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Stem Cell Therapies in Orthopaedic Trauma

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Abstract

Stem cells offer great promise to help understand the normal mechanisms of tissue renewal, regeneration, and repair, and also for development of cell-based therapies to treat patients after tissue injury. Most adult tissues contain stem cells and progenitor cells that contribute to homeostasis, remodeling and repair. Multiple stem and progenitor cell populations in bone are found in the marrow, the endosteum, and the periosteum. They contribute to the fracture healing process after injury and are an important component in tissue engineering approaches for bone repair. This review focuses on current concepts in stem cell biology related to fracture healing and bone tissue regeneration, as well as current strategies and limitations for clinical cell-based therapies.

Keywords

Stem Cells; Connective Tissue Progenitors (CTPs); Mesenchymal Stem Cells (MSC’s); Bone healing; Tissue Engineering

Introduction

In orthopaedic surgery, stem cells present a number of clinical opportunities, from tissue regeneration and modulation of immune function, to modeling rare diseases. Stem cells can be broadly divided between totipotent cells that are capable of forming all of the tissues in the body (e.g. embryonic stem cells), or adult stem cells with more limited potential. Here, we discuss the sources, applications, and limitations of adult stem cell populations for bone repair.

Conflict of Interest: All others have no conflicts.

Adult stem cells are generally capable of forming one or more embryonically-related tissue types. Stem cells are technically different from progenitor cells, yet the term “stem cell” is commonly, and often incorrectly, used to encompass both classes of cells. Stem cells are defined by their capacity to “self-renew.” This occurs by asymmetric division of stem cells that results in generation of two different cells. One daughter returns to a resting (G_0) state identical to the original stem cell, while the other cell gives rise to the rapidly proliferating, transit amplifying population of progenitor cells that generate the new tissues.

The term connective tissue progenitors (CTPs) is used to describe the heterogeneous system of stem cells and progenitor cells that are present in native tissues, which can proliferate and generate one or more connective tissues (e.g. bone, cartilage, fat, fibrous tissue, muscle, and blood).[1, 2] The prevalence and biological potential of CTPs differ from one tissue to another. No single marker or set of markers exist that identify all CTPs in native tissues. However, the concentration, prevalence and biological potential of CTPs can be estimated using established *in vitro* 2D and 3D colony-forming assays, which are generally tissue type specific.

The term mesenchymal stem cell (MSC) is found widely in the musculoskeletal literature. In contrast to tissue resident colony founding CTPs, the term MSC refers to populations of culture-expanded cells that have biological potential that may be relevant to orthopaedic applications. The International Society for Cellular Therapy has defined MSCs as culture-expanded cells that adhere to tissue culture plastic, retain the capability for tri-lineage (bone, cartilage, and fat) differentiate *in vitro*, express surface markers CD105, CD73, and CD90, and are negative for CD45, CD34, CD14 or CD11b, CD79a, or CD19, and HLA-DR.[3]

Stem Cells and Progenitor Cells: What are The Sources for Fracture Healing?

CTPs are resident in all musculoskeletal tissues, particularly bone and bone marrow.[2, 33] Work by Colnot has demonstrated that the periosteum and endosteum are rich sources of osteochondral progenitor cells during fracture healing.[4] Grafting experiments revealed that the transplanted periosteum generates both osteoblasts and chondrocytes during fracture repair, while transplanted endosteum generates primarily osteoblasts. The differentiated cells are located directly adjacent to the grafted tissue, indicating that the cells differentiate locally and do not migrate widely within the fracture callus. Moreover, bone morphogenetic protein-2 (BMP-2) stimulates chondrogenesis within the periosteum but not the endosteum, indicating that cells within the periosteum and endosteum may differ with respect to activation factors. Marrow may also contain more than one CTP population. Marrow-derived cells expressing leptin receptor,[5] gremlin,[6] and hyaluronan[36] give rise to bone that forms during repair. However, whether these cells are of the same populations is unknown.

While the origin of the CTPs that initially differentiate at the fracture site has been identified, the origin of later appearing cells is less well known. Most fractures heal through a combination of intramembranous and endochondral ossification. During endochondral ossification a cartilage template forms and is replaced by bone, and the osteoblasts that replace the cartilage are thought to be delivered to the fracture site by the invading blood

vessels.[7] However, recent work has demonstrated that hypertrophic chondrocytes in the fracture callus may persist and transdifferentiate into osteoblasts.[8] This is an under-appreciated mechanism in fracture healing, and it introduces the potential to therapeutically stimulate the conversion of chondrocytes into osteoblasts (e.g. hypertrophic non-union) once the mechanisms are understood.

Circulating CTPs, or CTPs that are mobilized into circulation following injury, may also contribute to fracture repair. However, under normal circumstances, data to date suggests that circulating cells contribute only a small number of cells in the facture callus.[9] Here again, therapeutic augmentation of this mechanism, by enhancing either mobilization or homing of circulating CTPs, represents a potential therapeutic opportunity.

CTPs and MSCs: How Can They Be Used to Influence Healing?

Due to the regenerative properties of CTPs and MSCs, there is great interest in developing therapeutic strategies to treat clinical conditions. Osteogenic cells constitute an important component of the 'diamond concept,' which illustrates the necessity for cells, growth factors, scaffolds, and the mechanical environment for bone regeneration.[10] Several studies have shown that implantation of stem cells, either alone or with other biological materials, to the fracture site of a patient with a delayed or nonunion, can stimulate healing.[11–13]

The most common source of CTPs is a bone marrow aspirate from the iliac crest. In addition to CTPs, bone marrow aspirates and reamings from long bone also contain endothelial progenitors.[14] With good technique, the concentration of CTPs will average about 1,000–2,000 CTPs per ml of aspirate.[15] Hernigou et al., processing marrow using density separation via centrifugation, has highlighted that successful treatment of atrophic diaphyseal nonunions with marrow-derived cells can be achieved as long as at least 50,000 CTPs (measured using "colony-forming units") are implanted at the site of the nonunion.[16] Other methods for CTP processing to enrich the desired cell type are also under development.[36]

Compared to the small number of CTPs in native tissues, the opportunity to use culture-expanded autogenous MSCs for bone repair is immense. MSCs may contribute to bone repair by: 1) differentiating into osteoblasts; 2) triggering the division and differentiation of native CTPs;[17] 3) modulating cells of the immune system;[18] 4) secreting trophic molecules that inhibit apoptosis and fibrosis and/or promote angiogenesis; and 5) homing to the fracture site via chemokine receptors, such as CCR1, CCR7, CCR9, and CXCR4-6[19, 20] and other pathways. However, these potential benefits require clinical evidence through relevant animal models and clinical trials, and they need to offset the expenses and risks associated with culture expansion.

Adult Progenitor Cell Populations: Which Might be the Most Useful?

Several preclinical studies have demonstrated the ability of MSCs to accelerate fracture healing,[21] or heal bone defects when combined with osteoconductive scaffolds[22] or proteins.[23] However, not all reports have demonstrated success,[24] and clinical data is

limited. Several clinical series describe the use of MSCs for therapy, but these have been level 4 studies lacking appropriate control groups.[25, 26]

While MSCs are the most frequently studied and characterized, other stem cell populations may be useful for therapeutic applications. In particular, endothelial progenitor cells (EPCs) offer unique opportunities. EPCs have been shown to contribute to bone and vasculature *in vitro*,[27] and in several preclinical studies the efficacy of EPCs in improving healing in bone defects has been demonstrated.[28–30] One recent study suggested that EPC therapy was superior to MSC therapy in a bone defect model in the rat.[31] Further, others have reported synergistic effects on angiogenesis and osteogenesis when EPCs and MSCs were combined in preclinical models,[32] suggesting that EPCs may be an important cell type for tissue engineering applications.

Developing Clinical Progenitor Cell Approaches to Bone Defects

All cell-based bone regeneration strategies are based on the paradigm that clinical success is limited by a suboptimal incidence of CTPs, or a reduction in the local environmental factors that regulate CTP survival and/or function.[33] Successful strategies must result in reliable bone formation in an acceptable time frame, while not resulting in undesired local or systemic consequences. While most engineering settings rely on highly purified and standardized reagents (e.g. titanium, polyethylene, and proteins), cell therapies do not have this luxury as the prevalence and potential of CTPs varies widely from patient to patient due to age, gender, genetic background, disease, or drug effects. Culture-expanded MSC populations also vary widely among tissues and batch, despite standardized nomenclature.[3]

There are many options for cell therapy, but these can be generally subdivided into therapies to target endogenous CTPs or to transplant CTPs.[33] Endogenous CTPs can be targeted with biological and/or physical factors, such as allograft bone matrix, bone graft substitutes, hBMP-2, and electrical stimulation.[34] Alternatively, the harvest and subsequent transplantation of the cells to sites requiring repair is being pursued for translational applications.[33] Improving CTP transplantation may include new methods to increase the purity or number of cells for transplantation, through density separation,[16] selective retention (using the tendency for CTPs to attach to bone or ceramic matrices),[35] or magnetic separation[36] based on surface markers.

Culture-Expanded Stem Cell Therapies: What Still Needs to be Overcome?

Despite significant theoretical advances, there are a number of barriers to translation before culture-expanded cells will be of clinical use in orthopaedics. Generating a reliably effective dose of cells that retain multi-lineage differentiation potential is a challenge. MSCs can be expanded *in vitro*. However, they have limited ability to proliferate beyond 20–40 doublings. Moreover, their differentiation potential is depleted during passage. Further, expanded cells may develop altered phenotypes, express aberrant markers or genes, or transition into senescence. Worse, they may develop genetic or epigenetic changes that impart undesired biological properties (e.g. transformation).

Controlled and Robust Differentiation

Lineage-specific differentiation of stem cells can be achieved by providing a chemically defined media containing growth factors. Protocols for MSC expansion and differentiation into osteocytes and chondrocytes are established and repeatable. However, these protocols differ depending on the *in vivo* niche from which the founding CTPs were isolated. Consequently, improving the regenerative capacity of MSCs requires quality control of the source of upstream CTPs, as well as the specific culture environment.

Effect of Aging on Stem Cells

The concentration and prevalence of CTPs decrease in frequency and function with age.[37] In theory, this may be partially overcome by rapid intraoperative processing to enrich for CTPs, or by *in vitro* expansion. Based on the concept that MSCs may be immunoprivileged, or hypo-immunogenic,[38, 39] many companies have tried to capitalize on the concept of using MSCs as allografts from a 'Universal MSC Donor.' To date, clinical trials (~200) examining transplantation of allogenic MSCs have consistently shown these cells to be safe;[40] however, contribution to formation of new tissue is limited.

Trophic Effect

The contribution of MSCs to new tissue formation is unclear, because long-term engraftment of transplanted MSCs has not been readily observed. Therefore, the effect of transplanted MSCs has largely focused on factors that MSCs may secrete. In concept, the secretome produced by MSCs may stimulate a regenerative response in injured tissue and modulates inflammation, possibly by suppressing an immune response and promoting an anti-inflammatory environment.[41] Even though the mechanisms are unclear, clinical trials have shown that MSCs reduce graft versus host disease.[42, 43] However, immunomodulation by MSCs has not been established in orthopaedic applications.

Injection Technique

Therapeutically, stem cells are often injected systemically or locally, but engraftment of cells delivered by this mechanism is generally low. The process itself may contribute to poor survival due to the high sheer forces experienced by the cells. Reducing sheer strain by injecting cells at a slow rate, using a larger needle, or employing a viscous vehicle may improve cell viability.[44] Thus, developing technologies to assist delivery of these cells may improve therapeutic outcomes.

Development and Testing of Cell-Scaffold Composites for Transplantation

A significant challenge to clinical application remains the optimization of scaffolds for delivery of stem cells. Scaffolds support attachment and retention of cells and provide a structure to guide cell migration and differentiation. The ideal scaffold would be resorbable and bioactive to accommodate tissue remodeling and presentation of factors that enhance CTP attachment, survival, proliferation, migration and differentiation. An extensive "toolbox" of sophisticated scaffolds has been developed, and optimizing these scaffolds demands rigorous, clinically relevant *in vivo* models to test these tissue-engineered composites.

Manufacturing for Culture-Expanded Cell Therapies

Underlying every culture-expanded cell therapy is the challenge of scaling from feasibility testing in small animals to treating humans. Cell harvest, expansion, and processing must occur in facilities that are certified under GLP (Good Laboratory Practice) and GMP (Good Manufacturing Practices). All products used in generating culture-expanded cells must be free of animal products, and synthetic or human-derived proteins/serums must be made in GLP/GMP facilities. All culture-expanded cell therapies will require highly regulated manufacturing and processing controls along with successful clinical trials, before FDA approval and broad clinical adoption can be attained.

Conclusions

Our understanding of the stem cell and progenitor cell in the biological process of fracture healing continues to grow. Many cell populations contribute to the natural process of fracture repair and bone regeneration. Harnessing the potential of these cells using advanced tissue engineering approaches holds great promise for improving current methods and developing new orthopaedic therapies.

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