Review Article: Stem Cell Therapy in Orthopaedic Surgery: Current Status and Ethical Considerations

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ABSTRACT

Regenerative medicine using stem cell therapy has sparked much interest in this 21st century not only because of the controversies that surround the ethics involving pluripotent stem cells but their potential for use in the clinic. The ability of stem cells to repair and regenerate new tissues and organs holds tremendous promise for the treatment of many serious diseases and injuries. This review provides a brief summary of the current status of research in stem cells with special emphasis on where we are in terms of the possible clinical application of stem cell therapy in orthopaedic surgery. We look at the available evidence and examine the ethical issues and considerations associated with the clinical use of stem cells.

WHAT ARE STEM CELLS

Stem cells have the capacity for self-renewal and the ability to differentiate into various types of tissues under certain conditions. Stem cells are classified based on their source into embryonic stem cells (ESCs), foetal stem cells (FSCs), and adult stem cells. Pluripotent ESCs are capable of differentiating into any tissue type. Adult stem cells are much more limited in their regenerative capability and are usually restricted to the tissues they reside in (i.e., liver hepatocytes and haemopoietic stem cells) (Figures 1 and 2).

It is currently highly risky to use ESCs in the clinic, as the issues of teratogenicity and immune reaction by the recipient have not been fully resolved. One technique for reducing the possibility of teratoma formation is to convert the pluripotent ESCs to multipotent mesenchymal stem cells (MSCs)[1]. The concern about immunogenicity has resulted in the quest for personalised pluripotent stem cells. In this regard, patient-specific cell lines have been developed by reprogramming adult cells to an embryonic state utilizing somatic cell nuclear transfer techniques (SCNT). More recently, Takahashi and Yamanaka have been able to create a pluripotent stem cell (iPSC) by the insertion of four transcription factors (Oct4, Klf4, Sox2 and c-Myc) into mouse skin fibroblasts[2]. Although these cells would be autologous and presumably non-immunogenic, there are still many potential problems as the induction is performed with viral vectors and the issue of teratogenesis still remains.

In the field of Orthopaedics, autologous stem cells such as mesenchymal stem cells (MSCs) are readily available and are amenable to harvesting and isolation from the bone marrow and other tissues of mesodermal origin. MSCs are already pre-programmed to differentiate into musculoskeletal tissue types. The challenge would be to expand them in adequate numbers and ensure that they are able to differentiate into the correct phenotype of tissue that they are intended to repair.

ORTHOPAEDIC APPLICATIONS OF STEM CELL THERAPY

The challenge in orthopaedics is to repair and regenerate damaged or diseased musculoskeletal tissues. The most commonly used stem cells are MSCs. These are non-hematopoietic, stromal cells that exhibit multilineage differentiation capacity, and are able to give rise to diverse tissues, including bone, cartilage, adipose tissue, tendon and muscle. These cells can be isolated from bone marrow or obtained under culture from various other sources, such as the periosteum, fat and skin[3]. Under controlled conditions, these cells can differentiate into multi-mesenchymal lineage (such as osteoblast, chondrocyte and adipocyte) and myoblast lineages, making them useful for cell and tissue engineering as well as gene therapy for Orthopaedic applications.

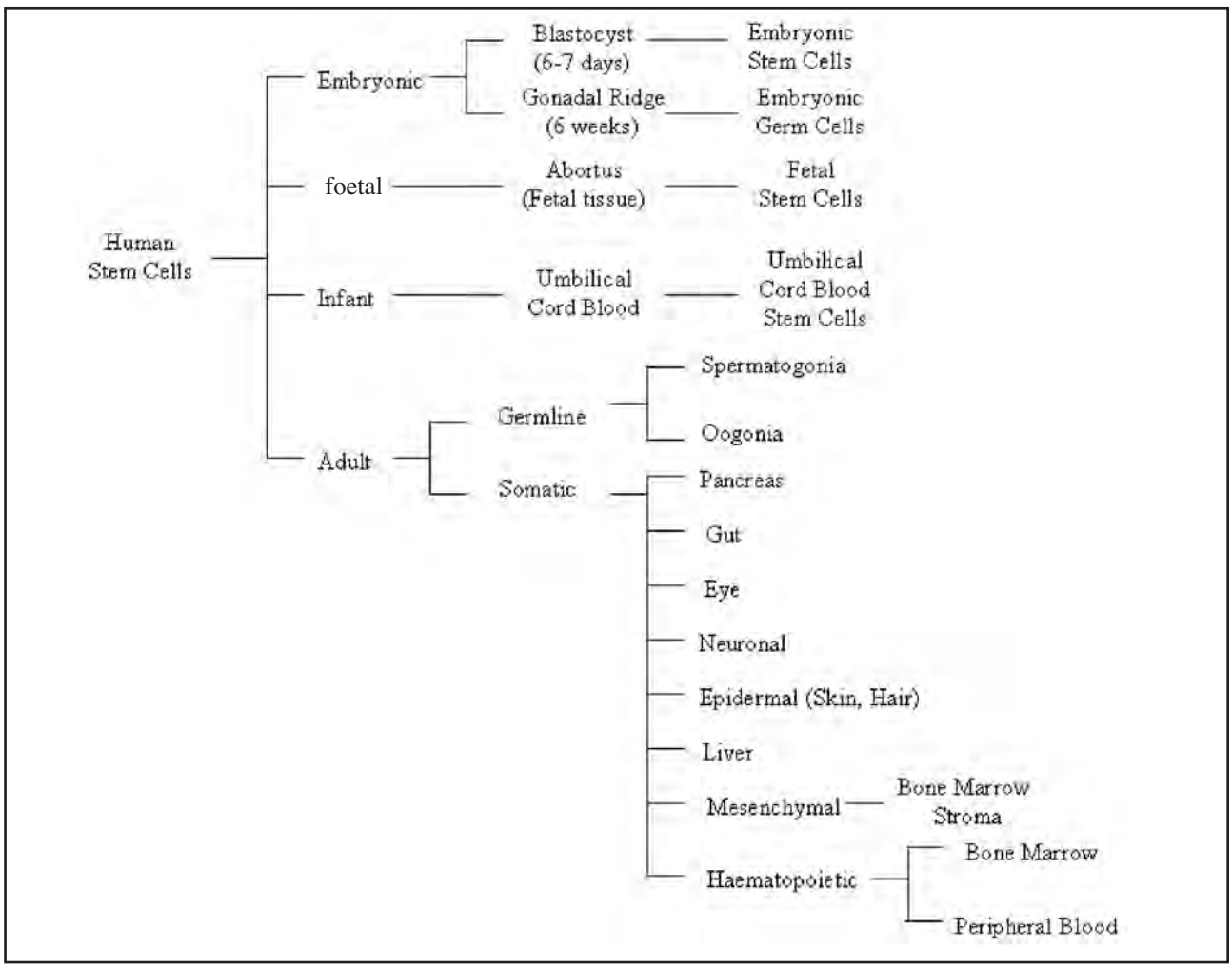

Fig. 1

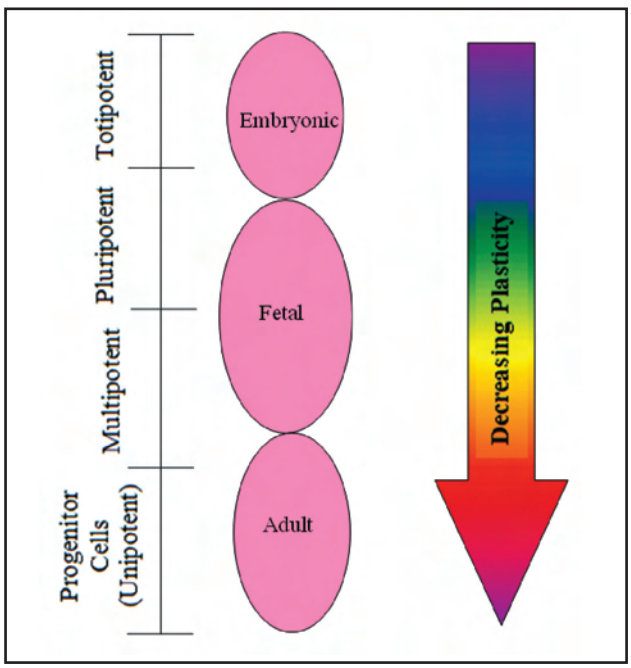

Fig. 2

Cartilage

Injured articular cartilage has poor potential for repair due to its avascular nature. Chondral damage is potentially a major cause of joint disease and disability as it can lead to osteoarthritis (OA), and it is well known that cartilage rarely heals spontaneously [4-9]. Procedures involving penetration of subchondral bone for traumatic chondral defects or ostechondritis dissecans, eventually produce healing due to the presence of fibrocartilage tissue and may give rise to good short to intermediate term results [10,11]. Fibrocartilage arising due to these techniques has poor mechanical properties compared to normal cartilage. Autologous chondrocyte implantation (ACI) is a biological attempt to regenerate normal articular cartilage.

While these techniques in biological resurfacing of cartilage defects require an open arthrotomy or arthroscopy, Lee *et al* investigated the possibility of direct intra-articular injection of MSCs suspended in hyaluronic acid (HA) as an alternative method. A partial-thickness cartilage defect was created in the medial femoral condyle of an adult minipig. MSCs

cultured from the iliac crest marrow of the same pig harvested and isolated in a separate procedure and suspended in 2 millilitres of hylan G-F 20 (Synvisc) were injected three times at weekly intervals intra-articularly after the creation of the defect. The cell-treated the pigs that received MSCs in HA showed improved cartilage healing both histologically and morphologically at 6 and 12 weeks compared with the controls [12]. In another study, Wakitani *et al* studied the effectiveness of autologous cultured bone marrow MSC transplantation in nine full-thickness articular cartilage defects of the patello-femoral joints (including two kissing lesions) in the knees of three patients. Six months after transplantation, the patients' clinical symptoms had improved and the improvements have been maintained over the follow-up periods (17-27 months) [13].

A recent study investigated the feasibility of using autologous uncultured bone marrow-derived mononuclear cell transplantation in large full-thickness cartilage regeneration in 48 rabbits [14]. The animals were divided into four groups: autologous uncultured bone marrow-derived mononuclear cells with fibrin gel (BMC), autologous uncultured peripheral blood-derived mononuclear cells with fibrin gel (PBC), fibrin gel alone (GEL), or nothing (CON) All cell group types were transplanted to the articular cavities of the rabbits seven days after the initial operation. Histological, histochemical grading and safranin O staining were used to evaluate the results. The BMC group was found to have superior cartilage repair compared to the other groups, and the PBC and CON showed similar inferior results, but were still slightly better than did the GEL group. Therefore, it was concluded that transplantation of autologous uncultured bone marrow-derived mononuclear cells contributes to articular cartilage repair has clinical potential. It is worthwhile to note that in this study the autologous uncultured peripheral blood-derived mononuclear cells showed inferior results when compared to the bone marrow-derived mononuclear cell group.

Bone

MSCs can be used to enhance bone regeneration and union in cases of critical bone defect, non-union, physis regeneration in children and to improve bone quality in osteogenesis imperfecta. For non-union cases, even though iliac crest bone grafts are still considered to be the gold standard due to their osteogenic, osteoinductive and osteoconductive properties [15], loading MSCs on an injectable carrier have been tested for efficacy as an alternative for open surgical procedures [16,17]. The simple, minimally invasive technique of percutaneous bone marrow grafting in patients suffering from tibial nonunion has been shown to result in union for most patients [18].

In critical bone defect there is loss of a portion of bone that then fails to heal and requires bone reconstruction to prevent a non-union. MSCs could facilitate osteogenesis in these settings, if loaded in scaffolds of predefined dimensions and shape to fit in the defect [19]. Quarto et al. used autologous MSCs in a porous ceramic scaffold to treat segmental bone defects in a limited group of patients for whom a traditional therapeutic alternative was very difficult or had already failed [20]. Human adipose tissue-derived MSCs [21] and muscle-derived MSCs have also been described as a source of osteoproductive cells for improved healing of critical bone defects [22].

Physeal injury in a growing child often results in formation of bony bridges that eventually lead to angular deformities or shortening. For angular deformities, various types of hemiepiphysiodeses can be used in skeletally immature patients, including stapling, percutaneous epiphysiodesis or the use of eight plates. For a skeletally mature adolescent, osteotomy can be considered. Excision of bony bridges and insertion of fat [23], polymeric silicone [24] or muscle [25] have been described to prevent bony bridges from reforming. However, these interposition techniques are only useful when the bony bridge is small (<30%). More recently, cultured autologous chondrocytes [26-28] and MSCs from bone marrow have been shown to repair large physeal defects leading to significant reduction in growth arrest [29,30].

Osteogenesis imperfecta (OI) is a genetic disorder caused by defects in type I collagen. Ideally, the treatment of OI should be directed toward enhancing bone strength by improving the structural integrity of collagen [31]. Pereira *et al.* infused bone marrow-derived MSCs from a normal mouse into irradiated transgenic recipient mice with an OI phenotype. Several months after transplantation the recipient mice demonstrated the presence of donor-derived MSCs in various organs, including bone, cartilage, lung and spleen. MSCs that homed to the bones differentiated into osteocytes and produced normal levels of collagen type I, with partial ablation of the Osteogenesis Imperfecta phenotype [32]. Similar results have been obtained after allogenic bone marrow transplantation in patients with OI. [33] MSCs transferred in a bone marrow graft may play a potential role in the cure for OI.

Tendons and Ligaments

Once injured, tendons and ligaments produce inferior quality repair tissue due to their limited regenerative ability. Use of biological grafts such as autografts, allografts and resorbable biomaterials can result various complications such as donor site morbidity, scar formation, risk of infection and tissue rejection. Thus, a biological solution using MSCs to regenerate tissues similar to the tissue of origin has attracted the interest of researchers in this field. Application of a collagen gel loaded with MSCs in a rabbit achilles and patellar tendon defect resulted in improvement of structure, biomechanics, and function [34,35].

Another challenging issue is the healing of the tendon graft to the bone (graft-host junction) in instances such as anterior

cruciate ligament reconstruction. The normal anatomy of the insertion site of the ACL is fibrocartilaginous tissue that has complex anatomy. Conventional free tendon transfers are unable to restore this complex anatomy within the first six months [36]. Rodeo et al showed that BMP-2 can augment tendon healing in a bone tunnel [37]. Lim *et al* studied the role of MSCs at the tendon-bone junction during reconstruction of the ACL in the rabbit and showed that applying MSCs to tendon grafts at the tendon-bone junction results in a zone of fibrocartilage at the junction which more closely resembled that of the normal ACL. These enhanced grafts have improved biomechanical properties compared to controls, and have exhibited a rapid and significant increase in load to failure and stiffness in the first eight weeks after ACL reconstruction [38]. Another recent study saw the use of synovial MSCs in the insertion of the Achilles tendon graft of rats into a bone tunnel from the tibial plateau to the tibial tuberosity. It was observed histologically that implantation of synovial MSCs into the bone tunnel accelerated healing and showed early remodeling of tendon-bone junction [39].

Meniscus

Injury to the inner third of the meniscus has limited healing potential due to its avascular nature. Meniscal repair is not possible in the avascular portion and meniscectomy has been shown to have a strong association with the subsequent development of osteoarthritis. This has led to investigations into the possibility of cell-based meniscal repair and regeneration. It has been demonstrated that isolated chondrocytes seeded on to meniscal matrices were able to bond separate pieces together. Histological and biomechanical analyses showed that the strength of the adhesion increased over time by the formation of a newly synthesised cartilaginous matrix [40]. Meniscal repair following intra-articular injection of MSCs has also shown improvement in meniscal wound healing; even in avascular areas, there was production of an abundant extracellular matrix contributed to meniscal repair [41,42].

A recent study tested a cell-scaffold combination for the repair of a critical-size defect of the rabbit medial meniscus [43], by compaing a hyaluronan/gelatin composite scaffold, and also scaffolds loaded with autologous marrow-derived MSCs, and empty scaffolds in the contralateral knees to untreated contralateral defect as control. Untreated defects had a muted fibrous healing response. Pre-cultured implants integrated with the host tissue and eight of 11 contained meniscus-like fibrocartilage, compared with two of 11 controls ($p < 0.03$). The mean cross-sectional width of the pre-cultured implant repair tissue was greater than controls ($p < 0.004$). This study demonstrated that repair of a critical size meniscal defect with a stem cell and scaffold based tissue engineering approach is a potential clinical application of MSCs [43].

Centeno *et al* conducted a study to determine if isolated and expanded human autologous MSCs could effectively regenerate cartilage and meniscal tissue when percutaneously injected into knees [44]. MSCs isolated from bone marrow aspiration of the iliac crest of a consenting volunteer were cultured ex-vivo and percutaneously injected into the subject's knee with MRI proven degenerative joint disease. At 24 weeks post-injection, the subject had statistically significant cartilage and meniscus growth on MRI, as well as increased range of motion and decreased modified VAS (visual analogue scale) pain scores. This has significant future implications for minimally invasive treatment of osteoarthritis and meniscal injury [44].

Muscles and Skin

Injured muscle fibres produce signals to promote myogenic differentiation [45] and drive MSCs to occupy the damaged area and regenerate damaged fibres [46]. In Duchenne Muscular Dystrophy (DMD), which is characterized by progressive muscular weakness and muscle wasting eventually leading to paralysis and death, intravenous injection of MSCs in models of immunodeficient mice with DMD has shown differentiation of MSCs into muscle fibres and partial restoration of dystrophin expression [47]. However, clinical applications require further advances in cell engineering and gene therapy. In the autologous transplantation of muscle-derived CD133+ cells in a 7-month, double-blind phase I clinical trial conducted on 8 boys with DMD, patients showed an increased ratio of capillary per muscle fibers with a switch from slow to fast myosin-positive myofibres [48].

MSCs have also been investigated for the treatment of chronic skin wounds [49]. Three patients suffering from chronic skin wounds of more than one year duration that did not respond to bioengineered skin applications or skin grafting with autologous skin were treated with application of autologous bone marrow-derived MSCs. In all three patients, complete wound closure was observed [49].

Spine, Spinal Cord and Neural Tissues

Repair of the spinal cord is a very complex process that includes restoring or enhancing local spinal reflex arcs and reconnecting regenerating axons [31]. Evidence of axonal regeneration and functional recovery has been seen in animal models of spinal cord injury. Akiyama et al have demonstrated that MSCs isolated in culture from the mononuclear layer of bone marrow can remyelinate demyelinated spinal cord axons after direct injection into the lesion. However, stem cells alone may not be enough to improve function in a damaged spinal cord [50].

Rosenfeld and Gillett have recently discussed problems associated with the use of stem cells in this context and cautioned against premature application of this technology. They warned against extrapolating the results of animal experiments directly to humans, as there may be a risk of further morbidity and even tumour growth [51]. Strategies for repair of the human spinal cord will, of necessity, be multifaceted, entailing enhancement of axonal growth and

reconnection, replacement of cellular elements, and the reversal of demyelination as necessary steps for success. The connective tissue matrix, the degree of glial scarring and the central myelin inhibitory factors, the elimination of which is required for axon outgrowth, are all important. The balance of these factors may be as important as the stem cells themselves, and very difficult to optimise [51].

Intervertebral disc degeneration, which is manifested by gradual loss of water and proteoglycans, can lead to back pain and other morbidity. Treated conservatively, approximately 90% of patients show improvement. After failure of conservative treatment, surgical options can be considered. Cell-based tissue engineering offers considerable promise for a biological alternative (transplantation to the intervertebral disc of mature autologous disc cells, chondrocytes or stem cells). Cell transplantation can potentially increase proteoglycan production, induce disc regeneration or slow the process of degeneration. In animal models, transplantation of autologous disc cells and chondrocytes derived from costal cartilage has been demonstrated to be feasible and may slow disc degeneration [52].

MSCs may potentially also contribute to the repair of peripheral nerves due to their ability to differentiate into neurons and glia [53]. Regeneration, functional recovery and healing of transected sciatic nerve has been demonstrated in several animal models [54].

ETHICAL CONSIDERATIONS

While prohibition of reproductive cloning is generally accepted, there is wide variability amongst different countries regarding the ethical guidelines and regulation of stem cell research and therapy. Recognising that these differences are inevitable, the International Stem Cell Forum (ISCF) was initiated in 2002 by Sir George Radda who was then the Chief Executive of the Medical Research Council (MRC) of the United Kingdom. The ICSF consists of delegates representing the funding agencies of countries involved in stem cell research. The aims of the ISCF are primarily designed to forge international collaborations in stem cell research by working to establish the standardisation of techniques, the sharing of cell lines, training, conferences, and information. Various subcommittees have been organised to discuss scientific issues, ethics and intellectual property.

In a similar vein, the International Society for Stem Cell Research (ISSCR) is a society for scientists involved in stem cell research. In addition to the publication of the *Guidelines for the Conduct of Human Embryonic Stem Cell Research* in December 2006, the ISSCR recently published its Guidelines for the Clinical Translation of Stem Cells, which is available online at http://www.isscr.org/clinical_trans/index.cfm [55]. These guidelines were developed by the Task Force for the Clinical Translation of Stem Cells, a multidisciplinary group of stem cell researchers, clinicians, ethicists, and regulatory officials from 13 countries. They highlight the scientific, clinical, regulatory, ethical, and social issues that should be addressed so that basic stem cell research is responsibly translated into appropriate clinical applications for treating patients. The Guidelines pertain to clinical translational research involving products from human embryonic or other pluripotent stem cells, novel applications of foetal or somatic (adult) stem cells, and hematopoietic or other stem cells used for applications outside established standards of care. The Guidelines address three major areas of translational stem cell research: (a) cell processing and manufacture; (b) preclinical studies; and (c) clinical research [55]. The ISSCR also provides information for patients and their doctors evaluating stem cell therapy in its Patient Handbook on Stem Cell Therapies [56]. These publications are very important guides and are highly recommended as reading material for those interested in using stem cells for therapeutic purposes.

While stem cell research holds tremendous promise for the development of new treatments for many serious diseases, nearly all stem cell therapies are new and highly experimental. Therefore, there is an urgent need to address the problem of unproven stem cell therapies being marketed directly to patients. Unfortunately, there are clinics around the world that exploit patients' hopes by offering "stem cell therapies", without credible scientific rationale, oversight or any form of patient protection. Lau et al analyzed websites in which stem cell clinics advertise via internet and appraised the relevant published clinical evidence of stem cell therapies to address three questions about these direct-to-consumer portrayals of stem cell medicine in this early market: i)What sorts of therapies are being offered?; ii) How are they portrayed?; and, iii) Is there clinical evidence to support the use of these therapies? Overall, they found that the portrayal of stem cell medicine on provider websites is optimistic and unsubstantiated by peer-reviewed literature [57].

CURRENT STATUS OF STEM CELL THERAPY

"Stem Cells: Hype or Hope?" Given the amount of hype that the media (and especially the internet) has given to stem cells as a cure for diseases that hitherto have not been successfully treated, it is the responsibility of every doctor to ensure that he/she has enough knowledge of the current status of stem cell therapies so that he/she can correctly advise his/her patient. At this time, it is fair to say that the research on ESCs and iPSCs is still fairly upstream and there should not be any attempt made to use these cells to treat musculoskeletal problems in patients. Both types of pluripotent cells share similar problems of possible teratogenicity which must resolved before these cells can be safely used in a clinical setting. Although there have been

attempts to reduce the possibility of teratoma formation by using MSCs derived from hESCs, this technique is still at the pre-clinical stage of research.

In general the use of adult stem cells is closer to the clinic as these cells are less potent than ESCs and more directed, so there is little or no likelihood of teratoma formation. There are now many pre-clinical studies in animals as well as a few clinical studies using bone marrow-derived MSCs to treat musculoskeletal problems. Most of these studies show promising results and are fairly safe as autologous cells are used. Bone marrow-derived MSCs have also been shown to be more directed towards forming cartilage than fat-derived MSCs, suggesting that these stem cells are pre-programmed to form certain tissues more efficiently than others [58]. Some studies have also shown that allogeneic MSCs do not evoke an immune response so there is potential for the use of allogeneic MSCs in the future [59].

The role of other stem cells in musculoskeletal regenerative medicine has not been as well investigated. Cord blood is generally used for haemopoietic disorders and has not been shown conclusively to be useful in the regeneration of musculoskeletal tissues. There are extremely few stem cells in peripheral blood and the use of peripheral blood for cartilage repair has been shown to be inferior to that of bone marrow-derived cells [14]. Similarly, aspirated bone marrow (without any further manipulation) has very few stem cells and is unlikely to produce any significant healing in cartilage or tendon defects.

When using stem cells in a clinical situation, it is very important that the cells have been derived and expanded under strict cGMP conditions. Informed consent must be obtained and the patient has to be followed closely to ensure his/her safety after stem cell transplantation. The ISSCR *Guidelines for the Clinical Translation for Stem Cells* and the *Patient Handbook on Stem Cell Therapies* provide the requisite information for the clinical use of stem cells [55,56].

Finally, it is important to note that as a doctor, the patient's safety and well-being must always come first. As pointed out by Lau *et al* [57], many of the advertised stem cell treatments on the Internet are not evidence-based. Patients must be protected from exploitation by such unscrupulous practices.

ACKNOWLEDGEMENT

The authors would like to express our appreciation for Miss Tai Beishan Sarah for her secretarial assistance.

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