Osteoarthritis and Cartilage 19 (2011) 1440–1448

Osteoarthritis and Cartilage

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Implantation of bone marrow-derived buffy coat can supplement bone marrow stimulation for articular cartilage repair

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ARTICLE INFO

Article history:
Received 1 October 2010
Accepted 4 July 2011

Keywords:
Articular cartilage repair
Bone marrow stimulation
Buffy coat

SUMMARY

Objective: Bone marrow stimulation (BMS) has been regarded as a first line procedure for repair of articular cartilage. However, repaired cartilage from BMS is known to be unlike that of hyaline cartilage and its inner endurance is not guaranteed. The reason presumably came from a shortage of cartilage-forming cells in blood clots derived by BMS. In order to increase repairable cellularity, the feasibility of autologous bone marrow-derived buffy coat transplantation in repair of large full-thickness cartilage defects was investigated in this study.
Methods: Rabbits were divided into four groups: the defect remained untreated as a negative control; performance of BMS only (BMS group); BMS followed by supplementation of autologous bone marrow buffy coat (Buffy coat group); transplantation of autologous osteochondral transplantation (AOTS) as a positive control.
Results: Repair of cartilage defects in the Buffy coat group in a rabbit model was more effective than BMS alone and similar to AOTS. Gross findings, histological analysis, histological scoring, immunohistochemistry, and chemical assay demonstrated that supplementation of autologous bone marrow buffy coat after BMS arthroplasty effectively repaired cartilage defects in a rabbit model, and was more effective than BMS arthroplasty alone.
Conclusion: Supplementation of autologous bone marrow-derived buffy coat in cases of BMS could be a useful clinical protocol for cartilage repair.

Introduction

Articular cartilage is a resilient load-bearing tissue that forms the articulating surface of diarthrodial joints and provides low friction, lubrication, and wearless characteristics necessary for repetitive gliding motion. However, because articular cartilage has neither vascular nor lymphatic channel supply and hence difficult access to stem cells, its self-healing potential is restricted[1,2]. Additionally low cell mobility due to surrounding matrix and a limited number of progenitor cells could be another contributing factors[3]. Until now, much effort has been placed on development of therapeutic approaches for cartilage repair.

Among the various strategies at present, the bone marrow stimulation (BMS) technique is one that has widely been used for repair of articular cartilage defects. This approach is aimed at creation of a suitable environment for new tissue formation, and utilization of the intrinsic healing ability of the body. BMS recruits mesenchymal stem cells (MSCs) or progenitor cells *via* subchondral penetration to the bone marrow, thereby stimulating cartilage repair. However, it was observed that fibrocartilaginous tissue with poor mechanical properties was obtained instead of the desired hyaline cartilage. This observation is likely a result of excessive loading conditions and a limited number of reparative MSCs available due to loss and dilution of bone marrow originated blood clots from the BMS region caused by the synovial fluid. To overcome this problem, some approaches to prevent leakage of bone marrow stem cells from the defect have been investigated. Breinan *et al.*

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doi:10.1016/j.joca.2011.07.012

covered the cartilage defect with a collagen film after performance of the microfracture technique in order to minimize the flow of bone marrow into the joint cavity[4]. Consequently, cartilage repair was better in the group with the covered defect compared to the group with the defect left exposed. In another approach, Taner *et al.* covered the defective cartilage area with autologous periosteum membrane following microfracture, and fixed the leg with an assistive device for reduction of overloading[5]. Breinan reported that addition of cultured autologous chondrocytes results in the same tissue types as those yielded by microfracture treatment[4].

In our previous study, a larger number of MSC drained from bone marrow produced a better quality of repaired cartilage in the defect[6]. Therefore, the effort to enhance the intrinsic cells should be exerted in order to obtain a more hyaline-like cartilage.

Bone marrow is known to contain multipotent stem cells that can differentiate not only into hematopoietic cells, but also into mesenchymal tissue. Azizi *et al.* reported that several investigators who are treating various intractable diseases (including ischemic myocardial diseases, osteogenesis imperfect, Duchenne's muscular dystrophy, and central nervous system disorders, such as Parkinson's disease) are focusing on the use of bone marrow stem cells[7]. Many studies have reported on the therapeutic potential of bone marrow-derived mononuclear cells for regeneration of various types of tissues[8]. These cells have been used for treatment of non-traumatic avascular necrosis of the femoral head[9], infarction of the myocardium[10], and cartilage damage[11] and have also been applied to a continuous spinal fusion mass[12]. In terms of cartilage repair, as bone marrow mononuclear cells (MNCs) can be fractionated to contain higher proportion of MSCs, they can potentially be used after microfracture to provide more MSCs in the defect area[11,12].

In this study, we hypothesized that implantation of autologous uncultured bone marrow cells from buffy coat into a full-thickness articular cartilage defect would generate tissue that more closely resembles hyaline cartilage. Extracellular matrix (ECM) membrane (ArtiFilm®, Regenprime Co., Ltd, Korea) was used to prevent leakage of injected cells and blood clots from the cartilage defect into the knee joint cavity.

Materials and methods

Experimental design and surgery

Use of animals in this experiment was approved by the Institutional Animal Experiment Committee of Ajou University. Seventy-two New Zealand white rabbits of 16 weeks old (an average weight of 3.0–3.5 kg) at which the growth plates are already closed were used in this study. Surgical procedures, including limb preparation and draping, were performed under general anesthesia with a mixture (0.2 ml/kg body weight) of Zoletil (50 mg/ml; Virbac Laoratoires-06516 Carros, France) and xylazine hydrochloride (Rompun; Bayer, Ansan, Korea) (Zoletil:Lompun = 1:2). An arthrotomy was made through a midline longitudinal incision on the medial parapatellar with the patella dislocated laterally to expose the femoral condyle. A drill 5 mm in diameter was used for creation of an osteochondral defect of 2 mm in depth in the patella groove[13]. BMS was performed using a 5 mm drill and 17-gauge needle.

For the *in vivo* study, we used a natural ECM membrane (ArtiFilm®, Regenprime Co., Ltd, Korea) derived from cultured young porcine chondrocytes to cover the cartilage defect (Fig. 1). ArtiFilm was fabricated by decellularizing a membrane-type cell/ECM composite produced by high density culture of porcine

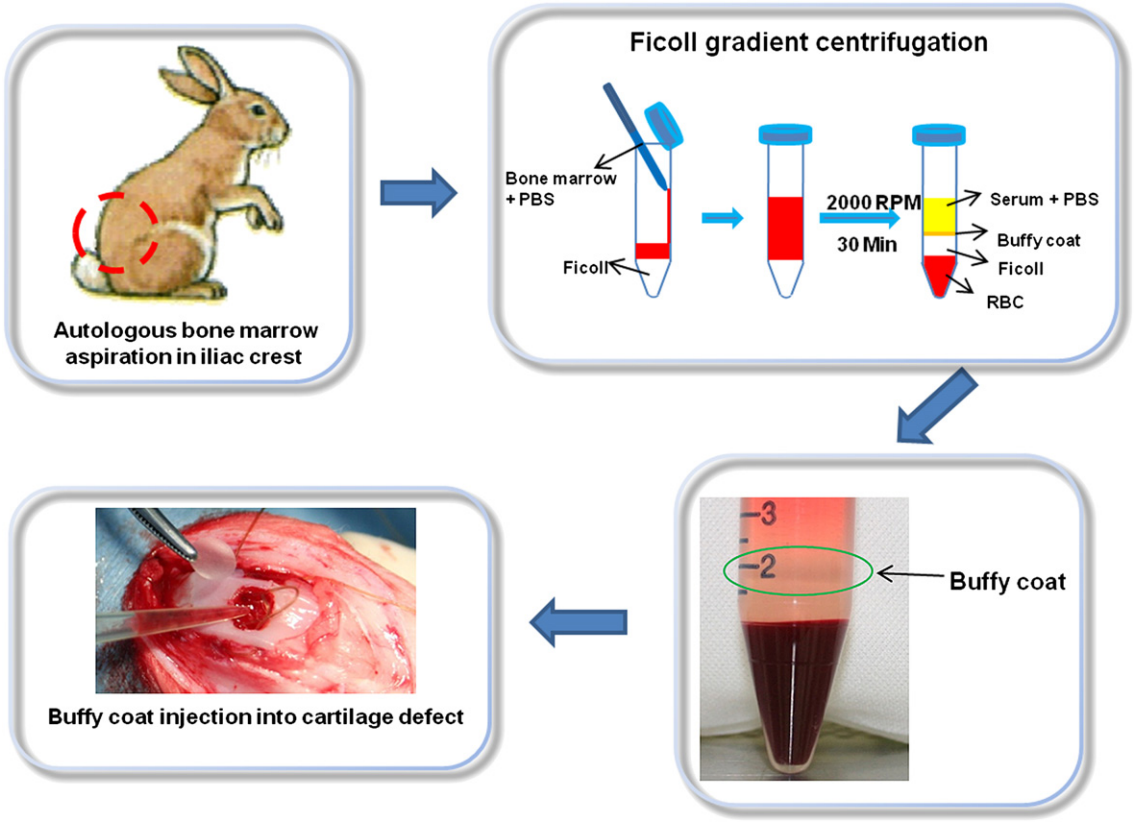

Fig. 1. Procedure for implantation of autologous buffy coat. Bone marrow was aspirated from rabbit iliac crest. Then, 20 μl of buffy coat was separated from bone marrow using a Ficoll gradient centrifugation system and injected into the BMS treated area of articular cartilage, followed by ECM membrane covering.

chondrocytes, and composed mainly of cartilage-enriched ECM components such as type II collagen and sulfated glycosaminoglycans (GAGs). The study group was divided at random into four groups ($n = 18$/group): (1) the defect was left untreated as a negative control (Defect group), (2) treated with BMS using a 17-gauge needle after making the osteochondral defect using a 5 mm drill (BMS group), (3) supplemented concentrated autologous buffy coat was isolated from the iliac crest after BMS (Buffy coat group) and (4) implanted with an autologous osteochondral graft (AOTS) as a positive control. In this study, the AOTS group was set up by re-inserting autologous osteochondral tissues isolated when making osteochondral defect using the AOTS system (Autograft diameter 6 mm, Arthrex, Germany)[14]. Cartilage defects in the Defect, BMS, and Buffy coat groups were subsequently covered with an ECM membrane with fixation using the cross suture method. Each operated knee joint was immobilized with an assistive device post-surgery. Repair of cartilage was evaluated by macroscopic, histological and immunohistochemical analyses for repaired tissues at 4 and 8 weeks post-operation and additionally by biochemical analysis for repaired tissues at 8-week post-operation.

The description on the experimental design is based on Animals in Research: Reporting *In vivo* Experiments (ARRIVE) guidelines[15].

Measurement of the number of MSC drained by the BMS technique and harvested at the iliac crest

Colony forming unit-fibroblast (CFU-F) analysis was performed for measurement of the number of MSCs present in bone marrow isolated from the iliac crest or blood clots after BMS on the cartilage defect. Briefly, 4 ml of bone marrow blood was harvested from the iliac crest and 100 μl of blood was drained from cartilage defects by microfracture. The samples were diluted in PBS and the buffy coat was isolated by centrifugation using a Ficoll density gradient (Amersham Biosciences, 17-1440-02, Sweden) at 1,000*g* for 30 min[16]. A total of 1 ml of buffy coat in serum was isolated from this suspension, and then concentrated into 20 ml. The buffy coat was resuspended in MSC culture medium consisting of α-MEM supplemented with 10% FBS, and subsequently plated in 6-well culture dishes. Bone marrow harvested from the blood clot obtained after BMS was resuspended in the same manner. After 6 days, non-adherent cells were discarded and adherent cells were replenished with fresh medium after washing. The cells were then cultured for about 12 days, with replacement of the culture medium every 3 days, and stained with 5% crystal violet solution in 100% methanol for 10 min for the CFU-F assay[17]. To minimize the effects of subjective bias three researchers counted the number of colonies greater than 2 mm in diameter in the blind manner.

Macroscopic and histological evaluations

At 4 and 8 weeks after surgery, the rabbits were euthanized by injection of an over-dose of pentobarbital. Femoral condyles were subsequently retrieved. Macroscopic images of the condyles were first observed; the samples were then paraffin-embedded, sectioned, and processed for routine safranin-O, Sirius red staining and immunohistochemical analysis[18]. For immunohistochemical analysis, the sections were incubated with mouse monoclonal anti-rabbit collagen type II antibody (Chemicon, Temecula, CA, USA) at 1:200 dilution for 1 h at room temperature. The sections were then incubated sequentially with biotinylated secondary antibody at 1:200 dilutions for 1 h and peroxidase-conjugated streptavidin solution for 30 min at room temperature (DAKO LSAB System, Carpinteria, CA, USA). After counterstaining with Mayer's hematoxylin (Sigma, St Louis, MO, USA), the sections were mounted with a mount solution prior to microscopic observation (Nikon E600, Japan)[19,20].

Histological scoring [International Cartilage Repair Society Score (ICRS)]

To minimize the effects of subjective bias three researchers independently evaluated the quality of the repaired articular cartilage in the defects, and a modified version of the histological grading scale system was used for the ICRS score[19–21]. The scale consists of seven categories and assigns a score ranging from 0 to 18.

Chemical assay of repaired tissue

For chemical assays of the repaired tissue, samples were harvested from the defect by surgical knife and curette. The harvested tissue was dried and digested in papain solution (5 mM L-cysteine, 100 mM Na_2HPO_4, pH 6.4, 5 mM EDTA, and 125 μg/ml papain type III) at 60°C for 24 h and then centrifuged at 12,000*g* for 10 min. For measurement of the GAG contents, the supernatant was subjected to 1,9-Dimethylmethylene Blue (DMB) colorimetric assay. DNA content was measured using a Qubit Fluorometer and the protocol given by the supplier (Invitrogen, Eugene, OR, USA)[20–22]. In all assays, normal cartilage from rabbit knee was used as a control. GAG contents were determined using chondroitin sulfate from shark cartilage (Sigma) as a standard[17–19].

Statistical analysis

Statistical analysis of the experimental data was carried out using one-way analysis of variance (one-way ANOVA) for multiple comparison and specific inter-data differences between mean values were identified using the Tukey-HSD test. *P* values less than 0.05 were regarded statistically significant and described in Results.

Results

CFU-F assay

Crystal violet staining showed that there was no significant difference in the number of MSC colonies between the same volume of blood clot located in the BMS region and harvested from the iliac crest. Average blood volume and number of MSCs after BMS in the cartilage defect area were 39.25 ± 9.8 μl and 23.88 ± 5.96 MSCs, respectively. The mean volumes of bone marrow and number of MSCs aspirated from the iliac crest were 4 ml and $2{,}431.2 \pm 270.8$ MSCs, respectively (Table I).

Gross findings and histological evaluation of cartilage defects

At week 4 after surgery, the defect area in the Defect group remained vacant while in the AOTS group, the defect was completely filled with smooth and glistening tissue. Repaired tissues in the BMS group partially filled the defect area and the extent of filling of the defect was much wider in the Buffy coat

Table I
Comparison of MSC frequencies between blood clot obtained from the BMS region and bone marrow obtained from the iliac crest. There was no difference in MSC frequency; however, as the achieved volume of bone marrow (4 ml) is bigger than the volume of the blood clot (40 μl) by 100 times, a centuple number of MSCs could be obtained from bone marrow through the buffy coat isolation method

	Volumes (μl)	Number of MSC (cells)	MSC frequency (cells/ml)	Number of MNC (cells)
Blood clot from BMS region	39.25 ± 9.8	23.88 ± 5.96	608.3 ± 151.88	
Bone marrow from iliac crest	$4{,}000 \pm 500$	$2{,}431.2 \pm 270.8$	607.8 ± 175.98	$9.2 \pm 3.9 \times 10^6$

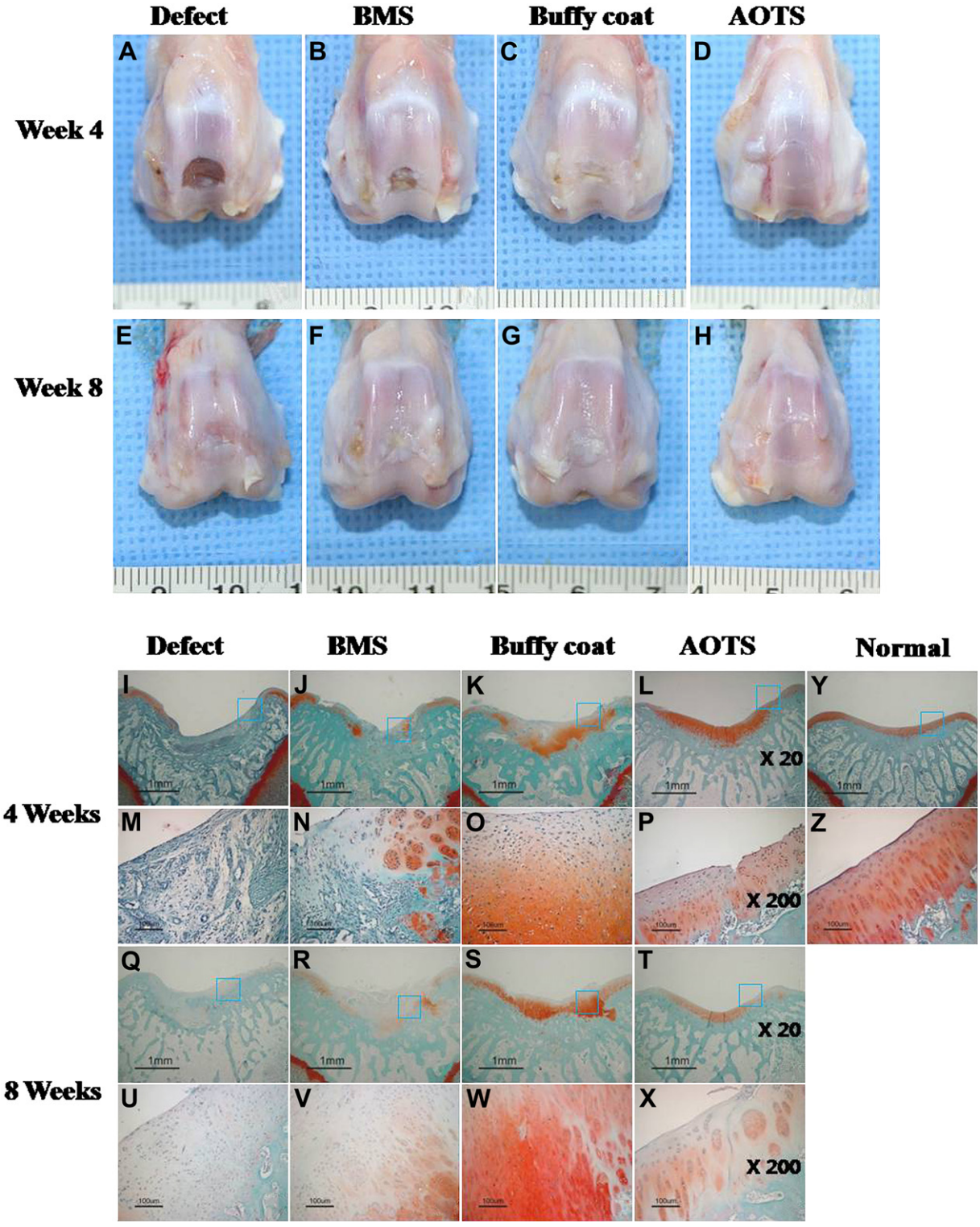

Fig. 2. Gross observation of the defect area of the cartilage at 4 weeks (A–D) and 8 weeks (E–H) after surgery. In the 4-week samples, C and D showed a smooth and glistening appearance, and continuity with the surrounding host cartilage tissue was also observed. In contrast, the defect was not repaired in picture A and was filled partially with fibrous tissues in picture B. At 8 weeks after surgery, a white and glistening appearance of repaired tissues was shown in all groups. Histological observation of repaired cartilage in the defect area of the experimental groups at 4 weeks (I–P) and 8 weeks (Q–X) after surgery. And Safranin-O staining image of normal cartilage was also provided (Y). The defect was partially filled with fibrous tissues in the Defect and BMS groups at week 4. In contrast, hyaline cartilage-like tissues were observed partially in the Buffy coat group and completely in the AOTS group. At week 8, fibrous/hyaline cartilage was regenerated in the Defect and the BMS groups. In contrast, hyaline cartilage tissues with mature matrix and columnar organization of chondrocytes were observed in the Buffy coat and AOTS groups. Magnification was ×20 (I–L, Q–T, Y), and ×200 (M–P, U–Z).

group, where the tissue looked a little whitish and was distinguished from the surrounding cartilage. At week 8, defects in all groups, except for the AOTS group, were observed to be filled with repaired tissue, but appeared different from the surrounding cartilage because of their white color. In the AOTS group, the defect area was filled with tissue that was indistinguishable from normal cartilage [Fig. 2(A–H)].

Safranin-O staining showed that defects in the Defect group were partially filled with fibrous tissue, while those in the AOTS group appeared to have normal cartilage, which had metachromatically stained ECM with well-aligned cells. However, graft tissue was not integrated with host tissue. In the BMS group, defects were filled with fibrous tissue minimally occupied with hyaline-like tissues at week 4. In contrast, most tissue, except for the surface layer, stained with safranin-O; however, its surface was quite rough and cells generally lacked orientation in the Buffy coat group. None of the groups, except for the AOTS group, showed subchondral bone remodeling. At week 8, fibrous tissue was filled in the Defect group, while the AOTS group showed good appearance of hyaline cartilage without evidence of degenerative change around the defect area. However, graft tissue still did not integrate with host tissue. Repaired tissue in the BMS group showed an increased area of metachromatic staining compared to that of 4 weeks; however, it was still occupied by much fibrous-like tissue covering the articular surface. In the Buffy coat group, repaired tissue was well organized with intense ECM; further, the cell distribution was composed of columnar and cluster cells with hyaline character, even though its surface and cartilage-bone were irregular. Except for the AOTS group, none of the groups showed the tide mark and columnar arrangement of cells [Fig. 2(I–Z)].

In Sirius red staining, collagen fiber was found only in small amounts in samples of the Defect and BMS groups at week 4, and no oriented pattern was observed. Collagen fibers were found in large amounts in the Buffy coat group. In the AOTS group, oriented collagen fibers perpendicular to the cartilage surface were found; however, integration was not observed between implanted cartilage tissue and host cartilage. At week 8 after surgery, the amount of collagen fibers was increased in all groups; however, oriented arrangement of collagen fibers was not observed in the Defect and BMS groups. In the Buffy coat and AOTS groups, a well-oriented arrangement of collagen fibers similar to normal cartilage were found at 8 weeks; however, integration between implanted tissue and host tissue was also not observed (Fig. 3).

The ICRS histological score showed significant improvement with time in all but the AOTS group. It was not until 8 weeks that the Buffy coat group and the BMS group showed a difference in ICRS score (Number of femoral condyles $(N) \geq 5$, $P < 0.001.018$, $P < 0.001$) (Fig. 4).

Expression of type II collagens

Expression of type II collagen increased gradually with time at the pericellular region in the repaired tissue of the BMS, Buffy coat, and AOTS groups. Collagen was diffusely distributed in territorial regions, as well as the pericellular area at week 4 in the BMS and Buffy groups. It was reorganized to converge into the pericellular area along with chondrocytes, similar to the AOT group at week 8. The Defect group showed minimal deposition of collagen, even at week 8 (Fig. 5).

Chemical assay of repaired tissue

At 8 weeks post-surgery, the amount of GAG in the Buffy group was significantly higher than those in the Defect and BMS groups, but not as much as in the AOTS and Normal groups, while there was no statistically significant difference in the amount of GAG in the

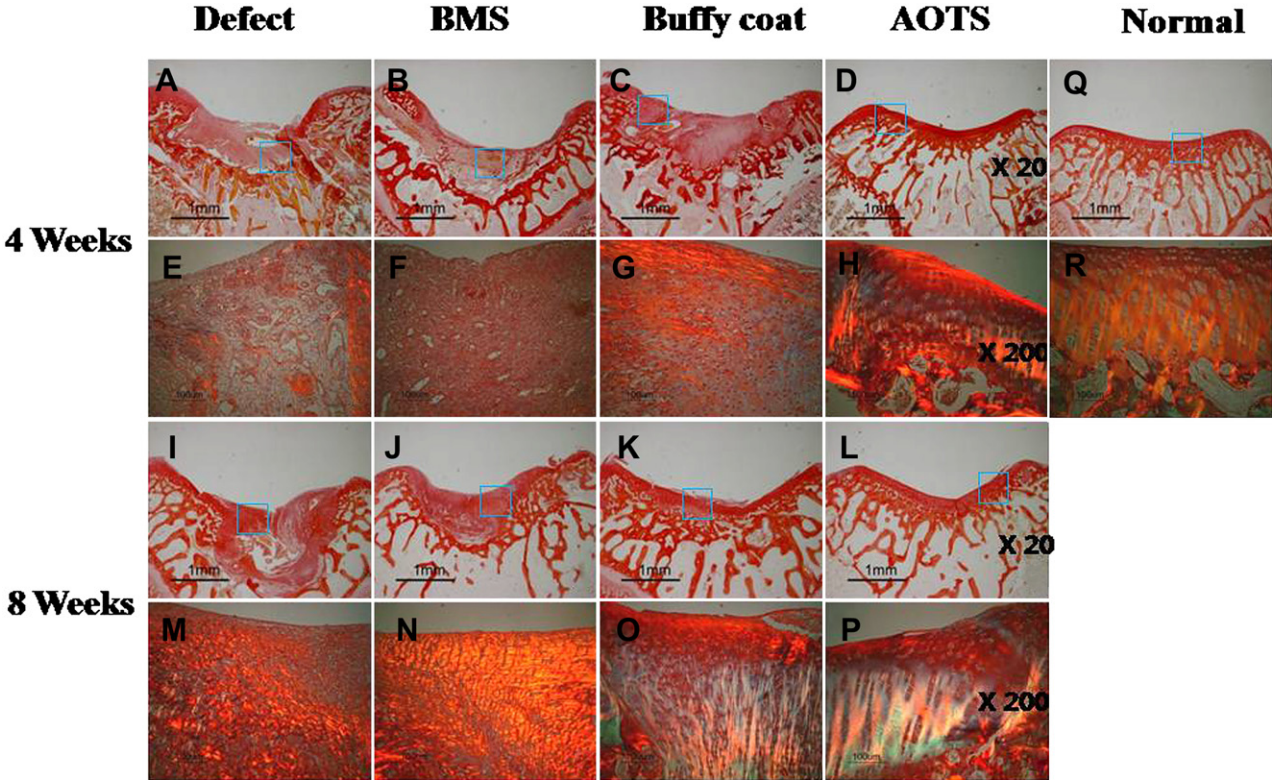

Fig. 3. Sirius red staining image at 4 weeks (A–H) and 8 weeks (I–P) after surgery. An image of normal cartilage was also provided (Q). Magnification was at ×100 (A–D, I–L) and ×200 (E–H, M–P), respectively.

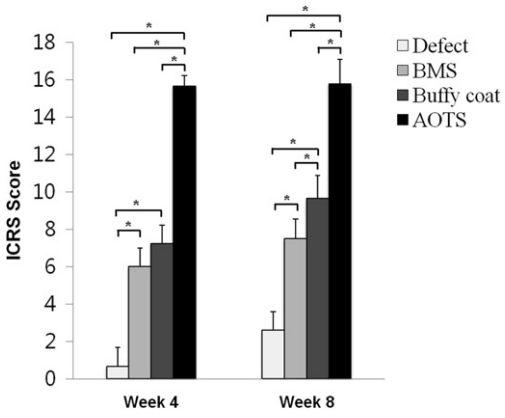

Fig. 4. ICRS scores at 4 weeks and 8 weeks after surgery. The total ICRS histological score increased significantly along with time in all groups. No significant difference was observed between the Buffy coat group and the BMS group at 4 weeks; however, at 8 weeks, a significant difference was shown in ICRS score. $N \geq 5$, $P = 0.018$, $P = 0$. (Error bars are standard deviation, $^*P < 0.05$).

Buffy coat group, AOTS group, and normal cartilage. This finding suggested that GAG formation of the Buffy coat group approached that of normal cartilage ($N \geq 5$, $P < 0.001.015$, $P < 0.001.047$) [Fig. 6(A)]. Lower levels of DNA content were detected in the Buffy coat group compared to the Defect and BMS groups. On the other hand, the AOTS and Normal groups did not show the significant difference. This finding means that repaired cartilage of the Buffy coat group was compatible to hyaline cartilage ($N \geq 5$, $P < 0.001.045$, $P < 0.001$) [Fig. 6(B)].

Discussion

We hypothesized that implantation of buffy coat provides a large number of bone marrow stem cells and other biochemical factors, and, in turn, can significantly enhance repair of cartilage defects. Several aspects of the results from this study support the hypothesis. Gross morphology of repaired tissue in the autologous buffy coat-supplemented group (Buffy coat group) resembled that of normal cartilage, having a glistening appearance in the defect area. In addition, hyaline cartilage-like tissue with mature matrix and columnar organization of chondrocytes was observed by histological evaluation. Similarly, the chemical assay showed that the GAG component in repaired tissue is almost the same as that in normal cartilage.

The amount of blood clots is not much after BMS. In addition, most of the blood clots consisted of blood cells that were less likely related to tissue regeneration, and the number of stem cells was only hundreds per mm^3 in defect area[6]. Given the fact that the number of chondrocytes in healthy cartilage is 100,000/mm^3, the number of stem cells obtained through BMS is relatively low[23]. The small number of MSCs may be a factor in the inclination of cartilage repair toward fibrocartilage after BMS. We found that an average of only 39.25 μl blood flowed out from the bone marrow through the BMS, which contained 23.88 MSCs (Table I) while 4 ml of bone marrow blood could be obtained at our discretion from the iliac crest, of which buffy coat contained approximately 2,431.2 MSCs (Table I). In the rabbit bone marrow, the MSCs frequency of the ficoll-isolated buffy coat (2.43 per 10^4 MNCs) was similar to that of the human bone marrow ranging 1 per 10^3–10^5 MNCs[24]. Resultantly, total number of MSCs in the defect area drastically increased with addition of buffy coat (approximately 2,400 MSCs). Therefore,

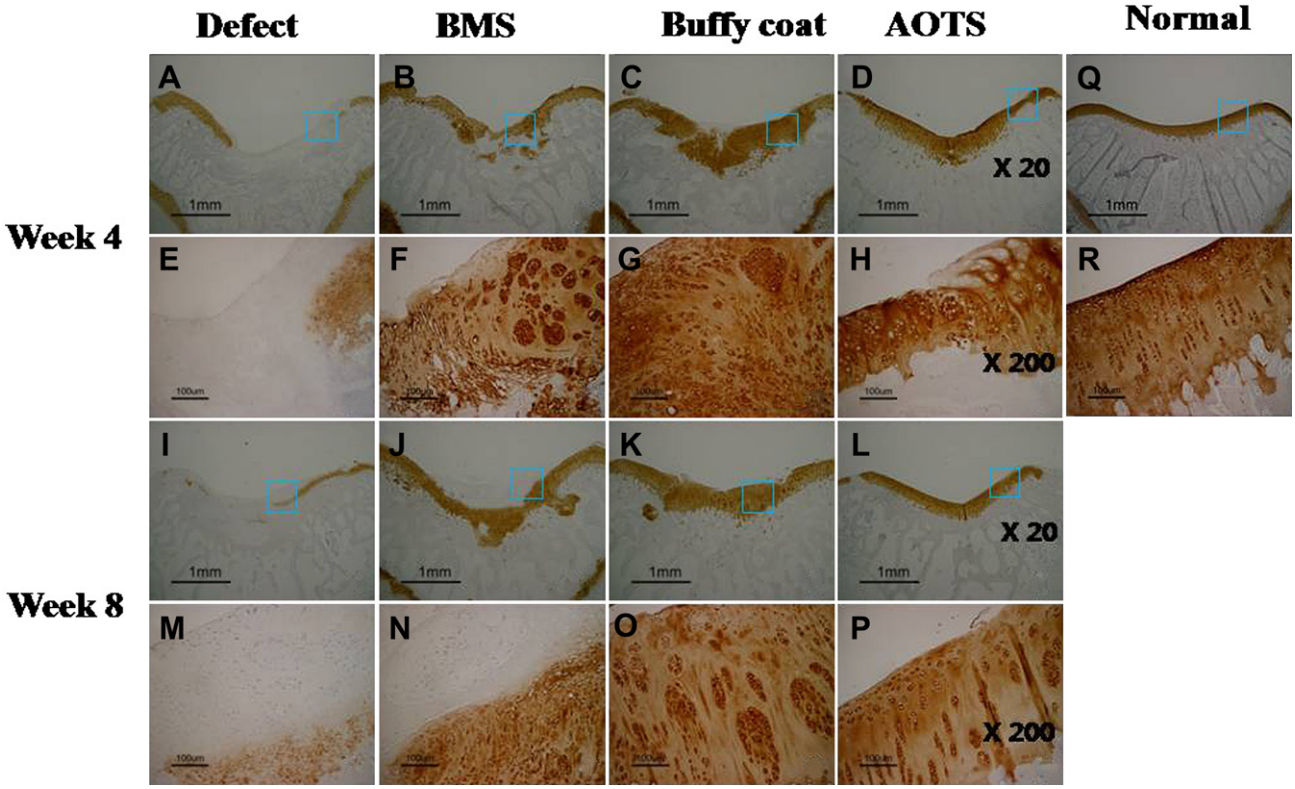

Fig. 5. Immunohistochemistry for collagen type II expression. At 4 weeks (A–H) and 8 weeks (I–P) after surgery. An image of normal cartilage was also provided (Q). (Magnification of ×20: A–D, I–L and magnification of ×200: E–H, M–P) Expression of type II collagen was gradually increased along with time at the pericellular region in repaired tissues of the BMS, Buffy coat, and AOTS groups. It was not significantly detected in the Defect group at both week 4 and 8. The most significant expression of type II collagen with dark brown color was observed in zonal-structure in the Buffy coat and AOTS groups at 8 weeks.

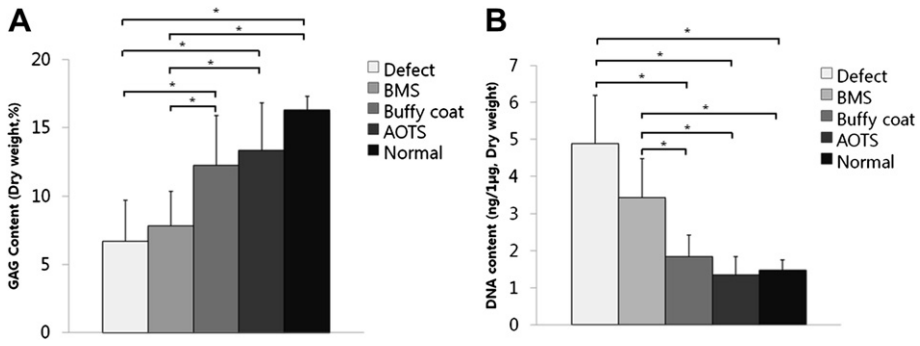

Fig. 6. (A) Changes and comparison of GAG contents among the experimental groups at 8 weeks after surgery. GAG contents of the Buffy coat group were higher than those of the Defect group and the BMS group. Moreover, they were significantly different in statistical analysis. However, the Buffy coat group showed no difference from those of the AOTS group and normal groups in statistical analysis. $N \geq 5$, $P = 0.015$, $P = 0.047$. (B) Changes and comparison of DNA contents among the experimental groups at 8 weeks after surgery. A lower level of DNA content was detected in the Buffy coat group compared with the Defect group and the BMS group. Moreover, they were significantly different in statistical analysis. However, the Buffy coat group showed no difference from those of the AOTS group and normal groups in statistical analysis. $N \geq 5$, $P = 0.045$, $P = 0$. (Error bars are standard deviation, $^*P < 0.05$).

a better result can be expected in cartilage repair if we supplement a larger number of cells. In the present study, we showed that implantation of autologous bone marrow buffy coat after BMS arthroplasty was more effective than the BMS alone and regenerated efficiently the cartilage defects in a rabbit model. Based upon the results of this study, the appropriate amount of buffy coat to be collected from the bone marrow when this technique is applied to a human subject can be approximated. In this study, the cartilage defect was 5 mm in diameter and 2 mm in depth, and its volume was approximately 40 μl, while buffy coat was approximately 20 μl. In case, for a human cartilage defect that is 10 mm in diameter and 4 mm in depth, 157 μl of buffy coat from 31 ml of bone marrow seems appropriate for the therapy.

More MSCs may be transplanted by extracting a lot of blood from the bone marrow and concentrating it. Hernigou *et al.* and Kasten *et al.* gained 108 CFU-Fs/ml and 1,480 CFU-Fs/ml in 306 ml and 297 ml aspirate, respectively, and finally concentrated them into 612 CFU-Fs/ml in 20 ml(4 times) and 2,579 CFU-Fs/ml in 18 ml (14 times) by decreasing the volume[25,26]. It is of note that the concentrated bone marrow aspirates were shown to repair osteochondral defect in a large animal of equine model[27]. Transplant of buffy coat has some advantages compared to the transplant of cultivated MSC. Transplant of autologous cell requires two steps for harvesting the patient's cell or tissue and transplanting after cultivation. In contrast, the transplant of buffy coat can be finished at a time without cultivation as well as reduce chance of contamination derived from it. In addition, it will reduce the cost of cultivation drastically.

Many reports have shown that bone marrow-derived MSCs have a high potential for the repair of mesenchymal tissue[28]. However, Matsumoto *et al.* reported that the cultured MSCs transplanted in the cartilage defect regenerated it but formed fibrocartilage[29]. Besides, uncultured MNCs that include both MSCs and HSCs, and the buffy coat have also been used widely as a cell source for cartilage regeneration[9–12]. MNCs or buffy coat can be used immediately without any risk related to their culture *in vitro*. It is also believed that T cells, B cells, macrophages, and cytokines that are secreted by platelets in buffy coat may influence defect healing.

One possible reason for the occurrence of fibro-cartilage repair is that bone marrow and blood clots could have been washed away by fluid within the knee joint cavity. To overcome this problem, the ECM membrane (ArtiFilm) made by cultured porcine chondrocytes was used in this study. The ECM membrane is comprised mainly of cartilage-enriched ECM molecules such as collagen type II and sulfated GAGs, and previously shown to have high biocompatibility, biodegradability, and cartilage affinity[30]. We speculate that the ECM membrane presumably can not only cover the cartilage defect to prevent loss of cells in the defect area, but can also interrupt influence from synovial fluid in order to prevent differentiation of stem cells in an unwanted direction[30]. Additionally it is very thin (approximately 30 μm) not to be caught in articulation. Accordingly, it is regarded as a suitable biomaterial for cartilage repair. Similar to our study, a collagen type I/III membrane has been also used recently to protect autologous bone marrow and successfully repair the cartilage defect[31].

It is widely accepted that scaffold biomaterials can also influence significantly the cartilage repair[32]. Because this study was designed to address the effect of BM-derived buffy coat, the ECM membrane was applied to all experimental groups to simply cover the defect area and prevent possible loss of MSCs. We speculate, however, that the ECM membrane could provide cells with a cartilage-like and favorable environment for cartilage repair. Although we have no direct results using the ECM membrane, a 3D scaffold made of the same ECM material was previously shown to be much superior to poly glycolic acid (PGA) scaffold in supporting cartilage tissue formation using chondrocytes and chondrogenesis of bone marrow MSCs *in vitro*[13,33]. Therefore, the ECM membrane could be a better choice over other biomaterial scaffolds for the cartilage repair.

Though AOT has been shown to lead cartilage regeneration fairly well in human or animal models, transplanted tissues have still problems arisen from different thickness and collagen fiber direction from surrounding ones. In this study, we set up new AOT protocol by inserting the isolated osteochondral tissue from the osteochondral defect back into its own site. It is expected to be optimal since the thickness and direction of collagen fiber of a transplanted tissue fits well into the surrounding tissues.

A major limitation of this study is that we did not compare implantation of buffy coat only without performance of microfracture. Because the number of MSCs from buffy coat was overwhelming that by microfracture, the effect of microfracture may be abolished. However, we speculate that in order to prove this concept, we should design another set of experiments for evaluation of the effect of microfracture, that is to say, the role of subchondral bone as a holder of blood clot or foundation to support repair of cartilage.

Conclusion

We observe that implantation of autologous bone marrow buffy coat after BMS repair of the cartilage defect in a rabbit model is

better than BMS alone. It is postulated that the implanted buffy coat provides a larger number of MSCs and plausible supporting humoral factors for repair of the defect and that the ECM membrane covering the defect played a role in prevention of the injected cells and blood clot from leaking into the joint fluid. As the method employed in this study is safe and easy, it is potentially feasible for clinical applications.

Author contributions

Long Hao Jin: Conception and design, analysis and interpretation of the data, drafting of the article, provision of study materials or patients, statistical expertise, collection and assembly of data.

Cheng Zhe Jin: Analysis and interpretation of the data, provision of study materials or patients, statistical expertise.

Byung Hyune Choi: Critical revision of the article for important intellectual content, provision of study materials or patients, statistical expertise, technical, or logistic support.

Young Jick Kim: Analysis and interpretation of the data, critical revision of the article for important intellectual content, statistical expertise, administrative, technical, or logistic support.

So Ra Park: Conception and design, critical revision of the article for important intellectual content, technical, or logistic support.

Byoung-Hyun Min: Conception and design, critical revision of the article for important intellectual content, final approval of the article, provision of study materials or patients, obtaining of funding, administrative, technical, or logistic support.

Conflict of interest

The authors indicate no potential conflicts of interest.

Acknowledgments

This study was supported by a grant of the Korea Healthcare Technology R and D project, Ministry for Health, Welfare and Family Affairs, Republic of Korea (A091120).

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