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

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Abstract

Objective: To test the hypothesis that platelet-rich fibrin glue (PR-FG) can be used clinically as a scaffold to deliver autologous culture-expanded bone marrow mesenchymal stem cells (BM-MSCs) for cartilage repair and to report clinical results 1 year after implantation of MSCs PR-FG. Patients and Methods: Autologous BM-MSCs were culture expanded, placed on PR-FG intraoperatively, and then transplanted into 5 full-thickness cartilage defects of femoral condyles of 5 patients and covered with an autologous periosteal flap. Patients were evaluated clinically at 6 and 12 mo by the Lysholm and Revised Hospital for Special Surgery Knee (RHSSK) scores and radiographically by x-rays and magnetic resonance imaging (MRI) at the same time points. Repair tissue in 2 patients was rated arthroscopically after 12 mo using the International Cartilage Repair Society (ICRS) Arthroscopic Score. Study Design: Case series; level of evidence 4. Results: All patients’ symptoms improved over the follow-up period of 12 mo. Average Lysholm and RHSSK scores for all patients showed statistically significant improvement at 6 and 12 mo postoperatively (P < 0.05). There was no statistically significant difference between the 6 and 12 mo postoperative clinical scores (P = 0.18). ICRS arthroscopic scores were 8/12 and 11/12 (nearly normal) for the 2 patients who consented to arthroscopy. MRI of 3 patients at 12 mo postoperatively revealed complete defect fill and complete surface congruity with native cartilage, whereas that of 2 patients showed incomplete congruity. Conclusion: Autologous BM-MSC transplantation on PR-FG as a cell scaffold may be an effective approach to promote the repair of articular cartilage defects of the knee in human patients.

Keywords
mesenchymal stem cells, platelet-rich plasma, fibrin glue, cartilage, repair

Introduction

Human articular cartilage has limited repair potential.1 Marrow-stimulation procedures as microfracture for cartilage repair rely on the formation of a primitive mesenchymal blood clot that forms fibrous tissue with variable outcome. Limitations of osteochondral grafts include limited donor site, morbidity, questionable cell viability, and fibrocartilage formation in between osteochondral plugs.2,3 Cell-based strategies have explored chondrocytes and mesenchymal stem cells (MSCs) with extensive basic science and preclinical studies.4-6 However, clinical strategies have focused on autologous chondrocyte implantation, yet the concept itself is not ideal; it is a 2-step procedure with donor site morbidity, the quantity of harvested chondrocytes is limited, and chondrocytes dedifferentiate into
fibroblasts when cultured ex vivo. Because of these limitations, the focus in cartilage repair is shifting toward bone marrow mesenchymal stem cells (BM-MSCs) and biodegradable scaffolds with or without growth factors. BM-MSCs are available in larger quantities, are easier to isolate and expand, and demonstrate strong chondrogenic potential.7

Platelet-rich preparation such as platelet-rich plasma (PRP) and platelet-rich fibrin glue (PR-FG) is a novel biological scaffold that has not been widely tested by itself as an MSC carrier in clinical chondrogenesis. It is nonimmunogenic, bioabsorbable, sterile, easily prepared, and set intraoperatively. The α-secretory granules of platelets contain transforming growth factor (TGF)-β1 and insulin growth factor-1 (IGF-1), which stimulate cartilage regeneration.8-11 It is expected that the biological effect of multiple growth factors on tissue regeneration is greater than that of a single growth factor. PR-FG has also been shown to provide sustained release and protection against proteolytic degradation of endogenous growth factors.12,13 Because the average baseline blood platelet count in an individual is 200,000 ± 75,000/µL, a platelet concentrate count of 1,000,000/µL (5-fold) has been commonly described for therapeutic platelet-rich preparations.14 Concentrations of growth factors in PR-FG are found to be in supraphysiological levels (5- to 27-fold, according to different processing methods).15 The concentrations of TGF-β1 and IGF-1 in PR-FG are in the range of 90 to 400 ng/mL and 50 to 200 ng/mL, respectively.16-18

Table 1. Characteristics of the 5 Patients with Cartilage Defects Treated with Bone Marrow Mesenchymal Stem Cells Transplanted in Platelet-Rich Fibrin Glue

| Case | Gender | Age | Disease | Site of lesion | Size of lesion (cm × cm) | Side | No. of previous operations | Associated surgery | Follow-up (mo) |
|------|--------|-----|---------|---------------|--------------------------|------|--------------------------|-------------------|---------------|
| 1    | M      | 24  | Degenerative OA due to neglected OCD | MFC | 3.5 × 3 | Rt | 1 | NA | 16 |
| 2    | F      | 21  | OCD | LFC | 2 × 1.5 | Rt | 1 | NA | 16 |
| 3    | M      | 24  | OCD | LFC | 1.8 × 2.1 | Lt | 1 | NA | 15 |
| 4    | M      | 21  | Traumatic | LFC | 2 × 3.2 | Rt | 1 | NA | 12 |
| 5    | M      | 37  | ACL tear | MFC | 2.2 × 2.4 | Lt | 2 | ACLR + MF | 12 |

Mean = 25.4 Mean = 5.8 cm² Mean = 14.2

Note: OCD = osteochondritis dissecans; OA = osteoarthritis; MFC = medial femoral condyle; LFC = lateral femoral condyle; Rt = right; Lt = left; NA = non applicable; ACL = anterior cruciate ligament; ACLR = anterior cruciate ligament reconstruction; MF = microfracture.

Patients and Methods

The study included 5 patients: 4 men and 1 woman (range = 21-37 y; mean = 25.4 y). Inclusion criteria were a full-thickness cartilage lesion in weightbearing areas of the femoral condyle (Outerbridge grade III or IV), size 3 to 12 cm², disabling symptoms not responding to conservative treatment, age between 18 and 50 y, and patients agreeing to comply with strict rehabilitation. Exclusion criteria were complex ligamentous knee instability, kissing lesions, axial malalignment (>10° varus/valgus) assessed clinically, osteochondral neoplasia, inflammatory joint disease, immune suppression, and symptomatic vascular/neurologic disorder of the lower extremities. All of the patients provided written informed consent according to regulations and after approval of the Ethical Committee and were operated on by the same surgeon (the 1st author). Patient details are provided in Table 1.

For clinical evaluation, we used 2 scores collected by an independent observer: the Lysholm score (subjective) and the Revised Hospital for Special Surgery Knee Score (subjective and objective). In addition, plain x-rays (AP, lateral ± tunnel views) and magnetic resonance images (MRIs; T1 weighted, T2-fat-suppressed weighted, proton density imaging) of the involved knee were performed using a 1.5T MRI machine (Philips, Amsterdam, the Netherlands) and evaluated by an independent senior musculoskeletal radiologist. The scores, x-rays, and MRIs were taken preoperatively and 6 and 12 mo postoperatively. Second-look arthroscopy was performed after 12 mo on 2 patients who consented to the procedure. The repair tissue was rated according to the International Cartilage Repair Society (ICRS) Arthroscopic Grading System. None of the patients consented to an arthroscopic biopsy.
Bone marrow (20 mL) was aspirated from the iliac crest under aseptic standard operative procedures and placed in heparinized tubes. Cell cultures were performed at the Molecular Biology and Tissue Engineering Unit at the same university. Briefly, nucleated cells were isolated with a density gradient (Ficoll-Paque; GE HealthCare, Waukesha, WI) and resuspended in culture medium (Delbecco’s Modified Eagle’s Medium) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (10,000 µg/mL). FBS used in this study was purchased from reliable countries without bovine virus traceability, where it is furthermore subjected to serial filtration and terminal sterilization with gamma irradiation and followed by bacterial and viral testing using validated procedures to ensure pathogen freedom. Cells were incubated at 37 °C in 5% CO2 for ~14 d. Culture medium was changed every 2 to 3 d. When cells reached 80% to 90% confluence, cultures were washed twice with phosphate-buffered saline (PBS), and the cells were trypsinized with 0.25% Trypsin in 1 mM EDTA for 5 min at 37 °C. After centrifugation, cells were resuspended in medium and subcultured for ~10 d, reaching an average count of 15 × 10⁶. MSCs in culture were characterized by their adhesiveness and fusiform shape, by flow cytometry for MSC surface markers (CD34⁺, CD45⁻, and CD73⁺) and for CD29 gene expression by reverse transcriptase polymerase chain reaction.¹⁹-²²

On the day of implantation, cells were trypsinized, collected, and resuspended in 1 mL PBS and transferred to the operating room in sterile tubes. Potential immunogenicity of less than 0.05 were accepted as significant.

Platelet concentrate was prepared in the hospital blood bank (El-Shabrawishi Hospital Blood Bank, Cairo, Egypt) according to standardized protocols previously reported elsewhere.²³ Briefly, using a cell saver centrifuge system, 500 mL volume of blood from healthy donors was collected into 70 mL of citrate-phosphate-dextrose anticoagulant. This was delivered into the 125-mL disposable bowl at 60 mL/min, with the centrifuge spinning at 2039 g until the red blood cells reached 1.25 cm from the collar of the bowl. The centrifuge speed was then reduced to 368 g, and a blood flow rate of 60 mL/min was used to continuously collect the PRP, from which 1 mL of platelet-rich concentrate was used with FG to form PR-FG. This method has been shown to yield a platelet count of 7.7 ± 1.1 × 10⁹/mL.²³

In a 2nd-stage implantation procedure, under a tourniquet, the knee joint was exposed via medial or lateral parapatellar arthroscopy. The defect was excised to a stable rim, and sclerotic bone was curetted. Multiple 3- to 4-mm-deep perforations in the subchondral bone were performed using a 1.2 Kirschner wire at 3- to 4-mm intervals. An autologous periosteal flap was harvested from the anteromedial ipsilateral proximal tibia and tailored to the defect size. The flap was sutured to surrounding cartilage with the cambium layer facing toward the subchondral bone using absorbable 4-0 sutures, leaving the 12 o’clock position open for introduction of the BM-MSCs/PR-FG mixture. The periosteal flap was additionally secured by FG. Allogenous PR-FG was supplied as 1 mL platelet concentrate, 1 mL fibrinogen, and 1 mL thrombin in separate syringes from the aforementioned blood bank. BM-MSCs were mixed with the PR-FG, and the mixture was left to gel and then introduced through the 12 o’clock position and subsequently sutured and sealed by FG (Fig. 1). The inoculum density of BM-MSCs at the time of implantation was 2 × 10⁵ cells/cm². The surgical wound was closed in layers. One drain without suction was placed intra-articularly.²⁴-²⁶

Continuous passive motion was initiated on the 2nd day following surgery. Weightbearing was restricted, whereas passive, active-assisted, and active range-of-motion exercises were initiated toward a full range equal to the contralateral knee. After 4 to 6 wk, partial weightbearing was initiated as tolerated. By 8 wk, full weightbearing, unlimited gym work (except treadmill jogging) was permitted. Light jogging was started at 5 mo.²⁴-²⁶

Statistical analyses were done using Statistical Package for Social Studies (SPSS) Software, version 11 for Windows. The clinical scores were shown as the mean ± standard deviation at 3 time points: preoperatively and 6 and 12 mo postoperatively. Data were analyzed statistically by Friedman’s test and the Wilcoxon signed-rank test. P values of less than 0.05 were accepted as significant.

Results

None of the cell cultures had bacterial or fungal infection, nor were there any perioperative complications in any of the patients. The clinical scores of the 5 patients are summarized in Table 2. Compared with preoperative status, all patients experienced marked improvement in symptoms and knee function, allowing them to return to normal daily activities. All 4 patients without preexisting radiographic signs of advanced degenerative changes returned to their previous sporting levels. Clinical scores 6 mo and 12 mo postoperative were statistically different from preoperative scores (P < 0.05). The difference between the 6- and 12-mo clinical scores did not reach statistical significance (P = 0.18; Fig. 2).

Radiologically, patient 1 had more advanced degenerative changes at the time of surgery (Fig. 3) than the other 4 patients, and his preoperative scores were also initially lower. Six- and 12-mo follow-up MRI of the operated knee showed partial defect fill, complete incongruity of the repair tissue with native cartilage, and lower signal intensity in relation to the surrounding subchondral bone. The MRI findings coincide with this patient’s relatively lower
Table 2. Details of the Clinical Scores of the 5 Patients Preoperatively, 6 and 12 Mo Postoperatively, as Well as the Arthroscopic Scores of the 2 Patients Who Underwent Follow-up Arthroscopy at 1 Y

| Case | Lysholm score | Revised hospital for special surgery knee score | International Cartilage Repair Society arthroscopic score |
|------|---------------|-----------------------------------------------|---------------------------------------------------------|
|      | Preop | 6 mo postop | 12 mo postop | Preop | 6 mo postop | 12 mo postop | 12 mo postop |
| 1    | 20    | 76          | 76           | 31    | 69          | 69           | 8            |
| 2    | 45    | 66          | 76           | 47    | 75          | 79           | 11           |
| 3    | 53    | 95          | 95           | 71    | 90          | 90           |              |
| 4    | 50    | 92          | 92           | 62    | 93          | 93           |              |
| 5    | 38    | 79          | 91           | 58    | 68          | 88           |              |
| Mean ± SD | 41.2 ± 13.14 (P = 0.042) | 81.6* ± 11.93 (P = 0.042) | 86.0* ± 9.25 (P = 0.042) | 31 ± 15.39 (P = 0.043) | 53.8 ± 15.39 (P = 0.043) | 79.0* ± 11.77 (P = 0.043) | 83.8* ± 9.78 (P = 0.043) |

*Statistically significant difference.

Figure 1. Autologous mesenchymal stem cell (MSCs) platelet-rich fibrin glue (PR-FG) implantation. (a) Cartilage defect curetted to a peripheral stable rim of cartilage and subchondral bone drilled. (b) Periosteal flap harvested from the proximal tibia. (c) Defect covered with periosteal flap, sutured, and then sealed with fibrin glue. (d) MSCs mixed with PR-FG intraoperatively to be introduced through the 12 o’clock position of the defect.
postoperative scores. X-rays of the patient at 6 and 12 mo showed no evidence of filling of the bony element of the defect nor restoration of the congruity of the femoral condyle. However, the patient reported significant relief of symptoms and was able to return to his laborious job after being incapacitated because of knee pain before surgery.

At 6 mo postoperative, MRI of patient 2 showed partial defect filling with incomplete congruity of the repaired articular surface. This persisted at 12-mo follow-up MRI. X-rays at 6 and 12 mo showed persistent radiolucency and sclerosis at the margins of the defect. Arthroscopic evaluation of the repair tissue showed 75% repair of defect depth, 75% graft integration, 25% with a border >1-mm wide, and a fibrillated surface all through. The overall ICRS score was 8/12 (nearly normal cartilage). The patient had described the outcome as “good.”

Patients 3, 4, and 5 described their outcomes as “excellent,” and all showed complete defect fill with no overgrowth or periosteal hypertrophy and congruity of the repair tissue with the native cartilage on 6 to 12-mo follow-up MRI (Fig. 4). X-rays showed complete filling of the defects with restoration of the congruity of the femoral condyles and no evidence of degenerative changes. In patient 4, 2nd-look arthroscopy after 12 mo showed the graft surface to be level with the surrounding cartilage, a demarcating border <1 mm at some zones (~25% of the circumference), and an intact smooth surface (Fig. 5). The overall ICRS score was 11/12 (nearly normal cartilage). The patient returned to high-impact sports after 9 mo.

Discussion
Numerous preclinical studies have reported development of hyaline-like tissue from autologous culture-expanded BM-MSCs, and some have shown superior functionality of BM-MSCs over chondrocytes. These results have
The image shows a page from a scientific article discussing the use of bone marrow stromal cells (BM-MSCs) in cartilage repair. The text includes details about the clinical trials using PR-FG as a cell carrier, mentioning its biodegradability, autologous nature, and the release profile of chondrogenic growth factors. The study reported significant improvement in functional knee scores and MRI findings for patients with osteochondral defects, with results maintained over 12 months postoperatively. The text also highlights the importance of early intervention in treating cartilage defects before degenerative changes become irreversible.

Further, the article discusses the variations in outcomes among patients with different degrees of degenerative changes in their native cartilage, suggesting that the condition of the native cartilage can affect repair outcomes. The text concludes with the need for further evaluation of this technique in patients with arthritic changes.

The figures in the article illustrate preoperative and postoperative MRI images, showing the repair cartilage filling the defect and the congruent surface with the native cartilage, indicating successful integration.

In summary, the article provides insights into the clinical application of BM-MSCs in cartilage repair, emphasizing the importance of early intervention and the role of native cartilage condition in the repair process.
Although patient 2 had the smallest defect size (3 cm²) in this case series, the relatively inferior outcome in this case compared with patients 3 to 5 could be attributed to a number of causes: First, the ongoing subchondral bone pathology due to osteochondritis dissecans evident as radioluency with surrounding bone sclerosis on plain x-rays at 1-y follow-up might have impeded proper tissue vascularization, with subsequent MSC death and poor graft integration. Second, the chondrogenic potential of BM-MSCs in this patient might have been quantitatively and qualitatively less than other patients. Payne et al.35 have recently reported that MSC chondrogenic potential shows both individual and age-dependent variations.

Although the age inclusion criterion for the study was 18 to 50 y, the patients in this pilot study were of relatively younger age (range = 21-37 y; mean = 25.4 y). This might have influenced the outcome of this cartilage repair procedure because the qualitative and quantitative difference in the metabolic activity of cells in the repair tissue is age dependent.36-41 It is well documented that MSCs age as they undergo more population doublings, and their synthetic activity and chondrogenic potential decline.42-46

The biopsy-induced injury and whether it heals spontaneously or further jeopardizes the repair limited its ethical justification to patients and remains a point of debate. This is of particular importance if long-term follow-up of the repair tissue is intended that should not be violated. MRI was used to evaluate the reparative tissue and is considered a good noninvasive method that was found to correlate well with 2nd-look arthroscopy and histology of cartilage repair tissue.47

FBS was used for *in vitro* culture expansion of MSCs. Steps were taken to minimize potential risks for pathogenicity and immunogenicity. Although these concerns cannot be completely eliminated, a phase I clinical study employing culture-expanded stem cells in FBS-containing media (at higher concentrations than used in our study) reinjected in patients with liver insufficiency similarly reported no complications or specific side effects related to the procedure.48

MSC implantation on PR-FG is a new technique and required an initial small number of patients enrolled in the pilot study, consequently limiting the sample size of this study. Because the sample size was small, we used nonparametric statistical methods, which do not require the assumptions of normality and equal variances (i.e., standard deviations). Friedman’s test was used to compare observations repeated on the same subjects as a group. It is similar to the parametric repeated-measures analysis of variance and is used to detect differences in treatments across multiple test attempts. The Wilcoxon signed-rank test was used to test for repeated measurements on a single sample. It can be used as an alternative to the paired Student’s *t* test when the population cannot be assumed to be normally distributed. Nonparametric tests are more conservative and are appropriate for hypothesis testing when the sample size is small. Because the sample size was small and there was no randomly assigned control group, a further randomized controlled clinical trial of this treatment modality, with larger numbers of patients and longer follow-up periods, is necessary to achieve even higher levels of validity.

This pilot study demonstrates feasibility and proof of principle that BM-MSCs PR-FG can be used clinically for the treatment of articular cartilage defects. This is consistent with basic science and preclinical *in vivo* studies reported in this field. We used standardized clinical outcome scores to assess the results of this treatment modality and MRI for assessment and follow up of the repair. The 2nd-look arthroscopies performed on 1 patient with partial defect fill by MRI and 1 patient with complete defect fill by MRI confirm the MRI assessments and support the use of MRI as a noninvasive postoperative assessment tool.

We conclude from this study that the transplantation of autologous culture-expanded BM-MSCs in PR-FG shows great promise in the treatment of full-thickness articular cartilage defects, particularly large-sized defects (>4 cm²). We have demonstrated that PR-FG successfully fixed the cultured MSCs within the defects and provided them with a suitable environment to synthesize a hyaline-like grossly appearing cartilaginous matrix. The positive 1-y clinical outcomes support further randomized controlled clinical trials of this treatment modality with larger numbers of patients and longer follow-up periods, as previously stated.

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**Declaration of Conflicting Interests**

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Clinical Trial Registration: www.clinicaltrials.gov ID NCT00891501.
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