FUTURE ORTHOPAEDICS: ROLE OF STEM CELLS

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INTRODUCTION

Today great hope is set on regenerative medicine in all medical fields. Leland Kaiser introduced the term "Regenerative medicine" in 1992. He forecasted that "a new branch of medicine will develop that attempts to change the course of chronic diseases and in many instances will regenerate tired and failing organ systems"[1] Since then, scientists all over the world try to develop cell-based approaches to regenerate damaged tissues, or even substitute whole organs[2].

Of course, regenerative medicine has developed to be of interest in orthopaedics. There, great hope was set on regenerative medicine to develop alternative therapies for cartilage damage, arthritis, large bone defects, or atrophic tendon ruptures during the last decade. These are all indications, which are treatable only insufficiently with conventional implants and surgical procedures[3-10]. Therefore, they frequently result in decreased function of the musculoskeletal system or even loss of patients' mobility. In the worst case, the mentioned diseases even result in a loss of autonomy for the patient.

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. But Despite rapid progress, significant challenges remain in the translation of these stem cell therapies for clinical applications.

In this review, a brief description of stem cells is provided, and the current status of stem cells in orthopaedic practice is discussed.

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. Embryonic stem cells (ESCs) are only found in early developmental stages of the organism. They represent the only cell type, which has the ability to renew itself indefinitely and it can differentiate into cells of all three germ layers. Adult stem cells are much more limited in their regenerative capability and are usually restricted to the tissues they reside in. The stem cell niche is an extracellular microenvironment in which the cell resides. The niche is an important regulator of the biochemical and physical signals that a stem cell receives, thereby impacting key aspects of activity, such as cell survival, proliferation and differentiation.[11-13] tissues such as bone, cartilage, and muscle naturally have distinct moduli,[14] and stem cells will preferentially differentiate toward certain cell types depending on the mechanical properties and nanostructure of the extracellular environment.[15-18]

From a legal and ethical point of view, research involving human embryonic cells is highly controversial. Besides the ethical concerns, the use of embryonic stem cells is problematic, as the

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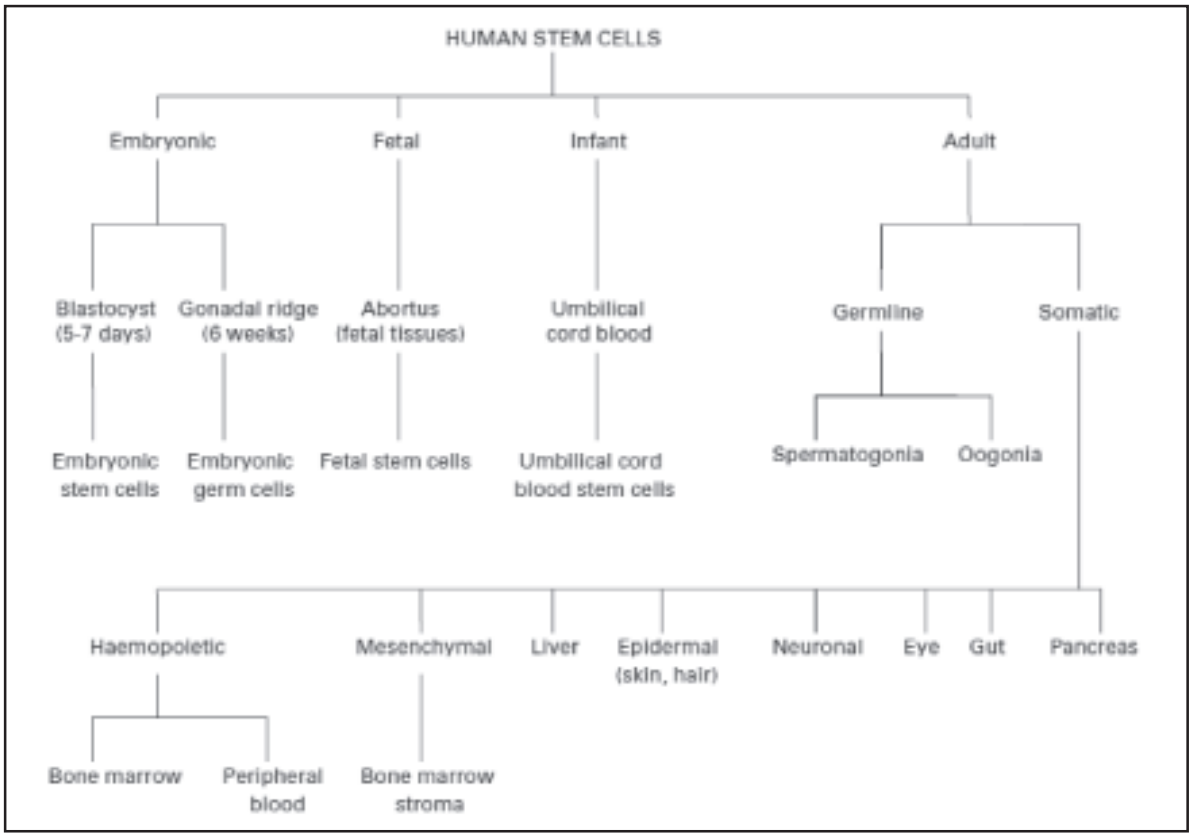

Figure 1: Differentiation of human stem cells

application of pluripotent cells inheres a distinct oncogenic potential, teratogenicity and immune reaction in the recipient. One technique for reducing thepossibility of teratoma formation is to convert the pluripotent ESCs to multipotent mesenchymal stem cells (MSCs). 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[19]. 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.

The use of adult stem cells raises less ethical concerns and has proved to be much safer than pluripotent stem cells. In addition, these cells have further advantages compared to ESCs, for example, a use for autologous cell therapies, using patients' own cells to reduce possible immune responses, is easier to realize. Nonetheless, the limited differentiation potential of adult stem cells narrows their applicability. Typically, adult stem cells can differentiate into the cell types of the tissue in which they reside. Mesenchymal stem cells have been found to be the most promising candidates, as they show good differentiation potential towards cartilage, tendon and bone cells. They can be isolated from a number of mesenchymal tissues as for example bone marrow, fat, synovial membrane, periosteum, and others[20]. Interestingly, these mesenchymal stem cells have been found to differ regarding their differentiation potential dependent on their tissue source[21].

ETHICAL CONSIDERATIONS IN THE USE OF STEM CELLS

There are different views regarding

therapeutic cloning and ESCs research. Many countries allow the use of ESCs derived from discarded or excess embryos from invitro fertilisation (IVF). However, there are differences of opinion regarding the derivation of new stem cell lines from embryos created specifically for research purposes. The United States does not allow the use of federal funds for research to create new ESC lines, but allows work on the ESC lines currently stored in the National Institute of Health (NIH). However, there is no regulation of research on ESCs carried out with private funding, resulting in the establishment of privately funded research centres.

Despite the discrepancy between the approaches of different governments, scientists from various countries havecome together to form the International Stem Cell Forum (ISCF). This was initiated in January 2003 by Sir GeorgeRadda when he was the Chief Executive of the Medical Research Council (MRC) of the United Kingdom. The ICSF aims to forge international collaboration in stem cell research by trying to establish the standardisation of techniques, the sharing of cell lines, training, conferences, and the exchange of information. It has also set up various subcommittees to discuss issues of scientific, ethical and intellectual property.

In May 2004 the United Kingdom set up a stem cell bank. This regulates the storing, characterisation and supplyof ethically approved quality-controlled stem cell lines for research. It includes all cell lines derived from embryonic, foetal and adult tissues and is hosted by the National Institute of Biological Standards and Control in South Mimms, Hertfordshire.

As ethical and safety concerns currently forbid application of iPSCs and ESCs in patients, we will focus on adult mesenchymal stem cells within the rest of the paper.

MESENCHYMAL STEM CELLS

The most commonly used stem cells are MSCs. These are nonhematopoietic, 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[22,23]. 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.

Two main types of MSCs have been used, Bone marrow derived and Adipose tissue derived. Bone marrow derived MSCs are more adept at Bone repair, Cartilage repair, and soft tissue repair[24,25]. Other Advantages of MSCs over ESCs are that they are Autologous hence no major ethical issues are there with their use. These can be expanded in vitro hence an unlimited supply of therapeutic cells is ensured. MSCs do not express HLA II antigens hence allotransplantation is possible.

APPLICATION IN ORTHOPAEDICS

Cartilage

Injured articular cartilage has poor potential for repair due to its avascular nature. Articular cartilage damage leads to Osteoarthritis. Procedures directed at the recruitment of stem cells from the marrow by penetration of the subchondral bone have been widely used to treat localised cartilage defects. The 'microfracture' technique is often used, but the fibrocartilage which results from these techniques has poor mechanical properties compared with normal cartilage[26]. More recently, attempts to 'regenerate' normal articular cartilage have been introduced in clinical practice with autologous chondrocyte implantation (ACI).

For autologous cartilage repair various two- and three-dimensional constructs are available. Most of the matrices consist of natural polysaccharides and proteins, such as alginate and collagen. Furthermore, synthetic polymers are also available for example, polyethylene glycol (PEG) or polylactic acid (PLA). Successful outcome of a

stem cell-based cartilage tissue engineering also depends on the design of extracellular matrix for a proper differentiation of MSCs into chondrocytes. Wakitani et al.[27] 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.[28] 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.[29] 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.

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[30], loading MSCs on an injectable carrier have been tested for efficacy as an alternative for open surgical procedures[31,32]

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.

Physeal Injuries

Physeal injury in a growing child often results in formation of bony bridges that eventually lead to angular deformities or shortening. Excision of bony bridges and insertion of fat, polymeric silicone[33] or muscle[34] 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[35-37] and MSCs from bone marrow have been shown to repair large physeal defects leading to significant reduction in growth arrest[38,39].

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[40]. 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.[41] MSCs transferred in a bone marrow graft may play a potential role in the cure for OI.[42]

Non-Unions

MSCs have osteogenic potential; they tend to differentiate along the osteogenic pathway in response to chemical stimulation.[43] 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.[44] Garg et al.[45], Kettunen et al, Hernigou et aland Goel et al[46] The application by these investigators has been on non-union of long bones especially tibia and also for diagnosed cases of pseudo-arthrosis. Fernandez et al[47] studied the effects of

autologous bone marrow mononuclear cells combined with allogenic bone graft for repair of pseudo-arthrosis 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"

Tendons & 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. Application of a collagen gel loaded with MSCs in a rabbit Achilles and patellar tendon defect resulted in improvement of structure, biomechanics, and function.

Another challenging issue is the healing of the tendon graft to the bone (graft-host junction) in instances such as anterior cruciate ligament (ACL) is the healing of the tendon graft to the bone. The normal anatomy of the insertion site of the ACL is fibro cartilaginous and consists of four distinct zones: ligament substance, unmineralised fibrocartilage, mineralised fibrocartilage and bone.[48] Conventional free tendon transfers are unable to restore this complex anatomy within the first six months.[49] Lim etal50studied the role of MSCs at the tendon-bone junction during reconstruction of the ACL in the rabbit. They 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 with controls, and have exhibited a rapid and significant increase in load to failure and stiffness in the first eight weeks after reconstruction of the ACL.[50]

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 remodelling of tendon-bone junction.

Meniscus

Tears in the avascular inner third of the meniscus have limited or no potential for repair as the reparative process cannot occur without the presence of vascularity. Meniscectomy has been shown to have a strong association with the subsequent development of osteoarthritis. Centeno et al[51] conducted a study to determine if isolated and expanded human autologous MSCs could effectively regenerate cartilage and meniscal tissue when percutaneously injected into knees[51]. 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 pain scores. A recent study tested a cell-scaffold combination for the repair of a critical-size defect of the rabbit medial meniscus, by comparing a hyaluronan/gelatin composite scaffold, and also scaffolds loaded with autologous marrow-derived MSCs, and empty scaffolds in the contra lateral knees to untreated contra lateral 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 has significant future implications for minimally invasive treatment of osteoarthritis and meniscal injury.[51]

Avascular necrosis (AVN)

Avascular Necrosis of femoral head leads to

the death of osteocytes present in the sub-chondral region and causes collapse of the femoral head; it alters the shape of the 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.[52] 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. Wang et al.[53] reported debridement, autogenous bone grafting and bone-marrow mononuclear cells im-plantation as an effective procedure in patients with small lesion, early-stage AVN of the femoral head. Limitation for the use of stem cells in this condition is the stage of presentation as once the collapse has started, the shape of femoral head cannot be returned back to normal by the stem cells.

Stem cells as fillers of bony voids

In cases of benign bone tumours such as simple bone cysts for which curettage has been done and in cases where there is a bone defect, a void is left behind in the bone. Marcacci et al.[54] used autologous MSCs that were expanded in vitro and seeded on hydroxyl-apatite scaffolds for filling of diaphyseal bone defects and reported good integration of the graft 7 years post-op without any secondary fractures.

Muscular Dystrophy

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. Wakitani et al[55] found that under certain conditions, in vitro bone marrow differentiates into contractile myotubes. Gussoni et al[56] showed that in immunodeficient mice a marrow-derived cell can migrate into areas of induced muscle degeneration, undergo myogenic differentiation and participate in the regeneration of the damaged fibres. The study showed that bone marrow- or muscle-derived stem cells appear to provide a means for systemic, rather than local, repair of muscle, as a consequence of the delivery of the cells throughout the vascular system. It is possible that in future the procedures for stem cell transplantation could be optimised to provide levels of engraftment of muscle that would be useful clinically.[57]

Spine & Neural Tissues

Degeneration of the intervertebral disc is a leading cause of back pain and morbidity. After failure of conservative management the surgical options for discogenic back pain are limited and usually invasive. Cell-based tissueengineering offers considerable promise for a more biological alternative by 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. Recently, Crevensten et al[58] explored the use of MSCs for intervertebral disc regeneration. They used an in vivo model to investigate the feasibilityinjected cells was observed, and their viability was 100%.

Repair of the spinal cord is a very complex process that includes restoring or enhancing local spinal reflex arcs and reconnecting regenerating axons.[59] 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.[60] However, the results of animal studies should not be directly extrapolated to human subjects. 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. Balance of these factors is necessary.

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.[43] and Gan et al.[44] 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.[45] have used lyophilised bone chips with platelet-enriched plasma with bone marrow aspirate in high tibial osteotomy and found an enhancement of healing.

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 ran-domised 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 iPS 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.

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