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RESEARCH ARTICLE

THE EFFICIENCY OF CO-APPLICATION OF PLATELET-RICH PLASMA AND BONE MARROW-DERIVED STEM CELLS IN REPAIRING SURGICALLY INDUCED ARTICULAR CARTILAGE DEFECT

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ABSTRACT

Background: Bone marrow-derived stem cells (BMSCs) have been applied in the treatment of many diseases including injured articular cartilage. Platelet-rich plasma (PRP) has been used to treat everything from plantar fasciitis to rotator cuff repair. But its effects differ, depending on the musculoskeletal structure and the composition of the PRP itself. The objective of this research was to compare the efficacy of single intra-articular injections of Preconditioned BMSCs with PRP and BMSCs without PRP in the treatment of surgically induced articular cartilage defect in animal model.

Methods: 18 adult dogs of both sexes, in good general health condition, weighing between 25-35 kg and aged 3-4 years were used in this study. BMSCs were isolated, cultured and expanded from canine bone marrow aspirate. PRP was collected and activated from canine peripheral blood. BMSCs preconditioned with or without PRP were transplanted into canine with surgical induced articular cartilage defect. Real time PCR was used to quantify transforming growth factor--β (TGF-β), collagen II and aggrecan genes expression in transplanted cartilage defects. Histopathology was performed to assess cartilage regeneration.

Results: PRP promoted BMSC differentiation into chondrogenic cells that highly significant expressed (P-value ≤ 0.001) TGF-β, collagen II and aggrecan compared to BMSCs transplantation alone. As a result, PRP-preconditioned BMSCs improved healing of surgical induced articular cartilage defect in canine models compared with that of unconditioned BMSCs.

Conclusion: Preconditioning of BMSCs with PRP transplantation is an efficient and important preclinical step toward the use of autologous BMSCs with PRP in the treatment of articular cartilage defects.

Glossary of abbreviations:

BMSCs: Bone marrow-derived stem cells
DMEM: Dulbecco's Modified Eagle's Medium
ECM: extra cellular matrix
FBS: fetal bovine serum
IGF-1:insulin growth factor-1
IL1: interleukin 1
OA: Osteoarthritis
PBS: phosphate-buffered saline
PRP: Platelet-rich plasma
RT–PCR: Real time PCR
TGF-β: transforming growth factor
TMJ: Temporomandibular joint
TMJ –OA: temporomandibular joint osteoarthritis
TNFα: tumor necrosis factor α

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INTRODUCTION

Osteoarthritis (OA) results from articular cartilage loss, induced by a complex interaction of genetic, metabolic, biochemical, and biomechanical factors with the secondary components of inflammation (Wearing *et al.,* 2006). Stress bearing joints of the body such as knee, hips, spine, and fingers are most commonly affected by OA (Jacofsky *et al.,* 2005). Osteoarthritis can also affect other joints in the body such as shoulder, ankle and Temporomandibular joint (TMJ). TMJ osteoarthritis affects the cartilage and other hard and soft tissues causing changes such as TMJ articular cartilage abrasion and deterioration (Jiao *et al.,* 2011 and Dijkgraaf *et al.,* 1997). Recently, it is believed that the disease progression results from an imbalance between pro inflammatory cytokines (including interleukin [IL]-1a, IL-1β, and tumor necrosis factor-α) and anti-inflammatory cytokines (including IL-4, IL-10, and IL-1ra) (Dennison and Cooper, 2003). This cytokine imbalance is thought to activate proteolytic enzymes, leading to the destruction of cartilage (Cook *et al.,* 2000). One of these cytokines are interleukin 1 (IL1) and tumor necrosis factor α (TNFα), which stimulate the production of proteinases and down-regulate aggrecan production (Peter, 2014).

Aggrecan is a large proteoglycan bearing numerous chondroitin sulfate and keratan sulfate chains that endow articular cartilage with its ability to withstand compressive loads (Kiani *et al.,* 2002). These inflammatory cytokines are associated with the production of aggrecanases and MMPs, which degrade the aggrecan core protein. The combined effect of increased degradation coupled with decreased synthesis results in aggrecan loss from the extra cellular matrix (ECM) lead to impairment of articular cartilage function (Kiani *et al.,* 2002). The main structural component of the cartilage tissue is collagen type II. Collagenases (MMP1 and MMP13) degrade the collagen fibrils of the tissue, initiating tissue fibrillation and eventual erosion. The imbalance between synthesis and degradation of collagen II leads to cartilage destruction (Troeberg and Nagase, 2012). The presence of degradation products of collagen II in urine correlate with the progression of articular damage in osteoarthritis (Meulenbelt *et al.,* 2006). TGF-βstimulates chondrocyte synthetic activity and decreases the catabolic activity of IL-1 (Blaney Davidson *et al.,* 2007).

TGF-β enhanced repair of cartilage defects in in rabbits(Diao *et al.,* 2009). Local delivery of mesenchymal stem cells to injured joints can stimulate regeneration of meniscal tissue and retards the progressive destruction normally seen in OA (Murphy *et al.,* 2003). Some clinical trials have proposed the approaches for OA treatment, which involve the intra-articular injection to deliver BMSCs directly into the synovial fluid compartment (Barry and Murphy, 2013). Although most clinical trials participated in the intervention of OA in knee joints, studies on the cartilage regeneration of BMSCs in TMJ OA have been largely investigated. (Chen *et al.,* 2013). Autologous platelet-rich plasma (PRP) has emerged as a treatment option for tendinopathies and chronic wounds. In addition to release of growth factors, PRP also promotes concentrated anti-inflammatory signals including interleukin-1, which has been a focus of emerging treatments for OA. (Sampson *et al.,* 2010). The α-secretory granules of platelets in PRP contain transforming growth factor (TGF)-β and insulin growth factor-1 (IGF-1), which stimulate cartilage regeneration (Schmidt *et al.,* 2006).

Platelet contains an abundance of growth factors and cytokines that are crucial in the healing process of soft tissues and bone mineralization. (Anitua *et al.,* 2006). It is expected that the biological effect of multiple growth factors on tissue regeneration is greater than that of a single growth factor. Platelet also discharges many bioactive proteins responsible for attracting macrophages, MSCs, and osteoblasts, which not only promote scavenging of necrotic tissue but also facilitate tissue regeneration and healing (Mackay *et al.,* 1998). PRP is composed of 3 to 8-fold greater concentration of platelets, as compared to whole blood, and contains a hyper-physiological content of autologous growth factors. Chondrogenesis was demonstrated in the model of dog knee cartilage defects when PRP was used with a scaffold (Sun *et al.,* 2010). The objective of the research was to compare the efficacy of single intra-articular injections of Preconditioned BMSCs with PRP and BMSCs without PRP in the treatment of partial thickness chondoral defects in animal model.

MATERIALS AND METHODS

Animals

Our experimental was case/control research study. All procedures were approved by the Ethics Committee of the Faculty of Veterinary Medicine, Cairo University, Egypt. 18 adult dogs of both sexes, in good general health condition, weighing between 25-35 kg and aged 3-4 years were used in this study. Animals were kept at the kennels of the Surgery Department, Faculty of Veterinary Medicine throughout the experiment. Materials and methods have been divided into laboratory, surgical and postsurgical follow up, macroscopic, histological and Molecular Assessment. Bone marrow aspiration was conducted at 20 days before the surgical induction of the femoral chondral defect. The PRP was prepared on the same day of injection.

Laboratory work

Harvesting and Characterization of MSCs

Bone marrow aspiration was conducted 14 days before the surgical induction of the chondral defect: Bone marrows (10 mL) were aspirated from the iliac crest of dog under aseptic standard operative procedures and placed in heparinized tubes. Nucleated cells were isolated with a density gradient (Ficoll-Paque; GE HealthCare, Waukesha, WI) and resuspended in culture medium DMEM (Delbecco's Modified Eagle's Medium) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (10,000 µg/mL). Cells were incubated at 37 °C in 5% CO_2. Culture medium was changed every 3 days. When cells reached 80% to 90% confluence, cultures were washed twice with phosphate-buffered saline (PBS), and the cells were trypsinized with 0.25% Trypsin in 1 mM EDTA for 5 min at 37 °C. After centrifugation, cells were resuspended in culture medium and subcultured reaching an average count of 5×10^6 (Yamazoe *et al.,* 2007). Cells were identified asmesenchymal stem cell morphological assessment using inverted microscope Undifferentiated MSCs reached 70-80% confluence.

Labeling of stem cells with PKH26 dye

MSCs were harvested during the 2nd passage and were labeled with PKH26 fluorescent linker dye. PKH26 was purchased from Sigma Company (Saint Louis, Missouri, USA).

Cells were centrifuged and washed twice in serum free medium. Cells were pelleted and suspended in dye solution.Cells were examined with a fluorescence microscope (Leica, Germany) to detect and trace the cells stained with PKH26. Cells were injected locally intraarticular in surgical induced cartilage defect. After 12 weeks, cartilage tissue was examined with a fluorescence microscope (Leica, Germany) to detect and trace the cells stained with PKH26.

Platelet-rich plasma (PRP) preparation

The PRP preparation was done on the same day of injection. The separation of the blood cell elements was performed using a laboratory centrifuge (Sigma, CA, USA). Dog PRP was derived from the peripheral blood of the same donor as the BM-MSCs. Briefly; 20 ml peripheral blood was collected into vacuum gel separator tubes with citrate phosphate dextrose as anticoagulant (figure 1A) and centrifuged at 800 × g for 10 minutes. This first centrifugation is resulting in two basic components: blood cell component (BCC) in the lower fraction and plasma component (PC) in the upper fraction. A mark was made to separate the BCC from the PC. Second centrifugation was done to increase the total amount of platelets; all content above this point was pipetted and transferred to another 5 ml vacuum plain tube without anticoagulant. The second centrifugation was at 1000 × g for 5 minutes to obtain a platelet pellet (figure 1B). Most of the plasma was then removed, leaving 3 ml plasma to resuspend the platelets. This preparation was inactivated PRP. Finally, PRP was activated by activating tubes containing 100 µl of 20% CaCl2 (Tatebe *et al.,* 2005).

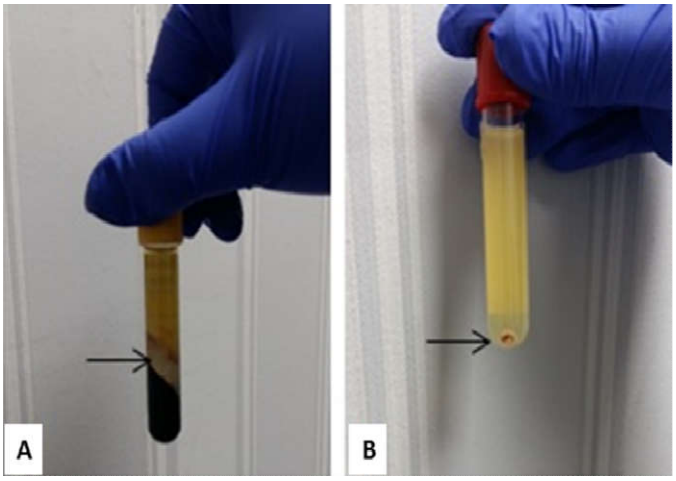

Figure 1. Vacuum gel separator tubes (figure 1A). platelet pellet (figure 1B).

D-Platelet count study

The platelets in the whole blood and PRP samples were counted by cell counter (Sysmex XT-4000*i* Automated Hematology Analyzer Lincolnshire, IL, USA). Obtained stem cells/PRP was injected back into the knee joint of its corresponding donor.

Surgical procedures

Surgical partial chondral defect was induced in the stifle of experimental dog by using a circular hand-made drill of 3mm diameter and 1mm depth after performing 3 cm lateral incision close to the lateral patellar ligament.

A partial thickness cartilage defect of weight-bearing articular surface was removed from the lateral femoral condyle without damaging the subchondral bone. Synovium and fasciae were sutured using 3/0 absorbable suture material. Skin was closed using 1/0 non absorbable suture material (Mokbel *et al.,* 2011). Animals were divided equally into three groups after the surgical induction of chondral defects. Group-I served as positive control. Group-II dogs were injected intra-articularly with 5 × 10^6MSCs/3ml PBS. Group-III dogs were injected intra-articularly with 5 × 10^6 MSCs/3ml PRP. surgical induced defect received a single injection of PRP+MSCs

Post-Surgical follow-up

During the first 5 days after surgery, all animals were given a systemic course of antibiotic. Skin stitches were removed after 7 days. All animals were exercised once daily by having them walk on a solid surface. All dogs were evaluated clinically for any abnormalities. At the end of the experiment after three months, dogs were put to sleep through intravenous injection of thiopental sodium.

Lameness of the affected limb

Clinical observation was applied once daily. Dogs were turned out on the ground on an area of 2 square meters. Their gait pattern was assessed by direct observation for 20 minutes individually. The lameness was defined as non-weight bearing of the affected limb and losing of typical flexion and extension cycle during hoping in comparison with the unaffected limb. The severity of the lameness was not quantified. The times to normal ambulation without the non-weight bearing lameness of the affected limb were recorded, and the lameness periods were calculated for each group. Two independent physiatrists, without prior knowledge of the experimental groups, performed the observation. During the first 5 days after surgery, all animals were given a systemic course of antibiotic. Skin stitches were removed after 7 days. All animals were exercised once daily by having them walk on a hard surface. All dogs were evaluated clinically for any abnormalities in gait according to "Evers" grading scale (Evers *et al.,* 1997). At the end of the experiment, dogs were put to sleep through intravenous injection of thiopental sodium, the distal femoral parts were isolated and cartilage samples were collected for histopathological evaluation.

Macroscopic Assessment

The knee joints were dissected after euthanasia. The knee goints were examined for gross morphologic changes.

Histological Assessment:

The lateral femoral condyle was fixed with 10% neutral buffered formalin and decalcified with 20% ethylenediamine-tetra-acetic acid. The decalcified condyle was embedded in paraffin and the standard frontal microsections with 5 μm width were prepared and stained with hematoxylin and eosin Histological evidence of cartilage degeneration was evaluated by the structural change of articular cartilage. (Bancroft *et al.,* 1990).

D- Real time PCR

Real Time PCR (quantitative PCR) will be done for aggrecan and TGF-β genes expression to assess and compare

quantitatively the reparative and therapeutic effects of injected labeled MSCs. Thirty mg cartilage tissue was homogenized by homogenizer (ART-MICCRA-Germany) and total RNA was isolated with RNAeasy Mini Kit (Qiagen). The mRNA expression level was quantified by qRT–PCR (Real time PCR). One µg of the total RNA from each sample were used for cDNA synthesis by reverse transcription using High capacity cDNA Reverse Transcriptase kit (Applied Biosystem, USA). The cDNA was subsequently amplified with the Syber Green I PCR Master Kit (Fermentas) in a 48-well plate using the Step One instrument (Applied Biosystem, USA) as follows: 10 minutes at 95^0C for enzyme activation followed by 40 cycles of 15 seconds at 95^0C, 20 seconds at 55^0C and 30 second at 72^0C for the amplification step. Changes in the expression of each target gene were measured relative to the mean critical threshold (CT) values of 18s RNA housekeeping gene by the ΔΔCt method. We used 1µM of both primers specific for each target gene (Table1).

Statistical analyses

Statistical package for social science (SPSS) version 12 was used for data management and analysis. To test the difference between quantitative variables for more than 2 groups Kruskall-Wallis test was used. While to compare quantitative variables between two groups Mann Whitney test was used, Bonferroni multiple comparison adjustment was done for pair wise comparison (P-value ≤ 0.001).

RESULTS

MSCs culture, identification, and labeling

Isolated and cultured MSCs reached 70–80% confluence at 14 days.

MSCs were morphologically identified in culture by their spindle shaped cells (figure 2A). The total number of MSCs injected was5 × 10^6 cells/ml and some of them were labeled with PKH26 dye (Figure 2B).

Platelet count

Platelet count results showed that the mean value of platelets count in the whole blood is 200000±75000and the mean value of platelets count in PRP samples is 1394000±60663 (5-folds) which has been commonly described for therapeutic platelet-rich preparations.(P ≤ 0.001) (Figure 3).

Post-surgical Clinical assessment

All operated animals exhibited immediate post-surgical lameness that was graded score 3. In the control, the 2nd degree lameness lasted for 6 weeks. On week 6 after surgery, animals were able to walk, bear weight but with an apparent degree of lameness. From week 7 animals kept on showing slight degree of lameness that was exaggerated by exercise.

In group-II, symptoms were similar to the control group for the first 4 weeks. After the injection of the MSCs at the end of the 4th week, animals showed slight degree of lameness that was exaggerated by exercise this lasted for the first 2 weeks post-injection. Between week 2 and 3 post-injection, the severity started to decrease, slowly but progressively, after the MSCs injection. From week 3-7 post-injection, animals were able to do more exercise with less pain. From week 7 post-injection, the lameness started to decrease more till it disappeared. In group-III, after the injection of the MSCs/PRP the severity started to be obviously decreased between the 6th and 7th day without the use of any pain killer.

Table 1. Primers sequence specific for each studied gene

Target gene	Primer sequence: 5`- 3`	Genebank accession number
Aggrecan	F: CCTGAACGACAAGACCATCGA R: TGGCAAAGAAGTTGTCAGGCT	U76615
Collagen II	F: AAGAAGGCTCTGCTCATCCAGG R: TAGTCTTGCCCCACTTACCGGT	X02420
TGF-β	F:GAAAT TGAGG GCTIT CGCCT R:AAGCA AT AGT TGGTG TCCAG	XM011527242.1
18s RNA	F: CAGCCACCCGAGATTGAGCA R: TAGTAGCGACGGGCGGGTG	JX132355.1

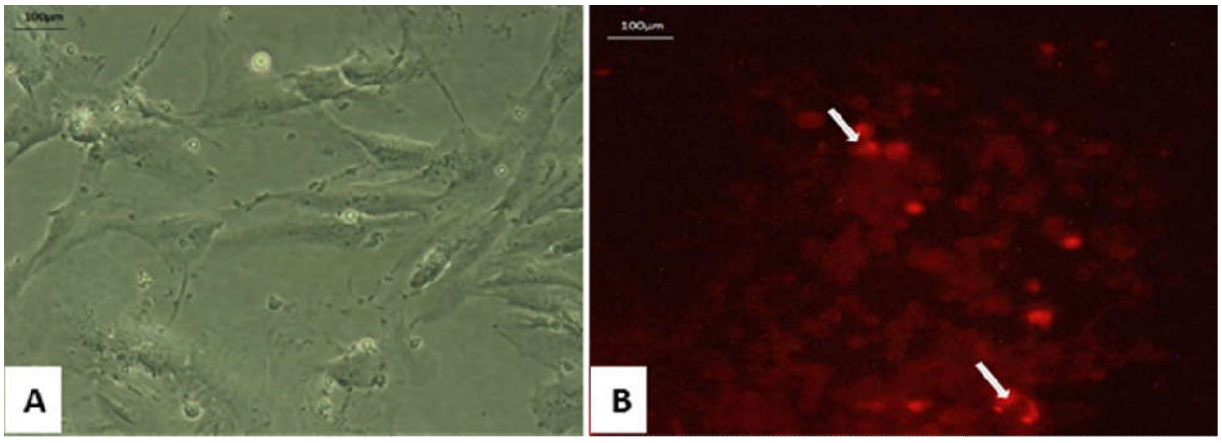

Figure 2: **Morphological observation of stem cells from bone marrow through phase contrast microscopy. (A) MSCs shows spindle shape cells with anastomosing interlacing cell processes. (B) PKH26 labelled injected stem cells visualized by flurosescent microscope in a formalin- fixed paraffin embedded tissue sections incorporated in the newly formed cartilage, indicating homing after injection**

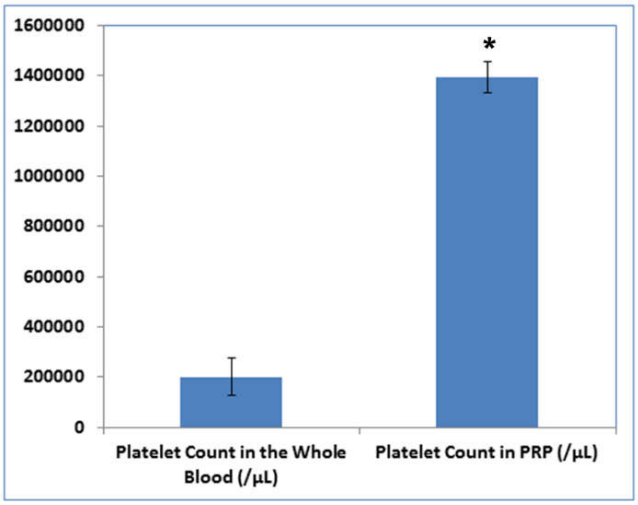

Figure 3. Bar chart showed high significance difference between the mean value of platelets count in PRP samples and the mean value of platelets count in the whole blood. (P ≤ 0.001)

By the end of the first week post-surgery, the lameness scale was graded score 2. This observation was continued for the following 4 weeks till end of the experiment. Between week 5 to 6, animals were able to bear weight. On week 7 the lameness started to decrease even on exercise till it was completely vanished.

Lameness period of the affected limb

The average time of recovery to normal ambulation was 20±3 days in group I , 16±2 days in group II and 14±3 days in group III , and. The average time to recovery of normal ambulation of group I was significantly longer than that of group I and II ($p<0.001$).

Macroscopic Findings

The higher articular discoloration, erosions and articular surface roughness was more in group-I than the other two groups. The articular surface showed the defect area occupied with a tough pale tissue that lacked the shiny appearance of the cartilaginous surface in group II and III .While the articular surface was filled with a smooth glistening tissue that resembled the surrounding intact cartilage in group II and III (figure 4).

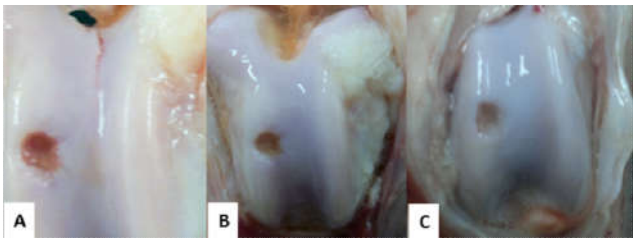

Figure 4. The articular surface showed the defect area occupied with a tough pale tissue. (figure 4 A).The articular surface was filled with a smooth glistening tissue that resembled the surrounding intact cartilage in group II and III (figure 4 B&C)

Microscopic findings

Group-I (surgical induced chondral defect): Articular cartilage of this group revealed areas of complete chondrocytes degeneration which presented by empty lacaunae. Multiple cystic cavities were also observed with apparent matrix fibrosis (Figure 5A&B).

Group-II (surgical induced defect received a single injection of MSCs): Showed an evidence of new cell formation and cell cloning, the newly differentiated cells showed chondrocyte-like phenotype (rounded form; surrounded by a lacuna). The repaired tissue showed diffuse cellularity with tangential and rounded cells. Furthermore, an appearance of a fibrocartilaginous tissue which extended about 1/3 of cartilage thickness was also noticed (Figure 5C&D).

Group-III (surgical induced defect received a single injection of PRP+MSCs): Presented an obvious repair of chondral defect with neocartilage . The repaired tissue showed diffuse cell growth and cell cloning in all layers. The cells appeared either in the form of chondrocyte-like phenotype (rounded form; surrounded by a lacuna) or clear chondrocyte clusters (Figure 5E&F).

Molecular Assessment

Real Time PCR (qRT-PCR) for quantitative expression of aggrecan, collagen II and TGF-β results showed the mean value of each as follows; Aggrecan gene expression in group I was 0.96±0.27 and in group II was 2.57±0.50 but in group III showed 3.38±0.68 mean value (Figure 6A). The mean value of collagen II expression in group I was 1.81±0.35 and in group II was 2.96±0.45 while in group III was 4.27±0.49 (Figure 6B). The mean value of TGF-β expression in group I was 0.57±0.08 and in group II was 1.66±0.48 while in group III was 2.53±0.79 (Figure 6C). Thus, Group-III showed the highest significant expression of TGF-β, collagen II and aggrecan as compared to group I and group II ($P \leq <0.001$).

DISCUSSION

Osteoarthritis (OA) is one of the most common joint diseases and is a significant cause of disability (Abramson and Attur, 2009). The majority of recently proposed therapeutic modalities for OA have a foundation in attempting to treat the imbalance between the pro-inflammatory and the anti-inflammatory cytokines (Iqbal and Fleischmann, 2007). This imbalance is thought to activate proteolytic enzymes, leading to the destruction of cartilage (Murphy *et al.,* 2003). In the current study, MSCs derived from bone marrow were used to initiate the process of cartilage defect repair as MSCs derived from bone marrow are the most promising cell source for cartilage repair (Hunziker and Rosenberg, 1996).

MSCs derived from bone marrow were used in the current study because they are relatively easy to obtain and will maintain their multilineage potential with passage (Hunziker, 2001). PRP therapy provides delivery of a highly concentrated cocktail of growth factors to accelerate healing in a simple, low cost and minimally invasive way. PR preparations as a cell carrier has the advantage of being autologous, nonimmunogenic, sterile, easily prepared and set intraoperatively. Biodegradable and contains chondrogenic growth factors with sustained release profiles. PRP is easily prepared and set intraoperatively (Sampson *et al.,* 2008). The ideal concentration of platelets in PRP is not yet clear. The average baseline blood platelet count in an individual is 200,000 ± 75,000/µL, a platelet concentrate count of 1,000,000/µL (5-fold) which has been commonly described for therapeutic platelet-rich preparations.

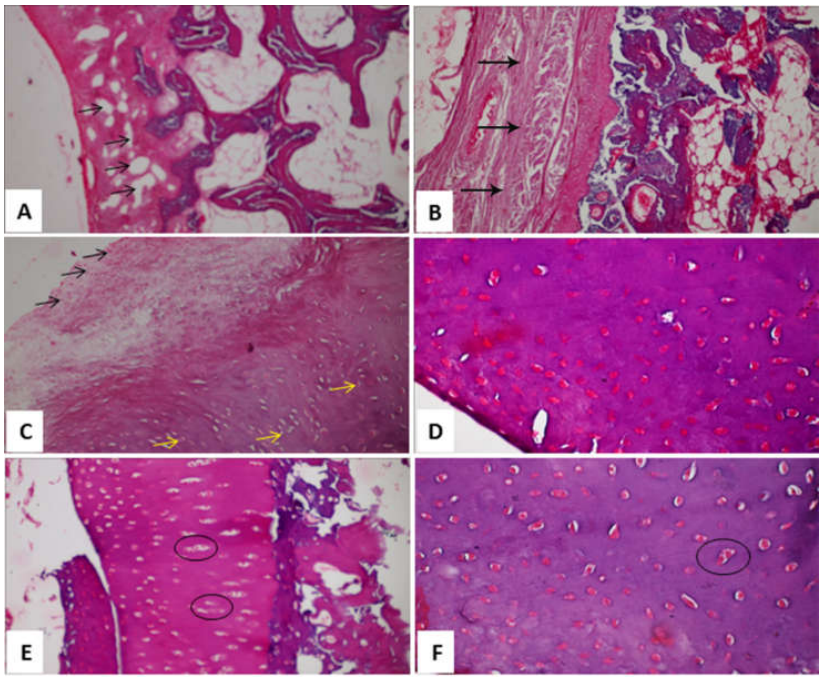

Fig. 5A. Articular cartilage of group I showing area of diffuse necrosis of chondrocytes with multiple cysts formation(arrows) (H & E X 200).
Fig. 5B. Articular cartilage of group I revealing irregularity of the articular surface with fibrillation of the matrix and loss of chondrocytes (arrows) (H & E X 200).
Fig. 5C: Articular cartilage of group II showing partial repair of chondral defect with neocartilage. Cells showed chondrocyte-like phenotype (rounded form; surrounded by a lacuna) (yellow arrows). The fibrillated superficial layer has tangential and rounded cells (black arrows). (H & E X 200).
Fig. 5D. Higher magnification of the articular cartilage of group group II showing replacement of the necrosed chondrocytes with new growth of cells and cell cloning (H & E X 400).
Fig. 5E Articular cartilage of group III revealing repair of chondral defect with neocartilage. Cells showed chondrocyte-like phenotype (rounded form; surrounded by a lacuna) with presence of chondrocyte cluster (circle) (H & E X 200).
Fig. 5F . Higher magnification of the articular cartilage of group III showing diffuse cell growth , cell cloning in all layers and chondrocyte clusters (circle). (H & E X 400)

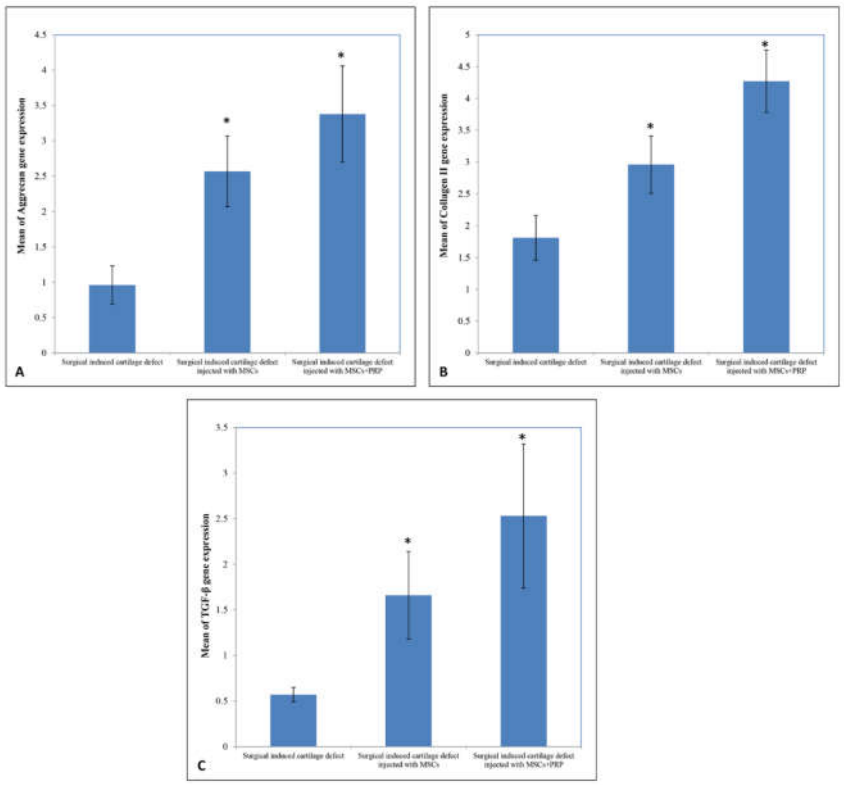

Figure 6. Bar chart of Group-III showed the highest significant expression of aggrecan as compared to group I and group II. (P ≤ 0.001) (Figure 6A). Group-III showed the highest significant expression of collagen II as compared to group I and group II. (P ≤ 0.001) (Figure 6B). Group-III showed the highest significant expression of TGF-β, as compared to group I and groupII. (P ≤ 0.001) (Figure 6C). *means significant difference (P ≤ 0.001)

This explains why we choose a specific number of platelets in our injections (Marx, 2004). Depending on the method used to process the PRP, it may also contain growth factor in white blood cell with concentrations above baseline values. (Pietrzak and Eppley, 2005). Platelets and white blood cells are sources of high concentrations of cytokines well documented to regulate anumber of processes related to healing and tissue regeneration (Eppley *et al.,* 2004). These processes include cell migration, cell proliferation, angiogenesis, inflammation mediation, and collagen synthesis (Werner and Grose, 2003). Dogs were used in this study as animal model, as they are comparatively available, easy to handle and maintain healthy. They are also known to maintain uniformity in their genetic characteristics. They have comparable organ sizes (to humans), which made them ideal for the study (Ostrander *et al.,* 2000 and Tsai *et al.,* 2007). Using a circular trephine with a predetermined diameter (3 mm) and depth (1mm) provided two major advantages. One, the rounded trephine made it easier to locate the area of interest for histo-sampling at the end of the experiment period.

Two, the predetermined depth was very successful in preventing the penetration of the subchondral bone in all operated cases so no involvement of the vasculature would occur Which simulate sequence of events happened in OA Consequently, progenitor cells in blood and marrow cannot enter the damaged region to influence or contribute to the reparative process to ensure that any chondral repair is entirely from the injected cells. In the present study, our Post-surgical Clinical assessment of experimental animals reveals that PRP-preconditioned BMSCs group experienced improvement in the functional knee movement. This is in agreement with previous published studies (Ali Soliman Hassan *et al.,* 2015). Our Post-Surgical Clinical assessment results are in agreement with our histological and genes expression results. According to the obtained histological results of the current study, PRP-preconditioned BMSCs showed the criteria of repair of the chondral defect.

This was presented in the form of neocartilage of the articular surface as well as the presence of chondrocyte-like cells and chondrocyte clusters. This suggested that PRP injection has improved the healing of articular cartilage defect and cartilage regeneration compared with that of unconditioned BMSCs. In the present study, TGF-β, collagen II and aggrecan were measured in order to assess the regenerative effects of PRP Real Time PCR results postulated that PRP promoted BMSC differentiation into chondrogenic cells that highly significant expressed TGF-β, collagen II and aggrecan compared to BMSCs transplantation alone. This suggests that intra-articular PRP injection could potentially serve as the endogenous source of chondroprotection and joint lubrication. The use of intra-articular injections of mesenchymal stem cells supplemented with PRP shown to be effective option for treating cartilage defects in OA. The results of the present study provide an effective treatment to temporomandibular joint osteoarthritis (TMJ -OA). Because of the limited self-healing potentials of avascular cartilage, little e□ective therapy is available for the repair of TMJ- OA disease. The conventional nonsurgical or surgical treatments cannot completely restore the TMJ function and reverse disease progression. MSCs, which have the multilineage di□erentiation potentials combined with PRP as in our study may provide an effective treatment for the cartilage damage in TMJ OA (Dixin *et al.,* 2017).

Conclusion

Preconditioning of BMSCs with PRP is an efficient and perfect method to apply BMSCs in cartilage regeneration. This study provides an important preclinical step toward the use of autologous BMSCs with PRP in the treatment of articular cartilage defects. Further studies are needed to evaluate the PRP effects, according to the different platelet concentration, injection time, and number of injection in knee OA and in severe knee OA for the achievement of the best and more durable results. Study supported financially by authors of manuscript.

Conflict of interest: All authors declare that there was no conflict of interest.

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