Stem Cells in Orthopaedics and Fracture Healing

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Abstract

Stem cell application is a burgeoning field of medicine that is likely to influence the future of orthopaedic surgery. Stem cells are associated with great promise and great controversy. For the orthopaedic surgeon, stem cells may change the way that orthopaedic surgery is practiced and the overall approach of the treatment of musculoskeletal disease. Stem cells may change the field of orthopaedics from a field dominated by surgical replacements and reconstructions to a field of regeneration and prevention. This review will introduce the basic concepts of stem cells pertinent to the orthopaedic surgeon and proceed with a more in depth discussion of current developments in the study of stem cells in fracture healing.

Stem cells are any undifferentiated, progenitor cells that are able to self-renew and differentiate into one or more different cell lineages. They undergo asymmetric division to produce cells that become more specialized, such as osteoblasts or chondroblasts, while maintaining the ability to self perpetuate.

Classification

Stem cells are classified in two ways: according to their plasticity and by their source. The ability of a stem cell to become multiple different cell types, or the degree of potential of a stem cell, is referred to as its plasticity. A stem cell can be classified as totipotent when it has the ability to become any cell type (including another totipotent cell). Cells with lesser degrees of plasticity are classified as pluripotent and multipotent. Pluripotent cells can become any cell type except for totipotent cells and multipotent cells are further specialized. Recently a group of researchers in Japan led by Dr. Shinya Yamanaka, an orthopaedic surgeon, as well as a group out of Wisconsin have described induced pluripotent stem cells (iPSCs), or cells whose plasticity is increased via transfection of genes. This discovery may change the concept of plasticity being a continuum to more of an infinite loop theory.

Stem cells are also classified according to their source, which also often correlates with their plasticity. Embryonic stem cells (ESCs) are cells isolated from the inner cell mass of a blastocysts (5- to 7-day-old embryo) and represent the only truly totipotent cell lineage. They were first described, in the early 1980s, as isolated from mouse embryos. Embryonic stem cells have the advantage of being able to replicate indefinitely in vitro and theoretically can become any cell type or an entire organism. Human embryonic stem cells were isolated in the late 1990s and have since become a political hot topic. Limitations on embryonic stem cell research have spurred further investigation into other sources of stem cells.

Fetal stem cells are cells extracted from fetal blood or extra-embryonal tissues. They are pluripotent, with decreased plasticity when compared to embryonic stem cells, and are being investigated in the possible treatment of disease in utero. Similarly, umbilical stem cells are isolated from umbilical cord blood at the time of birth. There is a high concentration of multipotent stem cells in umbilical cord blood, and they have already found use in the treatment of hematopoietic and bone marrow disorders. Recently,
Adult stem cells are of interest, have been the subject of the most investigations in musculoskeletal research, and may hold many important implications in the field of orthopaedics. Adult stem cells are undifferentiated cells found amongst specialized cells in the post natal state that are able to self-replicate and differentiate into various cell types. They are able to remain in a quiescent state until stimulated by a process that is poorly understood. Their main role is thought to be that of maintenance and repair of tissues. Adult stem cells are classified by the source tissue from which they are derived as well as the types of cells that can be derived from them. Adult stem cells are subcategorized by the cell types that they can become. Adult stem cells include hematopoietic stem cells, which are found most abundantly in the bone marrow and can differentiate into a variety of blood cell lineages, and neuronal stem cells, which can differentiate into neural structures. Most orthopaedic research has been on mesenchymal stem cells (MSCs), which is the focus of the remainder of this review. MSCs are derived from mesenchyme, which are cells of mesoderm origin of the human embryo, and are most pertinent to orthopaedics as they can differentiate into cells of the musculoskeletal system, including bone, tendon, muscle, and cartilage (Fig. 1). A great deal of new and exciting research is taking place looking at mesenchymal stem cells in relationship to musculoskeletal disease.

MSCs have been retrieved from a variety of tissues throughout the body, including periosteam, muscle, circulating blood, blood vessels, synovium, and fat; and thus they represent a readily available source of adult stem cells. They can be harvested from a patient and cultured (multiplied) in vitro and can thus act as autografts, eliminating the concerns of immunogenicity that exist with other graft sources. Some difficulties do exist with MSCs, as they have not been found in high concentrations in the adult body and, at this time, can only be multiplied outside of the body for about 1 year. This makes the process of culturing and harvesting MSCs arduous and time consuming. MSCs have the potential to become a variety of precursor cells; most pertinent to orthopaedics is their ability to become bone, cartilage, muscle, tendon, and ligament producing cells. Beyond their role in becoming precursors cells, recently it has been discovered that MSCs also produce a multitude of cytokines and growth factors that induce their own proliferation and differentiation.

The application of MSCs to musculoskeletal disease first warrants a discussion of the general principles and problems that occur with their use. The principles of isolation, proliferation, differentiation, and delivery describe the general goals of stem cell research and provide the basis of the potential of stem cell use in orthopaedics. Isolation is the process by which MSCs are harvested, identified, and separated from surrounding tissue. In most studies, MSCs have been harvested by bone marrow aspirates, an invasive procedure that often yields few stem cells. The discovery of stem cells in more peripheral tissue, such as subcutaneous fat, may provide an abundant source of stem cells, especially in the American population. Interestingly, it has been shown that the numbers of MSCs decrease with age by a process that is not understood. This makes the harvesting of MSCs for use in an elderly population much more difficult and has brought up the possibilities of MSC banking earlier in life. This decrease in the amount of MSCs in an aging population may also be ultimately involved in the multitude of degenerative processes that affect the musculoskeletal system and may become a target of therapies in the future.

After MSCs have been harvested many techniques have been described for their identification and isolation. These include flow cytometry, per plating, cell transfers and antibody-coated beads. The cell markers that identify human MSCs are not clearly defined and determination of cell lineages is not confirmed with human samples until further differentiated cells are induced, meaning that we cannot confirm the presence of human MSCs until cells or tissues induced from these cells are found to be of mesenchymal origin. This brings into question the possible differences in MSCs reported in studies with different isolation protocols.

Proliferation is the process by which MSCs are plated in vitro, most commonly on animal product, and grown in monolayers (one cell layer). This process requires constant, labor intensive replating to provide continuous multiplication of cells. As stated, the process of proliferation of adult MSCs at this time may be limited.

Differentiation is the process by which MSCs become more specialized cells. Although MSCs have been found
to differentiate into multiple cell lineages, they do not have the plasticity of embryonal stem cells. 16 The process of differentiation is very poorly understood and represents a major obstacle to the development and therapeutic use of MSCs. MSCs respond to a variety of stimuli, including hormonal, chemical, and mechanical factors to differentiate into a variety of specialized cell types. 23 In orthopaedics, it is known that cytokines and mechanical environments play a role in stimulating MSCs into bone, tendon, and cartilage precursors. BMP-4 has been shown to induce MSCs to become bone and articular cartilage precursors. 24 BMP-7 is a strong stimulator of MSCs to become bone precursors. 25 The mechanical stimulation in a fracture environment also stimulates the migration and differentiation of MSCs at a fracture site and is now known to be an integral part of fracture healing. The process of differentiation has proven to be extremely complex, and an exact replication of the body’s intricate stimulation of cells may not be possible.

The next obstacle in the use of stem cells in orthopaedics is the mode of delivery of stem cells for their therapeutic use. Orthopaedic injuries are most often localized, while degenerative processes are more generalized. Stem cell therapy has been studied in both local and systemic delivery, and the results are very promising. 26-28 A variety of possibilities exist in the delivery of stem cell therapeutics in orthopaedics. Studies have looked at the stimulation of in vivo stem cells, local implantation, systemic administration, the use of stem cells as a vector for gene therapy, the implantation of MSCs that have been differentiated in vitro, the in vitro creation and implantation of whole end organs, and tissue engineering as all distinct possibilities in the future of the orthopaedic use of MSCs. 29, 30

**Stem Cells in Fracture Healing**

The role of stem cells in fracture healing has now been well established. 31 Although stem cells have applications throughout the field of orthopaedics, a discussion of each topic is beyond the scope of this article. We will use the example of the role of stem cells in fracture healing as an example of the advancement in the knowledge and application of stem cells in orthopaedics.

Bone has the ability to heal itself when fractured. Bone healing is a complex and well-orchestrated process that depends on many factors: cellular, molecular, and mechanical. Unlike other adult tissues, which generate scar tissue at the site of an injury, the skeleton heals by forming new bone that is indistinguishable from uninjured bone. Recently, the role of stem cells of various origins has been demonstrated. The enhancement or exploitation of this can enhance bone repair and regeneration.

The osteogenic potential of MSCs has already been verified. 31, 32 Two approaches have been used for cell delivery: bone marrow aspiration and direct introduction at the lesion or expansion ex vivo before implantation. Percutaneous autologous bone marrow grafting has been shown to be an effective treatment for tibial diaphyseal nonunion in one study. 31 The efficacy is influenced by the amount of progenitor cells in the harvested graft, as harvested iliac crest bone marrow graft appears to contain a suboptimal concentration of cells.

In vitro studies have shown that myoblastic cell lines in mice can differentiate into osteoblastic lineage cells upon stimulation with bone morphogenetic protein. 25 Lee et al showed that their highly purified muscle derived MSCs (preplate technique) differentiated into osteogenic lineage, 33 suggesting that subpopulations of muscle-derived stem cells are capable of bone healing. Shen and colleagues 34 were able to show that IGF-1 transduced mesenchymal cells were able to return and repopulate the bone marrow with a preferential recolonization of the fracture site in a mouse in vivo model. They have also shown an accelerated fracture healing by demonstrating a greater average mineralized matrix and progression to osseous callus. This demonstrates, as earlier studies have shown, that MSCs are attracted to fracture sites and that there may be a role in systemic administration of stem cells in certain instances, for example, with fractures that have a relatively high non-union rate or in elderly patients who have been shown to have a decreased concentration of MSCs.

MSCs may also be used as conduits for gene therapy. MSCs have been used as a tool to deliver bone morphogenetic protein-2 (BMP-2), by in vivo and ex vivo approaches. 35-37 A study by Musgrave and colleagues showed that human skeletal-muscle derived cells can be successfully used in gene therapy for the deliverance of BMP-2 in an ex vivo fashion. 38 As stated, BMP-2 induces MSCs to become bone forming cells, so these altered cells may provide local stimulation of existing MSCs, increasing the therapeutic effect. Human skeletal muscle, which is readily available to the orthopaedic surgeon, is an attractive cell source that is responsive to BMP-2. Furthermore, the combination of osteogenic growth factor release (BMP-2) from polymer scaffolds, such as polyactic acid, and the addition of preosteogenic cells have further increased the possibility of engineering bone. 39, 40

MSCs have been shown to readily differentiate down an osteogenic pathway in response to chemical signals. 41 MSCs have been shown to be the primary source for endochondral bone formation. 42 and as such are ideal for future bone repair constructs. Sumanasinghe and associates 13 presented in their study of human MSCs that mechanical strain alone could induce osteogenic differentiation. The ability to create a three-dimensional osteogenic cell culture is an essential step in the creation of bioengineered three-dimensional osteogenic constructs for bone healing. Recent studies have shown that the combination of angiogenic and osteogenic factors can stimulate bone healing and regeneration. 44 Therefore, the ability to deliver a combined delivery system of growth factors at different rate kinetics locally from biodegradable scaffolds could enhance the reparative
mechanism of critical sized bone defects; thus, mimicking the in vivo bone repair conditions.

With better understanding of the biology of stem cells in the future and with enhancement of technologies that are capable to influence, modify, and culture these cells, a new field of regenerative skeletal medicine may emerge.

Conclusion and Summary
The treatment of musculoskeletal disease at this point of time concentrates on the concepts of reconstruction and replacement. A large amount of musculoskeletal pathology is thought to be due to inadequate or absent regenerative potential of a variety of musculoskeletal tissues. The field of stem cells has the potential to change this completely. Stem cells are far from replacing the orthopaedic surgery that we know today, but they will augment our ability to treat pathologic processes that continue to be problematic to our everyday practice. Developments in fracture healing will soon revolutionize the treatment of fracture care and have the ability to possibly eradicate the problem of non-unions. Other developments may be farther off, but the developments that are possible and that are being studied now should become familiar to all orthopaedic surgeons.

Disclosure Statement
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References


