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研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
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EVIDENCE-BASED ORTHOPAEDICS

Platelet-Rich Plasma Added to the Patellar Tendon Harvest Site During Anterior Cruciate Ligament Reconstruction Enhanced Healing

de Almeida AM, Demange MK, Sobrado MF, Rodrigues MB, Pedrinelli A, Hernandez AJ. Patellar Tendon Healing with Platelet-Rich Plasma: A Prospective Randomized Controlled Trial. Am J Sports Med. 2012 Jun;40(6):1282-8.

Question: In patients having anterior cruciate ligament (ACL) reconstruction, does the addition of platelet-rich plasma (PRP) to the patellar tendon harvest site improve tendon-healing?

Design: Randomized (allocation concealed), blinded (outcome assessor), controlled trial with 6 months of follow-up.

Setting: São Paulo University Medical School, São Paulo, Brazil.

Patients: 27 patients who were <45 years of age (mean age, 24.3 years; 89% men) with an ACL injury and skeletal maturity entered the study. Exclusion criteria included complex ligament lesions, osteoarthritis, previous surgery on the same joint, postoperative infection, arthrofibrosis, re-operation, and thrombocytopenia. 22 patients (81%) completed a 6-month follow-up.

Intervention: Patients were allocated to receive PRP (n = 12) or no PRP (control, n = 15). All patients underwent arthroscopic ACL reconstruction. During anesthesia, PRP was obtained from each patient in the PRP group with use of a cell separator with a platelet apheresis kit (Haemonetics, Braintree, Massachusetts). The peritendon was opened longitudinally and separated from the patellar tendon. A 1-cm wide bone-patellar tendon-bone graft was obtained for ACL reconstruction. The patellar tendon defect was completely filled with 20 to 40 mL of PRP gel in the PRP group; nothing was added to the control group. The tendon was closed to the fat pad with number 3-0 absorbable suture without closing the tendon itself. The peritendon was closed with number 3-0 absorbable suture. Postoperatively, suction drains were placed inside the knee joint and analgesia was carefully monitored. Patients were discharged after 24 hours. Early knee range of motion and progressive weight-bearing with crutches was allowed for 3 weeks.

Main outcome measures: The primary outcome was healing of the patellar tendon harvest site assessed by magnetic resonance imaging (MRI) at 6 months. Secondary outcomes were postoperative pain assessed with use of a visual analog scale immediately after surgery. Knee function assessed with use of the Lysholm, International Knee Documentation Committee, Kujala, and Tegner questionnaires, and isokinetic testing results (quadriceps peak torque deficit) were assessed at 6 months.

Main results: Patients who received PRP had better healing of the patellar tendon harvest site than control group patients did, as shown by a smaller gap area seen on MRI (Table). The groups did not differ with regard to measurements of the cross-sectional area of the patellar tendon or with regard to patellar height, as measured with use of the Insall-Salvati index (Table). Patients in the PRP group had less postoperative pain (Table). Knee function improved in both groups according to all questionnaires except the Tegner, with no between-group differences. The groups did not differ with regard to the isokinetic testing results.

Conclusions: In patients undergoing ACL reconstruction, the addition of platelet-rich plasma to the patellar tendon harvest site improved tissue-healing at the patellar tendon donor site and reduced pain immediately after surgery.

6-Month Outcomes for the Platelet-Rich Plasma [PRP] Group Versus the No PRP Group*

Outcomes	PRP	No PRP	P Value
Gap area (mm^2)	4.9	9.4	0.046
Cross-sectional area (mm^2)	173.0	176.3	0.856
Insall-Salvati index	1.0	1.1	0.806
Pain (VAS)	3.8	5.1	0.02

*Mean values are expressed. VAS = visual analog scale (higher scores indicate greater pain).

Source of funding: No external funding.

For correspondence: Dr. A.M. de Almeida, Department of Orthopedics and Traumatology, São Paulo University Medical School, FIFA Medical Centre of Excellence, R. Dr. Ovidio Pires de Campos, 333, Cerqueira César, 05403-010 São Paulo, Brazil. E-mail address: adrianoalmeida@usp.br

Commentary

PRP has emerged as a promising but still unproven treatment option in musculoskeletal tissue repair[1]. The randomized controlled trial by de Almeida et al. tested the effect of PRP gel on tendon-healing of the patellar tendon harvest site during ACL reconstruction by comparing the results after implantation of PRP gel with the results of no treatment.

It is not clear if patients were blinded to their treatment allocation. The authors claimed that patients who received PRP had better healing of the patellar tendon harvest site than the control group did, with a smaller gap area detected by MRI and less postoperative pain as evaluated with use of a VAS. It is not only the surgical procedure but also well-controlled postoperative rehabilitation that can affect the outcome; thus, a description of the postoperative rehabilitation regimen for the patients in both groups is important, but this information is lacking in this study. Furthermore, evaluation of only one axial MRI section is not sufficient to evaluate tendon-healing. Although established protocols were followed, it would be preferable if the authors reported the quantity of healed area throughout the length of harvested tendon as shown on MRI[2,3].

Also, the authors did not demonstrate that PRP treatment contributed to improved patient function as assessed by knee functional scores or isokinetic testing. The goal of ACL reconstruction is to improve the symptoms and function of the patient, including a return to strenuous activities. Since the two groups were similar with respect to outcome scores and isokinetic testing, it is unclear if patients truly derived benefit from the treatment. Lastly, a follow-up of six months may not be sufficient for the evaluation of patient function after ACL reconstruction.

Norimasa Nakamura, MD
Osaka University Graduate School of Medicine, Osaka, Japan

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Frictional Properties of Articular Cartilage-like Tissues Repaired with a Mesenchymal Stem Cell-based Tissue Engineered Construct*

Hiromichi Fujie, and Norimasa Nakamura

Abstract— **We have been developing a novel tissue engineering technique for cartilage repair using a scaffold-free tissue engineered construct (TEC) bio-synthesized from synovium-derived mesenchymal stem cells (MSCs). In the present study, the effect of TEC on the repair of chondral defect in the femoral condyle of immature and mature pigs were investigated. The permeability of TEC-treated repaired tissues was significantly higher than normal level at surface layer in immature animals, while the permeability was slightly higher than normal level at middle and deep layers in mature animals. In immature animals, the coefficient of friction of TEC-treated tissues against a glass plate was load-dependently increased, with a significantly higher value than normal level observed at a high load (280 kPa). In contrast, the coefficient of friction was load-dependently decreased in mature animals, with no significant differences from normal level observed at all loads (70, 140, and 280 kPa). It is suggested that the frictional properties of TEC-treated cartilage-like repaired tissues are recovered to normal level in mature animals, while they are unrecovered to normal level due to underdeveloped, permeable surface layer in immature animals.**

I. Introduction

The healing capacity of articular cartilage is limited [1], it is therefore required to develop effective cell-based therapies for cartilage repair. Although synthetic or animal-derived scaffolds are frequently used for cell deliveries long-term safety and efficiency of such scaffolds still remain unclear. We have been developing a new tissue engineering technique for cartilage repair using a scaffold-free tissue engineered construct (TEC) bio-synthesized from synovium-derived mesenchymal stem cells (MSCs) [2-4]. As the TEC specimen is composed of cells with their native extracellular matrix, we believe that it is free from concern regarding long term immunological effects. In the present study, the effect of TEC on the repair of partial chondral defect were investigated. The permeability and coefficient of friction of the TEC-repaired cartilage-like tissues were determined in immature and mature animal models.

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Hiromichi Fujie is with Tokyo Metropolitan University, Hino, Tokyo 191-0065, Japan, and Kogakuin Univeristy, Hachioji, Tokyo 192-0015, Japan (corresponding author to provide phone: +81-42-585-8628; fax: +81-42-585-8628; e-mail: fujie@sd.tmu.ac.jp).

Norimasa Nakamura, is with Osaka University Medical School, Suita, Osaka 565-0871, Japan (e-mail: norimasa.nakamura@ohsu.ac.jp).

II. Method

A. Preparation of TEC and chondral defect repair

Synovium-derived cells including MSCs obtained from the synovial membrane of immature porcine knee joints at the age of 3-4 months were cultured in a monolayer in DMEM (Fig.1). Cell proliferation was performed though 4 to 7 passages. When the cell density reached to 4.0×10^5 cells/cm^2 (6-cm dish), 0.2 mM of ascorbic acid 2-phosphate was added to the cell culture plates, and allowed to undergo active contraction for 8 hours to develop TEC specimens [2]. Three month-old immature and 12 month-old mature 24 pigs were used as donors in the present study. They were divided into 4 groups; TEC-treated immature group (n=6), TEC-untreated immature group (n=6), TEC-treated mature group (n=6), and TEC-untreated mature group (n=6). A cylindrically shaped, partial chondral defect of 8.5 mm in diameter and 1.5 mm in depth was created in the weight-bearing area of the medial condyle of distal femur in each animal. The defects were subjected to allografting implantation of the TEC specimen in TEC-treated immature and mature groups (n=12), while the defects were subjected to no implantation in TEC-untreated immature and mature groups (n=12). Six months after surgery, cylindrically-shaped plug specimens of 4 mm in diameter and 5 mm in height were harvested from the donor sites. For comparison, plug specimens of dimensions identical to above-described were harvested from the medial condyle near donor sites to serve as immature (n=6) and mature (n=6) normal cartilage groups.

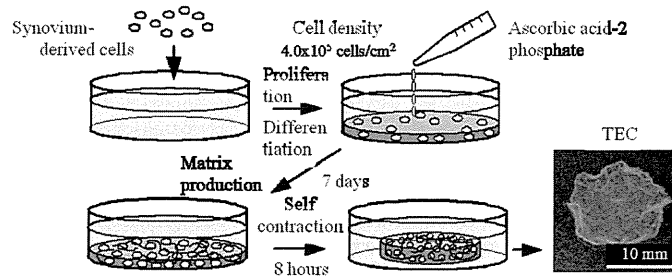

Figure1. Production of tissue engineered construct (TEC) from synovium-derived cells including MSCs.

B. Morphological observation

An atomic force microscope (AFM) (Nanoscope IIIa, Veeco Instruments, USA) was used in contact mode to scan the surface images of TEC-treated and -untreated mature and immature specimens (n=4) and control mature and immature specimens (n=2) soaked in saline solution at room

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temperature. Histological observation was also performed using safranine-O for the plug specimens.

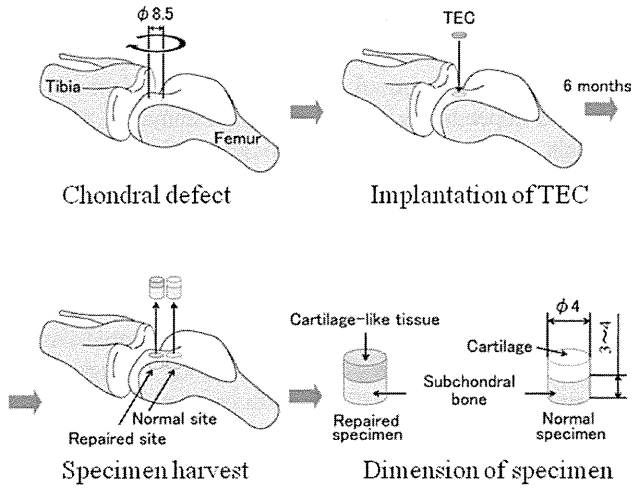

Figure 2.Allografting implantation of the TEC specimen to a chondral defect of porcine femur, and the dimension of plug-specimens harvested from donor sites.

C. Permeability tests and friction tests

Friction tests were performed for the specimens in a confined compression fashion on a flat glass plate soaked in saline solution at room temperature using a custom made micro friction tester [5]. The friction speed was 20 mm/s while the normal load was 70, 140, and 280 kPa. The coefficient of dynamic friction of the specimen was obtained immediately after frictional motion was applied. After the friction test, each plug specimen was sliced in parallel with surface to three layer specimens consisting of surface layer, middle layer, and deep layer. Thickness of the layers was between 250 and 300 μm. The permeability of the layer specimens were determined using a permeability tester under 30% of compression [6].

III. RESULTS

A. Morphological observation

Macroscopic observation indicated that the chondral defects were filled with cartilage-like tissues in TEC-treated groups, while the defects were filled with transparent tissues in TEC-untreated groups, in both the immature and mature animals (Fig.3). Histological observation indicated that the TEC-treated cartilage defects were filled with safranine-O stained tissues, while, the TEC-untreated defects had only partial coverage with safranine-O unstained tissues, in both the immature and mature animals (Fig.4). It was demonstrated that the TEC-treated cartilage was hyaline cartilage-like at intermediate-to-deep area but remained fibro cartilage-like at the surface in both the mature and immature animals. Atomic force microscopic observation indicated that TEC-treated repaired sites exhibited rough surfaces of approximately 1-2 μm in height, while the TEC-untreated sites exhibited rough surfaces of approximately 1-5 μm in height (Fig.5).

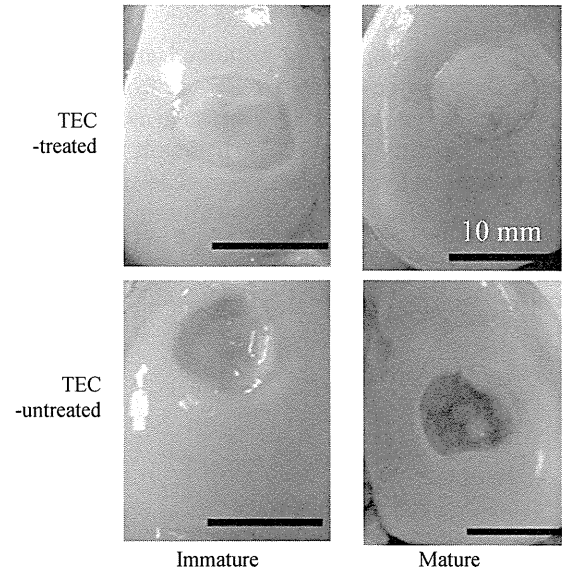

Figure 3. Macroscopic observation of TEC-treated and TEC-untreated repaired tissues of immature and mature porcine femurs.

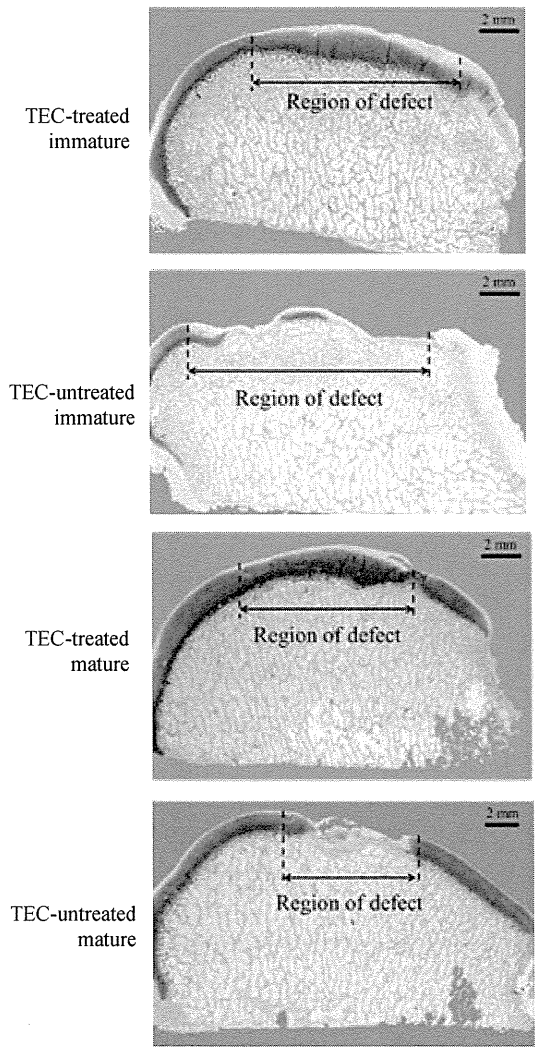

Figure 4. Histological observation of TEC-treated and TEC-untreated repaired tissues of immature and mature porcine femurs.

B. Permeability

The hydraulic permeability remained at low level in normal cartilage; between 4 and 8 x 10^{-15} m^4/Ns in immature animals, and approximately 1 x 10^{-15} m^4/Ns in mature animals. Note that, in immature animals, the hydraulic permeability was significantly increased versus normal level up to 18 x 10^{-15} m^4/Ns at surface layer. The permeability was also increased at middle and deep layers in mature animals although no significant differences was observed versus normal level.

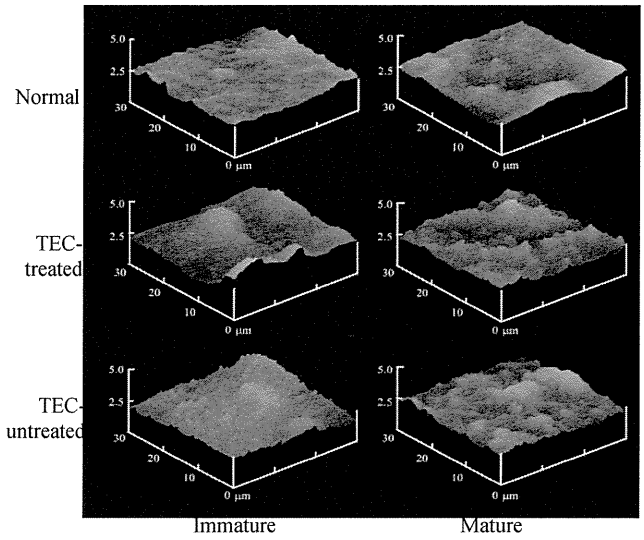

Figure 5. AFM observation of normal cartilage (upper), TEC-treated tissues (middle), and TEC-untreated tissues (lower) in chondral defects in immature (left) and mature (right) porcine femur.

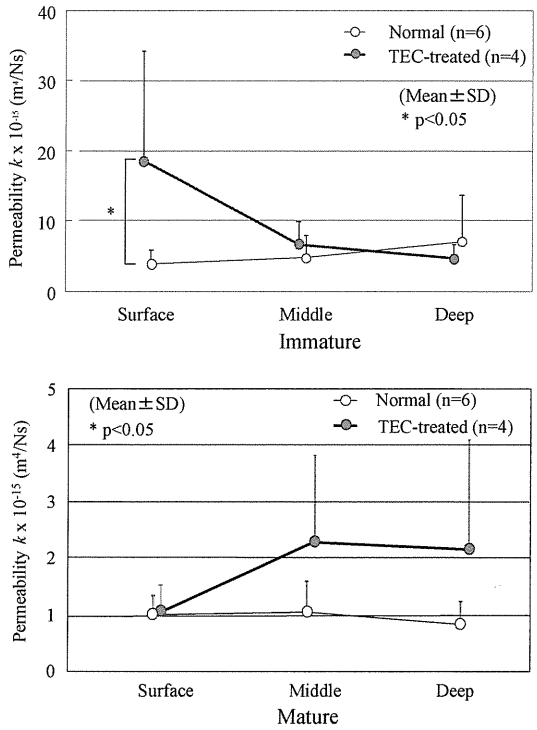

Figure 6. Permeability of the surface, middle, and deep layers of TEC-treated tissues under 30% of compression in immature (upper) and mature (lower) animals.

C. Coefficient of friction

The coefficient of friction of the TEC-treated tissues are shown in Fig.7. In normal cartilage, the coefficient of friction was load-dependently decreased in both immature and mature animals. However, the coefficient of friction of the TEC-treated tissues was load-dependently increased in immature animals. The coefficient of friction was significantly lower in TEC-treated tissues than in normal cartilage at 70 kPa, while the coefficient of friction was significantly higher in TEC-treated tissues than in normal cartilage at 280 kPa. In contrast, the coefficient of friction of TEC-treated tissues in mature animals was load-dependently decreased to approximately 0.12 at 280 kPa with no significant difference observed versus normal cartilage.

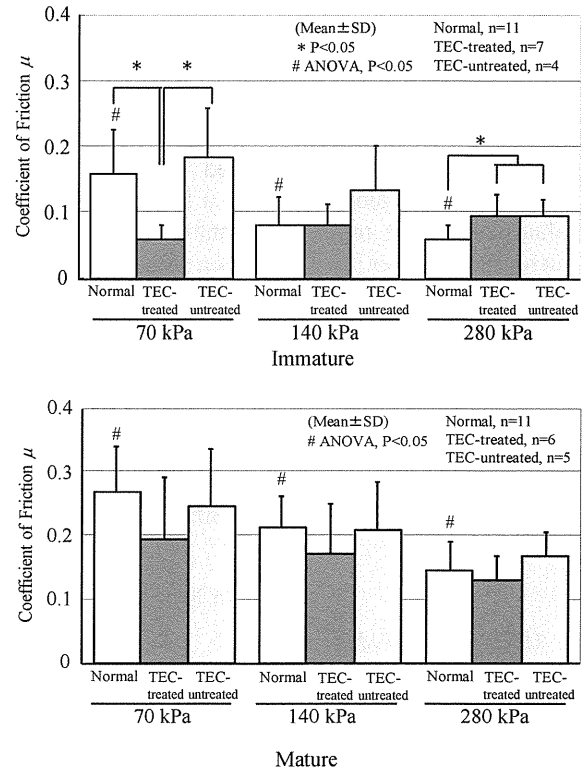

Figure 7. Coefficient of friction of TEC-treated and -untreated tissues against a glass plate at a speed of 20 mm/s as a function of applied load in immature (upper) and mature (lower) animals.

IV. Discussion and Conclusion

Partial chondral defects on the femoral condyle in pigs were repaired with synovium-derived MSCs-based TEC in the present study. As compared with tissues repaired with other methods, such as micro fracture [7], the TEC-treated repaired tissues exhibited more cartilage-like structure and properties. Morphological observations indicated that the defects were filled with hyaline cartilage-like repaired tissues, while the

TEC-untreated defects were filled with fibro cartilage-like tissues, 6 months after implantation. However, it was found that, even in TEC-treated tissues, the superficial region remained fibro cartilage-like, and the region was thicker in immature animals than in mature animals.

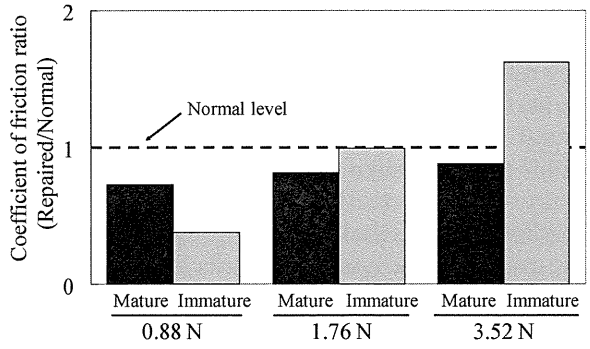

Figure 8. Ratio of coefficient of friction of TEC-treated and TEC-untreated tissues to normal level as a function of applied load.

Figure 8 indicated the ratio of frictional coefficient of the TEC-treated and TEC-untreated tissues to those of normal cartilage as a function of applied load. It is indicated that the frictional coefficient of TEC-treated tissues can be recovered closely to, or slightly better than, normal level in mature animals in all the loads. However, in immature animals, the frictional coefficient was 60% higher than normal level in TEC-treated tissues at a high load (3.52 N). This was maybe caused by the abnormality in composition found at the surface of TEC-treated immature animals. It is required to perform further studies for the improvement of cartilage repair using TEC, in particular, for the recovery of surface layer in immature subjects.

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Editorial

Cartilage Repair in Asia: Selected Reports on Research and Clinical Trials

The Asian orthopaedic community has always been in the forefront of basic science research. This, however, has not been replicated in the field of clinical research. In the last decade, awareness in the field of cartilage repair and collaborative efforts among countries has bridged this gap, and this has led to the formation of the Asian Cartilage Repair Society in 2011.

The purpose of this special collection of articles is to highlight the results of cartilage repair with and without cell-based therapy from selected Asian research and clinical teams. The section begins with a review of the research and clinical activities in the region, "Cartilage Repair: 2013 Asian Update," highlighting the difficulties confronted by local researchers and clinicians, which are both different from and in addition to those of their western counterparts. Even so, they have made their good contributions despite these issues. As noted in the article, in vitro research activities in Asia have concentrated on the sources of cells, growth-factor supplements, and scaffolds. A majority of the published reports are of animal studies. The following articles were taken from prominent teams as a representation of the efforts in the region. We hope that the subject matter matches the important topics as discussed in the review article.

Both cells and factors are needed for tissue regeneration. Currently, administration of cells genetically engineered to express high levels of selected cytokines is an active area of research. However, the regulatory requirements for gene delivery using retroviral vectors or other biological systems are difficult to satisfy. Hence the work of Shi et al.[1] is included. These investigators were able to demonstrate in an animal model (rabbit) that nanoparticles can be used to deliver a gene with therapeutic potential (bone morphogenetic protein 4 or BMP-4) into mesenchymal stem cells generated from adipose tissue without viral vectors. The cells had increased BMP-4 expression and retained their function after being administered into rabbits.

The next 2 papers are clinical studies, ranging from a retrospective review to a randomized controlled trial. Postprocedure monitoring is necessary to determine the repair process in terms of progress and extent. Li et al.[2] report that quantitative magnetic resonance imaging can be an effective tool to measure cartilage repair in younger patients with anterior cruciate ligament reconstructions. Using relaxation time T2 measurements and follow-up of patients for 2 years, the authors noted that T2 can suggest textural changes ahead of morphologic changes. It is possible that early signs of osteoarthritis might be inferred by the use of sequential longitudinal follow-up procedures as earlier shown. Finally, as a representation of clinical trials using mesenchymal stem cells in conjunction with surgery, Wong et al.,[3] in a randomized clinical trial, found that patients receiving cultured autologous bone marrow–derived mesenchymal stem cells for knee cartilage repair, when injected with hyaluronic acid after microfracture, experienced significant clinical and radiologic improvement 2 years postoperatively.

It is obviously impossible to cover all areas of research, development, and clinical application in Asia within a limited space. However, our intent is to highlight some of the important achievements in Asia, with the hope that readers can appreciate Asian research activities despite the problems specific to the area. We hope that established researchers will be encouraged to redouble their efforts and young investigators will be spurred on to a career in cartilage repair.

James H. P. Hui, M.B.B.S., F.R.C.S., F.A.M.S.
Singapore
Deepak Goyal, M.B., M.S.(Orth), D.N.B.(Orth), M.N.A.M.S.
Ahmedabad, India
Norimasa Nakamura, M.D., Ph.D.
Osaka, Japan
Mitsuo Ochi, M.D.
Hiroshima, Japan

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Cartilage Repair: 2013 Asian Update

James H. P. Hui, M.B.B.S., F.R.C.S., F.A.M.S., Deepak Goyal, M.D.,
Norimasa Nakamura, M.D., Ph.D., and Mitsuo Ochi, M.D., for the Asian Cartilage Society

Abstract: Despite financial and regulatory hurdles, Asian scientists and clinicians have made important contributions in the area of cartilage repair. Because it is impossible to include observations on all the published articles in one review, our attempt is to highlight Asian progress in this area during recent years (2005 to the present), reviewing research development and clinical studies. In the former, our discussion of in vitro studies focuses on (1) potential sources of stem cells—such as mesenchymal stem cells (MSCs) from marrow, cord blood, synovium, and mobilized peripheral blood—which are capable of enhancing cartilage repair and (2) the use of growth factors and scaffolds with and without cells. Our discussion of animal studies attempts to summarize activities in evaluating surgical procedures and determining the route of cell administration, as well as studies on matrices and scaffolds. It ranges from the use of small animals such as rats and rabbits to larger animals like pigs and dogs. The local adherent technique, enhancement of microfracture with poly(L-lactic-co-glycolic acid) scaffold, adenovirus-mediated bone morphogenic protein (BMP) genes, and MSCs—whether they are magnetically labeled, suspended in hyaluronic acid, or immobilized with transforming growth factor-β (TGF-β)—have all been able to engineer a repair of the osteochondral defect. Although published Asian reports of clinical studies on cartilage repair are few, the findings of relevant trials are summarized in our discussion of these investigations. There has been a long history of use of laboratory-derived MSCs for cartilage repair. Recent progress has suggested the potential utility of cord blood and mobilized peripheral blood in this area, as well as more injectable bone marrow (BM)-derived stem cells. Finally, we make a few suggestions on the direction of research and development activities and the need for collaborative approaches by regulatory agencies.

Asian scientists and clinicians have been active in the research of cell-based therapy for cartilage damage. They face major hurdles in clinical applications—including awareness, cost, and rehabilitation issues; the almost total absence of a regulatory framework in most countries; patchy research activities as well as few publications in regional or national languages; an absence of data sharing; and an absence of multicenter trials. In addition, the unfortunate rise in "stem cell tourism" has compounded the problems,[1,2] making regulatory agencies and institutional review boards even more cautious in their evaluation and approval of legitimate clinical studies and giving patients the wrong impressions about the utility of stem cells.[3] These issues were recognized by prominent faculties in Asia and guided them to search for common solutions together. The result was the formation of the Asian Cartilage Repair Society in 2011. At that time it was decided to initiate a basic analysis of various research activities done by different members, for example, laboratory studies, animal trials, clinical studies, case series, and multicenter trials. Despite all the difficulties, the effort by teams of investigators in various Asian countries had resulted in numerous quality publications. Any attempt at a comprehensive review is impossible. The purpose of this article is to discuss some of the important research and clinical activities in chondrocyte and MSC cartilage repair in Asia during recent years. The focus is on the use

From the Cartilage Repair Program, Therapeutic Tissue Engineering Laboratory, Department of Orthopaedic Surgery (J.H.P.H.), National University Health System, National University of Singapore, Singapore; Saumya: Center for Advanced Surgeries of the Knee Joint (D.G.), Ahmedabad, India; Department of Orthopaedic Surgery (M.O.), Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan; Department of Rehabilitation Science (N.N.), Osaka Health Science University, Osaka, Japan.

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Address correspondence to James H. P. Hui, M.B.B.S., F.R.C.S., F.A.M.S., Cartilage Repair Program, Therapeutic Tissue Engineering Laboratory, Department of Orthopaedic Surgery, National University Health System, National University of Singapore, 1E, Kent Ridge Road 119288, Singapore. E-mail: james_hui@nuhs.edu.sg

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of the cells for cartilage damage, with emphasis on human cells aiming for clinical use.

Research Development

A literature review on cell-based therapy for cartilage damage was performed for articles published from January 2005 to December 2012. To explore the present status of cartilage repair, an Internet search was carried out using various search engines in December 2012. Because there is no geotagging of articles; it is virtually impossible to find the source of various articles on cartilage repair. A PubMed search was carried out with "articular cartilage repair in 'name of country'" options. Multiple searches were done, changing names of various Asian countries each time. Articles published from January 2005 to December 2012 were included. A total of 290 articles were retrieved using the described method (Table 1).

Table 1. Research Articles from Asian Countries: A PubMed Search

Type of Study	Number of Articles	% of Total
In vitro	69	24
Animal model	144	50
Clinical	77	26
Total	290	100

In Vitro Studies

Cell Source

It is well known that human bone marrow (BM) MSCs can be induced to differentiate into chondrocytes in vitro. Recent studies in Asia confirmed this finding. The chondrocytic potential (along with osteogenic and adipogenic potential) was retained when BM was processed using a special device in a closed system without centrifugation. Ito et al.[4] reported that MSCs from human BM as well as from synovium were capable of chondrocytic differentiation in vitro, although morphological differences between the 2 sources could be seen during induction. In an earlier study, bone morphogenic protein (BMP)-2 was found to be more effective than BMP-4 and BMP-6 for inducing BM MSCs into in vitro cartilage formation.[5]

Various other sources of MSCs have been investigated in laboratories across Asia. The main sources of MSCs include synovium, cord blood, and adipose tissue. Sekiya and his team have been developing MSCs from synovium and synovial fluid (SF). Their results showed that SF MSCs from human as well as various animal models were at least comparable or superior to BM MSCs in terms of gene expression profile,[6] proliferation and chondrogenesis being superior to periosteum, adipose, and muscle.[7,8] Ichinose et al.[9] have also shown that in vitro chondrogenesis of BM MSCs, synovial MSCs, and chondrocytes have similar efficacy. The availability and utility of SF MSCs had been confirmed by other Asian scientists.[10] The collection, processing, culture procedure, and route of administration of SF MSCs were studied in human cells as well as in animal models.[11] The potential clinical efficacy was confirmed in animal models.[12,13]

Arguably, cord blood appeared to be the most mature alternative for generating MSCs.[14,15] In an earlier report, MSC development was shown to be successful in 23% of cord blood units, but an expansion of 1000-fold was observed in some.[16] Cord blood MSCs had been taken to phase III clinical trial as an allogeneic cell product by Medipost in South Korea, and accrual was completed in January 2011 (www.clinicaltrials.gov). This cell product has received regulatory approval by the South Korean government and is marketed as CARTISTEM (Medipost, Seoul, Korea) for indication in cartilage defects in that country.

The umbilical cord tissue can be a source of MSCs. Functional MSCs (in vitro, animal models, and human MSCs into immunocompromised animals) have been produced from umbilical cord tissue by many groups in Asia.[17,18] In addition, researchers in different Asian countries have shown that MSCs can be developed from fetal BM as well as other tissues.[19,20] Human functional MSCs were shown to be better than adult BM MSCs in osteogenesis,[20] and had been grown in a 3-dimensional bioreactor for potential allogeneic clinical applications in Singapore.[21,22]

MSCs derived from human adipose tissue are also capable of chondrocytic differentiation. Despite similar cell surface marker profiles, adipose cells were deemed to be inferior to those from BM in terms of cell size and cartilage matrix staining.[23] Similarly, when MSCs from matching human adipose tissue and BM were cultured in a chondrogenic cocktail, type II collagen and proteoglycans (an indication of functional chondrocytes determined by histological, immunocytochemical, and glycosaminoglycan evaluations) were synthesized only by BM MSCs, despite the observation that both cell types showed an increase in gene expression of collagen II.[24]

Other sources of human MSCs include embryonic stem cells[25] and peripheral blood.[26] Of particular interest, Chong et al.[26] discovered that MSCs from peripheral blood are characteristically similar to and have similar chondrogenic differentiation potential as those derived from BM.[26] Clinical use of peripheral blood stem cells (PBSCs) for cartilage was reported in a small local trial of 5 patients receiving unmanipulated autologous PBSCs.[27] The patients were given a dose of growth colony-stimulating factor 300 μg/d for 3 days for mobilization before apheresis collection of PBSCs. Each patient received one administration of fresh cells and 4 additional weekly cryopreserved and thawed

PBSCs. Flow cytometry showed that the PBSC collections contained $CD34^+$ (hematopoietic) and $CD105^+$ (mesenchymal) stem cells. Using the same mobilization protocol, the authors went on to a randomized controlled trial (recruitment completed and recently published) comparing hyaluronic acid with and without PBSCs in 50 patients (25 participants each arm) diagnosed with chondral injury to the knee joints. Although the patients in the study arms were unexpectedly younger, they also had better improvement in both International Cartilage Repair Society (ICRS) and magnetic resonance imaging scores.[28] There were no observed adverse events.

Growth Factors and Scaffolds

The goal of achieving optimal expansion of functional MSCs depends on the understanding of growth mechanisms as well as evaluating substrates and media. Substantial amounts of research and development have been devoted to 3-dimensional (3D) culture of MSCs, because it has the advantage of combining the effects of scaffold and MSCs. Scaffolds are implemented to facilitate articular cartilage regeneration. They do this by imitating the extracellular matrix and providing the mechanical support needed to recreate the desired 3D structure at the defect. Other functions of scaffolds include the stimulation of cell proliferation and differentiation. Ideally, the scaffolds induce total integration of neocartilage with native tissue. 3D structures that have supported or improved (or both) MSC proliferation or function (or both) include cartilage fragments and fibrin glue,[29] fibrin and hydrogel,[30] extracellular matrix taken from cartilage,[31] beads alone with dextran-based cell culture,[32] beads coated with anti-CD44 antibodies,[33] collagen in nanofiber[34] or with chondroitin sulfate C,[35] as well as other material such as poly(L-lactide-co-ϵ-caprolactone).[36]

Various agents have been shown to support and enhance MSC growth or differentiation, or both, in vitro. They are known broadly as growth factors. Some important compounds include fibroblast growth factor 2 (FGF-2),[37,38] TGF-β1,[39,40] heparin sulfate (which increased the fold-expansion of MSCs),[41] and ascorbic acid phosphate (SF MSCs).[42] Active expansion of an undifferentiated cell population mediated by FGF-2 is required to initiate and support a chondrogenic repair response in full-thickness defects of articular cartilage. Endogenous FGF-2 could not meet the requirements of growth signaling in the center of larger sized defects.[43] TGF-β1 is a powerful growth factor that fosters chondrogenic differentiation of MSCs. It initiates and accelerates regeneration and suppresses inflammation, immune response, and breakdown of the extracellular matrix.[44] It also assists with matrix production.[45]

Media comparison studies found that human serum was just as good if not better than fetal bovine serum.[46] Results of using hypoxic conditions for MSC culture were controversial; it could be better[47] or worse than normal oxygen.[48] The difference might be attributed to the source material (rabbit *v* rat). Ito et al.[4] developed a novel fabric filter of rayon and polyethylene and showed that BM (human and canine) stromal cells (containing MSC precursors) could be enriched by affinity (and not by size), and the MSCs after culture had multilineage potential as well as the ability of accelerated bone regeneration in dogs. Using flow cytometric cell sorting to study precursors, Hachisuka et al.[49] found that multipotential MSCs could be grown from mouse BM—adherent cells that were CD34-negative ($CD34^-$), $CD45^-$, $CD44^-$, and Sca-1—positive ($Sca\text{-}1^+$).[49]

However, growth factors are not without their disadvantages. They are costly and their short half-lives call for high dosages and frequent injections. In addition, high doses of growth factors can lead to other unforeseen detrimental effects.[50] For instance, high doses of TGFβ-1 can cause fibrosis and osteophyte formation. To solve this problem, Fan et al.[45] used microspheres to facilitate a controlled release of TGFβ-1. Despite these shortcomings, the use of growth factors is a promising method for enhancing cartilage repair.

Animal Studies

Animal studies are conducted to ensure that the MSCs and their progenitors are functional and can deliver the expected clinical effects. Their safety profile must also be established before human clinical trial protocols can be submitted to regulatory agencies. Most of the publications reported rat, rabbit, porcine and to a lesser extent dog, studies on (1) route of cell administration in cartilage defects, (2) validation of different cell types or culture conditions, or both, (see earlier), or (3) surgical procedure investigations. In addition, animal studies were used to confirm different sources of MSCs as discussed earlier.

For cell administration and delivery, some studies included direct injection of SF MSCs into meniscal defects (rat),[51] femoral condyle (minipig),[52] or disk cartilage (rabbit),[53] using magnetically labeled MSCs in rat[54] or rabbit.[55] Delivery of MSCs affects their viability, and Kobayashi et al.[55] investigated the possibility of accumulating magnetically labeled MSCs under the direction of an external magnetic force at the desired portion of osteochondral defects of the patellae after intra-articular injection of the MSCs.[55] In this study, MSCs were labeled magnetically and injected into rabbit knees under the influence of an external magnetic force. MSCs were successfully accumulated at the site of the defect. The magnetic approach provides a new less invasive way to accumulate small amounts

of MSCs to a specific area to avoid potential negative effects such as scar tissue and free bodies. This new system could potentially become a novel delivery method in humans. In another study by Motoyama et al.,[56] the team was able to gather TGF-β–immobilized magnetic beads under an external magnetic force, and chondrogenesis was achieved from the MSC–magnetic bead complexes in the presence of 1 ng/mL magnetic beads immobilized by TGF-β. For nonmagnetic delivery, direct injection is another established method. Lee et al.[52] performed a study to investigate the possibility of direct intra-articular injection of MSCs suspended in hyaluronic acid as an alternative to the much more invasive methods currently available. The team discovered that this is a viable option for treating large cartilage defects. Interestingly, Pi et al.[57] described a nonviral vector delivery of chondrocyte homing peptide that could improve MSC targeting to cartilage in rabbits.[57]

For investigation on culture conditions, Park et al.[58] observed that rabbit cartilage that was more mature in vitro produced better results in repair after administration,[58] and Kamarul et al.[59] showed that rabbit chondrocyte with glucosamine sulfate and chondroitin sulfate had better function. Cell types used in animal studies include allogeneic MSCs (rabbit)[60] or autologous chondrocytes (rabbit)[61] for cartilage damage, allogeneic MSCs for ligament damage (rabbit),[62] and BM MSCs (goats) for long-term estrogen deficiencies (lower proliferation rate and decreased osteogenic capacity).[63]

For procedural studies, Koga et al.[64] tested a "local adherent technique" using MSCs in rabbit. The team discovered that placing an MSC suspension on the cartilage defect for 10 minutes resulted in adherence of greater than 60% of synovial MSCs to the defect and promoted cartilage regeneration. This method makes it possible to adhere MSCs with low invasion, without periosteal coverage, and without a scaffold, and opens up new possibilities in scaffold-free repair. The use of microfracture in articular cartilage repair has also been explored. Shi et al.[65] integrated in situ BM stem cells (BMSCs) of rabbits with an implanted poly(L-lactic-co-glycolic acid) scaffold and subjected it to microfracture. The procedure rapidly and effectively promoted hyaline-like cartilage regeneration. Zhang et al.[66] reported a method for articular cartilage repair consisting of microfracture, a biomaterial scaffold of perforated decalcified cortical bone matrix (DCBM) and adenovirus-mediated bone morphogenetic protein-4 gene therapy. With this method, large areas of cartilage defect could be quickly repaired with regeneration of native hyaline articular cartilage. In another study involving scaffolds, Shao et al.[67] attempted to repair large osteochondral defects using hybrid scaffolds and BM-derived MSCs in a rabbit model. A hybrid scaffold created from porous polycaprolactone (PCL) and tricalcium phosphate–reinforced PCL for cartilage and bone repair, respectively. Defects were created and split into 2 groups: control (treated with hybrid scaffolds only) and BMSC (treated with scaffold and BMSCs). These defects were created at high–load-bearing areas to simulate a clinical situation. The results showed the BMSCs to be superior to the controls. There was firm integration, and although at 6 months there was some degradation in certain samples, remnants of scaffold could help in bone and cartilage formation. This is because foreign BMSCs survive at least 6 weeks in vivo. Overall, PCL-based hybrid scaffolds with BMSCs may be an alternative treatment for large osteochondral defects in high-load sites. In a study by Ho et al.,[68] the team evaluated a biphasic osteochondral implant coupled with an electrospun membrane in a large animal model. The results showed that osteochondral repair was promoted and host cartilage degeneration was arrested, as shown by superior glycosaminoglycan maintenance. This positive morphological outcome was supported by a higher relative Young's modulus, which indicated functional cartilage restoration. Bone ingrowth and remodeling occurred in all groups, with a higher degree of mineralization in the experimental group. Tissue repair was compromised in the absence of the implanted cells or the resurfacing membrane.

It has been recognized that the age of donors and recipients may affect the effectiveness of MSC-based therapy. Shimomura et al.[69] investigated whether skeletal maturity influences repair. A scaffold-free 3D tissue-engineered construct derived from synovial MSCs from immature and mature pigs was used to treat the defects of corresponding immature and mature pigs, respectively.[69] The tissue-engineered construct was scaffold free, promoted repair, elicited no immune reaction, and could potentially save time and money. Overall, the results not only show the feasibility of allogeneic MSC-based cartilage repair over generations but also may validate the use of an immature porcine model as clinically relevant to test the feasibility of synovial MSC-based therapies in chondral lesions. Another study done by Jin et al.[70] entailed the use of a rabbit model to evaluate the extent to which the maturity of engineered cartilage influenced the remodeling and integration of implanted extracellular matrix scaffolds containing allogeneic chondrocytes.[70] The results showed that in vivo engineered cartilage was remodeled when implanted; however, its extent to maturity varied with cultivation period. The importance of the in vitro cultivation period was highlighted because the more mature the engineered cartilage was, the better the repair of the osteochondral defect.

Clinical Development

Published reports from Asia on clinical studies are rare because of the financial and regulatory difficulties. The

paucity of multicenter trials is discouraging, but the huge number of basic research, animal, and laboratory studies is very encouraging. It suggests that in a few years from now, some of the research and development may be converted into clinical trials, and, hopefully, eventually into multicenter studies.

Much progress has been made in countries where cell therapy is available. Wakitani et al.[71] have been pioneers in this field, and in 2002 the team set out to use BM MSC transplantation to repair human articular cartilage defects in osteoarthritic knee joints. The study group consisted of 24 knees of 24 patients with osteoarthritis who underwent a high tibial osteotomy. Half of the study group underwent cell transplantation, whereas the other half served as controls. The results of the study were that although the clinical improvement was not significantly different, the arthroscopic and histological grading scores were better in the cell-transplanted group than in the cell-free control group. As one of the pioneering studies, this study undeniably contributed to the start of the use of cell therapy for cartilage repair in Asia.

Other comparative studies have also been done, including those undertaken to evaluate the efficacy of atelocollagen-associated autologous chondrocyte implantation. Tohyama et al.[72] conducted a multicenter study involving 27 patients with cartilage lesions in a femoral condyle or on a patellar facet who underwent transplantation with chondrocytes in a newly formed matrix of atelocollagen gel. The results were promising, except for detachment of the graft in 2 cases. The Lysholm score increased significantly from 60.0 ± 13.7 points to 89.8 ± 9.5 points ($P = .001$). Based on the ICRS grade for arthroscopic appearance, 6 knees (24%) were assessed as grade I (normal) and 17 knees (68%) as grade II (nearly normal). Takazawa et al.[73] reported on a multicenter study using autologous chondrocytes cultured in 3D atelocollagen gel. The improvement in clinical outcome of the 14 patients was maintained at 1 year and 6 years after implantation.

A matched cohort (single center) study conducted in Singapore (72 patients) by Nejadnik et al.[74] comparing the efficacy of autologous MSCs versus chondrocytes for cartilage repair found that there was essentially no difference in the clinical outcome between the patients receiving the 2 cell types, except that BMSCs were better for physical role functioning, with a greater improvement over time in the BMSC group ($P = .044$ for interaction effect).[74] This was supported by an animal (rabbit) study of another team.[60]

A study conducted by Tanaka et al.[75] in 6 patients aimed to characterize the spontaneous osteonecrosis of the knee (SONK) lesion histopathologically and to report on preliminary clinical results of autogenous osteochondral grafting for SONK. Short-term clinical results of osteochondral autografting were favorable, and histological results showed that subchondral fracture is the causative mechanism underlying SONK.[75]

The effectiveness of autologous osteochondral transplantation to treat cartilage defects was explored by Xu et al.[76] in China. Twenty-five patients with chondral and osteochondral defects of the weight-bearing surfaces were treated with autologous osteochondral transplantation for repair of the chondral and osteochondral defects of the non–weight-bearing surfaces using arthroscopy. The results were promising, with the advantages of being minimally invasive and also avoiding allograft rejections. Another team from China has also explored the effectiveness of intra-knee articular injection of platelet-rich plasma to treat knee articular cartilage degeneration in a group of 30 patients. Although the results were good (according to IKDC score, Western Ontario and McMaster Universities Osteoarthritis index, and the Lequesne index) at the 6-month time point, more data involving larger samples and long-term follow-up are required to confirm the safety and effectiveness of this technique.[77]

For small-scale studies involving 5 patients or less, Kasemkijwattana et al.[78] from Thailand showed that autologous chondrocyte implantation had good clinical potential in 5 patients, because they were able to return to normal activity levels. The same team also showed that autologous chondrocyte implantation could be used in a 3D collagen scaffold (single-patient case report).[79] In 2011, the team also confirmed the clinical utility of autologous MSCs in 2 patients.[80]

Another technique adopted by Saw et al.[27] from Malaysia was arthroscopic subchondral drilling followed by postoperative intra-articular injections of autologous (peripheral blood progenitor cells) PBPCs in combination with hyaluronic acid (HA). The team evaluated 5 patients who had undergone this procedure and found that it was possible to achieve articular hyaline cartilage regeneration in the knee joint. With these results, the group conducted a randomized clinical trial involving 50 patients with ICRS grades 3 and 4 lesions of the knee, with a total follow-up duration of 18 months. All these patients underwent arthroscopic subchondral drilling and were equally randomized to the control (HA) or intervention (PBPCs and HA) group. All patients received 5 weekly injections, with 3 additional injections at weekly intervals 6 months after surgery. They found that intra-articular injections of PBPCs with HA resulted in histological and radiological improvements in patients with grades 3 and 4 chondral lesions.[28]

The Republic of Korea has been particularly active in the clinical application of cell-based repair of cartilage damage. As described earlier, cord blood MSCs were used in a phase III clinical study sponsored by Medipost. There were at least 3 trials listed at www.clinicaltrials.gov sponsored by Sewon Cellontech (Seoul, Korea) using

autologous chondrocytes (Chondron; Sewon Cellontech). Accrual had been completed in all 3 studies, one of which was a phase III study.

Although the bulk of patients with cartilage defects belong to the older age group, the young and active, especially athletes, would also benefit from effective cartilage repair. Kuroda et al.[81] treated a full-thickness articular cartilage defect in the right femoral condyle of a 31-year-old male athlete with autologous BM stromal cells. Seven months after surgery, arthroscopy revealed the defect to be covered with smooth tissue. Histologically, the defect was filled with a hyaline-like type of cartilage tissue.[81] One year after surgery, the clinical symptoms had improved significantly. The patient had regained his previous activity level and experienced neither pain nor other complications. Another study involving even younger patients was conducted by Teo et al.[82] They recognized that although recent advances have been made in using chondrocytes and other cell-based therapy to treat cartilage defects in adults, it is unclear whether these advances should be extended to adolescent and young adult patients. They retrospectively reviewed 23 patients between 12 and 21 years of age (mean, 16.8 years) treated for osteochondritis dissecans lesions involving the patella from 2001 to 2008 and found that cell-based therapy was associated with short-term improvement in function in adolescents and young adults with patellar osteochondritis dissecans. Therefore, the transplantation of autologous BM stromal cells can promote the repair of large focal articular cartilage defects in young active patients.

With all these new clinical developments, the safety of trials has always been center stage. Wakitani et al.[83] reported on the follow-up of 41 patients who had undergone autologous BM MSC transplantation for cartilage repair for up to 11 years and 5 months. The results gave support to the safety of autologous MSC transplantation, because neither tumors nor infections were detected in the patients.

Conclusions

Because articular cartilage defects have limited potential for healing, development of new methods for treating them is of utmost importance. Asia has been very active in this respect, and a range of clinical and nonclinical trials has been attempted. The surgical methods attempted in the different Asian countries include autologous chondrocyte implantation, bone marrow stimulation, and mosaicplasty. Asia has been keenly exploring MSC-based cell therapy as an alternative for cartilage repair. MSCs are self-renewing and multipotent. Clinical trials show that MSC transplantation has good clinical potential and is durable, and thus far trials have shown it to be safe. MSCs are increasingly being used from a wider range of sources, and new more potent growth factors are being discovered. All these developments are promising not only for Asia but also for researchers and patients around the world.

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