

# Effects of Platelet-Rich Plasma & Platelet-Rich Fibrin with and without Stromal Cell-Derived Factor-1 on Repairing Full-Thickness Cartilage Defects in Knees of Rabbits

Soghra Bahmanpour<sup>1</sup>, PhD;  
Maryam Ghasemi<sup>2</sup>, PhD;  
Mohsen Sadeghi-Naini<sup>3</sup>, MD;  
Iraj Ragerdi Kashani<sup>4</sup>, PhD

<sup>1</sup>Laboratory for Stem Cell Research, Department of Anatomical Sciences, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran;

<sup>2</sup>PhD Student in Anatomical Sciences, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran;

<sup>3</sup>Tehran University of Medical Science, Tehran, Iran;

<sup>4</sup>Department of Anatomical Sciences, School of Medicine, Tehran University of Medical sciences, Tehran, Iran

## Correspondence:

Soghra Bahmanpour, PhD; Laboratory for Stem Cell Research, Department of Anatomical Sciences, School of Medicine, Zand Blvd, Shiraz, Iran  
Tel/Fax: +98 711 2304372

Email: bahmans@sums.ac.ir

Received: 21 August 2015

Revised: 30 September 2015

Accepted: 18 October 2015

## What's Known

- Implantation of a combination of collagen I scaffold and ultrapurified alginate gel containing stromal cell-derived factor-1 (SDF<sub>1</sub>) improved the repairing process by conferring better support for the migration of cartilage mesenchymal stem cells (C-MSCs) and synovial membrane mesenchymal stem cells (SM-MSCs).
- Adding platelet-rich fibrin (PRF) to bone allograft in sinus elevation surgery can reduce the healing time.
- Some studies have shown that if platelet-rich plasma (PRP) is applied in chondral lesions, signs of healing will be observed.

## What's New

- The effects of PRF+SDF<sub>1</sub> and PRP+SDF<sub>1</sub> were not studied so far.
- We used PRP and PRF scaffolds and SDF<sub>1</sub> to magnify their effects and reconstruct injured sites. These scaffolds are rich in slow-releasing growth factors and are easily harvested from autologous blood.
- There is similarity between PRF fibrin network and the natural one.
- SDF<sub>1</sub>, which is expressed by BMSCs, regulates the trafficking, homing, and migration of stem cells into the injury site.

## Abstract

**Background:** The purpose of this study was to create biomaterial scaffolds like platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) containing stromal cell-derived factor-1 (SDF<sub>1</sub>) as a chemokine to induce hyaline cartilage regeneration of rabbit knee in a full thickness defect.

**Methods:** We created a full thickness defect in the trochlear groove of thirty-six bilateral knees of eighteen mature male rabbits. The knees were randomly divided into six groups (group I: untreated control, group II: PRP, group III: PRF, group IV: Gelatin+SDF<sub>1</sub>, group V: PRP+SDF<sub>1</sub>, and group VI: PRF+SDF<sub>1</sub>). After four weeks, the tissue specimens were evaluated by macroscopic examination and histological grading, immunofluorescent staining for collagen type II, and analyzed for cartilage marker genes by real-time PCR. The data were compared using statistical methods (SPSS 20, Kruskal-Wallis test, Bonferroni post hoc test and P<0.05).

**Results:** Macroscopic evaluations revealed that international cartilage repair society (ICRS) scores of the PRF+SDF<sub>1</sub> group were higher than other groups. Microscopic analysis showed that the ICRS score of the PRP group was significantly lower than other groups. Immunofluorescent staining for collagen II demonstrated a remarkable distribution of type II collagen in the Gel+SDF<sub>1</sub>, PRP+SDF<sub>1</sub> and PRF+SDF<sub>1</sub> groups compared with other groups. Real-time PCR analysis revealed that mRNA expression of SOX9 and aggrecan were significantly greater in the PRF+SDF<sub>1</sub>, PRP+SDF<sub>1</sub>, Gel+SDF<sub>1</sub> and PRF groups than the control group (P<0.05).

**Conclusion:** Our results indicate that implantation of PRF scaffold containing SDF1 led to the greatest evaluation scores of full-thickness lesions in rabbits.

Please cite this article as: Bahmanpour S, Ghasemi M, Sadeghi-Naini M, Ragerdi Kashani I. Effects of Platelet-Rich Plasma & Platelet-Rich Fibrin with and without Stromal Cell-Derived Factor-1 on Repairing Full-Thickness Cartilage Defects in Knees of Rabbits. Iran J Med Sci. 2016;41(6):507-517.

**Keywords** • Platelet-rich plasma • Platelet rich fibrin • Chemokine CXCL12 • Cartilage • Knee

## Introduction

Adult articular cartilage has insufficient ability to repair after either erosion or damage and it is unlikely to be reformed to normal condition once it has been impaired.<sup>1</sup> Functional repair of articular

cartilage defects encountered a major challenge to cartilage tissue engineering.

Articular cartilage repair has been based on finding and resolving the primary reason for the injury, but it has been recognized that only treating the damage site is not adequate to gain good long-term functionality.<sup>2</sup> The matrix environment of hyaline cartilage is different from that of subchondral bone in biological, physical and chemical characteristics.<sup>3</sup> This led to the proposal that in order to gain positive results in tissue repair, the 'microenvironment' in which the repair process takes place should be retouched, such as those elements that play a pro-angiogenic, conductive, cell differentiation and chemotactic role.<sup>2</sup> This tendency has been the reason behind a growth in using genetic and tissue engineering in the examination of articular cartilage repair.<sup>4</sup>

A number of natural and artificial biomaterials have been used as scaffolds for cartilage reformation, but their biological effectiveness as well as safety remains unresolved. Therefore, scaffold biomaterials extracted from the patients' own body have long been a tempting choice.<sup>5</sup> Platelet-rich plasma (PRP), which can be easily harvested from autologous blood, has been used in clinical experiments for decades.<sup>6</sup> PRP is rich in growth factors, containing those that help the proliferation of chondrogenic cells and discharging of cartilaginous matrix, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF- $\beta$ ), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and insulin-like growth factor (IGF).<sup>6,7</sup>

A new member in platelet concentrated products is platelet-rich fibrin (PRF), which can be prepared easily without biochemical blood handling. PRF is similar to PRP except in biochemical architectures because of slow polymerization during PRF preparation.<sup>8</sup> The PRF fibrin network is similar to the natural one. Such a network leads to a more competent cell mobilization and repopulation and consequently healing.<sup>8</sup> Stromal cell-derived factor-1 (SDF<sub>1</sub>), a chemokine factor expressed by BMSCs, regulates the trafficking, homing and migration of stem cell by mediating chemokinesis and chemotaxis into the injury site.<sup>9</sup> Bone marrow, which is adjoining the chondro-osseous junction, discharges SDF<sub>1</sub>.<sup>10</sup>

We believe that autologous PRP and PRF could be used alone as a 3-D scaffold capable of cartilage tissue regeneration because of releasing endogenous growth factors. SDF<sub>1</sub> stimulates cartilage mesenchymal stem cells (C-MSCs), synovial membrane mesenchymal

stem cells (SM-MSCs), and bone marrow stromal cells (BMSCs) to migrate to the subchondral bone in full-thickness defects to make cartilage. The aim of the study was to evaluate tissue repair along with PRP and PRF or without treatment. The secondary objective was to evaluate the efficacy of PRP and PRF used with SDF<sub>1</sub>.

## Materials and Methods

### PRP Gel Preparation

Blood samples were taken from the hearts of six rabbits. The 9 mL of whole blood collected in a tube containing 1 mL an anticoagulant (3.8% sodium citrate solution) was centrifuged for 5 minutes at 2,000 rpm and the supernatant was placed in a new tube. The supernatant was centrifuged for 10 minutes at 3,000 rpm. The plasma at the top was discarded and the remaining 1 mL of liquid PRP precipitate was collected. After mixing PRP (200  $\mu$ L/defect) and 40 mmol/L calcium chloride (CaCl<sub>2</sub>), a gel was formed. The PRP gel bonded to the adjacent cartilage about 10 minutes after implantation.<sup>11</sup>

### PRF Preparation

The 10 mL blood samples collected from the hearts of six rabbits without anticoagulant were immediately centrifuged at 3,000 rpm for 10 minutes. In the middle of the tube, a fibrin clot was formed between supernatant acellular plasma and the lower red corpuscles.<sup>8</sup>

### SDF<sub>1</sub> Incubation

Before transplantation, 600  $\mu$ L of rhSDF<sub>1</sub> alpha (120 ng/mL Peprotech) was incubated with PRP gel, PRF and gelatin separately for 2 hours at 37°C.<sup>12</sup>

### Study Design and Animal Experimentation

A total of 18 adult male white Japanese rabbits (weighing 2.5-3.0 kg) were used in this study. The animals were housed in metal wire cages, in a temperature-controlled room under a 12:12 h light-dark cycle at 22–24°C and 50–60% relative humidity. They were fed ad libitum with standard laboratory chow and tap water. Animal interventional experiments were carried out at Animal Experiments of Tehran University of Medical Sciences under the rules and regulation of the Animal Care and Use Committee of Shiraz University School of Medicine.

The 36 bilateral knees of the 18 rabbits were randomly divided into 6 experimental groups of 6 knees per group. Each animal was sedated by intramuscular injection of ketamine hydrochloride 60 mg/kg and xylazine 6 mg/kg. In sterile conditions, a medial para-patellar arthrotomy

was made in both knees. A full-thickness cylindrical cartilage defect of 4 mm in diameter and 3 mm in depth was created in the patellar groove using a standard size stainless biopsy punch. The joints were thoroughly rinsed with sterile saline solution before transplantation. In group I, the defects were left unfilled (control group). In group II, the defects were filled with platelet-rich plasma only (PRP group). In group III, the defects were filled with platelet-rich fibrin only (PRF group). In group IV, the defects were filled with gelatin containing stromal cell-derived factor-1 (Gel+SDF<sub>1</sub> group). In group V, the defects were filled with platelet-rich plasma containing stromal cell-derived factor-1 (PRP+SDF<sub>1</sub> group). In group VI, the defects were filled with platelet-rich fibrin containing stromal cell-derived factor-1 (PRF+SDF<sub>1</sub> group) in a randomized manner. The rabbits were accommodated in separate cages and allowed unrestricted activity after surgery.<sup>9,11,12</sup>

**Gross Morphology**

Four weeks after implantation, the rabbits were sacrificed with an overdose of anesthesia. The distal parts of the femurs were excised, photographed and graded for cartilage repair, according to the international cartilage repair society score (ICRS) macroscopic assessment scores<sup>11</sup> (table 1).

**Histological and Immunohistochemical Analysis**

After gross evaluation, samples were fixed in 4% paraformaldehyde for 7 days, decalcified in 10% EDTA for 3 weeks and then embedded in paraffin and cut perpendicularly into 5 µm sections. Then, the sections were stained for general histology with hematoxylin and eosin (H&E) and with Toluidine blue to estimate the cartilaginous matrix distribution. The re-created tissue was scored blindly according to the ICRS scale<sup>9,13</sup> (table 2).

Immunofluorescent analysis was performed using a monoclonal antibody against Collagen II (Cat number: MA1-37493). The sections were deparaffinized by xylene and rehydrated by decreased concentrations of ethanol solutions. Heat-induced epitope retrieval was done in citrate buffer (sodium citrate, 10 mM; pH 6.0) in a pressure cooker for 4 minutes at full pressure. Subsequent to cooling, endogenous peroxidase was blocked using a 3% hydrogen peroxide solution for 20 minutes. The slides were then washed with phosphate buffer solution (10 mM; pH 7.4) and incubated with primary antibodies overnight at 4°C. The slides were washed again three times with PBS and subsequently incubated with an FITC-conjugated secondary

**Table 1: ICRS macroscopic evaluation of cartilage repair**

Categories	Score
<b>Degree of defect repair</b>	
In level with surrounding cartilage	4
75% repair of defect depth	3
50% repair of defect depth	2
25% repair of defect depth	1
0% repair of defect depth	0
<b>Integration to border zone</b>	
Complete integration with surrounding cartilage	4
Demarcating border <1 mm	3
3/4 of graft integrated, 1/4 with a notable border >1 mm width	2
1/2 of graft integrated with surrounding cartilage, 1/2 with a notable border >1 mm	1
From no contact to 1/4 of graft integrated with surrounding cartilage	0
<b>Macroscopic appearance</b>	
Intact smooth surface	4
Fibrillated surface	3
Small, scattered fissures or cracks	2
Several, small or few but large fissures	1
Total degeneration of grafted area	0
<b>Overall repair assessment</b>	
Grade I: normal	12
Grade II: nearly normal	11-8
Grade III: abnormal	7-4
Grade IV: severely abnormal	3-1

antibody (Cat number: 11555100) according to the respective manufacturer's instructions. The slides were washed three times with PBS and then covered with cover slips. Finally, samples were analyzed by florescent microscope.<sup>9</sup>

**Real-time PCR Analysis**

Total RNA was extracted from the regenerated tissues in the defect area. Total RNA extraction was performed using RNX-plus kit (CinnaGen) according to the protocol. The RNA samples were resuspended in 30 µL of nuclease-free water. The concentration and quantification of total RNA were measured with spectrophotometer, with the OD260/OD280 ratio of all RNA samples 1.9–2.0 and OD260/OD230 ratio up to 2. The first strand cDNA was synthesized with the first-strand cDNA synthesis kit (Bioneer kit, K-2101). For each reaction, 1 µg (1 µL) RNA was used for reverse transcription, in a mixture of 20 pmoles (1 µL) random primer, and 18 µL DEPC-D.W with a final volume of 20 µL. The mixture was incubated at 15°C for 1 min, 50°C for 60 minutes and heated at 95°C for 5 minutes to terminate the reaction. The cDNA was subsequently stored at -20°C. qPCR was performed in a volume 1 µL of primer and 1 µL from template also add 3 µL

**Table 2:** ICRS visual histological assessment scale

Feature	Score
I. Surface	
Smooth/continuous	3
Discontinuities/irregularities	0
II. Matrix	
Hyaline	3
Mixture: Hyaline/fibrocartilage	2
Fibrocartilage	1
Fibrous tissue	0
III. Cell distribution	
Columnar	3
Mixed/columnar-clusters	2
Clusters	1
Individual cells/disorganized	0
IV. Cell population viability	
Predominantly viable	3
Partially viable	1
<10% viable	0
V. Subchondral Bone	
Norma	3
Increased remodeling	2
Bone necrosis/granulation tissue	1
Detached/fracture/callus at base	0
VI. Cartilage mineralization (calcified cartilage)	
Normal	3
Abnormal/inappropriate location	0

DEPC.D.W with 5  $\mu$ L Master Mix (AccuPower® 2X GreenStar™ qPCR Master Mix, Bioneer kit). Real-time PCR was done by ABI (Applied Biosystems). All PCR reactions were in the following condition: initial 95°C for 15 minutes, followed by 35 cycles at 95°C for 15 s and 60°C for 30 s. After completing the qPCR, their products run on agarose gels for quality check and real-time data analyzed by Livak rules.<sup>14</sup> Beta actin was used for the internal control. The PCR primers for each gene are displayed below. Each sample was tested in duplicate. The sequences of primers used in real-time PCR analyses were as follows: Aggrecan forward GAGACCAAGTCCTCAAGCCC; reverse CTCTGTCTCCTGGCAGGTTT. Sox9 forward GCTCCGACACCGAGAATACA; reverse TTGACGTGGGGCTTGTCTT.<sup>13</sup>

#### Data Analysis

Data are expressed as mean $\pm$ SD (standard deviation). Significance was considered at the 0.05 level. Outcome variables were macroscopic evaluation scores, histological scores, and genes expression. A comparison between groups for each outcome was carried out with the Kruskal-Wallis test. Bonferroni post hoc tests were used for the comparison analysis of each two groups.

Data were analyzed using SPSS (version 20) software.

## Results

#### Macroscopic Observation

Four weeks after surgery, the regenerated regions of all groups were studied. The macroscopic evaluation of the treated areas in the PRF+SDF<sub>1</sub> group revealed complete repair and integrated well with the surrounding cartilage as compared with other groups (figure 1). A post-hoc analysis showed that ICRS scores of all experimental groups were significantly higher than those in the control group and the scores of the PRF+SDF<sub>1</sub> group were greater than those of the other groups (P<0.001) (figure 2).

#### Microscopic Observation

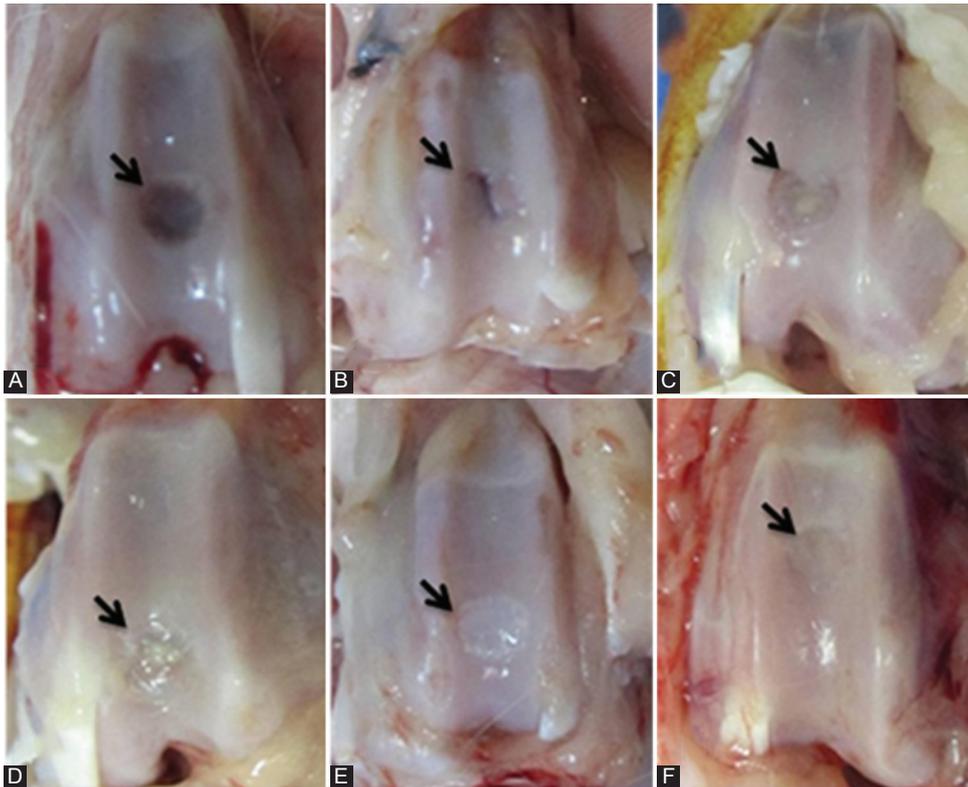
Four weeks after surgery, the regenerated regions were evaluated by H&E and Toluidine blue staining. The regenerated regions of the PRF+SDF<sub>1</sub> group were entirely filled by the repaired tissue and showed better results than the other groups (figures 3 and 4). A post-hoc analysis showed that ICRS scores of all groups, except for the PRP group, were significantly higher than the control group (P<0.001). The scores of the PRF+SDF<sub>1</sub> group were greater than the other groups, but were not significant when compared with the Gel+SDF<sub>1</sub> and PRP+SDF<sub>1</sub> groups. The scores of the PRP group were significantly lower than the other groups except for the PRF group, which was not significant (figure 2). The qualitative study of immunofluorescent staining of collagen II demonstrated that neo-cartilages were minimally positive in the control, PRP, and PRF groups. In contrast, the Gel+SDF<sub>1</sub>, PRP+SDF<sub>1</sub>, and PRF+SDF<sub>1</sub> groups exhibited a remarkable distribution of type II collagen (figure 5).

#### Real-Time PCR Analysis

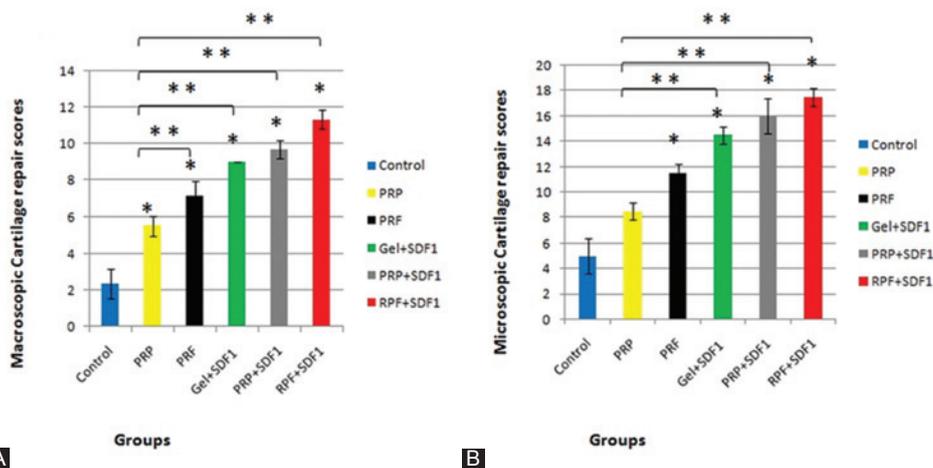
Four weeks after surgery, a real-time PCR analysis revealed that the mRNA expression of SOX9 was significantly greater in the regenerated tissue of all groups than the untreated control group (P<0.001). The level of gene expression of aggrecan in all groups was significantly higher than the control group, except for the PRP group that was not significant (P<0.001) (figures 6 and 7).

## Discussion

This study considered the effects of PRP and PRF with or without SDF<sub>1</sub> in full-thickness lesions in rabbits' knees. A literature review indicated



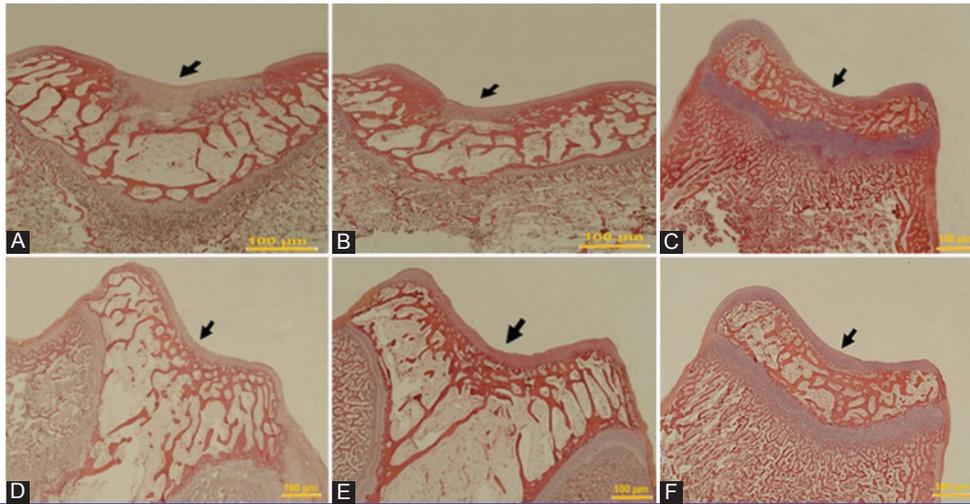
**Figure 1:** Gross appearance of defects in the trochlear groove at four weeks after surgery, respectively. A) Control group: Transplantation was not performed. The defect is partially vacant and clearly noticeable from the surrounding cartilage. B) PRP group: Healing tissue is thinner than normal surrounding cartilage and shows a large fissure. C) PRF group: Repaired tissue covers defect with a minor concavity. D) Gel+SDF<sub>1</sub> group: Repaired tissue covers the defect almost completely, but is thin and distinguishable from the surrounding cartilage. E) PRP+SDF<sub>1</sub> group: Healing tissue covers defect with smooth white tissue and distinguishable from the surrounding cartilage. F) PRF+SDF<sub>1</sub> group: Repaired tissue covers the defect completely and no obvious margin was notable.



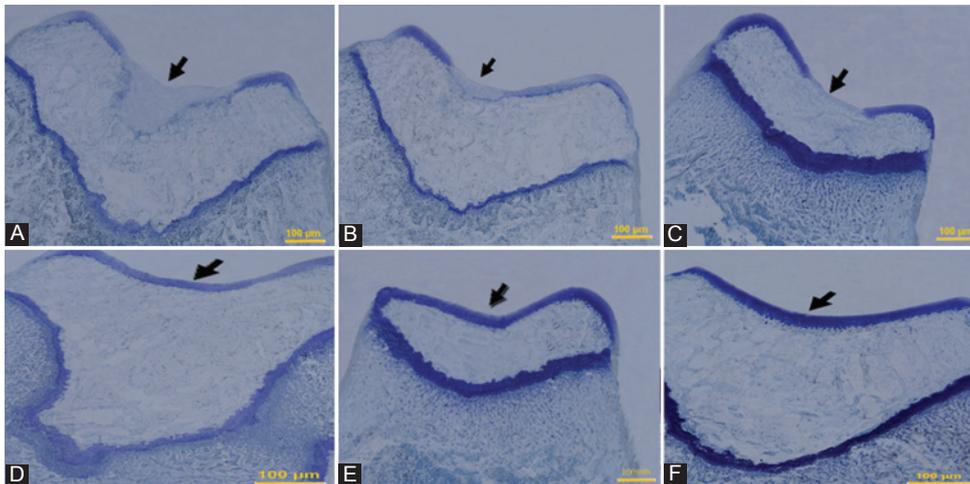
**Figure 2:** A) ICRS macroscopic and B) ICRS microscopic scores in the regenerated cartilage of defects in the trochlear groove at four weeks postoperatively. Data are presented as mean±SD. \*Significant difference with non-treated control ( $P<0.05$ ); \*\*Significant difference with RPP group ( $P<0.05$ ).

that the effects of PRF+SDF<sub>1</sub> and PRP+SDF<sub>1</sub> were not studied so far. We demonstrated that the implantation of PRF+SDF<sub>1</sub> led to the greatest yield scores of full-thickness lesions in rabbits' knee. Therefore, it can be concluded that the scaffolds containing SDF<sub>1</sub> have a better result on cartilage repair. Zhang W. et al.<sup>12</sup> implanted a combination of collagen I scaffold to mimic the

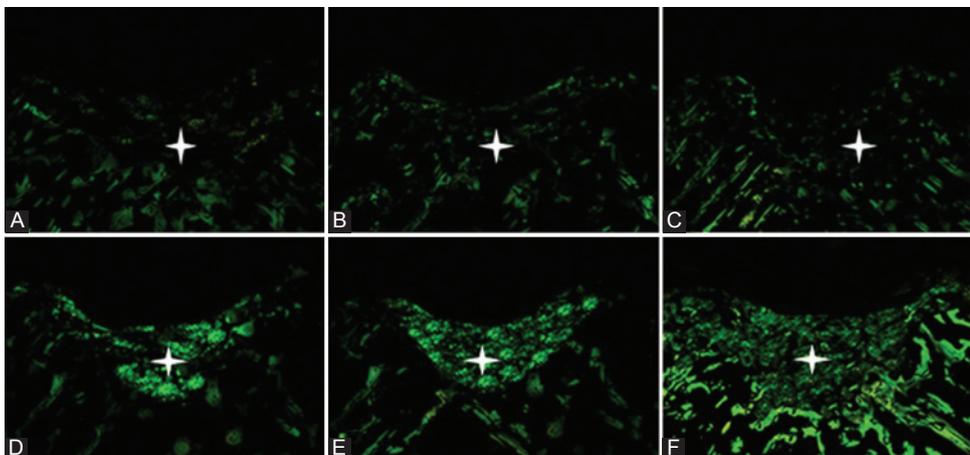
subchondral bone matrix environment containing SDF<sub>1</sub> in order to repair partial-thickness defects in rabbits' knee. The results demonstrated that collagen I scaffold containing SDF<sub>1</sub> improved the repairing process by a better support for C-MSCs and SM-MSCs migration and adhesion six weeks after transplantation. Some researchers used ultra-purified alginate gel containing SDF<sub>1</sub> to



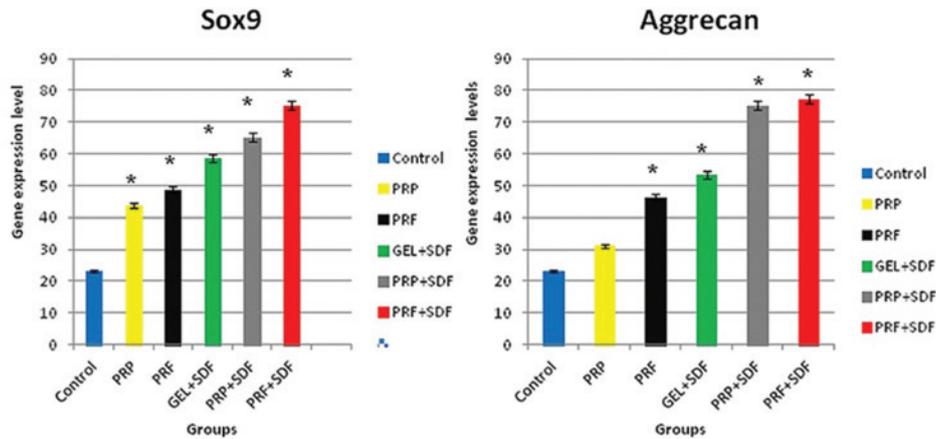
**Figure 3:** The microscopic appearance of H&E staining of defects in the trochlear groove at four weeks after surgery, respectively (Original magnification=200×). A) Control group: The defect is concave and a noticeable thin layer of non-cartilaginous tissue. B) PRP group: The defect is filled by fibrous tissue with a crack. C) PRF group: The defect is covered by fibrous tissue. D) Gel+SDF<sub>1</sub> group and (E) PRP+SDF<sub>1</sub> group: The defects in both groups are filled with a repaired tissue, which are thinner than the normal surrounding cartilage. F) PRF+SDF<sub>1</sub> group: The defect is entirely filled by the repaired tissue, resembling the healthy cartilage surrounding the tissue.



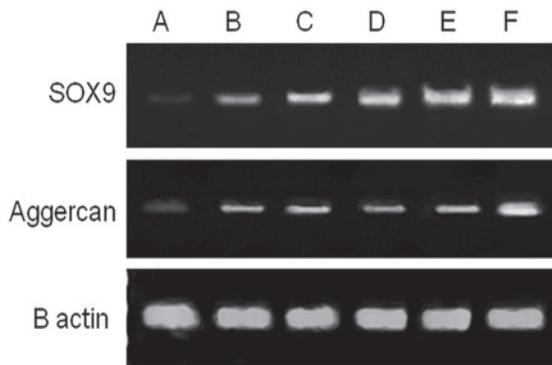
**Figure 4:** Histological appearance of Toluidine blue staining of defects in the trochlear groove at four weeks after surgery, respectively (Original magnification=200×). A) Control group: The defect is filled by no cartilage matrix. B) PRP group, and C) PRF group: The defects in both groups have poor staining fibrocartilaginous tissues and poorly organized ECM. D) Gel+SDF<sub>1</sub> group, and E) PRP+SDF<sub>1</sub> group: The repaired tissues show a good staining in both groups. F) PRF+SDF<sub>1</sub> group: The defect has an intense staining.



**Figure 5:** Immunofluorescent staining appearance of defects in trochlear groove at four weeks after surgery. A) Control group, B) PRP group, C) PRF group, D) Gel+SDF<sub>1</sub> group, E) PRP+SDF<sub>1</sub> group, and F) PRF+SDF<sub>1</sub> group. The neo-cartilages are minimally positive in A, B and C. The neo-cartilages are positive in D and E. A remarkable distribution of type II collagen is seen in F.



**Figure 6:** The amount of gene expression of SOX9 and aggrecan in the regenerated cartilage of defects in the trochlear groove at four weeks postoperatively. Data are presented as mean±SD. \*Significant difference with non-treated control (P<0.05).



**Figure 7:** The quality checking of RT-PCR for SOX9 and aggrecan from the regenerated cartilage of defects in the trochlear groove at four weeks after surgery. Lane A: Control group where transplantation was not performed; Lane B: PRP group; Lane C: PRF group; Lane D: Gel+SDF<sub>1</sub> group; Lane E: PRP+SDF<sub>1</sub> group, and Lane F: PRF+SDF<sub>1</sub> group.

improve osteochondral lesions in rabbits' knee. According to their studies, the repairing effect of osteochondral defects is elevated, because SDF<sub>1</sub> increases the migration of host cells (mainly BMSCs) to the site of injury, and this makes SDF<sub>1</sub> as a potential factor in cartilage repair.<sup>15</sup> Wei L. et al. used SDF<sub>1</sub> to induce chondrocyte hypertrophy. They studied the length of the hypertrophic zone by immunohistochemical analysis of tibia cartilaginous growth plates of chicken embryos, which were incubated with SDF<sub>1</sub> for 2, 4, and 6 days in organ culture. The results showed the length of the hypertrophic zone was significantly elevated after 4 and 6 days.<sup>3</sup> In injured tissue with the permissive matrix environment, endogenous stem cells can participate in corrective process. Some studies have introduced populations of stem/progenitor cells in the synovial membrane<sup>16,17</sup> and articular cartilage,<sup>18-20</sup> which show characteristic of stem cells and have the potential for cartilage

repair.<sup>21,22</sup> The findings of the above studies are in agreement with our results that show SDF<sub>1</sub> can facilitate the migration of C-MSCs and SM-MSCs to full-thickness defect sites. Although SDF<sub>1</sub> has been notified to promote inflammatory cell migration,<sup>23</sup> we could not find inflammatory cells in the newly formed tissue in PRF, PRP and Gel containing SDF<sub>1</sub> groups. In parallel with our data, a study on tendon repair by exogenous SDF<sub>1</sub> showed improvement of injury site and diminishing accumulation of inflammatory cells.<sup>9</sup> In PRF, the fibrin formation meshes, which are similar to biochemical architectures of the natural one,<sup>8</sup> can trap a significant amount of circulating cytokines. This is what renders PRF as a distinctive treatment in injury repair. This method increases the lifespan of these cytokines (as a long-term effect), without which, it will be only released and used at the time of the primary phase of matrix remodeling. As a result, when the cells begin extracellular matrix remodeling to reconstruct the injured site, the cytokines will be available in the affected area for the required longer period. Cytokines are important factors in the delicate balance of tissue homeostasis.<sup>9</sup>

In a pilot study, Haleem A.M. et al. showed that all patients with osteochondral defects who received BM-MSCs transplanted on platelet-rich fibrin glue (PR-FG) had a significant improvement in their functional knee scores and MRI findings after 6 months and remained stable 12 months post operation.<sup>1</sup> In agreement with this clinical study, Choukroun J. et al. revealed that filling a tooth socket by PRF can enhance the healing process without any complication.<sup>24</sup> In a supplementary study, they reported that adding PRF to bone allograft in sinus elevation surgery can lead to 4-month reduction in healing time by increasing the speed of bone allograft

maturation.<sup>25</sup> However, in contrast to our results, an in-vivo study by Shao X.X. et al. on osteochondral defects in rabbits' knee showed that BMSCs seeded in fibrin glue matrix forms a poor-quality cartilage-like tissue.<sup>26</sup> It can be concluded that the reason for varying results can be due to the differences in biomechanical conditions of defect types. Kelly D.J. et al. reported that differentiation of bone-marrow derived stem cells can be affected by mechanical signals.<sup>27</sup> Moreover, suitable recurring compressive stress significantly increases chondrocyte proliferation in addition to aggrecan and collagen synthesis by chondrocytes.<sup>28</sup> Some researchers reported that cell adhesion and migration can facilitate by some elements such as different cell type and substrate stiffness.<sup>29,30</sup> Most importantly, in a separate study, Wang H. et al. had compared the amount of bounded bovine chondrocytes on various surfaces of middle deep zone bone or calcified cartilage or hyaline cartilage. They reported that different contact surface stiffness give different ability to cells for migration and attachment.<sup>31</sup>

Opinions about autologous PRP usage in terms of its composition is controversial.<sup>32,33</sup> Activated PRP can be used in different forms like gel as a scaffold, liquid form injection or can be infiltrated in the tissue thickness.<sup>34</sup> The selection of each form relies upon the type of tissue and the existing problem.<sup>35,36</sup> By degradation of the fibrin skeleton of PRP, it can release endogenous growth factors without modifying genes or cytokine delivery technologies. Growth factors play a significant role in tissue repair, especially in the regenerative process of tissues with low cellular density and vascularization, such as cartilage.<sup>6,37,38</sup> A study showed that if PRP was applied in full-thickness chondral lesions, the signs of healing will be observed, but it did not show healthy or functional behavior.<sup>4</sup> Wu W. et al. reported cartilage formation into the PRP scaffold after implanting PRP gel as a carrier for cultured autologous chondrocytes in the subcutaneous tissue of rabbits' knees.<sup>39</sup> Another study suggested that transplantation of PRP gel along with synovial membrane derived mesenchymal stem cells (SDSCs) can facilitate the repair of osteochondral lesions in rabbits' knees.<sup>11</sup> Perhaps the underlying reason for different results is various compositions of this plasma fraction in different species, including rabbits.<sup>32,34,40</sup>

It should be noted that our study had a limitation. PRF was harvested from an autologous blood sample that had a limited volume and PRF tissue banks were not achievable.

## Conclusion

In the present study, in addition to using PRP and PRF as scaffolds for cell seeding, we used a mixture of SDF1 and PRP and PRF scaffolds to enhance the migration of host cells. In our research, the effects of PRP and PRF alone on the full-thickness defects were also studied separately. We found that the added SDF<sub>1</sub> stimulated cell or the combination of PRP, PRF, and SDF<sub>1</sub> stimulated the observed improvement.

## Acknowledgment

This work was supported by Shiraz University of Medical Sciences and Tehran University of Medical Sciences. The authors would like to thank Maryam Hatami for her assistance in developing the animal model. The present article was extracted from the thesis written by Maryam Ghasemi and was financially supported by Shiraz University of Medical Sciences, under grant number 93-7010.

**Conflict of Interest:** None declared.

## References

1. Haleem AM, Singergy AA, Sabry D, Atta HM, Rashed LA, Chu CR, et al. The Clinical Use of Human Culture-Expanded Autologous Bone Marrow Mesenchymal Stem Cells Transplanted on Platelet-Rich Fibrin Glue in the Treatment of Articular Cartilage Defects: A Pilot Study and Preliminary Results. *Cartilage*. 2010;1:253-61. doi: 10.1177/1947603510366027. PubMed PMID: 21170288; PubMed Central PMCID: PMC3002255.
2. Alford JW, Cole BJ. Cartilage restoration, part 1: basic science, historical perspective, patient evaluation, and treatment options. *Am J Sports Med*. 2005;33:295-306. doi: 10.1177/0363546504273510. PubMed PMID: 15701618.
3. Wei L, Kanbe K, Lee M, Wei X, Pei M, Sun X, et al. Stimulation of chondrocyte hypertrophy by chemokine stromal cell-derived factor 1 in the chondro-osseous junction during endochondral bone formation. *Dev Biol*. 2010;341:236-45. doi: 10.1016/j.ydbio.2010.02.033. PubMed PMID: 20206617; PubMed Central PMCID: PMC2862458.
4. Serra CI, Soler C, Carrillo JM, Sopena JJ, Redondo JI, Cugat R. Effect of autologous platelet-rich plasma on the repair of full-thickness articular defects in rabbits.

- Knee Surg Sports Traumatol Arthrosc. 2013;21:1730-6. doi: 10.1007/s00167-012-2141-0. PubMed PMID: 22918398.
5. Ahmed TA, Giulivi A, Griffith M, Hincke M. Fibrin glues in combination with mesenchymal stem cells to develop a tissue-engineered cartilage substitute. *Tissue Eng Part A*. 2011;17:323-35. doi: 10.1089/ten.TEA.2009.0773. PubMed PMID: 20799906.
  6. Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ. The role of growth factors in cartilage repair. *Clin Orthop Relat Res*. 2011;469:2706-15. doi: 10.1007/s11999-011-1857-3. PubMed PMID: 21403984; PubMed Central PMCID: PMC3171543.
  7. Sun Y, Feng Y, Zhang CQ, Chen SB, Cheng XG. The regenerative effect of platelet-rich plasma on healing in large osteochondral defects. *Int Orthop*. 2010;34:589-97. doi: 10.1007/s00264-009-0793-2. PubMed PMID: 19434411; PubMed Central PMCID: PMC2903145.
  8. 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:e37-44. doi: 10.1016/j.tripleo.2005.07.008. PubMed PMID: 16504849.
  9. Shen W, Chen X, Chen J, Yin Z, Heng BC, Chen W, et al. The effect of incorporation of exogenous stromal cell-derived factor-1 alpha within a knitted silk-collagen sponge scaffold on tendon regeneration. *Biomaterials*. 2010;31:7239-49. doi: 10.1016/j.biomaterials.2010.05.040. PubMed PMID: 20615544.
  10. Fermas S, Gonnet F, Sutton A, Charnaux N, Mulloy B, Du Y, et al. Sulfated oligosaccharides (heparin and fucoidan) binding and dimerization of stromal cell-derived factor-1 (SDF-1/CXCL 12) are coupled as evidenced by affinity CE-MS analysis. *Glycobiology*. 2008;18:1054-64. doi: 10.1093/glycob/cwn088. PubMed PMID: 18796646.
  11. Lee JC, Min HJ, Park HJ, Lee S, Seong SC, Lee MC. Synovial membrane-derived mesenchymal stem cells supported by platelet-rich plasma can repair osteochondral defects in a rabbit model. *Arthroscopy*. 2013;29:1034-46. doi: 10.1016/j.arthro.2013.02.026. PubMed PMID: 23726109.
  12. Zhang W, Chen J, Tao J, Jiang Y, Hu C, Huang L, et al. The use of type 1 collagen scaffold containing stromal cell-derived factor-1 to create a matrix environment conducive to partial-thickness cartilage defects repair. *Biomaterials*. 2013;34:713-23. doi: 10.1016/j.biomaterials.2012.10.027. PubMed PMID: 23107295.
  13. Matsuda H, Kitamura N, Kurokawa T, Arakaki K, Gong JP, Kanaya F, et al. Influence of the gel thickness on in vivo hyaline cartilage regeneration induced by double-network gel implanted at the bottom of a large osteochondral defect: short-term results. *BMC Musculoskelet Disord*. 2013;14:50. doi: 10.1186/1471-2474-14-50. PubMed PMID: 23369101; PubMed Central PMCID: PMC3564809.
  14. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001;25:402-8. doi: 10.1006/meth.2001.1262. PubMed PMID: 11846609.
  15. Sukegawa A, Iwasaki N, Kasahara Y, Onodera T, Igarashi T, Minami A. Repair of rabbit osteochondral defects by an acellular technique with an ultrapurified alginate gel containing stromal cell-derived factor-1. *Tissue Eng Part A*. 2012;18:934-45. doi: 10.1089/ten.TEA.2011.0380. PubMed PMID: 22097896.
  16. Fickert S, Fiedler J, Brenner RE. Identification, quantification and isolation of mesenchymal progenitor cells from osteoarthritic synovium by fluorescence automated cell sorting. *Osteoarthritis Cartilage*. 2003;11:790-800. doi: 10.1016/S1063-4584(03)00167-5. PubMed PMID: 14609532.
  17. De Bari C, Dell'Accio F, Vandenabeele F, Vermeesch JR, Raymackers JM, Luyten FP. Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane. *J Cell Biol*. 2003;160:909-18. doi: 10.1083/jcb.200212064. PubMed PMID: 12629053; PubMed Central PMCID: PMC2173757.
  18. Williams R, Khan IM, Richardson K, Nelson L, McCarthy HE, Anabalsi T, et al. Identification and clonal characterisation of a progenitor cell sub-population in normal human articular cartilage. *PLoS One*. 2010;5:e13246. doi: 10.1371/journal.pone.0013246. PubMed PMID: 20976230; PubMed Central PMCID: PMC2954799.
  19. Pretzel D, Linss S, Rochler S, Endres M, Kaps C, Alsalameh S, et al. Relative percentage and zonal distribution of mesenchymal progenitor cells in human osteoarthritic and normal cartilage. *Arthritis Res Ther*. 2011;13:R64. doi: 10.1186/

- ar3320. PubMed PMID: 21496249; PubMed Central PMCID: PMC3132059.
20. Koelling S, Kruegel J, Imer M, Path JR, Sadowski B, Miro X, et al. Migratory chondrogenic progenitor cells from repair tissue during the later stages of human osteoarthritis. *Cell Stem Cell*. 2009;4:324-35. doi: 10.1016/j.stem.2009.01.015. PubMed PMID: 19341622.
  21. Shimomura K, Ando W, Tateishi K, Nansai R, Fujie H, Hart DA, et al. The influence of skeletal maturity on allogenic synovial mesenchymal stem cell-based repair of cartilage in a large animal model. *Biomaterials*. 2010;31:8004-11. doi: 10.1016/j.biomaterials.2010.07.017. PubMed PMID: 20674010.
  22. Sekiya I, Muneta T, Koga H, Nimura A, Morito T, Shimaya M, et al. [Articular cartilage regeneration with synovial mesenchymal stem cells]. *Clin Calcium*. 2011;21:879-89. doi: CliCa1106879889. PubMed PMID: 21628803.
  23. de Vries-van Melle ML, Mandl EW, Kops N, Koevoet WJ, Verhaar JA, van Osch GJ. An osteochondral culture model to study mechanisms involved in articular cartilage repair. *Tissue Eng Part C Methods*. 2012;18:45-53. doi: 10.1089/ten.TEC.2011.0339. PubMed PMID: 21875392; PubMed Central PMCID: PMC3245721.
  24. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101:e56-60. doi: 10.1016/j.tripleo.2005.07.011. PubMed PMID: 16504852.
  25. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101:299-303. doi: 10.1016/j.tripleo.2005.07.012. PubMed PMID: 16504861.
  26. Shao XX, Huttmacher DW, Ho ST, Goh JC, Lee EH. Evaluation of a hybrid scaffold/cell construct in repair of high-load-bearing osteochondral defects in rabbits. *Biomaterials*. 2006;27:1071-80. doi: 10.1016/j.biomaterials.2005.07.040. PubMed PMID: 16129483.
  27. Kelly DJ, Jacobs CR. The role of mechanical signals in regulating chondrogenesis and osteogenesis of mesenchymal stem cells. *Birth Defects Res C Embryo Today*. 2010;90:75-85. doi: 10.1002/bdrc.20173. PubMed PMID: 20301221.
  28. Smith RL, Carter DR, Schurman DJ. Pressure and shear differentially alter human articular chondrocyte metabolism: a review. *Clin Orthop Relat Res*. 2004:S89-95. PubMed PMID: 15480081.
  29. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. *Science*. 2005;310:1139-43. doi: 10.1126/science.1116995. PubMed PMID: 16293750.
  30. Lo CM, Wang HB, Dembo M, Wang YL. Cell movement is guided by the rigidity of the substrate. *Biophys J*. 2000;79:144-52. doi: 10.1016/S0006-3495(00)76279-5. PubMed PMID: 10866943; PubMed Central PMCID: PMC1300921.
  31. Wang H, Kandel RA. Chondrocytes attach to hyaline or calcified cartilage and bone. *Osteoarthritis Cartilage*. 2004;12:56-64. PubMed PMID: 14697683.
  32. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-rich plasma: from basic science to clinical applications. *Am J Sports Med*. 2009;37:2259-72. doi: 10.1177/0363546509349921. PubMed PMID: 19875361.
  33. Milano G, Sanna Passino E, Deriu L, Careddu G, Manunta L, Manunta A, et al. The effect of platelet rich plasma combined with microfractures on the treatment of chondral defects: an experimental study in a sheep model. *Osteoarthritis Cartilage*. 2010;18:971-80. doi: 10.1016/j.joca.2010.03.013. PubMed PMID: 20433936.
  34. Anitua E, Sanchez M, Orive G, Andia I. The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. *Biomaterials*. 2007;28:4551-60. doi: 10.1016/j.biomaterials.2007.06.037. PubMed PMID: 17659771.
  35. Azofra SM, Anitua E, Andía I, Padilla S, Mújica I. Use of autologous plasma rich in growth factors in the treatment of a large, non-traumatic avulsion of articular cartilage a case report. *Med Sci Sport Exer*. 2002.
  36. Mizuta H, Kudo S, Nakamura E, Otsuka Y, Takagi K, Hiraki Y. Active proliferation of mesenchymal cells prior to the chondrogenic repair response in rabbit full-thickness defects of articular cartilage. *Osteoarthritis Cartilage*. 2004;12:586-96. doi: 10.1016/j.joca.2004.04.008. PubMed PMID: 15219574.
  37. Freyria AM, Mallein-Gerin F. Chondrocytes or adult stem cells for cartilage repair:

- the indisputable role of growth factors. *Injury*. 2012;43:259-65. doi: 10.1016/j.injury.2011.05.035. PubMed PMID: 21696723.
38. Vinatier C, Mrugala D, Jorgensen C, Guicheux J, Noel D. Cartilage engineering: a crucial combination of cells, biomaterials and biofactors. *Trends Biotechnol*. 2009;27:307-14. doi: 10.1016/j.tibtech.2009.02.005. PubMed PMID: 19329205.
39. Wu W, Chen F, Liu Y, Ma Q, Mao T. Autologous injectable tissue-engineered cartilage by using platelet-rich plasma: experimental study in a rabbit model. *J Oral Maxillofac Surg*. 2007;65:1951-7. doi: 10.1016/j.joms.2006.11.044. PubMed PMID: 17884521.
40. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemost*. 2004;91:4-15. doi: 10.1160/TH03-07-0440. PubMed PMID: 14691563.