Application of stem cells in orthopaedic conditions: what is the current evidence?

SK Tripathy1*, P Behera2, RK Sen2, T Goyal1

Abstract
Introduction
Stem cell therapy plays an important role in orthopaedic treatment. Although some studies have shown promising results in repair of bone, tendon and cartilage, few studies have demonstrated incoherent results. The bone and cartilage regeneration ability of stem cells have been demonstrated clinically, but the tendon regeneration capability is still in the experimental stage. Various factors including the stage of the disease, processing and concentration techniques, application and retaining methods control the disease outcome. Researchers have still not identified the best carrier of stem cells. This review focuses on basics of stem cells and their current recommendation in orthopaedic conditions.

Conclusion
Stem cell therapy looks to be an appealing new option but many of these studies have shown failures as well as successes. Many more long-term prospective randomised human trials need good results before the use of these cells can be recommended.

Introduction
Application of ‘regenerative medicine’ in orthopaedic practice has aroused a new ray of hope among surgeons. Myriads of orthopaedic conditions with limited therapeutic options could be benefited with technologies developed in regenerative medicine1. Growth factors, stem cells and products developed through genetic engineering are now being widely used for several orthopaedic conditions. Stem cells hold great potential in repairing damaged tissue, but the clinical application into the target site is still a major challenge. In this review, a brief description of stem cells is provided, and the current status of stem cells in orthopaedic practice is discussed.

Discussion
The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

Concept of stem cells
A stem cell is a cell that has two essential properties: self-renewal and ability to differentiate into a particular cell type. Thus, a stem cell is able to maintain its own population and at the same time is able to produce a particular lineage of cell2,3. The differentiation a stem cell would undergo depends on the type of the stem cell and the surrounding environment.

Broadly, there are two types of stem cells: embryonic and adult stem cells. Embryonic stem cells (ESCs), as the name suggests, are the cells present in the embryos, i.e. found only during the early developmental phase of an organism. These are considered to be purely pluripotent and capable of indefinite self-renewal. Being pluripotent, an ESC can give rise to any type of cell of the body and hence can regenerate a part or even a complete organ1. Harvesting an ESC, which is found in an embryo is not only technically demanding and difficult, but has many ethical and legal issues associated with it too. Induced pluripotent stem cells (iPS cells) were first reported by Takahashi and Yamanaka in 20064. They prepared these cells by transfecting embryonic growth factors into somatic cells to induce de-differentiation of the somatic cells to make them behave like ESCs. Thus, this process took away the consideration of ethics but yet the technique has not been brought into mass use, primarily because of the apprehension of the teratogenic potential of such cells5.

The adult stem cells are multipotent and found more or less in every tissue. These stem cells lack the pluripotent character of the ESCs but are capable enough to produce cells of a particular type of lineage; usually the tissue in which the stem cells reside determines the lineage. This narrows the clinical applicability of such stem cells but as the current techniques of stem cell application use autologous stem cells, it is definitely safer than the ESCs and is free from the ethical and legal considerations. Moreover, considering the cost, the application of adult stem cells would be cost effective than the ESCs. Among adult stem cells, mesenchymal stem cells (MSCs) are the most studied and considered as most competent stem
cells. Although these MSCs are found in bone marrow, fat, synovial membrane, periosteum and other locations, the bone marrow is considered as the favoured source of these cells. These MSCs can be induced in vitro and in vivo to differentiate into a variety of mesenchymal tissues, including bone, cartilage, tendon, fat and bone marrow stroma. Differentiation into other types of tissue-specific cells, such as cardiac myoblasts, endothelial cells, hepatocytes and neural cells has been demonstrated in experimental studies.

Based on the source of stem cells, two other types have been described. These have properties that are intermediate to those of the ESCs and the adult stem cells. Foetal stem cells are cells extracted from foetal blood or extra-embryonal tissues. They are pluripotent but have a decreased plasticity when compared with ESCs, and are being investigated in the possible treatment of diseases in utero. Similarly, umbilical stem cells are isolated from the umbilical cord blood at the time of birth. There is a high concentration of multipotent stem cells in umbilical cord blood, and they are being used in the treatment of hematopoietic and bone marrow disorders.

Application of stem cells

The MSCs are the most studied stem cells and hence are the most commonly applied too. Stem cells may be used in two ways. One is called as the cell therapy in which the stem cells are harvested and are applied as ‘cells’ to the concerned area of interest. A common source of MSCs is the bone marrow, especially from the iliac crest. Bone marrow is aspirated from the region of posterior superior iliac spine and the MSCs contained are used either by concentrating the aspirate to use the natively available stem cells or the cells are separated and cultured in laboratory media to increase their numbers before being used.

The other mode of application is more sophisticated and is called tissue engineering. Here, the stem cells are combined with a three-dimensional matrix to compose a tissue-like construct to substitute lost parts of the tissue, or even whole organs. But even though many successful constructs have been made, tissue engineering has been limited only to laboratories and clinical application is very limited. This has been attributed to the inability in constructing a vascular compliment of the modelled structure. MSCs are believed to act by two ways; one is being incorporated as the component of the tissue or the organ with deficient structure and the other as a stimulator of cytokine secretion to promote local tissue healing.

Application of stem cells in orthopaedics

Modern day orthopaedics has moved on from being a stream obsessed with bones to one which considers the muscles, tendon, ligaments and cartilage as an essential part of it. Their supportive and locomotive roles make these structures vulnerable to injuries. The relative lack of vascularity as compared with other tissues makes these structures incapable of quick healing like other body organs. Consequently, chronicity tends to become a hallmark of musculoskeletal injuries. Non-unions, avascular necrosis (AVN), bone defects, tendinosis and cartilage defects are among few musculoskeletal conditions for which effective treatment modalities are lacking and these are the areas where stem cells and regenerative medicine have a definite role.

Clinical use of stem cells has been done in cases of non-unions, AVN, as fillers of bone defects, for enhancing spinal fusions etc. The use of stem cells for enhancing tendon healing and for growth of cartilage is still in experimental stages and clinical application is very limited.

Use in non-unions

Bone is a marvellous tissue. The natural tendency of bone is to unite by forming bone when fractured. Still, many times a fracture may not unite at all or may have a fibrous union. There are numerous reasons for it and their discussion is beyond the scope of this article. At times, non-union results from a gap at the fracture site resulting from bone loss. The common method to treat non-union has been bone grafting to provide osteo-inductive, osteo-conductive substrates and to supply osteo-progenitor cells. Bone grafting is rather considered as the gold standard procedure for non-unions. However, the autogenous bone grafting technique tends to produce donor site morbidity and use of allograft has the tendency to produce immunological reaction.

MSCs have osteogenic potential, they tend to differentiate along the osteogenic pathway in response to chemical stimulation. MSCs have been shown to be the source of endochondral bone formation. The method of application of MSCs, which are usually harvested from the iliac crest, is usually by percutaneous injections to the non-union site.

Percutaneous injection of MSCs has shown to promote union in non-unions by Connolly et al., Garg et al., Kettunen et al., Hernigou et al. and Goel et al. The application by these investigators has been on non-union of long bones especially tibia and also for diagnosed cases of pseudo-arthritis. Fernandez et al. studied the effects of autologous bone marrow mononuclear cells combined with allogenic bone graft for repair of pseudo-arthritis of long bones. Bone marrow mononuclear cells (BM-MNCs) comprise of progenitor and stem cells with pro-angiogenic and pro-osteogenic properties. They concluded that, “Combination of autologous BM-MNCs and allogenic bone graft could constitute an easy, safe, inexpensive and efficacious attempt...”
to treat long-bone pseudoarthrosis and non-union by reproducing the beneficial properties of autologous bone grafting while restricting its disadvantages”. Thus, stem cells are helpful in promoting union in cases of non-unions when they are used alone or in combination.

**Use in avascular necrosis of femoral head**

Avascular necrosis of the femoral head is usually noticed in young patients following trauma, steroid intake, alcohol consumption etc. Loss of vascularity leads to the death of osteocytes present in the sub-chondral region and causes collapse of the femoral head; it alters the shape of femoral head and produce pain, limp and restriction of movements. Treatment options available till date primarily focus on reducing the intra osseous pressure by drilling channels into the head through the neck if presentation is early. In advanced disease, replacement arthroplasty is commonly opted for. Electrical stimulation, osteotomies and pharmacological management have been studied with varying results. MSCs have been applied for the re-growth of the dead area of the femoral head. A common method of application has been by the injection of bone marrow concentrate (Figures 1 and 2). The studies by Hernigou et al., Gangji et al. and Sen et al. have shown promising results. Recently, Wang et al. reported debridement, autogenous bone grafting and bone-marrow mononuclear cells implantation as an effective procedure in patients with small lesion, early-stage AVN of the femoral head. The concept of introducing osteo-progenitor cells into the area of dead bone seems logical and the results speak for themselves. Limitation for the use of stem cells in this condition is the stage of presentation as once the collapse has stated, the shape of femoral head cannot be returned back to normal by the stem cells. Overall, with proper patient selection the stem cells do appear to be a promising prospect for management of AVN of femoral head.

**Stem cells as fillers of bony voids**

Generally, following a surgical procedure there is a void left behind in the bone. Such a scenario is seen in cases of benign bone tumours such as simple bone cysts which curettage has been done and in cases where there is a bone defect. Although autogenous bone graft may be used to fill up these voids, use of stem cells in conjunction with bone grafts has been done by investigators. Park et al. and Zamzam et al. have used stem cells for filling the voids in simple bone cysts with good results. However, Wright et al. found the injections of intraluminal bone marrow into simple bone cysts to be inferior to intraluminal methyl prednisolone injections. Marcacci et al. used autologous MSCs that were expanded in vitro and seeded on hydroxyapatite scaffolds for filling of diaphyseal bone defects and reported good integration of the graft 7 years post-op without any secondary fractures. Jager et al. too concluded that the MSCs may be a promising alternative to autogenous bone grafts for volumetric bone defects. Thus, the studies conducted so far are supportive of using the MSCs in bone marrow as a viable and promising alternative to autogenous bone grafts.

**Use in cartilage defects**

Cartilage as a result of its avascularity is notorious for non-healing. Once damaged the ability to repair itself is very poor. Autologous cartilage transplantation and autologous chondrocyte transplantation have been used
for large cartilaginous defects with varying degrees of success. Abrasion chondroplasty in which drill holes are made into the cartilage to allow for the subchondral bone marrow to come out and layer the cartilage defects have shown good results. Such a procedure is followed by formation of fibro-cartilage at the cartilage defect site.

The encouraging results of MSC-based cartilage repair of full thickness cartilage defects in rabbit models reported by Shafiee et al. and Tay et al. led to similar use in clinical practices. Wakitani et al. reported a series of three cases of repair of articular cartilage defects in the patello-femoral joint with autologous bone marrow MSCs. They expanded the MSCs harvested from iliac crest in vitro for 4 weeks and then transplanted them to the site of defect using collagen gel and covered the defect with a periosteal flap. They reported satisfactory clinical and macroscopic results. The small sample size decreased the impact of the study. A cohort study was performed by Nejadnik et al. on a total of 36 patients. The patients underwent autologous cartilage transfer or MSCs implantation. At 24 months post-operatively, no significant difference of functional knee scores between the groups was noted.

Buda et al. used MSCs for treatment of osteochondral lesions of the femur and talus. They reported satisfactory clinical results and integration of cells in defects in both types of osteochondral lesions. Thus, use of MSCs in cartilaginous lesions has come to the clinical stages from experimental stages and the results have been encouraging.

Use in tendon repair

Tendons, which are primarily composed of collagen, are one of the less vascular structures of the body and once injured do not tend to heal quickly. This tendency of non-healing produces a condition called tendinosis. Scientists have been trying various ways to improve tendon healing. Use of growth stimulating substances such as platelet rich plasma has been used for enhancing tendon healing. Experimental laboratory studies on animal models using MSCs embedded on various types of scaffolds have returned some encouraging but inhomogenous results. Chong et al. used MSCs with fibrin sealant in a rabbit Achilles tendon model. In their study, no differences between fibrin and fibrin with MSC could be shown histologically. Gujotta et al. succeeded in enhancing tendon healing in rotator cuff model, applying transfected MSCs using the embryonic transcription factor membrane type 1–matrix metalloproteinase and the tendon transcription factor scleraxis. Recently, tendon-derived stem cells (TDSCs) have been identified within tendon tissues.23, TDSCs exhibit universal stem cell characteristics, such as clonogenicity, a high proliferative capacity, multi-differentiation potential, non-immunogenicity and immunosuppression and hence may become a potent agent of tendon repair once their clinical efficacy is demonstrated in laboratory and clinical settings.

Thus, the use of stem cells may be a good option for use in tendon injuries but the results so far have been limited to experimental studies only and clinical evidences are still awaited before one may recommend their use.

Other applications of stem cells in orthopaedics

Along with the above-discussed applications of the stem cells, some other conditions are also being investigated for their suitability for stem cell application. Enhancement of spinal fusion has been tried by applying the stem cells by Neen et al. and Gan et al. Gan et al. reported 95.1% of their patients to have had good fusion after 34.5 months but Neen et al. reported similar healing capacity as autologous cancellous bone grafting in posterolateral fusion and poor results in interbody fusions of the spine. Dallari et al. have used lyophilised bone chips with platelet-enriched plasma with bone marrow aspirate in high tibial osteotomy and found an enhancement of healing.

Silva et al. conducted a prospective randomised trial on 43 patients undergoing anterior cruciate ligament (ACL) reconstruction. Twenty patients in an experimental group received adult non-cultivated bone marrow stem cells and 23 patients in the control group did not receive stem cells. All patients underwent magnetic resonance imaging of the knee at 3 months after surgery to evaluate the signal-to-noise ratio of the interzone. They concluded that adult non-cultivated bone marrow stem cells did not seem to accelerate graft-to-bone healing in ACL reconstruction, hence stem cells have limited role in ACL reconstruction.

Neural tissues have long been considered to be devoid of regeneration potential. Once damaged, the chances of recovery of neural tissue in spinal cord injury tissue are very bleak. Still, no cure exists for neural damage. The stem cells through their ability to differentiate into various types of tissue may play a role in regeneration of neural tissue. Experimental studies on animal models conducted by Yasuda et al. have shown encouraging results. Other similar studies have shown mixed results. The type of stem cell, which would give better results and the actual mechanism by which these stem cells act are yet to be decided. Until long-term follow-up results come out it would be supportive of their use and the safety profile of the stem cells is established unequivocally, their use in spinal cord injury patient might not be recommendable.

Conclusion

Stem cell therapy is as an attractive option for the treatment of intractable diseases. Its use is based on sound biological principles. However, whether one should accept the stem
cell therapy in all the conditions discussed above is questionable. Many of these studies have shown good results but at the same time many have shown failures. This might also be linked to the patient selection, the type of cells used, the concentration of cells used, the method of application, duration of follow up and evaluation tools among others. Many more long-term prospective randomised human trials need to have good results before one may actually recommend the use of these cells. Establishing the safety profile of these is equally important, for many of the iPSC cells have been shown to be teratogenic. Thus, one should tread with caution the path of stem cell application but wherever a suitable case is available a trial should be taken of this treatment modality.

**Abbreviations**
AVN, avascular necrosis; BM-MNC, bone marrow mononuclear cell; ESC, embryonic stem cell; iPS cell, induced pluripotent stem cell; MSC, mesenchymal stem cell; TDSC, tendon-derived stem cell.

**References**
29. Zamzami MM, Abak AA, Bakarman KA, Al-Jassir FF, Khoshhal KI, Zamzami MM.


Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)