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Effect of platelet-rich plasma on temporomandibular joint cartilage wound healing: Experimental study in rabbits

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ABSTRACT

Purpose: The aim of the study is to evaluate the effect of platelet-rich plasma (PRP) injection on temporomandibular joint (TMJ) cartilage and subchondral bone healing.
Materials and methods: Sixteen New Zealand rabbits were divided into two groups, including single PRP and multiple PRP injection groups. Sodium mono-iodoacetate (MIA) was injected bilaterally into the TMJ of all rabbits to create osteoarthritis (OA). PRP was injected once into the right TMJ in the single PRP group and was injected three times (once a week) into the right TMJ in the multiple injection group 4 weeks after injection of MIA. At the time of each PRP injection, isotonic NaCl solution was injected into the left TMJ in the control groups. All animals were sacrificed 30 days after the first PRP injection.
Results: As a result of the histological evaluation, there was no statistically significant difference in cartilage and subchondral bone regeneration between the groups ($p > 0.05$).
Conclusions: Although there was no statistically significant difference between PRP and control groups, it was seen that improvement were better in PRP groups. According to the Results of our study, it seems that different methods should be tried to investigate the efficacy of PRP on the TMJ healing.
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1. Introduction

The temporomandibular joint (TMJ) is a synovial bilateral joint in which the most complex movements of the human body occur (Wang et al., 2015). Osteoarthritis (OA) can be seen as the clinical and pathological outcome of a range of disorders that result in structural and functional failure of synovial joints. In general, it has been considered a disease of articular cartilage. However, the current concept holds that OA involves the entire joint organ, including the subchondral bone, disc, ligaments, periarticular muscle, capsule, and synovium (Hunter and Felson, 2006). Temporomandibular joint OA is an important subgroup of TMJ disorders. The etiology of TMJ OA is multifactorial and complex and still uncertain (Tanaka et al., 2008; Kalladka et al., 2014; Wang et al., 2015). Although OA can affect anyone at any age, it has been linked to the aging process (Kalladka et al., 2014). The most common clinical symptoms in patients with TMJ OA are pain, limitation of function, and joint sounds. The quality of life of patients with TMJ OA decreased due to pain and TMJ dysfunction (Kalladka et al., 2014; Wang et al., 2015).

Platelet-rich plasma (PRP) is an autologous blood product that is obtained by centrifuging blood and contains more platelets than normal (Smyth et al., 2013). Platelet-rich plasma contain 3 to 5 times more growth factor than normal plasma (Foster et al., 2009). Platelets include growth factors as well as fibrin, fibronectin (Fn), vitronectin (Vn), and many biologically active specific molecules, which act as cell adhesion molecules (Marx, 2004). Studies have reported that it increases healing in soft tissue, hard tissue and nerve tissue and that it shows antimicrobial properties, thanks to the leukocytes and interleukins within it (Nikolidakis and Jansen, 2008; Plachokova et al., 2008; Garcia et al., 2010).

Platelet-rich plasma is considered as a new therapeutic agent in the treatment of degenerative disorders of the TMJ. Platelet-rich plasma contains high concentrations of growth factors that accelerate healing. Studies have shown that PRP stimulates cell proliferation and increases hyaluronic acid production in cartilage

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matrix and synovial cells. Platelet-rich plasma also increases glycosaminoglycan (GAG) synthesis, stabilizes joint angiogenesis and forms a framework for stem cell migration (Hegab et al., 2015). In the literature, PRP is used successfully for a long time in the treatment of OA of the knee joint (Kon et al., 2010a; Filardo et al., 2011; Wang-Saegusa et al., 2011). However, there are few studies in the literature on the efficacy and use of PRP in the treatment of TMJ OA.

In our study, we aimed to examine histopathologically the changes in TMJ cartilage and subchondral bone using by intra-articular injection of PRP in experimental animal models of TMJ OA.

2. Materials and methods

2.1. Study design

This study was approved by Karadeniz Technical University Animal Experiments Local Ethics Committee (Date: 22 April 2014, Protocol No: 2014/16). Sixteen adult male White New Zealand rabbits weighing between 3 and 3.5 kg were used in our study. All animals were evaluated for systemic infection and anatomic malformation before the study. The rabbits were housed in suitable cages at 22 ± 2 °C with a daily 12-h light/12-h dark schedule.

The rabbits were randomly divided into two groups of eight each.

Multiple PRP injection group (n = 8): OA was created bilaterally in the TMJ by sodium mono-iodoacetate (MIA) in eight rabbits in this group. A 0.8–1 ml quantity of PRP was injected three times at 1-week intervals into the right TMJ. A 0.8–1 ml quantity of isotonic NaCl solution was injected three times at 1-week intervals into the left TMJ with the same technique for control purposes (n = 16).

Single PRP injection group (n = 8): OA was created bilaterally in the TMJ by MIA in eight rabbits in this group. A 0.8–1 ml quantity of PRP was injected once into the right TMJ. A 0.8–1 ml quantity of isotonic NaCl solution was injected once into the left TMJ with the same technique for control purposes (n = 16).

2.2. Preparation of platelet-rich plasma

There is no any accepted standard centrifuge number or parameter in the literature. Different techniques are mentioned for preparing PRP. Standard PRP kits were used in our study. In this method, approximately 8–10 ml of blood is transferred into a tube containing sodium citrate during preparing PRP, and then PRP is obtained by centrifuging the blood in one or two steps. PRP obtained after centrifugation is not active and therefore a mixture of bovine thrombin and calcium chloride is added to it in order to activate PRP.

2.3. Experimental procedure

The rabbits were prepared in a similar manner before MIA and PRP injections. Arthrocentesis technique was used in both injections. Each animal was weighed before anesthesia and thus their body weights were determined. After appropriate dosage of anesthetic agent was calculated for each animal, 50 mg/kg ketamine hydrochloride (Ketalar flk, Pfizer, 50 mg/ml solution) and 5 mg/kg xylazine hydrochloride (Rompun enj 2% sol, Bayer, Germany) were intramuscularly administered to provide general anesthesia. The skin of the TMJ region was shaved following the anesthesia. The skin was disinfected with 10% povidone-iodine solution (Betadine sol, Kansuk, Turkey) for antisepsis. In intraarticular injections, it was entered into the joint space using a 29-gauge insulin needle with arthroscopic technique. After the right upper compartment of the rabbit TMJ was palpated, 8 mm posterior to the lateral canthus in the internal auditory canal was determined as the needle entry point. It was entered below the zygomatic process of the temporal bone and behind the condyle. It was moved in a medioanterior direction; then it was entered into the joint space.

Guler et al. created an experimental OA model in the rabbit TMJ. The dose of MIA used in their study was taken for reference. A 50 μl quantity of 3 mg/ml MIA solution was injected into both joints of the rabbits. After waiting 4 weeks for formation of OA, the PRP injection phase of the experiment began (Guler et al., 2011).

The rabbits were prepared for PRP injection in a manner similar to that for MIA injection. Standard PRP (Smart PRP Platelets Rich Plasma Separation Kit) kits were used in our study. In order to prepare PRP, 10 ml of blood was collected from the rabbit ear veins and then was transferred into a tube containing sodium citrate as anticoagulant. The obtained blood was centrifuged in one stage at 3200 rpm for 12 min in the swing centrifuge device.

After the centrifugation, the upper part of the tube contains platelet poor-plasma (PPP) and PRP is just below PPP. The separator gel was present below the PRP, and red blood cells were obtained at the bottom of the tube. After PPP was removed from the tube with the aid of a special needle, PRP in the middle was carefully drawn into the syringe and was made available. A 0.8–1 ml quantity of PRP was obtained through this process. Then 10% calcium chloride was added to it in order to activate PRP.

The obtained PRP was injected with a 29-gauge insulin needle into the right TMJ of the rabbits were previously created bilaterally TMJ OA. At the time of each PRP injection, 0.8–1 ml of isotonic NaCl solution was injected into the left TMJ with the same technique for control purposes. In the single PRP injection group, the rabbits were sacrificed 30 days after the first PRP injection. In the multiple PRP injection group, PRP was injected at days 1, 7 and 14 after formation of OA, and the rabbits were sacrificed 30 days after the first PRP injection.

After the rabbits were anesthetized with ketamine-xylazine mixture for sacrifice, an overdose (100 mg/kg) of thiopental sodium (Ekipental, Tum-Ekip Pharmaceutical Inc., Istanbul) was administered intracardially.

The TMJ in each rabbit was dissected bilaterally. It was milled under irrigation with a bur so that the joint region included the temporal bone and the condylar process of the mandible. En bloc excision was performed, to be 2 × 1 cm in size. The obtained tissue specimens were evaluated histopathologically.

2.4. Histopathological evaluation

The obtained samples were gently dissected and the remaining soft tissues were removed. They were individually placed in numbered containers containing 10% neutral formaldehyde solution. The bloody solution was changed 30 min later, and the tissues were fixed for 48 h.

After the fixation process was completed, 10% formic acid solution was used for decalcification of the tissues. During the decalcification process, the solution was changed every 2 days. The tissues were decalcified at room temperature for 20 days and then were washed in stream tap water for 4 h. They were passed through a graded series of alcohol for dehydration. After they were treated with xylene, they were embedded in paraffin.

The prepared paraffin blocks were cut into a thickness of 5 μm with a microtome (Leica RM2255, Japan) and then were taken on numbered slides.

After the sections taken on the slides were allowed to stand for 60 min at 50 °C, they were hydrated by the removal of paraffin through a graded series of xylene and alcohol. The sections prepared in this way were stained with regular hematoxylin-eosin

(H&E) staining. The sections, which were passed through a graded series of xylene and alcohol, were then covered with lamella by dripping Entellan on them.

The prepared sections were evaluated with a light microscope (Olympus BX51-Japan) equipped with a digital camera (Olympus DP71-Japan), and then were photographed (DP controller 3.3.1292 version Olympus Corporation-Japan).

All sections of all groups were evaluated for articular cartilage, osteochondral junction, chondrocyte appearance, and histological changes in subchondral bone structure. Each section was evaluated in terms of articular cartilage (normal, thickened, thinned), chondrocyte appearance (normal, hypocellular, clustered), osteochondral junction (normal, invaginated, weak junction and subchondral bone structure (normal, increase in trabecular bone)) (Duygu et al., 2011). In our study, thickening or thinning in articular cartilage, clustering or hypocellularity in chondrocyte appearance, invagination or weak junction in osteochondral junction, and increase in subchondral trabecular bone were considered pathologically.

2.5. Statistical analysis

SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software was used for the statistical evaluation of the histopathological data obtained. The descriptive statistics were calculated. Then, the Wilcoxon signed-rank test was used to analyze the difference between the control groups and the experimental groups, and the Mann–Whitney U test was used to compare the two experimental groups ($p < 0.05$). Histopathological parameters of each group was calculated by the Spearman rank correlation coefficient ($p < 0.05$). In all analyses, a p value < 0.05 was considered statistically significant.

3. Results

No rabbits included in the study died from infection or any cause other than sacrifice. No animals were left out of the study. Since the rabbits included in the study had standard age and weight, it was not examined whether there was a significant difference between the rabbits in terms of the characteristics such as age and weight. A total of 32 TMJs of 16 rabbits were evaluated in the study.

When the single PRP injection group was evaluated for articular cartilage, it was normal in 4 (50%) joints, thickened in 1 (12.5%) joint and thinned in 3 (37.5%) joints. When the control group (single injection) was evaluated for articular cartilage, it was normal in 3 (37.5%) joints, thickened in 1 (12.5%) joint and thinned in 4 (50%) joints. When the single PRP injection group was evaluated for osteochondral junction, it was normal in 3 (37.5%) joints, invaginated in 4 (50%) joints and weak junction in 1 (12.5%) joint. When the control group (single injection) was evaluated for osteochondral junction, it was normal in 3 (37.5%) joints, invaginated in 2 (25%) joints and weak junction in 3 (37.5%) joints. When the single PRP injection group was evaluated for chondrocyte appearance, it was normal in 5 (62.5%) joints and clustered in 3 (37.5%) joints. When the control group (single injection) was evaluated for chondrocyte appearance, it was normal in 4 (50%) joints, clustered in 1 (12.5%) joint and hypocellular in 3 (37.5%) joints. When the single PRP injection group was evaluated for histological changes in subchondral bone structure, it was normal in 4 (50%) joints and displayed an increased trabecular bone volume in 4 (50%) joints. When the control group (single injection) was evaluated for histological changes in subchondral bone structure, it was normal in 2 (25%) joints and displayed an increased trabecular bone volume in 6 (75%) joints (Table 1).

There was no statistically significant difference between the single PRP injection group and the control group (single injection) in terms of articular cartilage, osteochondral junction, chondrocyte appearance, and histological changes in subchondral bone structure ($p > 0.05$). Histological images of the single PRP injection group and the control group (single injection) are shown in Fig. 1.

When the multiple PRP injection group was evaluated for articular cartilage, it was normal in 4 (50%) joints, thickened in 1 (12.5%) joint and thinned in 3 (37.5%) joints. When the control group (multiple injection) was evaluated for articular cartilage, it was normal in 2 (25%) joints, thickened in 4 (50%) joints and thinned in 2 (25%) joints. When the multiple PRP injection group was evaluated for osteochondral junction, it was normal in 6 (75%) joints, invaginated in 1 (12.5%) joint and weak junction in 1 (12.5%) joint. When the control group (multiple injection) was evaluated for osteochondral junction, it was normal in 3 (37.5%) joints, invaginated in 4 (50%) joints and weak junction in 1 (12.5%) joint. When the multiple PRP injection group was evaluated for chondrocyte appearance, it was normal in 4 (50%) joints, hypocellular in 2 (25%) joints and clustered in 2 (25%) joints. When the control group (multiple injection) was evaluated for chondrocyte appearance, it was normal in 2 (25%) joints, hypocellular in 2 (25%) joints and clustered in 4 (50%) joints. When the multiple PRP injection group was evaluated for histological changes in subchondral bone structure, it was normal in 4 (50%) joints and displayed an increased trabecular bone volume in 4 (50%) joints. When the control group (multiple injection) was evaluated for histological changes in subchondral bone structure, it was normal in 2 (25%) joints and displayed an increased trabecular bone volume in 6 (75%) joints (Table 2).

There were no statistically significant differences between the multiple PRP injection group and the control group (multiple injection) in terms of articular cartilage, osteochondral junction, chondrocyte appearance, and histological changes in subchondral bone structure ($p > 0.05$). However, it was seen that the multiple PRP injection group was superior to the control group (multiple

Table 1
Numerical and percentage distribution of the single PRP injection group and the control group (single injection).

Changes in the Condyle		Single PRP Inj. Group (n/%) (n = 8)	Single PRP Inj. Control Group (n/%) (n = 8)	P Value
Articular Cartilage	Normal	4 (%50)	3 (%37,5)	p > 0,05
	Thickened	1 (%12,5)	1 (%12,5)	
	Thinned	3 (%37,5)	4 (%50)	
Osteochondral Junction	Normal	3 (%37,5)	3 (%37,5)	p > 0,05
	İnvaginated	4 (%50)	2 (%25)	
	Weak Junction	1 (%12,5)	3 (%37,5)	
Chondrocyte Appearance	Normal	5 (%62,5)	4 (%50)	p > 0,05
	Hypocellular	0	3 (%37,5)	
	Clustered	3 (%37,5)	1 (%12,5)	
Subchondral Bone	Normal	4 (%50)	2 (%25)	p > 0,05
	İncrease in Trabecular Bone	4 (%50)	6 (%75)	

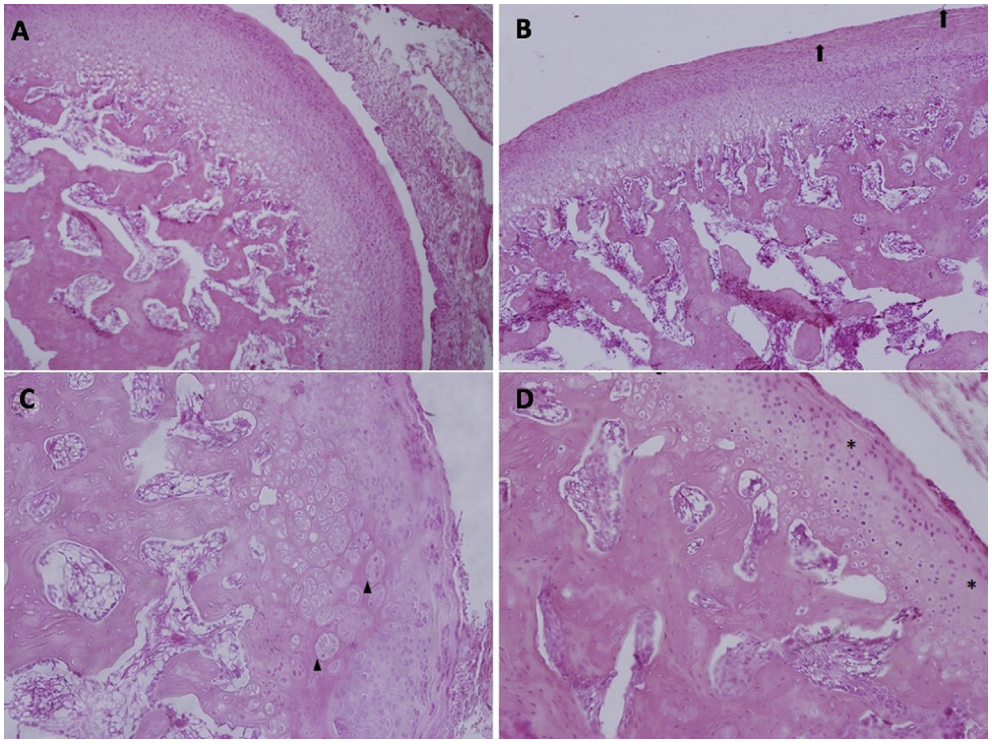

Fig. 1. (A) Thickened articular cartilage, normal chondrocytes and normal subchondral bone structure were observed in the single PRP injection group (H&E, 10x). (B) Normal articular cartilage, increased fibrocartilage and fibrillations (↑) were observed in the control group (single injection) (H&E, 10x). (C) Thickened articular cartilage and chondrocyte clusters (▲) were observed in the single PRP injection group (H&E, 20x). (D) Thinned articular cartilage and hypocellularity (*) were observed in the control group (single injection) (H&E, 20x).

injection) in terms of improvement. Histological images of the multiple PRP injection group and the control group (multiple injection) are shown in Fig. 2.

There were no statistically significant differences between the multiple PRP injection group and the single PRP injection group in terms of articular cartilage, osteochondral junction, chondrocyte appearance, and histological changes in subchondral bone structure ($p > 0.05$). A difference that was not statistically significant between the multiple PRP injection group and the single PRP injection group was observed in the osteochondral junction. When articular cartilage, chondrocyte appearance, and histological changes in subchondral bone structure were evaluated, similar Results were obtained. The osteochondral junction was normal in 6 (75%) joints in the multiple PRP injection group, and was normal in 3 (37.5%) joints in the single PRP injection group (Table 3).

In our study, the control groups were also compared with each other, and the effect of the number of injections (needle-induced trauma) on healing was evaluated. There was no statistically significant difference between them ($p > 0.05$). When the correlation between the data in all groups was evaluated, the relationship between the data was not statistically significant ($p > 0.05$).

4. Discussion

Platelet-rich plasma is referred to as autologous platelet concentrate and has been widely used in the treatment of bone and soft tissue defects in recent years. Many molecules such as coagulation factors, growth factors, cytokines, chemokines, and integrins are released through the activation of platelets (Sundman et al., 2011).

Our study was planned to determine whether the use of PRP in the treatment of TMJ OA has an effect on healing in degenerated joint cartilage and subchondral bone due to the high amount of different growth factors in it.

Table 2
Numerical and percentage distribution of the multiple PRP injection group and the control group (multiple injection).

Changes in the Condyle		Multiple PRP Inj. Group (n = 8)	Multiple PRP Inj.Control Group (n = 8)	P Value
Articular Cartilage	Normal	4 (%50)	2 (%25)	p > 0,05
	Thickened	1 (%12,5)	4 (%50)	
	Thinned	3 (%37,5)	2 (%25)	
Osteochondral Junction	Normal	6 (%75)	3 (%37,5)	p > 0,05
	İnvaginated	1 (%12,5)	4 (%50)	
	Weak Junction	1 (%12,5)	1 (%12,5)	
Chondrocyte Appearance	Normal	4 (%50)	2 (%25)	p > 0,05
	Hypocellular	2 (%25)	2 (%25)	
	Clustered	2 (%25)	4 (%50)	
Subchondral Bone	Normal	4 (%50)	2 (%25)	p > 0,05
	İncrease in Trabecular Bone	4 (%50)	6 (%75)	

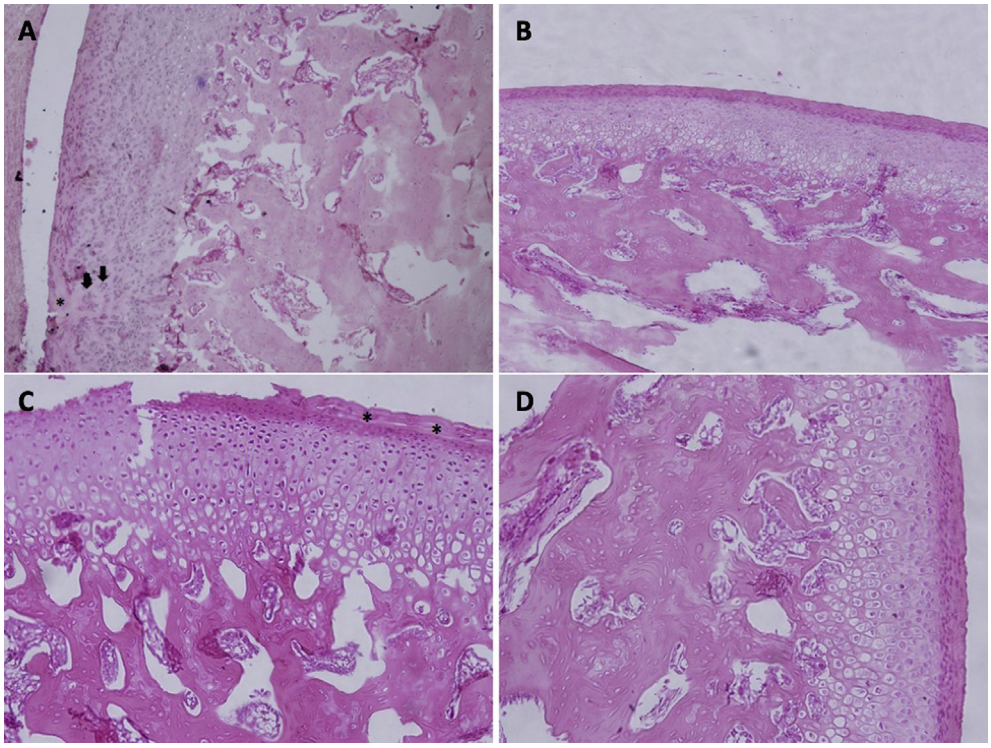

Fig. 2. (A) Thickened articular cartilage, fissures (*), chondrocyte clusters (⬇) and increased trabecular bone volume were observed in the control group (multiple injection) (H&E, 10x). (B) Thinned articular cartilage and normal osteochondral junction were observed in the multiple PRP injection group (H&E, 10x). (C) Thinned articular cartilage, irregularities, fibrillations and fissures (*) and normal subchondral bone structure were observed in the control group (multiple injection) (H&E, 20x). (D) Normal articular cartilage, normal chondrocyte appearance and normal osteochondral junction were observed in the multiple PRP injection group (H&E, 20x).

Table 3
Numerical and percentage distribution of the single PRP injection group and the multiple PRP injection group.

Changes in the Condyle		Single PRP Inj. Group (n = 8)	Multiple PRP Inj. Group (n = 8)	P Value
Articular Cartilage	Normal	4 (%50)	4 (%50)	p > 0,05
	Thickened	1 (%12,5)	1 (%12,5)	
	Thinned	3 (%37,5)	3 (%37,5)	
Osteochondral Junction	Normal	3 (%37,5)	6 (%75)	p > 0,05
	İnvaginated	4 (%50)	1 (%12,5)	
	Weak Junction	1 (%12,5)	1 (%12,5)	
Chondrocyte Appearance	Normal	5 (%62,5)	4 (%50)	p > 0,05
	Hypocellular	0	2 (%25)	
	Clustered	3 (%37,5)	2 (%25)	
Subchondral Bone	Normal	4 (%50)	4 (%50)	p > 0,05
	İncrease in Trabecular Bone	4 (%50)	4 (%50)	

Intraarticular injections of PRP are frequently and successfully applied to degenerative diseases of joints such as knee and hip. However, there are few studies on TMJ OA. Different treatment modalities show poor improvement in articular cartilage and degenerated chondrocytes (Kutuk et al., 2014). Recent studies have focused on tissue biology and have shown that growth factors have an effect on cartilage repair. Platelet-rich plasma is a natural source of growth factors. Studies have indicated that it has a healing effect on cartilage damage in degenerative knee diseases. In addition, studies have reported that its effect on the pain has continued for 1 year after intraarticular injection of PRP into the knee joint (Kon et al., 2010a; Sampson et al., 2010). In the protocols for PRP injection performed in the treatment of knee OA in the literature, Spakova et al., Napolitano et al. and Sanchez et al. performed PRP injection three times at 1-week intervals (Sanchez et al., 2008; Napolitano et al., 2012; Spakova et al., 2012). Whereas Halpern et al. performed PRP injection once, Kon et al. and Wang-Saegusa et al. performed PRP injection three times at 2-week intervals (Kon et al., 2011; Wang-Saegusa et al., 2011; Halpern et al., 2013). In our study, experimental OA model was created in the rabbits to evaluate and compare the effects of single and multiple PRP injections on cartilage and subchondral bone healing in TMJ OA. PRP was injected once in the single PRP injection group and was injected three times (once a week) in the multiple injection group. Thus, the effect of single and multiple PRP injections on healing in TMJ OA was also assessed.

Although different animal models provide important information about joint biology and OA pathology, differences between models make it difficult to compare studies. Spontaneous models develop slowly and are most similar to human disease. However, these are time-consuming, and disease development manifests individual differences as in humans. Although genetically modified animals are the most appropriate models for the functional role of specific molecules in OA pathology and cartilage hemostasis,

application of findings to human disease is questionable. Surgical models can also produce rapid and reproducible damage. However, these models are more suitable for the traumatic OA than the classic OA (Ameye and Young, 2006; Guler et al., 2011). Structural techniques are similar to the OA mechanism developed in humans and produce degeneration in a shorter time than other techniques. They are often preferred for reasons such as that they do not damage intraarticular and peripheral tissues, they are easy to apply, and degenerative changes to be generated can be adjusted by dose (Helminen et al., 2002; Kobayashi et al., 2003).

Osteoarthritic articular cartilage is characterized by chondrocyte clusters, thickened fibrous layer in the cartilage, and invasion of the connective tissue in subchondral bone in the early period. It has been reported that, in the late period, hypocellularity occurs due to apoptotic cell death, articular cartilage is replaced by fibrous tissue, subchondral bone emerges due to development of erosion, and necrosis develops (Cledes et al., 2006).

Cartilage degeneration develops in all species after intra-articular injection of MIA. The degree of degeneration changes according to the application frequency and concentration level. In these models, osteophytes develop besides cartilage degeneration. In addition, it has been shown that MIA injection produces changes similar to human OA in subchondral bone (Bendele, 2001; Guzman et al., 2003).

Cledes et al. applied 50 μl of 1.5 mg/ml MIA solution to the rabbit TMJ by intraarticular injection and examined its osteoarthritic effect on the rabbit TMJ at different time intervals (10, 20, 30, 40 days). On the 30th day, severe OA changes in the joint were observed histopathologically, and articular cartilage was completely replaced by fibrous tissue. On the 40th day, it was reported that five stages of OA including initial, repair, early, middle and late occurred and that articular cartilage disappeared completely and then subchondral bone exposed and that necrosis developed. It was reported in the same study that intraarticular injection of MIA caused both degeneration and repair response in the TMJ. This repair response is defined as hypertrophic cartilage reaction and chondrocyte migration surrounding the lesions developed in cartilage (Cledes et al., 2006).

Guler et al. evaluated histologically and radiologically the effect of MIA on formation of experimental OA at different doses (1.5, 2, 2.5, 3 mg/ml) and time intervals (2, 4 and 6 weeks) in rabbits. A 50 μl quantity of MIA solution was injected into the rabbit TMJ by arthrocentesis technique. At the 4th week, there was a normal image in the condyles of joint after injection of 1.5 mg/ml MIA. However, clustering was observed in chondrocytes after injection of 2.5 mg/ml MIA, and early changes such as thickening and thinning in cartilage and poor attachment and invagination in osteochondral junction were seen after injection of 3 mg/ml MIA. It was reported that 3 mg/ml MIA injected by arthrocentesis technique was effective on the development of early osteoarthritic changes in the rabbit TMJ at 4 weeks, and that technique applied has advantages such as causing degeneration in a short time and without damaging surrounding tissues (Guler et al., 2011).

Based on the Results obtained by Guler et al., MIA dose to be injected intraarticularly was determined to be 3 mg/ml in our study. Based on the findings of the studies referred to above, the time of PRP injection was determined as the 4th week, when early osteoarthritic changes became largely evident. Thus, the therapeutic role of PRP on OA could be assessed.

In our study, selecting for the control group the other joint of the same animal minimized the effect of anatomical differences. Injection of isotonic NaCl solution into the control group at the amount of PRP injected into the experimental group balanced the inflammation due to tissue tension and needle trauma. Thus, it allowed us to clearly assess the difference between the two groups.

When we examined the changes in chondrocyte appearance in the TMJ of rabbits that were sacrificed 30 days after the first PRP injection in our study, there was no statistically significant difference between the groups ($p > 0.05$). However, it was seen that the number of joints with normal chondrocyte appearance was higher in the multiple PRP injection group compared to the control group (multiple injection). Although the presence of chondrocyte clusters in all groups has indicated that repair response developed in the TMJ in the early OA, it was thought that hypocellularity may be associated with cell death.

There was no statistically significant difference between the groups when the changes in articular cartilage were examined and compared between the groups ($p > 0.05$). There was no statistically significant difference between the multiple PRP injection group and the single PRP injection group in terms of the parameters. Although there was no statistically significant difference in improvement between the PRP injection groups and the control groups, it was seen that the data for improvement were better in the PRP injection groups compared to the control groups. This can be attributed to the fact that growth factors in PRP stimulate healing and proliferation in chondrogenic cells and increase cartilage matrix synthesis on cartilage repair.

Kutuk et al. created surgically OA in bilateral TMJ in 16 New Zealand rabbits. They applied a single PRP injection to one side and infused isotonic NaCl solution to other side. They evaluated histopathologically the effect of PRP on TMJ OA. In that study, bone regeneration was statistically significantly higher in the PRP group compared to the control group. Moreover, although regeneration in hyaline cartilage and fibrous cartilage was higher in the PRP group compared to the control group, there was no statistically significant difference between the two groups (Kutuk et al., 2014). That study supports our study by showing that regeneration was higher in the PRP group, although there was no statistically significant difference in cartilage healing between the PRP and control groups.

When the changes in osteochondral junction were examined, there was no statistically significant difference between the groups, similar to the other findings ($p > 0.05$). Although there was no significant difference in changes such as invagination or weakening in osteochondral junction between the other groups except for the multiple PRP injection group, it was observed that normal osteochondral junction was higher in the multiple PRP injection group. This can be interpreted as that a high number of PRP injections can positively influence regeneration in the osteochondral junction.

In our study, although there was no statistically significant difference in improvement between the experimental and control groups, more improvement was observed in the PRP injections groups in terms of subchondral bone healing. This finding supports the positive effects of PRP on bone healing in the literature (Marx et al., 1998; Ogundipe et al., 2011; Poeschl et al., 2012). However, the reason for the inability to see the expected level of healing in the subchondral bone in our study can be attributed to the fact that PRP was administered into the joint space instead of in bone tissue, and diffusion could not be achieved adequately in injured bone tissue.

Cartilage is avascular, is aneural, lacks lymphatic drainage and has limited intrinsic regenerative capacity. It is far from the systemic circulation. This causes the normal inflammatory and reperative processes to fail during articular cartilage repair (van Buul et al., 2011; Dold et al., 2014). Animal studies show that the use of PRP restores cartilage damage during articular injuries. Theoretically, although the use of PRP is supported in osteochondral pathologies, its clinical efficacy is controversial (Kon et al., 2010b; Lee et al., 2012; Dold et al., 2014). In almost all published in vitro studies, the positive effects of PRP on chondrocyte

proliferation have been demonstrated (Xie et al., 2014; Akeda et al., 2006; van Buul et al., 2011).

Clinical studies have shown statistically significant positive Results of PRP on knee and TMJ OA in the literature (Filardo et al., 2011; Kon et al., 2011; Liu et al., 2014; Pihut et al., 2014; Kilic et al., 2015). In our study, the effect of PRP injection on healing of TMJ OA was not statistically significant. The fact that the knee joint has a wider area than the TMJ and that injection can reach more to the damaged area caused by OA and can be more effective locally, may be the reason for this difference. However, TMJ injections are performed in the upper joint space, and therefore the inadequate diffusion of injected PRP into the lower joint space may also play a role in the formation of this difference.

However, the number of studies that can explain this healing mechanism at the histological level is rather low. Therefore, it is difficult to arrive at the conclusion that the successful clinical outcomes observed in the above-mentioned studies are due to the healing properties of the PRP on tissues. Although this success observed in clinical studies may be attributed to lubrication effect of both PRP and other co-applied agents, the effect of PRP on histological wound healing and its effect on clinical parameters may not be parallel.

PRP has become increasingly popular and has been widely used in recent years. There are unanswered questions regarding to whom it can be applied, when it can be performed, how it can be applied, and through which mechanism should it be obtained. It is necessary to establish standardization as a result of basic scientific and clinical studies in the past and future both. Different products can be obtained with different commercial kits, leading to problems in the evaluation of treatment efficacy in clinical trials. There are many methods for preparing PRP in the literature, and there is no standardization in the preparation of PRP. This makes it difficult to compare the Results and to perform an accurate assessment.

As the roles of growth factors in wound healing and their interactions with each other are clarified, their applications, either individually or in combination, will open the way for biological treatments. It is important to find treatments that have predictable Results in wound healing and that can provide rapid tissue repair and functional improvement. In this context, PRP constitutes an important step.

5. Conclusion

In our study in which the efficacy of PRP on TMJ OA was histologically evaluated, although there was no statistically significant difference in improvement between the PRP injection groups and the control groups, articular cartilage, osteochondral junction, chondrocytes, and subchondral bone improved more in the PRP injection groups. This can be attributed to the fact that the high amounts of growth factors in PRP stimulate proliferation and differentiation on fibroblasts, osteoblasts, and chondrocytes, and increase extracellular matrix synthesis from chondrogenic cells during cartilage repair. Platelet-rich plasma provides the formation of inflammatory response and a more appropriate environment for healing in the TMJ thanks to its anti-inflammatory properties. In addition, we think that PRP may increase quality of life by providing an analgesic effect and increasing function in the treatment of TMJ OA in clinical practice. Platelet-rich plasma can be used safely because it is obtained autologously and does not have a specific side effect. Despite the positive effects of PRP on bone and cartilage regeneration, there is a need for more controlled clinical and animal studies that reflect long-term Results in the TMJ, for the elimination of uncertainties regarding the preparation and implementation strategies.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcms.2018.12.004.

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