Indian Journal of Orthopaedics (2020) 54:720–725
https://doi.org/10.1007/s43465-020-00067-w

ORIGINAL ARTICLE

Treatment of Full-Thickness Cartilage Defects with Pedunculated and Free Synovial Grafts: A Comparative Study in an Animal Model

Bahtiyar Haberal[1] · Orcun Sahin[1] · Aysen Terzi[2] · Ekin Kaya Simsek[1] · Ates Mahmuti[1] · İsmail Cengiz Tuncay[1]

Published online: 27 April 2020

Abstract

Aims and Objectives The purpose of this study was to compare the potential effects of pedunculated and free synovial grafts in the repair of full-thickness articular cartilage defects on an animal model with histological and immunohistochemical analysis.

Materials and Methods A comparative study in an animal model was performed with 24 rabbits, divided into two groups. Full-thickness cartilage defects were created bilaterally on the knees of all rabbits. Pedunculated and free synovial grafts were applied to the right knees of Group 1 and Group 2, respectively. Left knees were left as the control group. Six rabbits from each group were randomly selected for euthanasia 4 and 8 weeks postoperatively. All samples were examined histologically with a cartilage scoring system. For immunohistochemical analysis, the degree of collagen 2 staining was determined using a staging system. All data were statistically compared between the study groups with Student's *t*-test or Mann–Whitney U-test. The correlations between categorical variables were analyzed with Fisher's exact test and Chi-square test.

Results In Group 1, the mean defect size had significantly decreased at 8 weeks postsurgery. It was also significantly smaller than that of Group 2. Both pedunculated and free synovial grafts had significantly better histological and immunohistochemical outcomes compared with the controls. Contrastingly, the results of comparison between the study groups (Group 1 vs. 2) at the 4th and 8th week were not statistically significant with regard to histological scores and immunohistochemical staining.

Conclusion Synovial tissue, whether pedunculated or free, provided much better cartilage recovery compared with the control. It can be used as a mesenchymal stem cell (MSC) source, and synovium-derived MSCs have the chondrogenic potential for the *in vivo* treatment of full-thickness cartilage defects.

Keywords Animal model · cartilage defects · synovium · treatment

Introduction

It is well documented that early and effective treatment of hyaline cartilage damage prevents osteoarthritis (OA) progression [1]. Although there is a range of different methods for the treatment of hyaline cartilage damage, the gold standard method still remains controversial [2, 3].

Various studies in the last decade have found positive outcomes associated with the use of synovial tissue in the treatment of full-thickness articular cartilage defects. This has provided new hope in the treatment and prevention of OA [4]. Although there have been various clinical and experimental studies in the literature analyzing the use of synovial tissue for the treatment of articular cartilage injuries, the data for its application are still scarce and inconclusive because of the heterogeneous methodologies and incomparable outcome measures [3–6]. The effectiveness of the pedunculated synovial graft for the treatment of full-thickness cartilage defects in an animal model was previously reported in the literature by the senior author of this study and proved to be an efficient method for treating focal cartilage defects [4]. Contrastingly, previous studies almost always use free synovial grafts for experimental purposes. For this reason, we hypothesized that the synovial tissue in

✉ Bahtiyar Haberal
bahtiyarhaberal@hotmail.com

[1] Department of Orthopaedics and Traumatology, Faculty of Medicine, Baskent University, Yukari Bahçelievler Mah, Maresal Fevzi Çakmak Cd. 10. Sok. No: 45, Bahçelievler, Çankaya, 06490 Ankara, Turkey

[2] Department of Pathology, Faculty of Medicine, Baskent University, Ankara, Turkey

its pedunculated form has better potential as a mesenchymal stem cell (MSC) source and hence has a greater effect in the treatment of full-thickness cartilage defects compared with the free form. To our knowledge, there is no *in vivo* study comparing the efficacy of pedunculated versus free synovial grafts for the treatment of cartilage defects. Therefore, to understand the real potential effect of synovial tissue in the treatment of articular cartilage defect, we have designed a comparative study in an animal model.

The aims and main objectives of this study are (i) to compare the potential effect of pedunculated and free synovial grafts for the repair of full-thickness articular cartilage defects on an animal model with histological and immunohistochemical analysis and (ii) to determine the best possible methodology for the application of synovial tissue to chondral defects.

Materials and Methods

Study design

An experimental animal model was conducted at our University Animal Research Laboratory with minimum 1-year-old New Zealand White rabbits which had an average weight of 3850.5 g (range 2900–4300 g). *A priori* power analysis was done to determine the minimum number of rabbits for statistical significance. From this analysis, 24 rabbits were assigned to the study. The rabbits were provided with tap water and food (at least 15% crude protein and around 10% digestible protein, 50 g per 1 kg of body weight daily) *ad libitum*. They were kept in separate cages and allowed to move freely within these. The animals were acclimatized for 3 weeks and weighed and handled daily for 4 days preoperatively. No animals were excluded from the study during this preoperative acclimatization period. The animal experiments and the study protocol have been approved by The Ethical Institutional Review Board (DA 14/21) of Baskent University, Ankara, Turkey.

The subjects were split evenly between two groups (12 rabbits in each group):

- Group 1: The "pedunculated" synovial graft group for knees of the right rear limb (left knees of the rear limbs were left as controls)
- Group 2: The "free" synovial graft group for knees of the right rear limb (left knees of the rear limbs were left as controls).

All right knees of the rear limbs were assigned randomly to one study group, and either a pedunculated (Group 1) or free synovial graft (Group 2) was performed to treat focal cartilage defects. The same cartilage defect was also created in the left knee of all rabbits' rear limbs.

All were left untreated as "Control Groups 1 and 2" for pedunculated and free synovial grafts, respectively. Six rabbits from each group were randomly selected 4 and 8 weeks postoperatively. These rabbits were euthanized, and both knees were harvested for gross and histological evaluation.

Surgical procedure for the study groups

This *in vivo* rabbit experiment was performed by two investigators experienced in animal models. It was carried out in an affiliated animal laboratory under the supervision of the Department of Orthopaedics. The same surgical approach was used for both knees in all rabbits. Once they were anesthetized, the knees of the rabbits were surgically scrubbed and draped. The approach to the stifle was through a midline incision, with a longitudinal incision over the patellar region. After a medial parapatellar arthrotomy, the patella was dislocated laterally and the femoral condylar region exposed with trochlea. When adequate exposure had been achieved, a full-thickness cartilage defect of minimum 5 mm was created on the medial trochlear region of the knee joint with a 1.2-mm drill [Figure 1]. Then, for the rabbits in Group 1, the medial pedunculated synovial tissue (still integrated with the medial trochlear region) [Figure 2a] was freed from the medial trochlear peridefect region of the femur and used as a graft to cover the cartilage defect [Figure 2b]. The same surgical procedure was also performed for Group 2, but this time the synovial graft was completely freed (completely dissected) from the medial trochlear region [Figure 3]. To stabilize the synovial grafts, a 0.75-mm diameter microdrill was used to create two holes for the sutures to pass through. A 5-0 nonabsorbable monofilament suture was used to attach

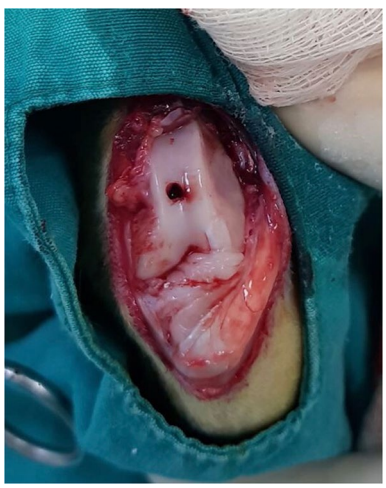

Fig. 1 The full-thickness cartilage defect on the medial trochlear region of the rabbit right knees

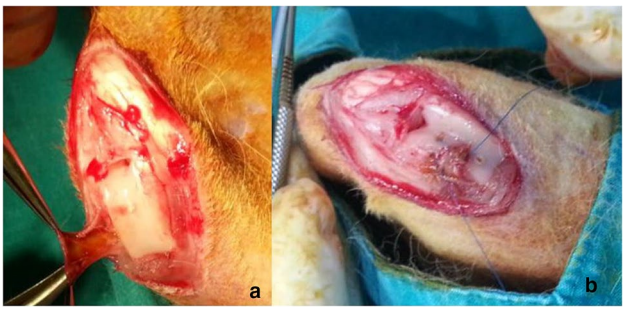

Fig. 2 The intraoperative view of Group 1. Note that the pedunculated synovial graft was freed from the medial trochlear region (a) and sutured over the chondral defect (b)

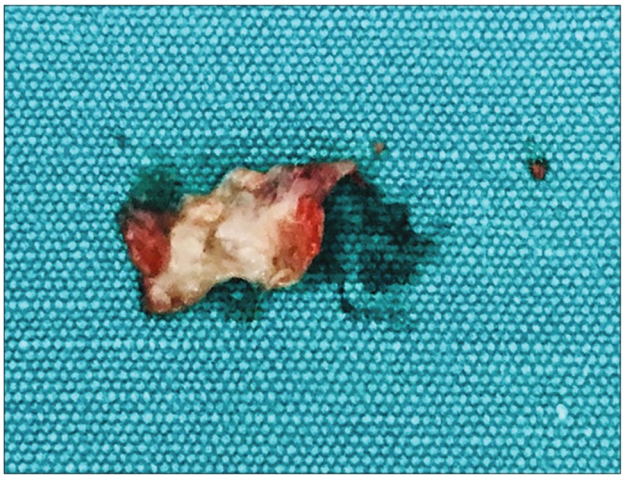

Fig. 3 The intraoperative view of Group 2. Note that the chondral lesion was covered with free synovial graft and sutured

the graft onto the cartilage defect. In the control knees (left knee of each rabbits' rear limb), the full-thickness cartilage defects were left untreated. After the procedure, the incision site was closed. On recovery, the rabbits were allowed to move around freely in clean cages. Antibiotic prophylaxis was administered through intramuscular injections of 50 mg/kg amikacin twice a day for 5 days.

Histological and immunohistochemical analysis

Following euthanasia, bilateral *en bloc* knee samples were fixed in 10% formaldehyde and transferred to 20% formic acid for decalcification. The specimens were embedded in paraffin and sections of 5-µ thickness were prepared on slides. After deparaffinization, the sections were stained with hematoxylin and eosin and safranin O stains. The quality of the newly formed hyaline cartilage was scored with a histological scoring system previously used in the literature [7, 8]. In this scoring system, chondral recovery on different categories for each knee (including the control group) was evaluated and scored ranged between 0 and 31 (0 for best recovery, 31 for worst recovery).

For immunohistochemical analysis, deparaffinized sections were prepared and primary antibodies for collagen 2 were stained with avidin-biotin-peroxidase (Vectastain Elite ABC kit; Vector Laboratories, Peterborough, UK). After the staining process, collagen type 2 staining stages were evaluated with a scoring system defined in previous studies [4]. Accordingly, pale and focal staining pattern compared with the adjacent cartilage tissue was defined as stage 1, and similar staining pattern compared with the adjacent normal cartilage tissue was defined as stage 3.

Sections were evaluated with a light microscope (Olympus BX51) after histological and immunohistochemical staining, and images were recorded with a digital camera (Olympus DP72).

Statistical analysis

Statistical analyses were performed using SPSS for Windows (Version 17, Chicago, IL, USA). Defining statistics for categorical and continuous variables were given in this study (mean, standard deviation, median, range, and percentile). The "Shapiro–Wilk" test was used to quantify the normal distribution of the data within each study group, and Levene's test was used to assess similarities in the variance of each test group. The difference between two study groups was compared using Student's *t*-test or Mann–Whitney U-test. The correlations between categorical variables were analyzed with Fisher's exact test and Chi-square test. The criterion for statistical significance was $P < 0.05$.

Results

All surgeries were performed without any intraoperative complications, and there were no anesthesia-related problems. In addition, there were no complications or loss of rabbits during the postoperative followup period.

Analysis of defect sizes

Full-thickness defect sizes at the 4^{th} and 8^{th} weeks postoperatively for Groups 1 and 2 and their statistical comparison results are summarized in Table 1. Although there was no significant difference in Group 2 with regard to defect sizes at the 4^{th} and 8^{th} weeks postoperatively ($P = 0.093$), in Group 1, the mean defect size was significantly decreased at 8 weeks postoperatively ($P = 0.015$). Statistical comparison between the study groups at 4 and 8 weeks postoperatively revealed that the defect size for Group 2 was significantly larger than Group 1 at the 8-week followup only ($P = 0.018$).

Table 1 Mean values and statistical comparison results of osteochondral defect sizes for the study groups

Study groups	Defect size (mm) (minimum-maximum)		
	4^{th} week	8^{th} week	*P* (4^{th} vs.8th)
1	3.92 (2-4.5)	1.83 (1-4)	0.015*
2	2.83 (2-3.5)	3.83 (3-5)	0.093
P (1 vs. 2)	0.042*	0.018*	N/A

N/A Not applicable

*Statistically significant $P < 0.05$

Histological and immunohistochemical results

For all study groups, the mean histological analysis scores for every subparameter of the scoring system with minimum and maximum values are summarized in Table 2.

The total histological scores and immunohistochemical analysis (the stages of collagen 2 staining) for the study groups without controls are summarized in Tables 3 and 4, respectively. There was no statistically significant difference with regard to total histological scores and collagen 2 staining between or within the study groups at 4 and 8 weeks postoperatively.

The mean values of the histological scores for the study groups with the controls and the statistical comparison outcomes are summarized in Table 5. The total histological scores of Groups 1 and 2 were statistically lower than those of the controls at 4 weeks postoperatively ($P = 0.026$ and 0.027, respectively). This means that there was significantly better cartilage recovery in the study groups compared with the controls after 4 weeks of followup Conversely, there was no statistically significant difference between the study groups and controls with regard to total histological scores at 8 weeks postoperatively ($P = 0.093$ and 0.5, respectively) [Figure 4]. The statistical comparison outcomes for the collagen 2 staining between the study groups and controls revealed that the study groups had more collagen 2 staining compared with the controls at both 4 and 8 weeks of followup ($P < 0.05$).

Table 3 Mean total histological scores and statistical comparison results of the study groups

Study groups	Total score (minimum-maximum)		
	4^{th} week	8^{th} week	*P* (4^{th} vs. 8^{th})
1	16 (9-21)	11.5 (6-17)	0.132
2	12.17 (8-17)	12.67 (9-17)	0.937
P (1 vs. 2)	0.132	0.589	N/A

N/A Not applicable

Table 4 Mean scores and statistical comparison results of immunohistochemical collagen 2 staining for the study groups

Study groups	Collagen 2 (minimum-maximum)		
	4^{th} week	8^{th} week	*P* (4^{th} vs. 8^{th})
1	2.5 (1-3)	0.75 (1-3)	0.49
2	2.5 (1-3)	2.83 (2-3)	0.589
P (1 vs. 2)	0.999	0.083	N/A

N/A Not applicable

Table 5 Mean scores and statistical comparison results of immunohistochemical collagen 2 staining for the study groups

Study groups	Collagen 2 (minimum-maximum)		
	4^{th} week	8^{th} week	*P* (4^{th} vs. 8^{th})
1	2.5 (1-3)	0.75 (1-3)	0.49
2	2.5 (1-3)	2.83 (2-3)	0.589
P (1 vs. 2)	0.999	0.083	N/A

N/A Not applicable

In addition, the mean value of the total histological scores for Groups 1 and 2 together was found to be 13.08 (6–21). This score was 20.33 (0–27) for the control group. There was a statistically significant difference between these two parameters ($P < 0.001$), i.e., the synovial graft whether pedunculated or free had a positive healing effect for the osteochondral lesion compared with the control group.

Table 2 Mean histological analysis scores of the study groups with minimum and maximum values for every microscopic parameter

Histological analysis	Control	Group 1	Group 2
Filling of defect relative to surface of original cartilage	0.72 (0-2)	0.16 (0-1)	0.5 (0-1)
Integration of repair tissue with surrounding articular cartilage	2 (1-3)	1.5 (0-3)	1.5 (0-3)
Safranin O fast green staining of cartilage above original tidemark	3 (1-4)	2.3 (0-3)	1.16 (0-3)
Cellular morphology of cartilage above original tidemark	4.63 (1-5)	2.91 (1-5)	1.66 (1-4)
Architecture within entire defect	3.18 (0-4)	1.41 (0-4)	1.25 (0-4)
Architecture of surface	2.5 (1-3)	1.75 (1-3)	1.75 (1-3)
Percentage replacement of subchondral bone	3.22 (0-4)	1.5 (0-3)	2.58 (0-4)
Reformation of tidemark	3.4 (2-4)	2.16 (1-4)	2 (1-4)

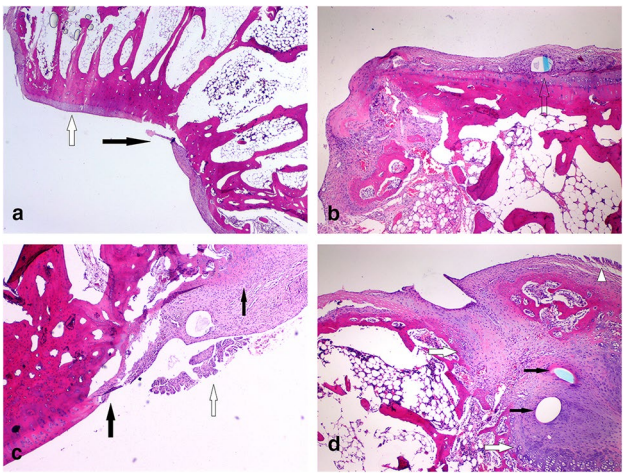

Fig. 4 The histological sections of Group 1 (a) and Group 2 (b) (H and E, ×40). (a) The full-thickness cartilage defect in the control group. Note that a complete defect, unfilled yet at the 4th week (black arrow) next to the intact joint cartilage (white arrow); (b) The chondral defect filled with newly formed cartilage tissue at the 4th week, in Group 1. Note that there is a section of the surgical suture material in the healing tissue (arrow); (c) The osteochondral defect filled with fibroblastic and chondroblastic proliferation, at week 4, in Group 2. Free synovial graft over the healing tissue (white arrow). Note that tidemark formations (black arrows) are on the osteochondral line; (d) The deeper osteochondral defect filled with exaggerated healing tissue at the 8th week, in Group 2. Two sections of surgical suture (black arrows) that surrounded by fibroblastic and chondroblastic proliferation and ossification. The formation of tidemark (white arrow) in the bottom of the defect and free synovial graft (white triangle) on the upper

Discussion

This is one of the first studies to compare free and pedunculated synovial tissue in an animal model as a new method in the treatment of focal cartilage defects. Although various methods have been described in the literature, to date, the gold standard treatment of focal cartilage defects is still under dispute [5, 9–14]. In the last decade, treatment methods using MSCs from various sources such as adipose tissue, bone marrow, or periosteum have gained popularity. It has also been proven that synovial tissue is a valuable source of MSC [12].

Synovium-derived MSCs are believed to possess superiority in chondrogenesis with a greater proliferative ability than other sources of MSCs. In a study by Hunziker and Rosenberg, it was observed that partial-thickness defects in the articular cartilage of rabbits were filled with a continuous layer extending from the synovial membrane to contribute to the repair of the cartilage [3]. Miyamoto *et al*. also reported that synovial tissue had the capacity to fill the cartilage defects in the transition zone between joint cartilage and synovial membrane in rats [13]. Hence, in the current study, synovial tissue in the pedunculated and free forms was used to treat full-thickness cartilage defects. In our clinical practice, synovial chondromatosis in which cartilage is formed in the pathological synovial tissue is a common pathological process. For these reasons, we believe that the synovium has great potential as a source of MSCs for chondrogenesis.

To our knowledge, the largest cartilage defect size created in a rabbit model was 3 mm. However, the defect sizes of subchondral bone substantially differ between studies [15–17]. Consequently, the outcomes of rabbit studies are not comparable with respect to chondral defect size. In our study, the average osteochondral defect size was approximately 3 mm, which is comparable with the literature. Other studies using rabbit models have a large range of followup periods postoperatively. The average followup period is 16 weeks [13, 18]. This highly complicates obtaining comparable, long term results of treatment. In our study, the longest followup period was 8 weeks, which is inconsistent with the literature. We believe that 8 weeks of followup is sufficient. Longer periods of followup result in higher costs and greater animal losses.

It is accepted in the literature that the ideal treatment for cartilage injury must result in complete integration of the newly formed cartilage with the surrounding tissues, sufficient mechanical stability, and complete refilling of the defect. In addition, the regenerated cartilage should provide joint contour and should be rough enough to bear weight to be biologically acceptable. None of the techniques including autologous chondrocyte implantation, microfracture, or mosaicplasty could offer a treatment modality that would result in ideal hyaline cartilage [5, 13, 19, 20]. In most instances, newly formed tissue contains a variety of histologicallymixed cartilage types (hyaline and fibrous). Hence, we believe that analyzing the effect of synovium-derived MSCs on cartilage healing is of the utmost importance to have a definitive treatment option which results in hyaline cartilage repair.

MSCs integrate better to the surrounding tissue and can withstand physiological mechanical weights longer. MSCs also have the potential to differentiate into subchondral bone [12]. They are multipotent and can regenerate easily *in vivo*. Synovium-derived MSCs have higher chondrogenic potential than stem cells obtained from the bone marrow or periosteum. Under proper *in vivo* conditions, they can migrate to cartilage defects and chondrogenically differentiate.

Narayanan *et al*. stated that MSCs from various tissues (synovium, periosteum, skeletal muscle, and fat) differ in capacity and multipotent ability in cartilage tissue engineering [21]. The literature has a general agreement that synovium-derived MSCs show greater chondrogenic differentiation ability and proliferative capability than other MSCs both *in vitro* [6, 12, 21] and *in vivo* [19]. A recent study by Ivirico *et al*. demonstrated that both fibrous synovium and adipose synovium have higher chondrogenic potential

than subcutaneous fat, and therefore, they are highly suitable sources for cartilage regeneration [10].

There are some limitations to our study. First, the maximum followup period was 8 weeks. Longer followup periods may provide better understanding of the long term effect of the synovium on cartilage repair. Second, there is no three-dimensional analysis of the defect size before and after surgery. There is also no biomechanical analysis of the newly formed repair tissue, so it is impossible for us to compare the mechanical strength of the study groups.

In addition, the mobility/weightbearing status of the rabbits and limping after surgery were not analyzed in this study. Finally, a detailed immunohistochemical evaluation other than collagen 2 staining and the quantity and quality of the synovial tissue was not analyzed in our study. Hence, it is not possible to prove that the MSCs recruited from the synovial tissue.

Conclusion

Compared with the control group, synovium, whether pedunculated or free, provided much better cartilage recovery in both of our study groups. Although pedunculated synovial grafts have better chondrogenic potential at 4 weeks postoperatively than free synovium, after 8 weeks both have the same chondrogenic effect.

Financial support and sponsorship Nil.

Conflicts of interest There are no conflicts of interest.

References

1. Redondo ML, Naveen NB, Liu JN, Tauro TM, Southworth TM, Cole BJ. Preservation of knee articular cartilage. Sports Med Arthrosc Rev 2018;26:e23-30.
2. Welton KL, Logterman S, Bartley JH, Vidal AF, McCarty EC. Knee cartilage repair and restoration: Common problems and solutions. Clin Sports Med 2018;37:307-30.
3. Hunziker EB, Rosenberg LC. Repair of partial-thickness defects in articular cartilage: Cell recruitment from the synovial membrane. J Bone Joint Surg Am 1996;78:721-33.
4. Tuncay IC, Ozdemir BH, Demirörs H, Karaeminogullari O, Tandogan NR. Pedunculated synovium grafts in articular cartilage defects in rabbits. J Invest Surg 2005;18:115-22.
5. Getgood A, Bhullar TP, Rushton N. Current concepts in articular cartilage repair. Orthop Trauma 2009;23:189-200.
6. Rodrigues MT, Gomes ME, Reis RL. Current strategies for osteochondral regeneration: From stem cells to pre-clinical approaches. Curr Opin Biotechnol 2011;22:726-33.
7. Rutgers M, van Pelt MJ, Dhert WJ, Creemers LB, Saris DB. Evaluation of histological scoring systems for tissue-engineered, repaired and osteoarthritic cartilage. Osteoarthritis Cartilage 2010;18:12-23.
8. Nagura I, Fujioka H, Kokubu T, Makino T, Sumi Y, Kurosaka M. Repair of osteochondral defects with a new porous synthetic polymer scaffold. J Bone Joint Surg Br 2007;89:258-64.
9. Hunziker EB. Growth-factor-induced healing of partial-thickness defects in adult articular cartilage. Osteoarthritis Cartilage 2001;9:22-32.
10. Ivirico J, Bhattacharjee M, Kuyinu E, Nair L, Laurencin CT. Regenerative engineering for knee osteoarthritis treatment: Biomaterials and cell-based technologies. Engineering 2017;3:16-27.
11. Kim MJ, Son MJ, Son MY, Seol B, Kim J, Park J. Generation of human induced pluripotent stem cells from osteoarthritis patient-derived synovial cells. Arthritis Rheum 2011;63:3010-21.
12. Labusca L, Zugun-Eloae F. Stem cell therapy for the treatment of cartilage defects and osteoarthritis. Bone and Cartilage Regeneration. Springer International Publishing: Switzerland; 2017. p. 11-45.
13. Miyamoto A, Deie M, Yamasaki T, Nakamae A, Shinomiya R, Adachi N. The role of the synovium in repairing cartilage defects. Knee Surg Sports Traumatol Arthrosc 2007;15:1083-93.
14. Wakitani S, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg Am 1994;76:579-92.
15. Buma P, Pieper JS, van Tienen T, van Susante JL, van der Kraan PM, Veerkamp JH. Cross-linked type I and type II collagenous matrices for the repair of full-thickness articular cartilage defects – A study in rabbits. Biomaterials 2003;24:3255-63.
16. Han CW, Chu CR, Adachi N, Usas A, Fu FH, Huard J. Analysis of rabbit articular cartilage repair after chondrocyte implantation using optical coherence tomography. Osteoarthritis Cartilage 2003;11:111-21.
17. Rudert M, Wilms U, Hoberg M, Wirth CJ. Cell-based treatment of osteochondral defects in the rabbit knee with natural and synthetic matrices: Cellular seeding determines the outcome. Arch Orthop Trauma Surg 2005;125:598-608.
18. Hui JH, Chen F, Thambyah A, Lee EH. Treatment of chondral lesions in advanced osteochondritis dissecans: A comparative study of the efficacy of chondrocytes, mesenchymal stem cells, periosteal graft, and mosaicplasty (osteochondral autograft) in animal models. J Pediatr Orthop 2004;24:427-33.
19. Keeney M, Lai JH, Yang F. Recent progress in cartilage tissue engineering. Curr Opin Biotechnol 2011;22:734-40.
20. 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.
21. Narayanan G, Bhattacharjee M, Nair L, Laurencin C. Musculoskeletal tissue regeneration: The role of the stem cells. Regen Eng Transl Med 2017;3:133-65.