Published in final edited form as:

Bioessays. 2013 March ; 35(3): 220–230. doi:10.1002/bies.201200068.

The promise and challenges of stem cell-based therapies for skeletal diseases: stem cell applications in skeletal medicine: potential, cell sources and characteristics, and challenges of clinical translation

S Diederichs, KM Shine, and RS Tuan*

Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Abstract

Despite decades of research, remaining safety concerns regarding disease transmission, heterotopic tissue formation, and tumorigenicity have kept stem cell-based therapies largely outside the standard-of-care for musculoskeletal medicine. Recent insights into trophic and immune regulatory activities of mesenchymal stem cells (MSCs), although incomplete, have stimulated a plethora of new clinical trials for indications far beyond simply supplying progenitors to replenish or re-build lost/damaged tissues. Cell banks are being established and cell-based products are in active clinical trials. Moreover, significant advances have also been made in the field of pluripotent stem cells, in particular the recent development of induced pluripotent stem cells. Their indefinite proliferation potential promises to overcome the limited supply of tissue-specific cells and adult stem cells. However, substantial hurdles related to their safety must be overcome for these cells to be clinically applicable.

Keywords

mesenchymal stem cells; orthopaedics; pluripotent stem cells; regenerative medicine; skeletal repair

Introduction

Despite decades of progress in the surgical treatment of damaged or diseased skeletal tissues, structural and functional tissue complexity, large-scale defect sizes, and post-injury degeneration continue to be major challenges to the field. The development of new tissue repair strategies is therefore urgently required. Recent advancements in our fundamental understanding of cell and tissue biology have expanded the focus of orthopedic medical research beyond traditional implant development and graft transplantation to include more novel repair and regeneration therapies¹. Indeed, the blossoming fields of tissue engineering and regenerative medicine hold great promise for overcoming the current barriers that limit long-term, successful clinical outcomes². Ideally, such strategies would allow for the in

*Corresponding author: Rocky S. Tuan; ; Email: rst13@pitt.edu

vitro or in vivo generation of biomimetic tissues that are indistinguishable in structure and function from their native counterparts. Moreover, the use of living cells will hopefully allow for active tissue remodeling, currently a major inadequacy inherent to inert implant devices and devitalized tissue grafts. While the practical formulation of cell-based tissue engineering and regenerative medicine therapies remains a difficult task, the great potential of such techniques has elicited a high level of interest in the development of cell-based strategies for skeletal tissue repair.

While the concept of using cells to restore damaged tissue seems intuitive based on their native role in tissue development and homeostasis, determining the optimal cell source(s) and method(s) by which to apply cells for this purpose is decidedly more complex. The interactive “diamond” concept of tissue engineering and regenerative medicine (Fig. 1) suggests that in addition to cell type, three-dimensional structure/architecture, mechanical/physical signals, and bioactive factors in the environment are critical and act in concert to direct tissue repair and regeneration³. While each of these areas is currently under active investigation for skeletal tissue repair, this paper will focus on the cells and the complexity of their possible therapeutic application. Cellular activity is however dynamically regulated by the other key cornerstones of the diamond.

A main challenge in cell-based skeletal therapies is optimal cell sourcing. Current options being considered include autologous and allogeneic tissue-specific cells, adult multipotent stem cells, and pluripotent stem cells (PSCs). The advantages and drawbacks of each are summarized in Fig. 2 and will be discussed in further detail in the following sections.

Tissue-specific cells are in principle the ideal cell types for cellular repair strategies and were in fact the first to translate into clinical practice (e.g. autologous chondrocyte implantation (ACI) for repair of articular cartilage lesions⁴). However, limitations in the amount of tissue that can be harvested for cell extraction, morbidity at the tissue harvest site, and finite proliferative capacity in vitro have prompted investigation of more readily available cell types. Indeed, stem and progenitor cells offer the advantages of harvest from alternative sites with potentially less tissue morbidity and degeneration as well as greater proliferation capacity and therefore higher yields. However, this is balanced by the potential difficulty both in guiding differentiation of these cells into specific skeletogenic lineages as well as preventing ectopic tissue overgrowth. Bone marrow-derived mesenchymal stem cells (BM-MSCs) are currently the best characterized stem cell population for skeletal tissue repair and closest to clinical translation. Yet, the invasiveness of tissue harvest and the known decline in marrow activity with age⁵ have prompted investigation of alternative sources of stem cells, including those derived from adipose, umbilical cord, muscle, and other tissues. However, given the vast amounts of cells anticipated to be needed for clinical cell-based repair procedures, stem cells from these sources may not be sufficient in number. Thus, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), which both possess unlimited proliferative capacity, are also under investigation as potential cell sources.

Cellular therapies already are a reality in clinical practice: Status and limits

In addition to the direct implantation of cells in suspension or as part of a tissue engineered construct, cellular therapies in the broadest sense cover all strategies involving cells as active regenerative agents. These strategies include autologous and allogeneic tissue transplantation techniques, as well as cell recruitment and homing strategies. Currently, the practice of orthopaedics utilizes a diverse array of such grafting strategies, which are summarized in Table 1. Some of these techniques are widely employed in bone repair ⁶, as the significant endogenous osteoconductive properties of bone tend to promote integration with host tissue. Similar grafting approaches are also applied to the repair of cartilage defects (e.g. osteochondral auto- and allografts; see review by Farr et al. ⁷), as well as tendon and ligament, where grafting represents the only current active regeneration option (see review by Rodrigues et al. ⁸). The main drawbacks of grafting include limitations in the amount of tissue available, donor site morbidity connected with autografts, and the risks imposed by an additional harvest surgery, as well as the danger of disease transmission and immunogenic reactions possibly induced by allografts.

In view of these limitations, alternative strategies have sought to either recruit cells from neighboring tissues or from the circulation or enhance the regenerative capabilities of local tissue stem cells. Implantation of bioactive scaffolds, currently applied primarily for bone regeneration, falls into this category. Biomaterial scaffolds as substrates for native cell migration, proliferation, differentiation, and matrix formation offer the advantage of being limitless in supply. Biomaterials such as demineralized bone matrix, hydroxyapatite and β -tricalcium phosphate, natural and synthetic polymers, treated titanium, and glass ceramics have been shown to possess osteoconductive and some osteoinductive properties ⁹. Unfortunately, without the additional osteoinductive properties offered by osteoprogenitor cells present in autografts, these materials show reduced efficacy. Selected scaffolds are therefore currently being modified to include inductive growth factors such as bone morphogenetic proteins (BMPs), including BMP-2 (InductOs®, Wyeth), and BMP-7 (Osigraft®, StrykerBiotech) to enhance bone formation ^{10, 11}. However, high growth factor concentrations, costs, and concerns over ectopic bone formation have limited their clinical utility ¹².

Another important cell recruitment strategy is the microfracture procedure, which is the current gold standard for the treatment of small cartilage lesions. For this technique, an awl is used to create small clots derived from 1 to 2 mm fractures in subchondral bone. Thus, multipotent cells are recruited, primarily from the marrow, to form new cartilage-like tissue in the defect area under appropriate conditions ¹³. The main drawbacks of this technique, however, are the high variability of clinical outcomes and the formation of fibrocartilaginous tissue with inferior mechanical properties to hyaline cartilage, thus leading to long-term complications ¹⁴.

The only actively applied cell implantation strategy in clinical musculoskeletal medicine is ACI as treatment for articular cartilage defects. In this two-stage procedure, tissue is harvested from non-load bearing surfaces and enzymatically digested to obtain chondrocytes ¹⁵. After culture expansion in vitro, usually requiring >6 weeks, the cells are injected beneath a periosteal or collagen flap covering the defect site. Other modifications include

seeding the patch with chondrocytes or allowing cells to grow on a scaffold [e.g. matrix-assisted autologous chondrocyte implantation (MACI®), Genzyme Biosurgery]. Similar cellular strategies utilizing tissue-specific cells after ex vivo expansion are currently in the clinical trial phase for tendon and intervertebral disc regeneration (i.e. autologous tenocyte implantation and autologous disc cell transplantation, respectively). A major drawback of this technique is the harvesting operation, which creates a new defect in a tissue with limited intrinsic regeneration capacity. Moreover, chondrocyte de-differentiation in culture ¹⁶ limits already low cell availability even more.

The limitations of current surgical repair technologies for skeletal tissues thus underscore the timely need for the development of regenerative approaches, ideally consisting of the combined use of cells, preferably autologous in origin, biomimetic scaffolds, and tissue inductive agents.

Sophisticated tissue engineering techniques currently under preclinical investigation will have important impact on future orthopaedic practice

Skeletal engineering techniques that are currently in clinical use or in clinical trials (summarized in Table 1) mainly involve direct combinations of cells and scaffolds. More recent technological developments in the field of biomaterials (reviewed in ¹⁷) encompass nanofabrication and surface modifications that bring biomaterials one step closer to actually mimic native extracellular matrix. 3D printing techniques are being applied to create constructs with a specific shape and internal structure and composition. Moreover, sophisticated or “smart” scaffolds have been developed to contain chemically linked drugs, biomolecules, and peptide sequences recognized by integrins and other cell adhesion molecules. These structures are capable of temporally and spatially controlled delivery of drugs and growth factors to facilitate cell attachment. These developments are especially promising to improving cell recruitment strategies. Indeed, recruitment of cells from the surrounding tissues or the blood system might be easier to translate to the clinic than actual cell-implantation strategies. A recent example is the application of transforming growth factor-beta (TGF- β)-containing hydrogels for cartilage repair. In a rabbit study TGF- β has been suggested to act beyond its well established strong chondrogenic induction as a chemo-attractant for progenitor cells ¹⁸. Increasing knowledge is currently being gained about growth factors and chemokines that enhance stem cell migration, among which stromal cell-derived factor 1 alpha (SDF-1 α) [chemokine (C-X-C motif) ligand 12 (CXCL12)], ligand of the chemokine (C-X-C motif) receptor 4 (CXCR4) surface receptor, is currently regarded to be the most promising candidate as homing factor for regenerative medicine ¹⁹. For example, SDF-1 α has been shown to improve BMP-2 induced bone formation in a mouse model ²⁰.

There is also extensive progress in direct cell-implantation strategies that do not rely on recruiting a sufficient amount of cells but rather aim to fill the defect with actively regenerating tissue. Taken together, the advances in biomaterial sciences and stem cell biology should enable the engineering of tissues of higher complexity, such as osteochondral constructs and intervertebral discs. Although not dealing with skeletal tissue, probably the most groundbreaking success in this field has been achieved by Atala et al. who engineered

autologous bladder constructs utilizing cells obtained autologously from a bladder biopsy seeded on collagen-based scaffolds after in vitro expansion²¹. Apart from material science, the major factor under permanent debate is the choice of the optimal cell source. We will therefore discuss possible cell sources in the following sections in detail. Because of their extensive proliferative potential, stem cells are currently thought to be the answer to the supply shortness of differentiated tissue cells.

What is impeding mesenchymal stem cell (MSC) translation into clinical practice?

Multipotent cells derived from adult tissues (termed MSCs by Caplan²²) were identified in bone marrow in the 1960s²³. Since their discovery, MSCs have increasingly been recognized for their potential to contribute to tissue regeneration not only by differentiating into skeletogenic tissues²⁴ but also by regulatory activities. MSCs inhibit scarring and apoptosis, as well as stimulate angiogenesis and proliferation of tissue-intrinsic stem or progenitor cells by autocrine and paracrine mechanisms². Indeed, they are known to secrete a wide variety of growth factors and cytokines that enable this so-called “trophic activity”²⁵. Moreover, MSCs are thought to be immunoprivileged and exhibit immune modulatory and suppressive properties²⁶.

Why then are MSCs, after almost half a century of intense studies, not currently a standard-of-care in musculoskeletal medicine? The complete answer to this question may be complex, but the basic reason can be reduced to the surprisingly inconclusive state of knowledge on the identity of the “MSC”. In addition, there remain uncertainties regarding the type and gravity of the risks connected with MSC therapies.

Despite almost five decades of investigations, our understanding of how MSCs contribute to tissue regeneration as well as their immune properties and homing potential remains incomplete. Moreover, the true origin and identity of the “MSC” is still being debated, and MSC-related research continues to be hampered by the variable characteristics of non-identical cell populations isolated using diverse protocols in different laboratories. While recent evidence associating MSCs with the perivascular niche²⁷ has shed some light on MSC identity, the debate remains open.

Furthermore, the highly promising results obtained from studies of MSCs in vitro and in animal models in vivo have yet to be matched by similar groundbreaking achievements in human patients. In fact, human MSCs seem to have inferior tissue formation capacity when compared to those from other species²⁸, and their abundance and functional capacity may decline even more with age and in patients with skeletal or metabolic diseases²⁹. Interestingly, a set of gene-based biomarkers has been proposed to predict the differentiation potential of adult stem cell preparations^{30, 31}. Their use to enhance the outcome of MSC osteogenesis and chondrogenesis is currently being explored. Given the biological variability of MSC activity, in vitro assays that can reliably correlate with and predict outcomes of tissue formation in vivo are urgently needed to ensure optimal graft performance.

Safety concerns remain despite years of clinical MSC applications

The main safety concerns connected with MSC therapies are tumorigenicity and heterotopic tissue formation. Indeed, malignant transformation has not been completely ruled out.

Spontaneous formation of tumor-like cells has been reported in human MSC long-term cultures³², while rodent MSCs have been reported to possibly transform as early as passage 3³³. Jeong et al. recently reported tumor formation in mice after transplantation of short-term cultured MSCs into the heart³⁴. Although to date no case of tumor formation has been published in human patients, clinical long-term data are needed to prove the safety of MSC therapy.

The concern about heterotopic tissue formation is most impressively underscored by the discovery of calcifications in mouse heart infarctions treated with bone marrow MSCs³⁵. It has generally been accepted that MSCs should be pre-differentiated in vitro and screened for the absence of any undifferentiated cells before implantation to minimize spontaneous differentiation into unrelated tissues. Interestingly, TGF- β mediated MSC chondrogenesis in vitro, instead of forming stable hyaline cartilage, results in a program characterized by cellular hypertrophy in the early phase³⁶ and eventually leading to in vivo calcification (reviewed by Hellingman et al.³⁷). These facts imply that osteogenesis, or at least matrix calcification, might constitute a default differentiation pathway of bone marrow-derived MSCs. That pathway has been suggested to be connected to their bone-related origin, but has also been observed for MSCs from adipose tissue³⁸ and even ESCs³⁹. In line with this, in vitro osteogenic pre-differentiation, although utilized frequently in many studies, has not been demonstrated to significantly improve bone formation when compared to undifferentiated cells.

Whole bone marrow preparations and allogeneic transplantation are attractive alternatives to culture-expanded autologous MSCs

Besides the aforementioned uncertainties, cellular therapies employing autologous cells also have to meet the accompanying logistical (and costly) challenges to manage a primary harvesting operation followed by an ex vivo phase, during which the cells are expanded and possibly combined with a structuring scaffold material. Throughout this phase, all cell manipulations will generally have to comply with requirements of good manufacturing practice (GMP) similar to those for pharmaceutical production. These include strict conditions of sterility, specific reagents devoid of heterologous proteins, and quality controls such as microbiological, immunological, and functional tests⁴⁰. Consequently, researchers have attempted to avoid the cumbersome ex vivo expansion step via the administration of whole bone marrow preparations to repair large bone defects⁴¹. The value of this approach lies in its experimental simplicity and safety and that it meets the requirement of the US Food and Drug Agency (FDA) for minimally manipulated cells. However, since high abundance of active multipotent stem cells within the marrow preparation is critical for successful clinical outcome⁴², the efficacy of the procedure may be compromised. Several techniques exist to enrich bone marrow preparations for the mononuclear cell fraction intra-operatively, including centrifugation⁴³, selective cell attachment to implantable matrices⁴⁴, or the “buffy coat” technique⁴⁵. However, despite some published successes⁴², acquisition of sufficient bone marrow to obtain the required number of progenitor cells remains a major challenge.

A less logistically demanding approach is the application of allogeneic cells that are available on demand from tissue or cell banks. In fact, GMP-compliant cell banks already exist (e.g. cord blood banks in connection with hematopoietic stem cell transplantation as curative therapy for many malignant and non-malignant conditions affecting children and adults ⁴⁶, and others ⁴⁷). However, allogeneic transplantation generally requires donor matching and significant pharmacologic intervention for immunosuppression. Interestingly, MSCs have been shown to be immunoprivileged, likely because of the lack of histocompatibility antigen (HLA) class II and the co-stimulating molecules, CD40, CD80, and CD86 ²⁶, such that even upon activation by HLA class I antigens, the T-cells will remain anergic. Successes of treatment of graft-versus-host diseases (GvHD) with MSCs to ameliorate the immune reaction induced by allogeneic hematopoietic stem cell transplantation have shown great promise in this regard ⁴⁸. On the other hand, it is still under debate whether MSCs might change their immunological state upon differentiation, and controversial results have been published ^{49–51}. For allogeneic MSC transplantation it must be ruled out that the remaining transplanted cells evoke an immune reaction upon differentiation. However, MSC immunoprivileges have also been challenged several years ago by mouse studies where an MSC-evoked memory T-cell response was observed to lead to immune rejection in vivo ⁵². This suggests that MSCs might not be as immunoprivileged as previously assumed. Despite this ongoing controversy, allogeneic MSC transplantation procedures are considered a clinically viable option and are currently investigated in clinical trials (e.g. Chondrogen™, Osiris Therapeutics; Trinity™, Blackstone Medicals).

The clinical translation of MSCs has thus been challenging, as a result of the lack of definitive evidence that these cells are the actual effectors of tissue regeneration and the difficulty to completely foretell their in vivo behavior, homing potential and disposition to neoplastic transformations. Nevertheless, the last 10 years have also witnessed the initiation of a large number of clinical trials to test the potential of MSCs for skeletal tissue regeneration, following the first case studies employing human culture expanded MSCs for bone ⁵³ and cartilage ⁵⁴ defects (reviewed recently by Gómez-Barrena et al. ⁵⁵, and Shenag et al. ⁵⁶ among others). Currently, over 230 clinical trials employing MSC transplantation are listed on the official website of the US National Institutes of Health (NIH; www.clinicaltrials.org). Interestingly, less than half of these studies are directly related to skeletal diseases, including indications for filling of bone void defects, osteonecrosis, distraction osteogenesis in limb-length discrepancy, osteogenesis imperfecta, spinal fusion, osteoarthritis and rheumatoid arthritis, cartilage lesions, and intervertebral disc degeneration. The majority of studies are in fact inspired by the trophic activities of MSCs and particularly their immunomodulatory activity, for the treatment of multiple sclerosis, amyotrophic lateral sclerosis, GvHD, Crohn's disease, spinal cord injury, Parkinson's disease, diabetes mellitus, acute myocardial infarction, stroke, asthma and chronic obstructive pulmonary disease, acute kidney failure, liver fibrosis, radiation syndrome, burns and wound healing, inflammatory bowel disease, and sepsis. Indeed, the discovery of the trophic activities of MSCs has greatly broadened their spectrum of possible indications, including now various severe and life-threatening diseases. Conversely, it is anticipated that the application of MSCs for such severe disorders will further accelerate clinical translation of MSC-based therapies. In fact, one very encouraging result after the first 10 years of individual case studies and clinical

trials employing MSCs is that so far none of the respective published reports has mentioned adverse effects such as inflammation or excessive tissue growth.

Alternative MSC sources can overcome shortcomings of bone marrow MSCs

Bone marrow-derived MSCs are currently the best characterized stem cell population for skeletal tissue repair and might be considered the gold standard for adult MSCs. However, cells with similar properties have been discovered in almost all tissues. The invasiveness of bone marrow harvest and the known decline in marrow activity with age⁵ have prompted increased interest in alternative sources of multipotent stem cells. Among these, adipose tissue⁵⁷ and umbilical cord⁵⁸ are the most prominent. Isolation of MSCs from these tissues avoids the invasive procedure of bone marrow aspiration in favor of a far less invasive liposuction or the use of surgical “waste” materials, such as fat tissue resulting from corrective surgeries and umbilical cords. Additional advantages of these sources are the generally higher cell yields from adipose tissue on the one hand and the developmentally younger origin of stem cells derived from umbilical cord blood and tissue. In particular, the latter promise both a broader differentiation spectrum and reduced genetic aberrations naturally accrued during the human lifespan. While these multipotent stem cell populations are largely similar, they also possess distinct tissue-specific characteristics. For example, the surface marker profile of distinct MSC populations is under continuous debate⁵⁹. Moreover, although multilineage differentiation has been proven, the potential varies between MSC populations from different origins⁶⁰. An important example is the reduced chondrogenic potential of adipose tissue derived MSC that has been attributed to an altered TGF- β receptor and BMP profile and can be overcome with BMP-6³⁸. Characterization of multipotent stem cells from both umbilical cord and adipose tissue is however less developed compared to bone marrow MSCs. Nevertheless, it is reasonable to expect that much of the experience gained from BM-MSC studies can be readily transferred to other related adult stem cell populations to accelerate their progression toward clinical translation.

Pluripotent stem cells – a potentially unlimited cell source complicated by yet-to-overcome challenges

Given the large quantities of cells frequently required for clinical cell-based therapies, stem cells from adult tissue sources with limited in vitro proliferative capacity may be insufficient in number. Thus, ESCs and iPSCs, which both possess unlimited proliferative capacity, have also been intensely investigated for regenerative medicine purposes. These PSCs are unique in their ability to differentiate into virtually any cell type derived from the three embryonic lineages (i.e. ectoderm, mesoderm, and endoderm), including those that are not achievable using MSCs, such as insulin-producing pancreatic beta cells⁶¹, hepatocytes⁶², and neurons⁶³. However, the developmental immaturity of PSCs also renders their guided differentiation in vitro into specific tissue forming cells – a process that requires the coordinated development of a number of well-defined intermediate cell types – particularly challenging⁶⁴.

The true attractiveness of iPSCs for regenerative medicine lies in their somatic cell origin, which allows for autologous cell derivation, thus avoiding the legal and ethical constraints associated with ESCs. However, safety remains a fundamental hurdle to overcome before

clinical application, as pluripotency-inducing genetic modifications are required in the cell reprogramming process. In addition, incomplete reprogramming as well as genetic and epigenetic aberrations potentially accrued during iPSC derivation and long-term culture^{65, 66} may possibly evoke immune response, even with the use of autologous cells. In fact, Zhao et al. recently observed rejection of syngeneic iPSCs, but not ESCs, in a mouse model⁶⁷, related to the abnormal overexpression of a group of minor antigens in iPSC-derived teratomas. However, after many reports of genetic aberrations in iPSCs^{65, 66}, a recent study that applied whole-genome sequencing to investigate the scope of such changes for the entire genome⁶⁸ concluded that episome-mediated reprogramming might not inherently be mutagenic during integration-free iPSC induction.

The process of reprogramming cells all the way to pluripotency to “regain” differentiation and proliferative capacities and validation of their genetic and epigenetic normality is enormously costly and labor-intensive. In response, researchers have sought to develop a method to directly convert one somatic cell type into another by combining traditional reprogramming factors and tissue specific master regulation factors⁶⁹. By avoiding the pluripotent state, the risk of teratoma formation in vivo may be eliminated altogether. Moreover, months of in vitro and in vivo pluripotency assays may be avoided [e.g. embryoid bodies formed in suspension culture and teratomas formed in severe combined immunodeficiency (SCID) mice containing cells of all three germ layers]. However, the trade-off for these advantages is that the potentially unlimited cell supply is lost, and that the choice of a highly abundant but preferably autologous cell source becomes an issue yet again.

Despite the challenges described above and the relatively new entry of PSCs, many pre-clinical implantation studies into animal models are already being performed. ESCs have taken the lead in these studies headed by the first publication on human ESCs in 1998⁷⁰, while the first reprogramming to pluripotency by defined factors was published in 2006⁷¹. Exciting results with regard to new treatment possibilities of neurodegenerative diseases (e.g. Parkinson’s disease⁷² and stroke damage⁷³), cardiovascular diseases (e.g. myocardial infarction⁷⁴) and diabetes⁶¹, have gained much publicity. PSC-based treatments are also under investigation for skeletal tissue defects, especially in the bone area (reviewed in detail by Gamie et al.⁷⁵). In vitro directed differentiation of PSCs into specific cell lines for tissue regeneration has however proven rather challenging. Indeed, the first convincing in vivo bone formation by PSCs was not published until 2008, when Jukes et al. successfully described endochondral bone formation by murine ESCs³⁹. This report was followed shortly thereafter by the first human ESC treatment of a craniofacial defect in SCID mice⁷⁶ and bone non-unions in rats⁷⁷. Although the results of these studies were promising, the osteogenic capacity of the cells was not sufficient to completely fill the defects.

The first proof-of-concept study with human iPSCs was reported recently by Deyle et al. who isolated and modified MSCs from discarded bone fragments of osteogenesis imperfecta patients undergoing corrective surgery⁷⁸. The mutant collagen gene was inactivated by virus-mediated gene targeting in vitro and the cells were reprogrammed into the pluripotent state, thus overcoming limitations in cell supply posed by gene targeting of MSCs alone. Thus, the group established a theoretically unlimited cell source from which mesenchymal

cells and osteoprogenitors were obtained. These mesenchymal cells were then shown to produce normal collagen and form bone in vivo in an ectopic mouse model.

In comparison to bone, studies on other skeletal tissues are lagging behind. Although in vivo cartilage formation by human iPSCs⁷⁹ and direct conversion of fibroblasts into chondrocytes⁸⁰ have been demonstrated, the observed results have been either modest or hampered by tumor formation.

Another aspect of the study by Deyle et al.⁷⁸ is the possibility to generate mesenchymal cells from PSCs, which could overcome limited MSC supply. Indeed, mesenchymal cells derived from both ESCs and iPSCs have been shown to express known MSC surface markers and exhibit tri-lineage differentiation potential⁸¹. Microarray data suggest that these mesenchymal cells might be developmentally younger than bone marrow MSCs⁸² and possess a higher proliferative potential. While using reprogramming of somatic cells as a means to derive abundant autologous mesenchymal cells will probably prove to be overly laborious and impractical, a recent report on immune privileges of such cells⁸³ is promising with regard to allogeneic applications. However, the published derivation methods for these cells are highly variable and result in diverse offspring. More in-depth studies are clearly needed to understand these cells within their developmental context.

Interestingly, two ESC studies have already made it into FDA-approved clinical trials. In 2010, Geron started a Phase I clinical trial utilizing human ESC-derived oligodendrocyte progenitors in patients with spinal cord injuries⁸⁴. The study faced regulatory confusion resulting in repeated interruptions, one of which was the finding of microscopic cysts in transplanted mice. Although later proved not to be harmful or teratoma, these findings resulted in investigations evaluating the cyst-forming potential of ESC preparations⁸⁵. Despite the fact that the treatment seemed to be well tolerated without substantial adverse effects, Geron discontinued the study in 2011 based on business-driven decisions⁸⁶. Regardless, the value of this first ESC-based clinical study was the discovery of practical challenges for ESC applications that must be addressed in the future. The second and only current ESC-related clinical trial involves using hESC-derived retinal pigment epithelial cells for the treatment of Stargardt disease and age-related macular degeneration, a Phase I/II clinical trial initiated by Advanced Cell Technology. Schwartz et al. recently published the preliminary results at four months of follow-up⁸⁷, reporting that the first two patients experienced no harmful side effects and appeared to have slightly improved vision.

Overall, the PSC field is at a considerably more primitive stage than the adult stem cell field, which has a 40-year lead. The future employment of PSCs and directly trans-differentiated cells for clinical applications will depend on the feasibility of producing large quantities of well-defined and functional cells. Thus, improving on or overcoming current ineffective reprogramming with precise control of lineage-specific differentiation to eliminate the risk of teratoma formation, and ensuring cell engrafting and optimal graft performance without heterotopic tissue formation will be necessary. While this goal has yet to be achieved, PSCs offer additional value as model systems for diseases and drug/toxicology screenings. Here, the availability of human PSCs, their unlimited proliferative potential, and multi-lineage

developmental capacity provide innovation and versatility unmatched by existing immortalized human cell lines and animal models.

Conclusions

Although tissue-specific autologous cells represent the current source for cell-based regenerative therapies, their low abundance and in vitro senescence, and the risk of donor site morbidity represent realistic limitations to their applicability. Isolating stem cells with high proliferative capacity from more readily regenerating tissues may help to overcome such limitations. At present, BM-MSCs represent the best-characterized stem cell population for musculoskeletal tissue regeneration, but several yet-to-be-resolved challenges have complicated their clinical translation. Most prominently, the in vivo biological identity of the MSC, and techniques to ensure optimal MSC performance remain elusive. Recent insights into trophic and immune regulatory activities of MSCs, although incomplete, have stimulated a plethora of new clinical trials for indications far beyond simply supplying progenitors to replenish or re-build lost/damaged tissues. Moreover, new MSC banks are currently being established and a number of MSC-based commercial products are in active clinical trials. These activities should rationally guide the development of MSCs toward standard-of-care clinical applications. Knowledge to be gained on the fundamental biology of stem cells may also contribute to the design of robust assays capable of predicting the behavior of implanted MSCs in vivo.

The choice of autologous versus allogeneic cell sourcing for skeletal tissue regeneration ultimately depends on a number of factors, particularly related to their immunogenicity. Provided issues such as immune response can be managed satisfactorily, allogeneic cells should remain a viable source. Finally, given the multi-lineage capability of MSCs to give rise to all skeletal cell types, the more comprehensive differentiation capacity of PSCs may not offer any immediate advantage. However, the unlimited proliferation potential of PSCs makes them an attractive candidate cell type for future skeletal regenerative technologies. Thus, the need to continue to investigate both MSCs and PSCs is self-evident.

Acknowledgments

Supported in part by Deutsche Forschungsgemeinschaft (DI-1684/1-1; SD) and the Commonwealth of Pennsylvania Department of Health (RST).

Abbreviations

ACI	autologous chondrocyte implantation
BM-MSC	bone marrow-derived mesenchymal stem cell
BMP	bone morphogenetic protein
CXCL12	chemokine (C-X-C motif) ligand 12
CXCR4	chemokine (C-X-C motif) receptor 4
ESC	embryonic stem cell

FDA	(US) Food and Drug Agency
GMP	good manufacturing practice
GvHD	graft-versus-host disease
HLA	histocompatibility antigen
iPSC	induced pluripotent stem cell
MACI	matrix-assisted autologous chondrocyte implantation
MSC	mesenchymal stem cell
PSC	pluripotent stem cell
SCID	severe combined immunodeficiency
SDF-1α	stromal cell-derived factor 1 alpha
TGF-β	transforming growth factor-beta

References

1. Noth U, Steinert AF, Tuan RS. Technology insight: adult mesenchymal stem cells for osteoarthritis therapy. *Nat Clin Pract Rheumatol*. 2008; 4:371–80. [PubMed: 18477997]
2. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol*. 2007; 213:341–7. [PubMed: 17620285]
3. Giannoudis PV, Einhorn TA, Marsh D. Fracture healing: the diamond concept. *Injury*. 2007; 38:S3–6.
4. Peterson L, Minas T, Brittberg M, Nilsson A, et al. Two-to 9-year outcome after autologous chondrocyte transplantation of the knee. *Clin Orthop Relat Res*. 2000; 374:212–34. [PubMed: 10818982]
5. Stolzing A, Jones E, McGonagle D, Scutt A. Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies. *Mech Ageing Dev*. 2008; 129:163–73. [PubMed: 18241911]
6. Bueno EM, Glowacki J. Cell-free and cell-based approaches for bone regeneration. *Nat Rev Rheumatol*. 2009; 5:685–97. [PubMed: 19901916]
7. Farr J, Cole B, Dhawan A, Kercher J, et al. Clinical cartilage restoration: evolution and overview. *Clin Orthop Relat Res*. 2011; 469:2696–705. [PubMed: 21240578]
8. Rodrigues MT, Reis RL, Gomes ME. Engineering tendon and ligament tissues: present developments towards successful clinical products. *J Tissue Eng Regen Med*. 2012; in press. doi: 10.1002/term.1459
9. Tanner KE. Bioactive composites for bone tissue engineering. *Proc Inst Mech Eng H*. 2010; 224:1359–72. [PubMed: 21287825]
10. Friedlaender GE, Perry CR, Cole JD, Cook SD, et al. Osteogenic protein-1 (bone morphogenetic protein-7) in the treatment of tibial nonunions. *J Bone Joint Surg Am*. 2001; 83-A:S151–8. [PubMed: 11314793]
11. Starman JS, Bosse MJ, Cates CA, Norton HJ. Recombinant human bone morphogenetic protein-2 use in the off-label treatment of nonunions and acute fractures: A retrospective review. *J Trauma Acute Care Surg*. 2012; 72:676–81. [PubMed: 22491552]
12. Okubo Y, Bessho K, Fujimura K, Konishi Y, et al. Osteoinduction by recombinant human bone morphogenetic protein-2 at intramuscular, intermuscular, subcutaneous and intrafatty sites. *Int J Oral Maxillofac Surg*. 2000; 29:62–6. [PubMed: 10691147]

13. Steadman JR, Rodkey WG, Briggs KK. Microfracture to treat full-thickness chondral defects: surgical technique, rehabilitation, and outcomes. *J Knee Surg.* 2002; 15:170–6. [PubMed: 12152979]
14. Mithoefer K, McAdams T, Williams RJ, Kreuz PC, et al. Clinical efficacy of the microfracture technique for articular cartilage repair in the knee: an evidence-based systematic analysis. *Am J Sports Med.* 2009; 37:2053–63. [PubMed: 19251676]
15. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, et al. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med.* 1994; 331:889–95. [PubMed: 8078550]
16. Stokes DG, Liu G, Dharmavaram R, Hawkins D, et al. Regulation of type-II collagen gene expression during human chondrocyte de-differentiation and recovery of chondrocyte-specific phenotype in culture involves Sry-type high-mobility-group box (SOX) transcription factors. *Biochem J.* 2001; 360:461–70. [PubMed: 11716775]
17. Christenson EM, Anseth KS, van den Beucken JJ, Chan CK, et al. Nanobiomaterial applications in orthopedics. *J Orthop Res.* 2007; 25:11–22. [PubMed: 17048259]
18. Lee CH, Cook JL, Mendelson A, Moiola EK, et al. Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study. *Lancet.* 2010; 376:440–8. [PubMed: 20692530]
19. Lau TT, Wang DA. Stromal cell-derived factor-1 (SDF-1): homing factor for engineered regenerative medicine. *Expert Opin Biol Ther.* 2011; 11:189–97. [PubMed: 21219236]
20. Higashino K, Vigneswarapu M, Bargouti M, Liu H, et al. Stromal cell-derived factor-1 potentiates bone morphogenetic protein-2 induced bone formation. *Tissue Eng Part A.* 2011; 17:523–30. [PubMed: 21043834]
21. Atala A, Bauer SB, Soker S, Yoo JJ, et al. Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet.* 2006; 367:1241–6. [PubMed: 16631879]
22. Caplan AI. Mesenchymal stem cells. *J Orthop Res.* 1991; 9:641–50. [PubMed: 1870029]
23. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. *Transplantation.* 1968; 6:230–47. [PubMed: 5654088]
24. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, et al. Multilineage potential of adult human mesenchymal stem cells. *Science.* 1999; 284:143–7. [PubMed: 10102814]
25. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem.* 2006; 98:1076–84. [PubMed: 16619257]
26. Nauta AJ, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. *Blood.* 2007; 110:3499–506. [PubMed: 17664353]
27. da Silva Meirelles L, Caplan AI, Nardi NB. In search of the in vivo identity of mesenchymal stem cells. *Stem Cells.* 2008; 26:2287–99. [PubMed: 18566331]
28. Chatterjea A, Meijer G, van Blitterswijk C, de Boer J. Clinical application of human mesenchymal stromal cells for bone tissue engineering. *Stem Cells Int.* 2010; 2010:215625. [PubMed: 21113294]
29. Murphy JM, Dixon K, Beck S, Fabian D, et al. Reduced chondrogenic and adipogenic activity of mesenchymal stem cells from patients with advanced osteoarthritis. *Arthritis Rheum.* 2002; 46:704–13. [PubMed: 11920406]
30. De Bari C, Dell'Accio F, Karystinou A, Guillot PV, et al. A biomarker-based mathematical model to predict bone-forming potency of human synovial and periosteal mesenchymal stem cells. *Arthritis Rheum.* 2008; 58:240–50. [PubMed: 18163504]
31. Dell'Accio F, De Bari C, Luyten FP. Molecular markers predictive of the capacity of expanded human articular chondrocytes to form stable cartilage in vivo. *Arthritis Rheum.* 2001; 44:1608–19. [PubMed: 11465712]
32. Rubio D, Garcia-Castro J, Martin MC, de la Fuente R, et al. Spontaneous human adult stem cell transformation. *Cancer Res.* 2005; 65:3035–9. [PubMed: 15833829]
33. Furlani D, Li W, Pittermann E, Klopsch C, et al. A transformed cell population derived from cultured mesenchymal stem cells has no functional effect after transplantation into the injured heart. *Cell Transplant.* 2009; 18:319–31. [PubMed: 19558780]

34. Jeong JO, Han JW, Kim JM, Cho HJ, et al. Malignant tumor formation after transplantation of short-term cultured bone marrow mesenchymal stem cells in experimental myocardial infarction and diabetic neuropathy. *Circ Res.* 2011; 108:1340–7. [PubMed: 21493893]
35. Breitbach M, Bostani T, Roell W, Xia Y, et al. Potential risks of bone marrow cell transplantation into infarcted hearts. *Blood.* 2007; 110:1362–9. [PubMed: 17483296]
36. Pelttari K, Winter A, Steck E, Goetzke K, et al. Premature induction of hypertrophy during in vitro chondrogenesis of human mesenchymal stem cells correlates with calcification and vascular invasion after ectopic transplantation in SCID mice. *Arthritis Rheum.* 2006; 54:3254–66. [PubMed: 17009260]
37. Hellingman CA, Koevoet W, van Osch GJ. Can one generate stable hyaline cartilage from adult mesenchymal stem cells? A developmental approach. *J Tissue Eng Regen Med.* 2011; in press. doi: 10.1002/term.502
38. Hennig T, Lorenz H, Thiel A, Goetzke K, et al. Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGFbeta receptor and BMP profile and is overcome by BMP-6. *J Cell Physiol.* 2007; 211:682–91. [PubMed: 17238135]
39. Jukes JM, Both SK, Leusink A, Sterk LM, et al. Endochondral bone tissue engineering using embryonic stem cells. *Proc Natl Acad Sci USA.* 2008; 105:6840–5. [PubMed: 18467492]
40. Sensebe L, Bourin P, Tarte K. Good manufacturing practices production of mesenchymal stem/stromal cells. *Hum Gene Ther.* 2011; 22:19–26. [PubMed: 21028982]
41. Connolly JF, Guse R, Tiedeman J, Dehne R. Autologous marrow injection as a substitute for operative grafting of tibial nonunions. *Clin Orthop Relat Res.* 1991:259–70. [PubMed: 2019059]
42. Hernigou P, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. *J Bone Joint Surg Am.* 2005; 87:1430–7. [PubMed: 15995108]
43. Kasten P, Beyen I, Egermann M, Suda AJ, et al. Instant stem cell therapy: characterization and concentration of human mesenchymal stem cells in vitro. *Eur Cell Mater.* 2008; 16:47–55. [PubMed: 18946860]
44. Muschler GF, Nitto H, Matsukura Y, Boehm C, et al. Spine fusion using cell matrix composites enriched in bone marrow-derived cells. *Clin Orthop Relat Res.* 2003; 407:102–18. [PubMed: 12567137]
45. Sakai S, Mishima H, Ishii T, Akaogi H, et al. Concentration of bone marrow aspirate for osteogenic repair using simple centrifugal methods. *Acta Orthop.* 2008; 79:445–8. [PubMed: 18626810]
46. Samuel GN, Kerridge IH, O'Brien TA. Umbilical cord blood banking: public good or private benefit? *Med J Aust.* 2008; 188:533–5. [PubMed: 18459926]
47. Tooth cell service launched. *Br Dent J.* 2011; 211:452. [PubMed: 22116223]
48. Ringden O, Uzunel M, Rasmusson I, Remberger M, et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation.* 2006; 81:1390–7. [PubMed: 16732175]
49. Niemeyer P, Kornacker M, Mehlhorn A, Seckinger A, et al. Comparison of immunological properties of bone marrow stromal cells and adipose tissue-derived stem cells before and after osteogenic differentiation in vitro. *Tissue Eng.* 2007; 13:111–21. [PubMed: 17518585]
50. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, et al. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol.* 2003; 31:890–6. [PubMed: 14550804]
51. Huang XP, Sun Z, Miyagi Y, McDonald Kinkaid H, et al. Differentiation of allogeneic mesenchymal stem cells induces immunogenicity and limits their long-term benefits for myocardial repair. *Circulation.* 2010; 122:2419–29. [PubMed: 21098445]
52. Nauta AJ, Westerhuis G, Kruisselbrink AB, Lurvink EG, et al. Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting. *Blood.* 2006; 108:2114–20. [PubMed: 16690970]
53. Quarto R, Mastrogiacomo M, Cancedda R, Kutepov SM, et al. Repair of large bone defects with the use of autologous bone marrow stromal cells. *N Engl J Med.* 2001; 344:385–6. [PubMed: 11195802]

54. Wakitani S, Imoto K, Yamamoto T, Saito M, et al. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage*. 2002; 10:199–206. [PubMed: 11869080]
55. Gómez-Barrena E, Rosset P, Muller I, Giordano R, et al. Bone regeneration: stem cell therapies and clinical studies in orthopaedics and traumatology. *J Cell Mol Med*. 2011; 15:1266–86. [PubMed: 21251219]
56. Shenaq DS, Rastegar F, Petkovic D, Zhang BQ, et al. Mesenchymal progenitor cells and their orthopedic applications: forging a path towards clinical trials. *Stem Cells Int*. 2010; 2010:519028. [PubMed: 21234334]
57. Zuk PA, Zhu M, Mizuno H, Huang J, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*. 2001; 7:211–8. [PubMed: 11304456]
58. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol*. 2000; 109:235–42. [PubMed: 10848804]
59. Szpalski C, Barbaro M, Sagebin F, Warren SM. Bone tissue engineering: current strategies and techniques-part II: cell types. *Tissue Eng Part B, Rev*. 2012; 18:258–69. [PubMed: 22224439]
60. Al-Nbaheen M, Vishnubalaji R, Ali D, Bouslimi A, et al. Human stromal (Mesenchymal) stem cells from bone marrow, adipose tissue and skin exhibit differences in molecular phenotype and differentiation potential. *Stem Cell Rev*. 2012; in press. doi: 10.1007/s12015-012-9365-8
61. Jeon K, Lim H, Kim JH, Thuan NV, et al. Differentiation and transplantation of functional pancreatic beta cells generated from induced pluripotent stem cells derived from a type 1 diabetes mouse model. *Stem Cells Dev*. 2012; in press. doi: 10.1089/scd.2011.0665
62. Choi D, Oh HJ, Chang UJ, Koo SK, et al. In vivo differentiation of mouse embryonic stem cells into hepatocytes. *Cell Transplant*. 2002; 11:359–68. [PubMed: 12162376]
63. Buhnenmann C, Scholz A, Bernreuther C, Malik CY, et al. Neuronal differentiation of transplanted embryonic stem cell-derived precursors in stroke lesions of adult rats. *Brain*. 2006; 129:3238–48. [PubMed: 17018551]
64. Wu SM, Hochedlinger K. Harnessing the potential of induced pluripotent stem cells for regenerative medicine. *Nat Cell Biol*. 2011; 13:497–505. [PubMed: 21540845]
65. Lister R, Pelizzola M, Kida YS, Hawkins RD, et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature*. 2011; 471:68–73. [PubMed: 21289626]
66. Gore A, Li Z, Fung H-L, Young JE, et al. Somatic coding mutations in human induced pluripotent stem cells. *Nature*. 2011; 471:63–7. [PubMed: 21368825]
67. Zhao T, Zhang ZN, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. *Nature*. 2011; 474:212–5. [PubMed: 21572395]
68. Cheng L, Hansen NF, Zhao L, Du Y, et al. Low incidence of DNA sequence variation in human induced pluripotent stem cells generated by nonintegrating plasmid expression. *Cell Stem Cell*. 2012; 10:337–44. [PubMed: 22385660]
69. Han DW, Tapia N, Hermann A, Hemmer K, et al. Direct reprogramming of fibroblasts into neural stem cells by defined factors. *Cell Stem Cell*. 2012; 10:465–72. [PubMed: 22445517]
70. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, et al. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998; 282:1145–7. [PubMed: 9804556]
71. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–76. [PubMed: 16904174]
72. Wernig M, Zhao JP, Pruszak J, Hedlund E, et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc Natl Acad Sci USA*. 2008; 105:5856–61. [PubMed: 18391196]
73. Oki K, Tatarishvili J, Woods J, Koch P, et al. Human induced pluripotent stem cells form functional neurons and improve recovery after grafting in stroke-damaged brain. *Stem Cells*. 2012; 30:1120–33. [PubMed: 22495829]
74. Nelson TJ, Martinez-Fernandez A, Yamada S, Perez-Terzic C, et al. Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells. *Circulation*. 2009; 120:408–16. [PubMed: 19620500]

75. Gamie Z, Tran GT, Vyzas G, Korres N, et al. Stem cells combined with bone graft substitutes in skeletal tissue engineering. *Expert Opin Biol Ther.* 2012; 12:713–29. [PubMed: 22500826]
76. Arpornmaeklong P, Brown SE, Wang Z, Krebsbach PH. Phenotypic characterization, osteoblastic differentiation, and bone regeneration capacity of human embryonic stem cell-derived mesenchymal stem cells. *Stem Cells Dev.* 2009; 18:955–68. [PubMed: 19327009]
77. Undale A, Fraser D, Hefferan T, Kopher RA, et al. Induction of fracture repair by mesenchymal cells derived from human embryonic stem cells or bone marrow. *J Orthop Res.* 2011; 29:1804–11. [PubMed: 21674605]
78. Deyle DR, Khan IF, Ren G, Wang PR, et al. Normal collagen and bone production by gene-targeted human osteogenesis imperfecta iPSCs. *Mol Ther.* 2011; 20:204–13. [PubMed: 22031238]
79. Medvedev SP, Grigor'eva EV, Shevchenko AI, Malakhova AA, et al. Human induced pluripotent stem cells derived from fetal neural stem cells successfully undergo directed differentiation into cartilage. *Stem Cells Dev.* 2011; 20:1099–112. [PubMed: 20846027]
80. Hiramatsu K, Sasagawa S, Outani H, Nakagawa K, et al. Generation of hyaline cartilaginous tissue from mouse adult dermal fibroblast culture by defined factors. *J Clin Invest.* 2011; 121:640–57. [PubMed: 21293062]
81. Barberi T, Willis LM, Socci ND, Studer L. Derivation of multipotent mesenchymal precursors from human embryonic stem cells. *PLoS Med.* 2005; 2:e161. [PubMed: 15971941]
82. Barbet R, Peiffer I, Hatzfeld A, Charbord P, et al. Comparison of gene expression in human embryonic stem cells, hESC-derived mesenchymal stem cells and human mesenchymal stem cells. *Stem Cells Int.* 2011; 2011:368192. [PubMed: 21941565]
83. Trivedi P, Hematti P. Derivation and immunological characterization of mesenchymal stromal cells from human embryonic stem cells. *Exp Hematol.* 2008; 36:350–9. [PubMed: 18179856]
84. Alper J. Geron gets green light for human trial of ES cell-derived product. *Nat Biotechnol.* 2009; 27:213–4. [PubMed: 19270655]
85. Strauss S. Geron trial resumes, but standards for stem cell trials remain elusive. *Nat Biotechnol.* 2010; 28:989–90. [PubMed: 20944564]
86. Frantz S. Embryonic stem cell pioneer Geron exits field, cuts losses. *Nat Biotechnol.* 2012; 30:12–3. [PubMed: 22231081]
87. Schwartz SD, Hubschman JP, Heilwell G, Franco-Cardenas V, et al. Embryonic stem cell trials for macular degeneration: a preliminary report. *Lancet.* 2012; 379:713–20. [PubMed: 22281388]
88. Arvidson K, Abdallah BM, Applegate LA, Baldini N, et al. Bone regeneration and stem cells. *J Cell Mol Med.* 2011; 15:718–46. [PubMed: 21129153]
89. Dimitriou R, Jones E, McGonagle D, Giannoudis PV. Bone regeneration: current concepts and future directions. *BMC Med.* 2011; 9:66. [PubMed: 21627784]
90. Jones E, Yang X. Mesenchymal stem cells and bone regeneration: current status. *Injury.* 2011; 42:562–8. [PubMed: 21489533]
91. Fong EL, Chan CK, Goodman SB. Stem cell homing in musculoskeletal injury. *Biomaterials.* 2011; 32:395–409. [PubMed: 20933277]
92. Aroen A. Stem cell therapy for articular cartilage defects. *Br Med Bull.* 2011; 99:227–40. [PubMed: 21821628]
93. Sakai D. Stem cell regeneration of the intervertebral disk. *Orthop Clin North Am.* 2011; 42:555–62. viii–ix. [PubMed: 21944591]
94. Young M. Stem cell applications in tendon disorders: a clinical perspective. *Stem Cells Int.* 2012; 2012:637836. [PubMed: 22448174]
95. Isaac C, Gharaibeh B, Witt M, Wright VJ, et al. Biologic approaches to enhance rotator cuff healing after injury. *J Shoulder Elbow Surg.* 2012; 21:181–90. [PubMed: 22244061]
96. Gulotta LV, Chaudhury S, Wiznia D. Stem cells for augmenting tendon repair. *Stem Cells Int.* 2012; 2012:291431. [PubMed: 22190960]
97. Horie M, Driscoll MD, Sampson HW, Sekiya I, et al. Implantation of allogenic synovial stem cells promotes meniscal regeneration in a rabbit meniscal defect model. *J Bone Joint Surg Am Volume.* 2012; 94:701–12.

98. Gwathmey FW Jr, Golish SR, Diduch DR. Complications in brief: meniscus repair. Clin Orthopa Relat Res. 2012; 470:2059–66.
99. Brophy RH, Matava MJ. Surgical options for meniscal replacement. J Am Acad Orthop Surg. 2012; 20:265–72. [PubMed: 22553098]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.

Conceptual depiction of the components of functional tissue regeneration. The diamond concept of tissue regeneration proposed by Giannoudis et al. 3 points out the highly dynamic regulatory interactions among the four cornerstone components (i.e. cells, bioactive molecules, three-dimensional surroundings, and physical signals). Some of the key features and activities of each of the components are indicated in the respective boxes.

Figure 2.

Cell sourcing for tissue engineering and regenerative medicine, starting with autologous versus allogeneic sources. Considerations are given to the advantages () and limitations and drawbacks () of each cell source or cell type.

Table 1Cell therapies for skeletal tissue repair: procedures in current clinical practice and in clinical trials^{a)}

Tissue type	Gold standard	Current cellular therapies	In clinical trials	Reviews
Bone	Autologous/allogeneic bone graft	1. Autologous bone grafting	Immunodepleted allogeneic MSCs on DBM (Trinity™; e.g. NCT00851162)	88_90
		2. Allogeneic bone grafting	Allogeneic bone marrow transplantation for osteogenesis imperfecta (e.g. NCT00705120)	
		3. Bone transport	Autologous BM-MSC (e.g. NCT01210950)	
		4. Growth factor augmented scaffolds/ceramics with BMP	PREOB® autologous differentiated osteoblastic cells (NCT01529008)	
Cartilage	Microfracture	1. Marrow stimulation/microfracture	Fresh non-culture expanded autologous BM-MSC derived intraoperatively with a cell separator and stimulated by a protein matrix (NCT01159899)	7, 91, 92
		2. Fresh osteochondral allograft	Autologous culture-expanded BM-MSC as alternative for autologous chondrocytes in ACI (e.g. NCT00891501)	
		3. Autologous chondrocyte implantation	Autologous ATSCs as alternative for autologous chondrocytes in ACI (NCT01399749)	
		4. Osteochondral autografts	Allogeneic ex vivo expanded BM-MSCs (NCT01586312)	
			Allogeneic ex vivo expanded umbilical cord-derived MSCs (NCT01547091)	
			Neocartilage Implant/ DeNovo® ET: allogeneic cultured juvenile human cartilage cells as engineered tissue graft (NCT01400607)	
			Cartistem®: allogeneic-unrelated umbilical cord blood-derived MSCs (NCT01041001)	
			TissueGene-C: allogeneic chondrocytes expressing TGF-β1 (NCT00599248)	
Intervertebral disc	Spinal fusion (possibly with autografts)	1. Spinal fusion possibly in combination with autografting	Culture-expanded autologous BM-MSC fixed in allogeneic human bone tissue (XCEL-MT-OSTEO-	93

Tissue type	Gold standard	Current cellular therapies	In clinical trials	Reviews
			ALPHA; NCT01552707) for spinal fusion	
		2. Disc arthroplasty	Culture-expanded autologous BM-MSC on phosphate ceramic for spinal fusion (NCT01513694)	
			Trinity Evolution™ viable cryopreserved cellular bone matrix (allograft, minimally manipulated; NCT00951938)	
			NeoFuse™: allogeneic BM-MSCs combined with MasterGraft® Matrix (calcium phosphate/ collagen ; e.g. NCT00996073)	
			PureGen™ BM-MSC allograft without ex vivo expansion (e.g. NCT01293981)	
Tendon/Ligament	Reconstruction using grafts	1. Autologous tendon grafting	Autologous tenocyte implantation (NCT01343836)	8, 94_96
		2. Allogeneic tendon grafting		
Meniscus	(Partial) surgical removal	1. Meniscal allografts	Chondrogen®: allogeneic ex vivo expanded BM-MSCs (NCT00702741)	97_99

^{a)} BM-MSC, bone marrow-derived mesenchymal stem cells; DBM, demineralized bone matrix; ACI, autologous chondrocyte implantation, ATSCs, adipose tissue-derived mesenchymal stem cells; TGF-β1, transforming growth factor-beta 1.