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Cartilage Repair With Autologous Bone Marrow Mesenchymal Stem Cell Transplantation: Review of Preclinical and Clinical Studies

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Abstract

Clinical trials of various procedures, including bone marrow stimulation, mosaicplasty, and autologous chondrocyte implantation, have been explored to treat articular cartilage defects. However, all of them have some demerits. We focused on autologous culture-expanded bone marrow mesenchymal stem cells (BMSC), which can proliferate without losing their capacity for differentiation. First, we transplanted BMSC into the defective articular cartilage of rabbit and succeeded in regenerating osteochondral tissue. We then applied this transplantation in humans. Our previous reports showed that treatment with BMSC relieves the clinical symptoms of chondral defects in the knee and elbow joint. We investigated the efficacy of BMSC for osteoarthritic knee treated with high tibial osteotomy, by comparing 12 BMSC-transplanted patients with 12 cell-free patients. At 16-month follow-up, although the difference in clinical improvement between both groups was not significant, the arthroscopic and histological grading score was better in the cell-transplanted group. At the over 10-year follow-up, Hospital for Special Surgery knee scores improved to 76 and 73 in the BMSC-transplanted and cell-free groups, respectively, which were better than preoperative scores. Additionally, neither tumors nor infections were observed in all patients, and in the clinical study, we have never observed hypertrophy of repaired tissue, thereby guaranteeing the clinical safety of this therapy. Although we have never observed calcification above the tidemark in rabbit model and human histologically, the repair cartilage was not completely hyaline cartilage. To elucidate the optimum conditions for cell therapy, other stem cells, culture conditions, growth factors, and gene transfection methods should be explored.

Keywords

cartilage, bone marrow mesenchymal stem cell, cell transplantation, chondral defect, tissue engineering

Introduction

Articular cartilage covers the ends of bones that form diarthrodial joints, and works as a lubricant and shock absorber. Histologically, articular cartilage is a subset of hyaline cartilage tissue where the extracellular matrix exhibits a collapsed structure lacking blood, lymphatic, or nerve supply, and therefore has poor repair potential. In general, cartilage defects are hardly repaired if they do not penetrate subchondral bone (partial-thickness defects), but could be repaired along with heterogeneous tissue, from fibrous tissue to fibrocartilage, when penetrating subchondral bone (full-thickness defects). However, the reparative tissue, even if appearing as hyaline cartilage histologically, would lack the biochemical capability to express some cartilage-specific molecules, and its biomechanical durability is substantially inferior to that of age-matched normal articular cartilage.[1]

Regarding the prognosis of damaged articular cartilage, defects have not been considered a major problem among many clinicians because they cause few clinical symptoms, at least in the short term. Recently, however, reports have revealed that clinical symptoms or radiological changes

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caused by articular cartilage defects are getting worse when observed for more than 10 years.[2,3] Thus now, articular cartilage defects are thought to need repairing to prevent subsequent osteoarthritis (OA) progression. In fact, articular cartilage defects are indeed a major clinical problem.

To date, we are only partially happy with the repair of articular cartilage defects using current clinical procedures. These procedures usually involves a bone marrow stimulation technique, in which subchondral bone is broken to facilitate cartilage repair from bone marrow–derived cells and growth factors, and consists of multiple perforations,[4] abrasions,[5] and micro-fractures.[6] However, with this procedure, cartilage defects are most often repaired with fibrocartilage, which is biochemically and biomechanically different from normal hyaline cartilage, and this tissue subsequently undergoes degeneration.[1]

Recent studies report the benefits of autologous chondrocyte implantation (ACI)[7] and mosaicplasty.[8,9] Although we can repair small articular cartilage defects using these techniques, their effectiveness is still disputed. Even after ACI and mosaicplasty, some defects continue to persist in the articular cartilage, albeit not in the main weightbearing portions of the joint. In ACI, there is only weak evidence to show the effectiveness[10] and we are forced to sacrifice normal cartilage tissue for harvest, and so an alternative method to obtain autologous cells is preferable.

Further investigations into the repair of articular cartilage defects, using certain other types of cells, have been performed worldwide. Osteochondral progenitor cells or mesenchymal stem cells exist in many kinds of tissue, such as bone marrow, synovium, muscle, and fat (adipose). Autologous cells of these tissues are easily obtained. Within these cells, synovial cells have the best capacity for chondrogenesis, and are a promising source of cells for clinical application.[11] Adipose-derived mesenchymal stem cells are also a noteworthy source for cell therapy.[12,13]

We focused on bone marrow–derived mesenchymal stem cells (BMSC) as a cell source, to explore a new method of cartilage regeneration by studying transplantation of autologous BMSC for articular cartilage defects. Here, we review our work on autologous BMSC transplantation into animals and humans, and show the long-term follow-up outcomes of autologous BMSC transplantation for patients with unicompartmental osteoarthritis containing articular defects in femoral condyle.

Bone Marrow Mesenchymal Stem Cells

In 1966, adherent cells in bone marrow were transplanted into cutaneously formed osteochondral tissue.[14] Since then, cells isolated from postnatal mammalian bone marrow have been shown to have the potential to differentiate into specific cells of mesenchymal tissue, such as bone and cartilage, when implanted in vivo.[15,16] Thus, adherent cells in bone marrow blood contain progenitor cells for bone and/or cartilage. We assumed that these cells were suitable to repair osteochondral joint defects because they could differentiate into both bone and cartilage. We therefore performed autologous culture-expanded BMSC transplantation in a rabbit model.[17]

Preclinical Study

In our rabbit osteochondral defect model, we first collected autologous osteochondral progenitor cells from bone marrow. Next, they were culture expanded and embedded into a collagen gel. These cellular grafts were then transplanted into large (3 mm × 6 mm × 3 mm) full-thickness defects in the weightbearing articular surfaces of 68 rabbits. These transplants were then observed for up to 6 months after surgery.

As early as 2 weeks after transplantation, the defect was mostly replaced with cartilage. The replacement of this repaired cartilage began in the deeper portion of the defect with vascularized bone. By 4 weeks after transplantation, the deeper portion of the defect was almost completely replaced with bone, and 24 weeks after transplantation, subchondral bone was completely repaired without loss or alteration of the overlying articular cartilage. We assume that BMSC preparations rapidly and quantitatively differentiate into chondrocytes in the rabbit distal medial femoral chondyle defect, as has been observed in subcutaneous implantation samples. We hypothesize that these donor chondrocytes and the cartilage tissue that they form, are replaced by host-derived vascular and bone-forming cells up to the bone articular cartilage junction.

We confirmed the effectiveness of BMSC transplantation in the repair of osteochondral joint defects in a rabbit model. Next, we explored whether this technique could be applied in humans. BMSC have a number of suitable properties. First, it is easy to obtain autologous cells. This can be achieved by the aspiration of blood from bone marrow using local anesthesia, without major side effects. Second, we can cause these cells to proliferate without losing their capacity for differentiation, which can then be applied to large articular cartilage defects.

Repair of Articular Cartilage Defects in Humans

All our clinical studies were performed in accordance with the ethical standards of our hospital committee on human experimentation. All subjects enrolled in these studies gave their informed consent, as approved by the institutional committees on human research, who also found these protocols to be acceptable.

In applying BMSC transplantation to chondral defects in humans, the same cell preparation was performed. Briefly, after aspiration of bone marrow blood from iliac crest, nucleated cells were cultured. When the attached cells had reached subconfluence, they were subcultured to expand in culture. Adherent cells were subsequently collected, embedded in a collagen gel, transplanted into the articular cartilage defect in patellae, and covered with autologous periosteum.

Patellar Case Report (First in Humans)

Two patients presented to our clinic because their knee pain prevented them from walking normally.[18] The first case was a 26-year-old woman and the second was a 42-year-old man. After thorough examination, we concluded that the knee pain was due to injured articular cartilage, because there was no other abnormality in their knees. There were no improvements in clinical symptoms despite conservative treatment for several months, so we decided to repair the defect with BMSC transplantation. Three weeks before transplantation, bone marrow was aspirated from the iliac crest of each patient. The cultured cells were subsequently collected, embedded in a collagen gel, transplanted into the articular cartilage defect in the patella, and covered with autologous periosteum. As early as 2 months after transplantation in the first case, we performed arthroscopy and biopsy and found that the defects were covered with tissue showing slight metachromatic staining. Six months after transplantation, clinical symptoms (pain and walking disability) improved considerably, and the improvement persisted for 9 years posttransplantation in one case, and 7 years in the other (at the time of report preparation); both patients are satisfied with the outcome. Two years after the first and 1 year after the second transplantation, arthroscopy revealed that the defects had been repaired with fibrocartilage. We confirmed that autologous BMSC transplantation had been an effective approach for promoting the repair of articular cartilage defects. Now, 16 years following transplantation in the first case and 14 years in the second case, no clinical problem has been reported.

Patellofemoral Joints

In addition, we reported BMSC transplantation into osteochondral defects in 5 knees (femur and patellae) from 3 patients. A 31-year-old woman (bilateral knees), a 46-year-old man, and a 42-year-old man (bilateral knees), underwent BMSC transplantation in their patellofemoral joints. All patients had suffered from pain and clicking in their patellofemoral joints on motion. Because magnetic resonance imaging (MRI) revealed articular cartilage abnormalities in the patellofemoral joints, we performed arthroscopy to confirm the lesions, followed by autologous BMSC transplantation another day. In these cases, we found articular cartilage damage in both the femur and patellae. We removed the damaged articular cartilage, transplanted BMSC embedded in the collagen gel, and covered the transplanted tissue with autologous periosteum. Clinical symptoms improved in all patients.[19]

Femoral Condyle

Kuroda *et al*.[20] reported that transplantation of BMSC into a 20- to 30-mm, full-thickness articular cartilage repair defect in the weightbearing area of the medial femoral condyle of a 31-year-old judo player was effective.[20]

Elbow

We applied this technique to repair osteochondral defects in 3 elbows (humeral capitellum) on three 14-year-old boys.[21] All patients were throwing-athletes and had been suffering from elbow pain during throwing motion. Range of motion was slightly restricted. In radiographs, separated bone fragment was observed in capitellum and diagnosed osteochondral dissecans. Because the separated fragment was large, unstable, and divided into small pieces, meaning it is impossible to reattach this fragment, and we decided to remove the fragment and to transplant autologous BMSC. Clinical symptoms after surgery were much improved in all patients.

Safety of Autologous BMSC Transplantation in Humans

The transformation of cultured cells is a major problem in cell therapy. We have never observed tumor formation in any of our numerous animal experiments or in clinical cases of BMSC transplantation. Although the possibility cannot be excluded, human somatic cells have limited capacity for cell division, and the transformation of cultured adult human BMSC is considered to be rare.

To confirm the safety of BMSC transplantation, we investigated records of all 41 patients who together had received 45 transplantations, including cases mentioned above between January 1998 and November 2008 until their last visit to clinic. Neither tumors nor infections were observed between 5 and 137 (mean of 75) months of follow-up. Therefore we conclude that autologous BMSC transplantation is safe.[22]

Comparative Study for Patients With Knee Osteoarthritis

Results of Our Previous Report

In order to apply this technique to the repair of articular cartilage defects in human osteoarthritic knees, we

transplanted culture-expanded autologous BMSC into the cartilage defects of osteoarthritic knee joints when patients were undergoing high tibial osteotomy (HTO). We then observed the repair tissue at second-look arthroscopic exams when patients were undergoing surgery for removal of the Steinmann pins and staples that fixed the separated proximal tibia.[23] Twenty-four patients with knee OA who underwent HTO were included in this study. Fifteen were female and 9 were male. The patients' average age was 63 years (range 49-70 years). Twelve received autologous bone marrow cell transplants, and 12 were cell-free controls. BMSC were prepared in the same manner. The mean transplanted cell number was 1.3×10^7. HTO was performed using dome osteotomy, fixed with 2 pins with a Charnley clamp and 2 staples. At the time of HTO for OA of the knee, we transplanted these cells embedded in collagen gel into the medial femoral condyle, where articular cartilage was lost and subchondral bone eburnation was exposed. We abraded the eburnated subchondral bone, transplanted cells with collagen, and covered the lesion with autologous periosteum harvested from the anteromedial surface of the tibia. The mean size of the abraded area was 14 mm × 35 mm. The mean follow-up period was 16 months. Before and after surgery, all patients rated their pain (30 points), function (22 points), range of motion (18 points), muscle strength (10 points), flexion deformity (10 points), and instability (10 points), using the Hospital for Special Surgery knee-rating scale.[24]

For the cell-transplanted group, the mean total score was 65.0 points before surgery and 81.3 after surgery, which was significantly improved. For the cell-free group, the mean total score was 66.3 before surgery and 79.2 after surgery, which was also significantly improved. Although the difference in clinical improvement between the groups was not significant, the arthroscopic and histological grading score was better in the cell-transplanted group than in the cell-free control group. As early as 6.3 weeks after transplantation, defects were covered with white soft tissue, in which metachromasia was partially observed, and 42 weeks after transplantation, the defects were covered with white soft tissue that was much harder than that observed at 6.3 weeks, but was still softer than the surrounding normal cartilage. In most areas of the repair tissue, metachromasia was observed, and the tissue appeared similar to hyaline cartilage.

Long-Term Results of the Comparative Study

We analyzed the clinical results 64 months after transplantation. We could follow 9 out of 12 cell-transplanted patients and 8 out of 12 control patients. As one patient in each group had received total knee replacement, we followed the remaining 8 cell-transplanted and 7 control patients. The mean clinical scores (standard deviation) of the cell-transplanted group and cell-free group were 74 (14) and 76 (16), respectively, which is not a significant difference. Both scores were lower than those of the first report but higher than those before surgery.

Recently, we investigated the long-term clinical results of these patients. At final follow-up, 7 cell-transplanted patients and 7 control patients were available for review. The final follow-up period was 120 and 130 months, respectively. One patient in the cell-transplanted group received total knee arthroplasty. Final HSS scores of the remaining 7 patients in the cell-transplanted group and 7 patients in the cell-free group were 76 (19) and 73 (11), respectively. This may be because some patients were suffering age-related cerebral infarction or femoral neck fracture, and having reduced activity. However, the knee function of these patients was comparable to that at short-term follow-up.

Discussion of BMSC Transplantation

As the clinical symptoms of most patients were improved by autologous culture-expanded BMSC transplantation, this procedure would appear to be effective in the repair of articular cartilage defects, although no direct evidence is available. In this comparative study, the difference in clinical improvement between BMSC-transplanted and control groups was not significant 10 years after the transplantation. HTO itself was effective enough to explain why there was no significant difference for this period. Although the difference in clinical improvement between both groups was not significant, the arthroscopic and histological grading score was better in the cell-transplanted group than in the cell-free control group. This repair was found to occur much earlier and was better than reported in HTO only or HTO with abrasion.[25,26] Moreover, we want to emphasize that the untreated tibial articular cartilage defects were not repaired with hyaline cartilage at all.

As we showed in the animal experiment, BMSC became chondrocytes and replaced by the host bone under the tidemark. We want to stress that we have never observed calcification above the tide mark in rabbit model. In the original cartilage area, cartilage was formed and in the original bone area, bone was formed. Although we do not know the mechanism, calcification or replacement by bone does not occur above the tidemark. When BMSC are implanted into a weight bearing region, they respond to the mechanical environment in an appropriate way.

In the clinical study, we have never observed hypertrophy of repaired tissue. In some cases where we performed biopsy or MRI, we have never observed calcification above the tidemark in humans, just like in rabbit model.

We also showed that autologous BMSC transplantation is a safe procedure because neither tumors nor infections were observed between 5 and 137 months (mean 75 months) of follow-up.

The important advantage of the technique described here is clear from the data provided. Although these progenitor cells are not abundant, we have been able to mitotically expand them in culture. These approaches have considerable relevance to the treatment of human cartilage defects, and provide the starting point for refinement of a repair technology that is capable, in principle, of regenerating large areas of articular cartilage.

It has been reported that cells isolated from human bone marrow aspirates could be induced to differentiate into other mesenchymal lineages, such as adipocyte, chondrocyte, or osteocyte, in vitro.[27,28] These cells were therefore called mesenchymal stem cells. Furthermore, they also differentiate into cells other than mesenchymal, ectodermal (neurocyte)[29] and endodermal (hepatocyte)[30] tissues (transdifferentiation). Recently, these cells have been considered as a useful source to repair some kinds of tissues, such as bone, cartilage, tendon, muscle, heart, small vessel, liver, nerve, and others.

The number of reports of BMSC transplantation in human articular cartilage is limited. Beside the reports described here, we have found reports from scientific meetings elsewhere in the world. Nejadnik *et al*.[31] reported BMSC transplantation into 36 articular cartilage defects and followed up for 24 months comparing the results with those of 36 ACI. They concluded that BMSC transplantation showed results comparable to ACI, and that it was a good procedure because it required one less step of surgery, reduced costs for patients, and minimized donor site morbidity. However, there are far fewer reports of BMSC transplantations than those of ACI. This is because ACI was explored first and made available for clinical approval very early on by some developed countries. Even in ACI, evidence of effectiveness compared with other procedure is still controversial.[32-34] A long-term follow-up clinical trial with high statistical power is needed to verify the safety and efficacy of new cartilage joint therapy. To date, only the randomized controlled trial in BMSC transplantation compared with ACI, mentioned above, has been reported. This report showed that the clinical effectiveness of BMSC transplantation is comparable to the results of ACI, while BMSC transplantation had superiority in some procedures.

Agung *et al*.[35] reported the BMSC via intra-articular injection was mobilized into the osteochondral defect in the knee joint to explore less invasive procedure than ACI. Wong *et al*.[36] compared HTO combined with injectable BMSC to HTO alone in human osteoarthritic knees. According to this report, the cell-recipient group was superior to the cell-free group in MRI examinations and some clinical evaluations.[36] Thus, the injectable BMSC procedure is effective for osteoarthritis. However, even now, further long-term follow-up studies with high statistical power are needed to establish more evidence.

Other options using cell therapy for cartilage repair are explored at the experimental level. To date, repairable cartilage tissues have not been completely composed of hyaline cartilage histologically, even with the cell combination therapy. Theoretically, hyaline cartilage is preferable with respect to mechanical properties related to durability. Mesenchymal stem cells could be driven into the chondrogenic lineage using cytokine[37-39] or gene transfection,[40,41] and the resulting artificial autogenetic chondrocytes would be transplanted into cartilage defects for improved outcomes. Allogeneic cell transplantation has also been explored in animal models. We have reported that cartilage-like tissue, generated ectopically by muscle-derived cells in a diffusion chamber using bone morphogenetic protein-2, is effective in repairing articular cartilage defects in rats.[39] We also reported that effectiveness of articular cartilage repair using cartilage-like tissue, generated ectopically by amnion-derived cells with bone morphogenetic protein-2.[42] These methods may constitute a new technique of tissue engineering for the repair of articular cartilage defects. Embryonic stem (ES) cells or inducible pluripotent stem cells are the most promising cell sources for many kinds of tissue repair. These cells should also be applicable to the repair of osteochondral defects; however, it is difficult to induce these cells exclusively into chondrocytes. When we transplanted ES cells into joint spaces, they formed a teratoma and subsequently destroyed the joint.[43] However, we have also reported that when transplanted into osteochondral defects, ES cells form cartilage and promote the repair process.[44] The mechanism of this phenomenon is unclear and the use of ES cells might be expected in the future. We also reported that when we transplanted ES cells into an osteochondral defect and fixed the joint by a pin, they formed a teratoma, while cell transplantation without fixation did not induce teratoma but did induce cartilage repair.[45]

Conclusion

Various procedures, including bone marrow stimulation, mosaicplasty, and ACI, have been clinically tried to assess their effectiveness in repairing articular cartilage defects. However, all of them have some demerits. Progenitor cells can proliferate without losing their capacity for differentiation, and we have used this property by transplanting autologous culture-expanded BMSC into articular cartilage defects in human. Our previous reports showed that BMSC treatment improved clinical symptoms, and the safety of this therapy was guaranteed in clinical use. Furthermore, in the clinical study, we never observed hypertrophy of repaired tissue. Although we have never observed calcification above the tidemark in the rabbit model and in humans histologically, the repair cartilage was not completely hyaline cartilage. To elucidate the optimum conditions for cell therapy, different culture conditions, mechanical stresses,

growth factors, and gene transfection methods have been explored, but none of these approaches have been applied clinically. In the future, less invasive administration such as intra-articular injection will be explored and less invasive and more accurate evaluation of cartilage damage will be required.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

This study was approved by our institutional review board.

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