ^{b}	3.0±0.0 ^{b}	2.8±0.5 ^{b}	3.0±0.0 ^{b}	I
C		2.8±0.5 ^{b}	3.0 ±0.5 ^{b}	2.8±0.5 ^{b}	2.8±0.5 ^{b}	3.0±0.0 ^{b}	3.0±0.0 ^{b}	2.5±0.6 ^{a,b}	2.8±0.5 ^{b}	2.8±0.5 ^{b}	I
D		3.0±0.0 ^{b}	3.0±0.0 ^{b}	3.0±0.0 ^{b}	3.0±0.0 ^{b}	3.0±0.0 ^{b}	3.0±0.0 ^{b}	3.0±0.0 ^{b}	3.0±0.0 ^{b}	3.0±0.0 ^{b}	I

Means with different superscript litters for each parameter at each time point are significantly different at P≤0.05

SA=Surface architecture, TM= Tissue morphology, CD= Cell distribution, CV= Cell population viability, SBA= Subchondral bone abnormalities, AC= Abnormal calcification, CMS= Collagen type II matrix staining, SMS= Safranin O matrix staining, Overall= Overall histopathological score.

4. DISCUSSION

Articular cartilage has inadequate ability for regeneration due to absence of blood and nerve supply. As soon as it is injured, it usually repaired with fibrous tissue especially in elderly which devoid the characters of the normal cartilage [22]**.** The treatment methods of damaged cartilage are limited and the traditional treatments are not ideal [23]**.** Osteochondral defects in the trochlear groove of rabbits are usually used for assessment of cartilage regeneration methods [24]. Great stress has been stated to encourage fibrous tissue development, suppress chondrogenesis and hyaline cartilage formation [25] So, in the present study, an osteochondral defect was performed in the trochlear groove of the femur after flexion of the joint by 90^{o} to decrees stress on the defect site. The same procedure was mentioned by [16].

Grossly, The healing of the defect in group C is better than control group which may be attributed to that transplanted BMNCs have T, B cells, macrophages, MSCs and TGF-β, which stimulate the chondrogenesis and differentiation of BMNCs into chondrocyte. Similar finding was obtained by [16]. There are superior healing of the defect in group B compared with control and BMNCs groups which could be attributed to cytokines and growth factors in PRF, which have the ability to stimulate cell differentiation, cell proliferation, cell motility, and matrix production [26]. Moreover**,** the better gross healing in group D compared to other treated groups is attributed to that PRF via its 3 D fibrin network, which acts as excellent scaffold for BMNCs which stimulates differentiation of BMNCs into chondrocyte, regulates MSCS proliferation and reduces cell mortality and apoptosis**.** Similar finding obtained by [9] who used PRF in combination with MSCs in repair of osteochondoral defect in canine.

Histologically, BMNCs treated group showed better healing compared with control group which is attributed to exogenous source of BMNCs and possibly MSCs found in the transplanted cells as well as the interaction between both types of cells with normal and damaged chondrocytes at the defect and synovial fluid. The injured chondrocytes may release different cytokines that could encourage better proliferation and differentiation of MNCs into chondrocyte [16]. In PRF treated group, the regenerated tissue was hyaline cartilage that characterized by smooth, continuous surface and columnar shaped cell arrangement with normal chondroid matrix and high amount of proteoglycan. This is in coincidence with [27]. The histological quality of regenerated tissue was superior in group D than other treated groups. because PRF is act as scaffold for BMNCs that encourage its proliferation and differentiation [9].

5. Conclusion

The results of the present study indicate that, combination of PRF with BMNCs encourage early healing of osteochondral defect with better collagen type II and proteoglycan deposition in the regenerated tissue rather than when each one was used alone. Further studies on clinically affected cases need to be done to get evidence on the beneficial effects of PRF and BMNCs combination.

Conflict of interest

The authors declare that there is no any conflict of interest in the current research work

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not for profit sectors

Authors´ contributions

M. G., A. R. and E. M. conceived of the idea, contributed to its design and coordination, helped in acquisition of the data, performed the statistical analysis of the data and drafted the manuscript, M.H. performed and interpreted the histopathological section, G. K. and A. Z. contributed to the analysis and interpretation of the data, and revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Acknowledgment

Many grateful thanks for Prof. Dr. Sabry El-Khodery Professor of Internal Medicine; Faculty of Veterinary Medicine, Mansoura University for his help in the statistical analysis.

5. REFERENCES

[1] Fossum TW. Small Animal Surgery Textbook-E-Book: (4th ed) Elsevier Health Sciences; 2013, St. Louis: 1143-1315.

[2] Musumeci G. The effect of mechanical loading on articular cartilage. *J Funct Morphol Kinesiol* 2016; *1*: 154-161. https://doi.org/10.3390/jfmk1020154

[3] Erggelet C, Endres M, Neumann K, Morawietz L, Ringe J, Haberstroh K, et al. Formation of cartilage repair tissue in articular cartilage defects pretreated with microfracture and covered with cell-free polymer-based implants. J Orthop Res 2009;27:1353-60. https://doi.org/10.1002/jor.20879

[4] Burks RT, Greis PE, Arnoczky SP, Scher C. The use of a single osteochondral autograft plug in the treatment of a large osteochondral lesion in the femoral condyle: an experimental study in sheep. Am J Sports Med 2006;34:247-55. https://doi.org/10.1177/0363546505279914

[5] Custers R, Saris D, Dhert W, Verbout A, Van Rijen M, Mastbergen S, et al. Articular cartilage degeneration following the treatment of focal cartilage defects with ceramic metal implants and compared with microfracture. JBJS 2009;91:900-10. https://doi.org/10.2106/JBJS.H.00668

[6] Souza TF, Andrade AL, Ferrreira G, Sakamoto SS, Albuquerque VBd, Bonfim S, et al. Healing and expression of growth factors (TGF-β and PDGF) in canine radial ostectomy gap containing platelet-rich plasma. Vet Comp Orthop Traumatol 2012;25:445-52. https://doi.org/10.3415/VCOT-10-10-0146

[7] Hopper N, Wardale J, Brooks R, Power J, Rushton N, Henson F. Peripheral blood mononuclear cells enhance cartilage repair in in vivo osteochondral defect model. PLoS One 2015;10:e0133937. https://doi.org/10.1371/journal.pone.0133937

[8] Gille J, Behrens P, Schulz AP, Oheim R, Kienast B. Matrix-associated autologous chondrocyte implantation: a clinical follow-up at 15 years. Cartilage. 2016;7:309-15. https://doi.org/10.1177/1947603516638901

[9] Kazemi D, Shams Asenjan K, Dehdilani N, Parsa H. Canine articular cartilage regeneration using mesenchymal stem cells seeded on platelet rich fibrin: macroscopic and histological assessments. Bone Joint Res. 2017;6:98-107. https://doi.org/10.1302/2046-3758.62.BJR-2016-0188.R1

[10] Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg Oral Med Oral

Pathol Oral Radiol Endod 2006;101:e37-e44. https://doi.org/10.1016/j.tripleo.2005.07.008
[11] Bai M-Y, Wang C-W, Wang J-Y, Lin M-F, Chan WP. Three-dimensional structure and cytokine distribution of platelet-rich fibrin. Clinics. 2017;72:116-24. https://doi.org/10.6061/clinics/2017(02)09
[12] Samuel S, Ahmad RE, Ramasamy TS, Manan F, Kamarul T. Platelet rich concentrate enhances mesenchymal stem cells capacity to repair focal cartilage injury in rabbits. Injury 2018;49:775-83. https://doi.org/10.1016/j.injury.2018.02.020
[13] Barbon S, Stocco E, Macchi V, Contran M, Grandi F, Borean A, et al. Platelet-Rich Fibrin Scaffolds for Cartilage and Tendon Regenerative Medicine: From Bench to Bedside. Int J Mol Sci 2019;20:1701. https://doi.org/10.3390/ijms20071701
[14] Schächinger V, Erbs S, Elsässer A, Haberbosch W, Hambrecht R, Hölschermann H, et al. Intracoronary bone marrow–derived progenitor cells in acute myocardial infarction. N Engl J Med 2006;355:1210-21. https://doi.org/10.1056/NEJMoa060186
[15] Lovell MJ, Yasin M, Lee KL, Cheung KK, Shintani Y, Collino M, et al. Bone marrow mononuclear cells reduce myocardial reperfusion injury by activating the PI3K/Akt survival pathway. Atherosclerosis 2010;213:67-76. https://doi.org/10.1016/j.atherosclerosis.2010.07.045
[16] Tiwary R, Amarpal, Aithal HP, Kinjavdekar P, Pawde AM, Singh R. Effect of IGF-1 and uncultured autologous bone-marrow-derived mononuclear cells on repair of osteochondral defect in rabbits. Cartilage. 2014;5:43-54. https://doi.org/10.1177/1947603513499366
[17] Bhansali S, Dutta P, Yadav MK, Jain A, Mudaliar S, Hawkins M, et al. Autologous bone marrow-derived mononuclear cells transplantation in type 2 diabetes mellitus: effect on β-cell function and insulin sensitivity. Diabetol Metab Syndr 2017;9:50. https://doi.org/10.1186/s13098-017-0248-7
[18] Amarpal X, Kinjavdekar P, Aithal H, Pawde A, Singh J, Udehiya R. Evaluation of xylazine, acepromazine and medetomidine with ketamine for general anaesthesia in rabbits. Scand. J. Lab. Anim. Sci. 2010;37:223-9.
[19] Miki S, Takao M, Miyamoto W, Matsushita T, Kawano H. Intra-articular injection of synovium-derived mesenchymal stem cells with hyaluronic acid can repair articular cartilage defects in a canine model. J Stem Cell Res Ther. 2015;5:2. https://doi.org/10.4172/2157-7633.1000314
[20] Bancroft JD, Gamble M. Theory and practice of histological techniques: Elsevier health sciences; 2008.
[21] Mainil-Varlet P, Van Damme B, Nesic D, Knutsen G, Kandel R, Roberts S. A new histology scoring system for the assessment of the quality of human cartilage repair: ICRS II. Am J Sports Med. 2010;38:880-90. https://doi.org/10.1177/0363546509359068
[22] Messent E, Ward R, Tonkin C, Buckland-Wright C. Osteophytes, juxta-articular radiolucencies and cancellous bone changes in the proximal tibia of patients with knee osteoarthritis. Osteoarthritis Cartilage. 2007;15:179-86. https://doi.org/10.1016/j.joca.2006.06.020
[23] Coates EE, Fisher JP. Engineering superficial zone chondrocytes from mesenchymal stem cells. Tissue Eng Part C Methods 2014;20:630-40. https://doi.org/10.1089/ten.tec.2013.0224
[24] Chu CR, Szczodry M, Bruno S. Animal models for cartilage regeneration and repair. Tissue Eng Part B Rev 2010;16:105-15. https://doi.org/10.1089/ten.teb.2009.0452
[25] Kelly DJ, Prendergast P. Mechano-regulation of stem cell differentiation and tissue regeneration in osteochondral defects. J Biomech. 2005;38:1413-22. https://doi.org/10.1016/j.jbiomech.2004.06.026
[26] Kazemi D, Fakhrjou A, Mirzazadeh Dizaji V, Khanzadeh Alishahi M. Effect of autologous platelet rich fibrin on the healing of experimental articular cartilage defects of the knee in an animal model. Biomed Res Int. 2014;2014. https://doi.org/10.1155/2014/486436
[27] Abd El Raouf M, Wang X, Miusi S, Chai J, Mohamed AbdEl-Aal AB, Nefissa Helmy MM, et al. Injectable-platelet rich fibrin using the low speed centrifugation concept improves cartilage regeneration when compared to platelet-rich plasma. Platelets. 2019;30:213-21. https://doi.org/10.1080/09537104.2017.1401058