Review Article

Mesenchymal Stem Cells in Musculoskeletal Tissue Engineering: A Review of Recent Advances in National University of Singapore

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Abstract

A key factor in the tissue engineering approach to tissue repair and regeneration is the use of appropriate cells. Mesenchymal stem cells (MSCs) are derived from bone marrow stroma or connective tissues and they have the potential to differentiate into various mesenchymal cell lines in vitro and in vivo. These cells hold great promise for musculoskeletal tissue engineering. This review is based mainly on the work which has been done in the National University of Singapore on the use of MSCs for engineering cartilage, growth plate, bone and tendon/ligament as well as the clinical trail of autologous chondrocyte implantation. It can help to shape future research on musculoskeletal tissue engineering.

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Key words: Cartilage and bone, Cell source, Tendon/ligament, Tissue repair and regeneration

Introduction

Stem cells are cells which are capable of self-renewal and differentiation into various tissues, depending on the type of stem cells. Pluripotent embryonic stem cells are capable of differentiating into any tissue type, while adult stem cells are much more limited in their regenerative capability and are usually restricted to the tissues they reside in (liver hepatocytes and haemopoietic stem cells). Mesenchymal stem cells (MSCs), isolated from other cells in bone marrow via their propensity for adhering to the plastic walls of tissue culture containers, also display such multipotent characteristics. Under controlled conditions, these cells can differentiate into multi-mesenchymal lineage (such as osteoblast, chondrocyte and adipocyte) and myoblast lineages.[1] Such cells are also easily obtained under culture from various sources, such as the periosteum, fat and skin,[2] making them prime candidates for use in cell and gene therapy.

Current research on human adult stem cells indicates significant potential for use in the development and regeneration of tissues, particularly in the field of transplantation. Due to the minute possibility of such cells being rejected by the patient, it would be extremely advantageous to isolate cells from the patient, direct the specialisation of the cells and transplant them back into the patient. Now, MSCs are being expected to treat a variety of clinical conditions, including large segmental defects, bone fractures or wounds that have severe scarring, infections, avascular tissue with a poor blood supply, non-union situations where bones are not fully joined and to treat the effects of irradiation and chemotherapy.[1,2]

In Singapore, current research in musculoskeletal tissue engineering, which includes the repair of physeal growth defects, cartilage defects, tendon/ligament and bone, is focussed on the potential use of adult stem cells as this field has tremendous potential in revolutionising the practice of medicine.

Articular Cartilage

Cartilage lesions are potentially a major cause of joint disease and disability as they can lead to osteoarthritis (OA), and they rarely heal spontaneously.[3-8] Full-thickness chondral injuries secondary to trauma from work or sports are common. Injuries and degenerative changes in the articular cartilage are, in essence, a significant cause of morbidity and diminished quality of life, with arthritis ranking second only to cardiovascular disease as a cause of work-related disability.[9]

A retrospective review of 31,516 knee arthroscopies by Curl et al[10] revealed an incidence of 41% Outerbridge grade III chondral lesions (fragmentation and fissuring of more than half an inch in diameter) and 19.2% Outerbridge grade IV lesions (erosion of cartilage down to subchondral bone). Overall, there was a prevalence of 20% grade IV chondral

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lesions to the medial femoral condyle, with 72% of the cases belonging to patients >40 years old.

It is thought that chondral injuries often exhibit degenerative changes with time,[4,8,11] leading to OA. In response to traumatic injury, chondrocytes appear to undergo an apoptotic response, which is believed to involve the degenerative process, and may be important in the capacity for repair.[12-14] Apoptosis of chondrocytes can, in fact, be inhibited with apoptotic inhibitors such as caspase.[14] Replacement with autologous cells or tissue is thought to provide a more durable hyaline-like cartilage. Experimental studies have demonstrated the ability of these tissues to produce a hyaline cartilage repair response,[15-19] and continuous passive motion has been shown to improve the quantity of repair tissue.[20]

In vitro cultured autologous chondrocytes, which were reimplanted under a periosteal patch, were first used by Brittberg et al[3] to treat unipolar lesions in non-arthritic knees. The pilot study showed that patients who had their femoral condyles treated fared best, with 14 of 16 having good to excellent results, and 11 of 15 biopsies showing hyaline cartilage. When the patella was treated, only 2 of 7 patients had good and excellent results, and only 1 of 7 had hyaline repair tissue. Treatment results for patients with patellar and tibial chondral injuries indicated that 70% to 80% of them improve symptomatically, while 85% to 90% of those with femoral weight-bearing condylar and trochlear injuries improved.[21] The same series showed that 89% of patients with osteochondritis dissecans also improved symptomatically.[22]

In a clinical evaluation of 244 patients followed for 2 to 10 years, Brittberg et al[23] showed that subjective and objective improvements were seen in a large number of patients with a femoral condylar lesion or osteochondritis dissecans. The percentage of good to excellent results was high (84% to 90%) for patients with different types of single femoral condylar lesions, whereas it was lower (mean, 74%) for those with other types of lesions. To study the long-term durability of autologous chondrocyte transplantation, 61 patients were followed for 5 years to 11 years (mean, 7.4 years) after the surgery. At 2 years, 50 of the 61 patients had a good or excellent result; at the 5- to 11-year evaluation, 51 of the 61 patients had such a result. The total failure rate was 16%, with a mean of 7.4 years. All failures of autologous chondrocyte implantation (ACI) occurred in the first 2 years, so a high percentage of the patients who had a good to excellent result at 2 years had such a result at the time of long-term follow-up as well.[23]

The extensive investigations[4-14] on the pathology of cartilage injury provide a solid basis for cartilage repair. Numerous animal[15-20,22] and clinical work[3,21,23,24] exhibits the promise of ACI. However, there are minimal data on the efficiency of ACI in an Asian population. The authors' institution carried out ACI in 1999 with a prospective cohort evaluation in an NMRC (National Medical Research Council)-funded and an IRB (Institutional Review Board)-approved clinical trial (unpublished data). Fifty-two patients, aged 13 to 55 years, have received treatment to date. Thirty-two patients have had more than 12 months' follow-up. Patients were assessed clinically and all had preoperative magnetic resonance imaging (MRI) and arthroscopy to diagnose the stage of the chondral defect, and postoperative MRI to assess the extent of the regeneration of cartilage. The chondral defects treated ranged from 4.2 cm^2 to 6.25 cm^2. Overall, 90.2% of patients improved symptomatically. In 7 patients who volunteered for a second-look arthroscopy at 1 year post-operation, good repair tissue with integration with surrounding host cartilage was observed. There was no adverse reaction related to surgery and no case of infection reported. These data on ACI in an Asian population are consistent with the results in Europe and the United States.[3,21,23,24] However, due to the relatively small sample size, it is difficult to stratify the data further according to the site of cartilage defects. Another shortcoming is the lack of a randomised control trial. Future studies will need to overcome these limitations, with the recruitment of more patients for such clinical trials.

Although ACI exhibited its efficiency for cartilage repair, large numbers of chondrocytes are unavailable from the limited donor source in some patients. Furthermore, chondrocytes are terminally differentiated and have limited lifespan. These disadvantages prompted researchers to investigate the use of MSCs for cartilage repair.[25] Wakitani et al[25] used bone marrow- or periosteal-derived MSCs dispersed in a type I collagen gel to repair a large full-thickness chondro-defect. They found that as early as 2 weeks after transplantation, the progenitor cells had uniformly differentiated into chondrocytes throughout the defects. At 24 weeks post-transplantation, the subchondral bone was completely repaired without loss of overlying articular cartilage. The reparative tissue of both the bone marrow and the periosteal cells was stiffer and less compliant than the tissue derived from the empty defects, but less stiff and more compliant than normal cartilage. There was no apparent difference between the results obtained with the cells from the bone marrow and those from the periosteum. In order to compare the stem cell therapy with current clinical methods, the authors' institution carried out a comprehensive study[26] which investigated the efficacy of periosteal graft, osteochondroidal autograft, autologous chondrocyte and MSC transplants in the treatment of chondral lesions in animal models. Full-thickness articular cartilage defects were created in the weight-bearing surface of the medial femoral condyle in 20-week-old New Zealand White (NZW) rabbits. A total of 56 knees were randomly

divided into 4 groups as follows: transfer of cultured chondrocytes; transfer of cultured MSCs; repair by periosteal graft and mosaicplasty. All of the contralateral knees served as control. Gross, histologic and biomechanical examinations at 36 weeks postoperation showed that the cultured MSCs had comparable enhancing effects as cultured chondrocytes on the repair of chondral defects in advanced osteochondritis dissecans (Figs. 1a and 1b), whereas mosaicplasty did well initially and periosteal graft did less favourably.

Besides the potential for chondro-defect repair, Murphy et al[27] also found that MSCs exhibited promise for retarding the process of OA. In their study, OA was induced unilaterally in the knee joint of donor animals by the complete excision of the medial meniscus and resection of the anterior cruciate ligament. A single dose of 10 million autologous MSCs suspended in a dilute solution of sodium hyaluronan was delivered to the injured knee by direct intra-articular injection 6 weeks after OA creation surgery. The results showed that local delivery of adult MSCs to injured joints stimulates the regeneration of meniscal tissue and retards the progressive destruction normally seen in this model of OA. These experiments implicated that MSCs hold exciting promise for OA treatment.

Physis (Growth Plate)

In a growing child, physeal injury can result in varying degrees of angular deformity and leg length discrepancy. Such deformity can be corrected using epiphysiolysis, stapling, epiphysiodesis or osteotomy, depending on the age of the child and the severity of the deformity. However, the clinical approach in treating severe growth arrest involves the use of free physeal transfers from the iliac apophysis and cultured chondrocytes derived from the iliac apophysis or articular cartilage. This approach requires the sacrifice of a significant amount of iliac apophysis or articular cartilage. In addition, the cultured chondrocytes display very limited regenerative capabilities. Recently, in the authors' institution, studies on repair of large growth defects were conducted. MSCs were cultured from periosteum harvested from the tibias of NZW rabbits.[28] An experimental model for growth arrest was created by excising the medial half of the proximal growth plate of the tibia of 6-week-old NZW rabbits. The cultured MSCs were embedded in agarose and transferred into the growth-plate defect after excision of the bony bridge in established growth arrest. Transfer of agarose alone and a periosteum flap without cells served as controls. In cases of transfer of MSCs, growth arrest with angular deformation and loss of length of the tibia were corrected (Fig. 2). Transfer of agarose alone and a periosteum flap yielded poor results. The high proliferation rate of MSCs makes them a good source of donor cells. Clinically, harvesting a piece of periosteum would also be less invasive. However, further studies need to be conducted to determine the factors involved in influencing the development of MSCs towards chondrogenesis in the physis. Full correction of angular deformity and length of the tibia clinically also remains a challenge and requires further research.

Bone

Critical-sized bone defects, whether caused by trauma, tumour excision, congenital malformation or aseptic loosening of prosthesis, often require the transplantation of bone tissue or substitutes to restore bone integrity. So far, autologous bone grafts are considered the "gold standard" in bone repair.[29] However, it is difficult to obtain large amounts of autografts in certain patients. Free bone grafts with microsurgical vascular anastomosis have also been successfully used for the repair of bone defects,[30,31] but the limited availability of donor sites and patient morbidity are disadvantages of this technique. In contrast, the application of allogenic bone material avoids these problems; however, it has a potential risk of infections and is also more expensive.[32,33]

Repair or the restoration of the function of traumatised, damaged or lost bone is a major clinical need, and bone tissue engineering has been heralded as an alternative to autografts. Numerous studies have investigated the use of MSCs for bone tissue engineering in vitro and in vivo.[34-36] There is no doubt about the efficiency of MSCs for bone regeneration, but an ideal delivery scaffold has not been found because the degradation rate, microstructure and strength of current scaffolds still cannot fulfill the requirement of bone regeneration.[37] Therefore, a multidisciplinary group at the National University of Singapore[38,39] designed and fabricated novel scaffolds by fused deposition modelling (FDM), which offers the possibility of designing and fabricating highly reproducible bioresorbable three-dimensional scaffolds with a fully interconnected pore network and mechanical properties in the range of cancellous bone. Arranged in a regular manner, interconnected three-dimensional channels are produced and the design of a pore morphology, which varies across the scaffold structure, is allowed. Scaffolds with a porosity of 60% to 65% have mechanical properties which are in the range of cancellous bone and have slow degradation kinetics (loose mechanical properties after 6 months and are completely metabolised after 24 months to 36 months).[40,41]

Polycaprolactone (PCL) scaffolds of different sizes and shapes have been studied in combination with osteoblasts and precursor cells in tissue engineering bone in several small,[42,43] medium and large[44] animal models. Most recently, Rai et al[45] were able to evaluate the parameters to process

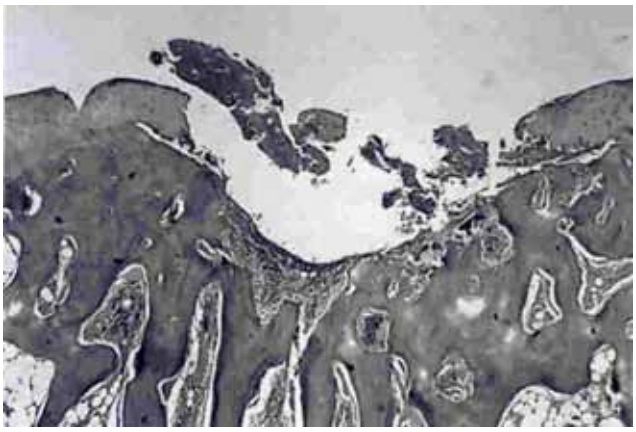

Fig. 1a. Histology shows the cartilage defect in the femoral condyle of a 36-week-old rabbit treated with normal saline injection (haematoxyclin and eosin stain x40).

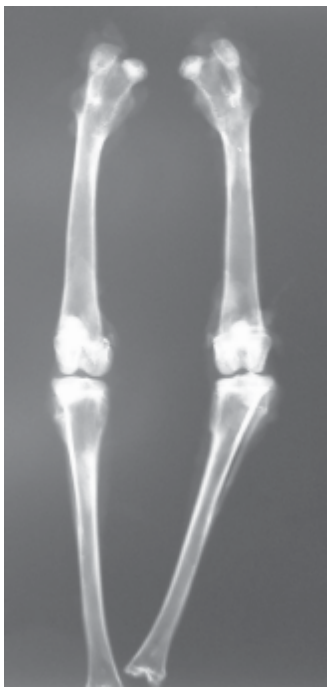

Fig. 2a.

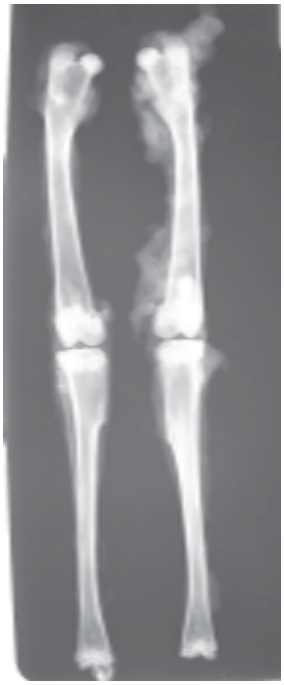

Fig. 2b.

Fig. 2a. Radiograph shows the control group with severe varus angulation, displays retardation of growth and a large difference in medial and lateral tibia height.

Fig. 2b. Radiograph shows the group injected with stem cells with mild varus deformity and a small difference in medial and lateral tibia height.

Fig. 3a. The authors' institution has designed and fabricated bioresorbable scaffolds for burrow hole application in cranioplasty.

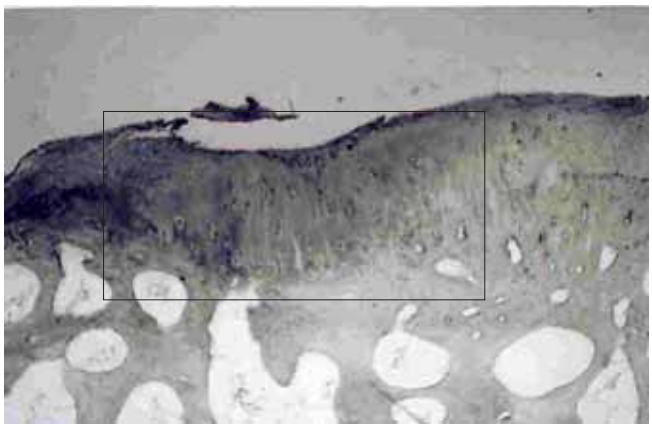

Fig. 1b. Histology shows a similar defect (marked with frame) in the femoral condyle of a 36-week-old rabbit treated with an injection of mesenchymal stem cells (haematoxyclin and eosin stain x40).

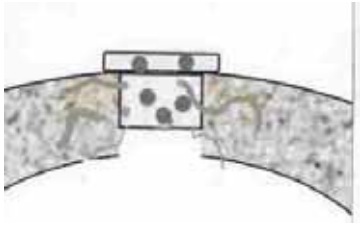

Fig. 3b. The design is based on a burr plug snap-fit mechanism and the implant is made from a bioresorbable polycaprolactone (PCL) polymer which has been proven to be biocompatible, degrades slowly and allows cells to attach and proliferate and deposit mineralised matrix.

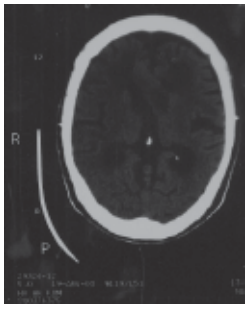

Fig. 3c. Computed tomograph showing a radiographic assessment at 3 days postoperatively.

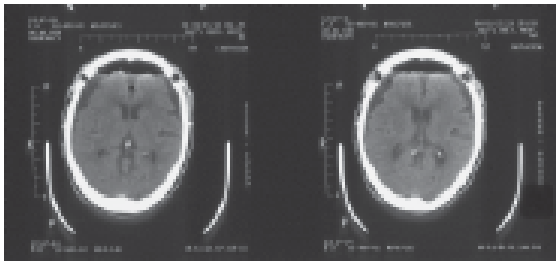

Fig. 3d.

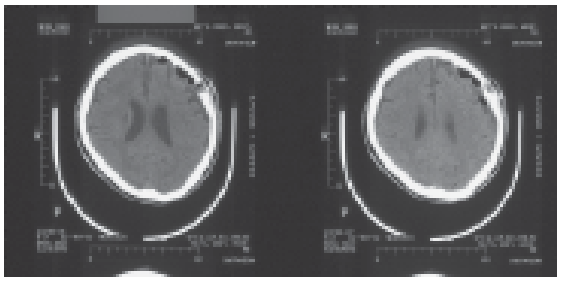

Fig. 3e.

Fig. 3d. Computed tomograph showing a radiographic assessment at 3 months postoperatively.

Fig. 3e. Computed tomograph showing a radiographic assessment at 9 months postoperatively.

their so-called second generation of scaffolds for bone engineering based on PCL/tri-calcium phosphate (TCP) and PCL/HA (hydroxyapatite) by FDM.

For regeneration of large and complex-shaped bone defects, scaffolds must possess several structural features that are difficult to achieve using conventional scaffold design and fabrication technologies. Precise control of scaffold porosity and internal pore architectural parameters (such as pore geometry, size, interconnectivity, orientation and branching) will be necessary to maximise nutrient diffusion, interstitial fluid to control cell growth and function, and to optimise scaffold mechanical function with regenerated tissue mechanical properties. The ability to design and manufacture utilising a range of materials will be essential to control chemical and physical properties that strongly influence cellular biology and, ultimately, tissue formation within scaffold architecture. The utilisation of computer-aided technologies in tissue engineering has evolved with the development of holistic technology platforms that allow the creation of complex shapes and custom-made, patient-specific scaffold/cell constructs. A multidisciplinary team from the National University of Singapore[46] was among the first to develop and execute a clinically driven, hard tissue engineering programme which utilises medical imaging, computational modelling, rapid prototyping, bioresorbable scaffolds, MSC-derived osteoblast-like cells and innovative transplantation surgery to treat complex craniofacial bone defects (Fig. 3).

Tendon and Ligament

Tendons and ligaments are arguably the least complex of the connective tissues with respect to their composition and architecture. This might reasonably lead to the expectation that they would be more amenable to tissue engineering approaches than other tissues. However, decades of experience have shown how difficult it is for tendons and ligaments to regenerate after injury. This limited capability of tendons to regenerate poses a challenge to tendon tissue engineering.

So far, few studies have been done on tendon tissue engineering compared to the extensive work on bone and cartilage tissue engineering. Several studies have investigated the use of gel and braided scaffold, with and without cells, for tendon/ligament repair; however, the inferior mechanical strength of the gel carrier and the poor tissue ingrowth associated with the braided scaffold has limited its success. Many other issues have yet to be addressed, such as:

1. The fate of implanted MSCs at the tendon site has not yet been studied;
2. No general principles have been established to select material for MSC delivery;
3. The role of MSCs in tendon repair has been arguable due to the lack of appropriate controls in previous studies; and
4. Limited attention has been placed on the tendon-to-bone healing when engineering tendon graft for tendon repair.

It is clear that the continued development of tendon tissue engineering will depend on the identification and characterisation of appropriate sources of cells as well as the development of new scaffolds. The identification of an optimal cell source for a particular tissue engineering application will depend on rigorous characterisation with regard to plasticity, propagation and control of differentiation. To guide the organisation, growth and differentiation of cells in tissue-engineered constructs, appropriate scaffolds are needed to provide mechanical support as well as physical, chemical and mechanical cues in forming functional tissues.

In the authors' institution, a series of studies have been carried out on the use of MSCs for tendon/ligament repair.[47-52] The results of the first study showed that the MSCs isolated by short-term plastic adhesion were able to differentiate into multi-mesenchymal lineages, such as osteo-, chondro- and adipo-lineages.[47] The second study illustrated that the implanted allogeneic MSCs could survive for as long as 8 weeks and were able to differentiate into spindle-shaped cells 5 weeks after implantation at rabbit patella tendon window wound site.[48] The third study selected the optimal material for MSC delivery by verifying that poly-lactide-co-glycolide (PLGA) was more likely to allow MSCs to adhere and grow as compared to the other 5 synthetic biodegradable polymers.[49] The fourth study showed that the composite of MSCs and knitted PLGA scaffold could improve the structure and biomechanics of tendon repair in a rabbit Achilles tendon model.[50,51] Besides showing the ability to accelerate tendon tissue formation, the MSCs exhibited the potential to restore the native structure at the tendon to bone interface healing in the fifth study in which hallucis longus tendons were translated into 2.5-mm-diameter calcaneal bone tunnels.[47,51] Furthermore, the sixth study proved that MSCs enhanced the strength of tendon graft-to-bone healing in an anterior cruciate ligament reconstruction model.[52] In all, these sequential studies proved that MSCs were good seed cells for tendon repair; the knitted PLGA scaffolds possessed optimal material and structural properties for MSC delivery and tendon tissue formation; and the composite of MSCs and knitted PLGA could be a potential substitute for tendon repair.

However, these studies verified that it is hard to fully regenerate tendon. The neotendons were similar to native tendon, but were not identical to native tendon. It implied that MSCs and knitted PLGA could only partially initiate

tendon regeneration. This outcome may be due to 2 reasons. The first reason is that the regeneration ability of tendon tissue is limited. Unlike bone, which can heal by regenerating normal bone in most cases, injured tendon often heal by scar tissue. The other reason is the lack of knowledge about the tissue-specific differentiation factors for tendons that have a similar function, like bone morphological protein (BMP-2) for bone regeneration. Further studies need to be conducted. It may be helpful to obtain some clues from the study of embryonic tendon development.

Conclusion

Cell-based tissue engineering for musculoskeletal tissues repair and regeneration hold great promise for the future. The cellular component of the tissue engineering paradigm is arguably the most important piece of the complex task of regenerating or repairing damaged or diseased musculoskeletal tissue. MSC population may be well suited for this task, as demonstrated by a number of studies. Future research should be directed at better characterisation of this cell population, including identifying unique markers and mapping lineage development. With the development of stem cell biology, MSCs will play an important role in tissue engineering and their clinical applications. The next several years will bring phase I and II studies using adult stem cells for engineered tissue constructs.

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