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REGENERATIVE MEDICINE

Concise Review: Bridging the Gap: Bone Regeneration Using Skeletal Stem Cell-Based Strategies—Where Are We Now?

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ABSTRACT

Skeletal stem cells confer to bone its innate capacity for regeneration and repair. Bone regeneration strategies seek to harness and enhance this regenerative capacity for the replacement of tissue damaged or lost through congenital defects, trauma, functional/esthetic problems, and a broad range of diseases associated with an increasingly aged population. This review describes the state of the field and current steps to translate and apply skeletal stem cell biology in the clinic and the problems therein. Challenges are described along with key strategies including the isolation and ex vivo expansion of multipotential populations, the targeting/delivery of regenerative populations to sites of repair, and their differentiation toward bone lineages. Finally, preclinical models of bone repair are discussed along with their implications for clinical translation and the opportunities to harness that knowledge for musculoskeletal regeneration. STEM CELLS *2014;32:35–44*

INTRODUCTION

Medical advances have led to a welcome increase in world population demographics. However, increased aging populations pose new challenges and emphasize the need for innovative approaches to augment and repair tissue lost through trauma or disease. To meet this demand, tissue regeneration strategies that build on advances in our understanding of postnatal skeletal stem cells (SSCs) and their role in bone development and repair promise to deliver specifiable replacement tissue. Thus, stem cell-based therapies have emerged as the likely contenders for bone repair and regeneration in nonunion fractures, healing of critical-sized segmental defects and regeneration of tissues in degenerative joint diseases.

The term "stem cell" can be applied to a diverse group of cells that share two characteristic properties: a capacity for prolonged or unlimited self-renewal under controlled conditions, and the potential to differentiate into a variety of specialized cell types. The term SSC is used in this review to refer specifically to the self-renewing stem cell of the bone marrow stroma responsible for the regenerative capacity inherent to bone. The heterogeneous population of cultured plastic adherent cells isolated from the bone marrow, which remain the most commonly used (if not acknowledged) population by researchers in the field of bone regeneration, will be referred to as bone marrow stromal cells (BMSCs).

This terminology has come to represent a particular conceptualization of postnatal stem cell biology that stands in contradistinction to that typically implied by the more commonly used term, mesenchymal stem cell (MSC). The term MSC was originally coined in reference to a hypothetical common progenitor of a wide range of "mesenchymal" (nonhematopoietic, nonepithelial, and mesodermal) tissues [1]. On the basis of a combination of in vitro assays and surface phenotyping [2], it has been widely accepted that MSCs exist in a broad range of postnatal tissues and organs, with a broad spectrum of lineage potentialities (extending to, e.g., skeletal muscle) [3]. This concept of a ubiquitous MSC with broad differentiation potential has, however, been subject to robust criticism for lacking the necessary in vivo experimental support and theoretical grounding [4]. Indeed, recent discussions of the therapeutic benefits observed in translational studies of MSC transplantation have increasingly emphasized alternative mechanisms independent of stem cell or tissue

progenitor function (centered on nonprogenitor potential), suggesting the MSC concept to be somewhat on the decline [5].

The alternative conception adopted in this review conceives of skeletal postnatal stem cells as thus having an organ specific ontogeny and in vivo identity and thus an organ specific progenitor function and therapeutic regenerative utility [6]. In the case of SSCs, this ontogeny, identity, function, and utility relate specifically to the various tissues and cell types that compose the bone organ, namely bone, cartilage, adipocytes, fibroblasts, and stromal tissue.

The capacity of bone to regenerate is evidence of the presence of a stem cell in bone. However, while this regenerative capacity has long been recognized, the in vivo identity of the responsible population has only recently been confirmed [7, 8]. Over the last 5 years a consensus has emerged that the SSC is a perivascular cell located in association with the microvasculature of the bone marrow stroma and which functions within this niche to contribute to the regulation of hematopoiesis [7]. In terms of ontogeny, SSCs can thus be understood as cells recruited early during bone development in service of the nascent hematopoietic microenvironment which retain (in the adult) the potential to reinitiate the developmental cascade from which bone, cartilage, and the marrow organ arose during development, as occurs naturally (albeit to a limited degree) in postnatal repair. Thus, SSC-based bone regeneration can be conceptualized as an attempt to harness and enhance the innate regenerative potential of bone to meet the clinical needs of an aging population [6, 9].

This review provides a critically selective, rather than comprehensive, overview of the application of SSCs for bone regeneration. We seek to identify the challenges facing clinical efficacy and translation, highlight certain recent approaches that suggest promise in meeting these challenges, and describe steps taken toward the translation of skeletal tissue engineering from bench to clinic for SSC-based bone regeneration.

SSCs: Cell Selection and Characterization

Friedenstein et al. first confirmed the presence of fibroblast-like clonogenic precursor cells (colony-forming unit-fibroblastic/CFU-F) in the tissue culture plastic (TCP) adherent nonhematopoietic fraction of bone marrow aspirate (BMA) [10]. Within the TCP-adherent human BMSC population there remains considerable heterogeneity. For example, a study assessing the ability of 185 BMSC clones to differentiate into the three main lineages demonstrated that only one third of the clones exhibited osteo-chondro-adipogenic differentiation potential characteristic of the tripotent primitive MSC population, while the majority of clones (almost 80%) exhibited a differentiation potential restricted to osteo-chondrogenic lineages typical of early osteoprogenitor cell populations [11]. Thus, it would appear incorrect to label the entire TCP-adherent nonhematopoietic fraction of bone marrow as MSCs or SSCs given cultures of BMSCs established solely on the basis TCP adherence contain both stem and differentiated cell populations (Fig. 1A).

"Stemness" of SSCs is assayed by the ability of clonal cell populations to regenerate bone and stroma and, critically, to establish a hematopoietic microenvironment upon in vivo transplantation [12] (Fig. 1B). In contrast, osteoprogenitor cells are only able to form bone following ectopic implantation with a suitable osteoinductive carrier in immunocompromised mice [13] (Fig. 1B). Over the years, a number of studies have attempted to isolate relatively homogenous populations of human SSCs based on the expression of one or more cell-surface markers that are characteristic of the SSC phenotype, including the STRO-1 antigen, CD29, CD73, CD90, CD105, CD106, CD166, CD146, CD44, and CD271 or, by negative selection for hematopoietic markers such as CD34, CD45, CD19, CD14, CD11b, CD79α, and HLA-DR surface molecules [14]. Notably, the comprehensive study lead by Bianco has provided compelling evidence that human $CD146^{+}$ $CD45^{-}$ stromal cells located in the perivascular spaces as subendothelial cells surrounding the vascular sinusoids in the bone marrow are self-renewing, clonogenic SSCs and, are able to regenerate bone and stroma, and establish the hematopoietic microenvironment following subcutaneous transplantation with hydroxyapatite/tri-calcium phosphate particles in immunocompromised mice [7].

Applying SSCs in Bone Regeneration

The unique in vivo capabilities of isolated and transplanted SSCs suggest for them a critical role in maintaining bone's innate capacity both for remodeling in response to mechanical stimuli and regeneration upon damage. In seeking to apply SSCs in therapeutic bone reconstruction therefore, we are attempting both to harness and enhance natural bone regeneration—"bridging the gap" as it were between the natural capacity of bone to regenerate and the clinical scenarios where mechanical or metabolic restrictions necessitate bone augmentation [9].

Autologous bone grafting (ABG) represents the gold-standard approach to harnessing bone's natural regenerative capacity in the clinic. ABG allows for the transplantation of SSCs with other supportive osteogenic populations integrated within an existing osteoinductive and vascularized environment. However, significant donor-site morbidity and volume restrictions prohibit its widespread application. As an alternative, BMA constitutes a relatively rich source of SSCs and has been successfully applied in the treatment of nonunions and bone cysts [15, 16]. Results have been variable however and correlate with CFU-F estimations of osteoprogenitor concentrations which are both low (typically $<0.005\%$ of total nucleated cells) and highly variable [17, 18]. In isolation from the ideal conditions of ABG, direct application of aspirated SSCs appears unlikely to be sufficient for robust skeletal regeneration. The respective limitations of ABG and BMA thus emphasize the need for improved strategies that seek not only to harness, but further enhance bone's natural regenerative capacity (Fig. 2). Such enhancements can be broadly classified as cellular enhancements, that is, those focused on the cells directly (e.g., involving their enrichment, expansion, priming for differentiation, and/or targeting to the site of regeneration), and extracellular enhancements, that is, those focused on providing an optimal extracellular environment for cell-mediated regeneration (through matrices/scaffolds that sustain the provision of appropriate mechanical, chemical, and biological environmental cues).

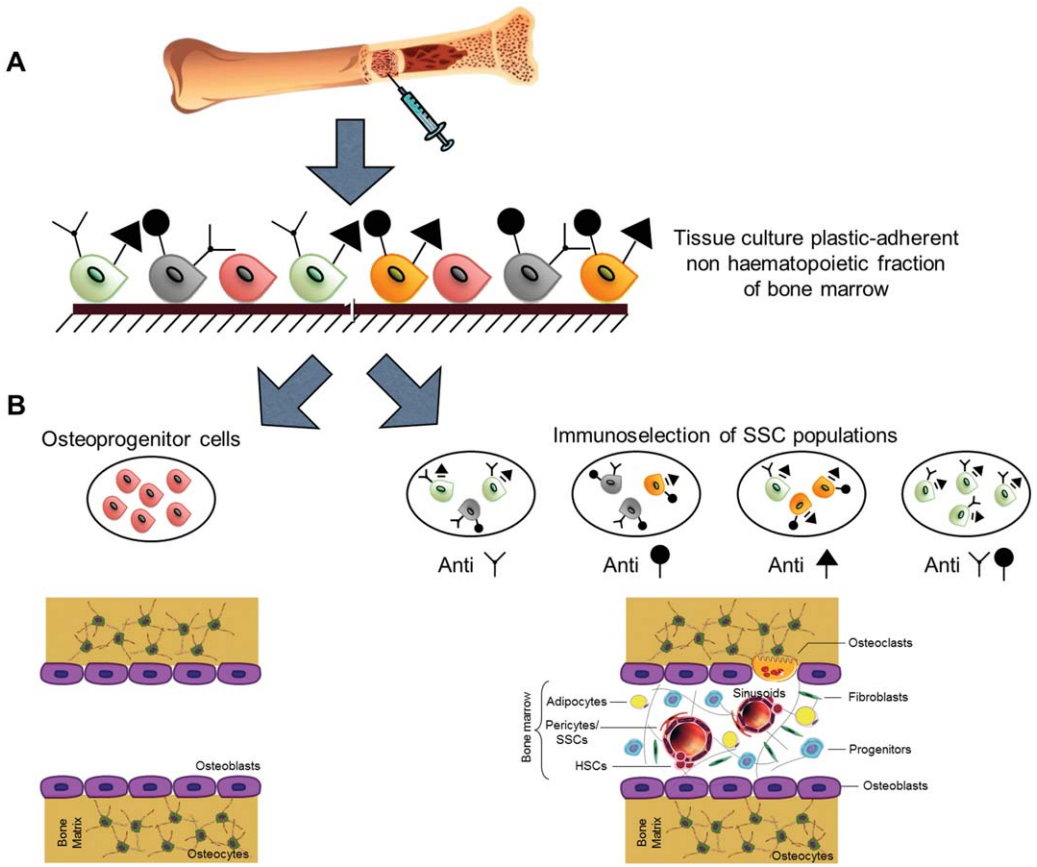

Figure 1. Isolating skeletal stem cells. **(A):** The tissue culture plastic-adherent nonhematopoietic fraction of bone marrow is heterogeneous as it contains both osteoprogenitors and SSCs. **(B):** Upon ectopic implantation with a suitable osteoinductive carrier in immunocompromised mice, osteoprogenitor cells are only able to form bone, while relatively homogenous populations of SSCs, immunoselected on the basis of expression of one or more SSC-surface markers, are able to regenerate bone and stroma and, critically, establish a hematopoietic microenvironment. Abbreviation: SSC, skeletal stem cell; HSC, haematopoietic stem cell.

BMA Enhancement

Significant clinical and preclinical studies have established the importance of CFU-F concentration in BMA for bone repair. Of particular note, a retrospective study by Hernigou et al. revealed that, of 60 patients treated for bone reconstruction surgery via percutaneous injection of iliac crest BMA, the 7 that failed to heal had significantly lower numbers and concentration of CFU-F [18]. In response to this observation, several approaches have been developed to concentrate freshly obtained BMA for the CFU-F-containing cellular component (e.g., [19]). Another strategy has been to explore alternative sites from which to source SSCs. For example, filtered aspirate obtained during the reaming of long bones allows a substantially larger harvest volume with a corresponding increase in the total number of CFU-F obtained [20, 21].

Ex Vivo Expansion of Skeletal SSCs

Despite advances in bone marrow aspiration techniques, clinical exploitation of SSCs for tissue regeneration seems likely to require ex vivo expansion of the cells under defined conditions to generate sufficient numbers while maintaining cell phenotype and genotype. This is a challenge however since ex vivo BMSCs characteristically exhibit a limited capacity for cell proliferation displaying "replicative senescence" as a consequence of successive subculture [22]. Critically, BMSCs from elderly human donors exhibit accelerated senescence phenotype following ex vivo cultures [22] suggesting limited autologous (and allogeneic) usefulness for transplantation in this aged cohort, although, evidence for altered stem cell number with ageing is conflicting.

Two strategies have been explored to enhance the proliferative potential of BMSCs in ex vivo cultures. The first approach seeks to delay senescence through improved culture conditions by applying growth factors or small molecules, extracellular matrix (ECM) substrates, and dynamic culture environments [23]. In this context, recent development of the use of dynamic systems that expand BMSC using a rotary reactor, spinner flasks with microcarrier-based stirred culture system, or disposable culture system, demonstrates potential for a several fold increase in the growth of BMSCs while maintaining a stable phenotype [24, 25]. The second approach deploys a genetic strategy with for example overexpression of human telomerase reverse transcriptase gene (*hTERT*) to increase BMSC telomerase activity and remove the replicative senescence phenotype [26]. However, this latter approach is unlikely to be suitable for clinical application given concerns over potential development of a transformed phenotype in transgenic cells.

Key in the implementation of a cell-based clinical approach are cell expansion strategies to circumvent issues of limited oxygen and nutrient diffusion and cell viability over

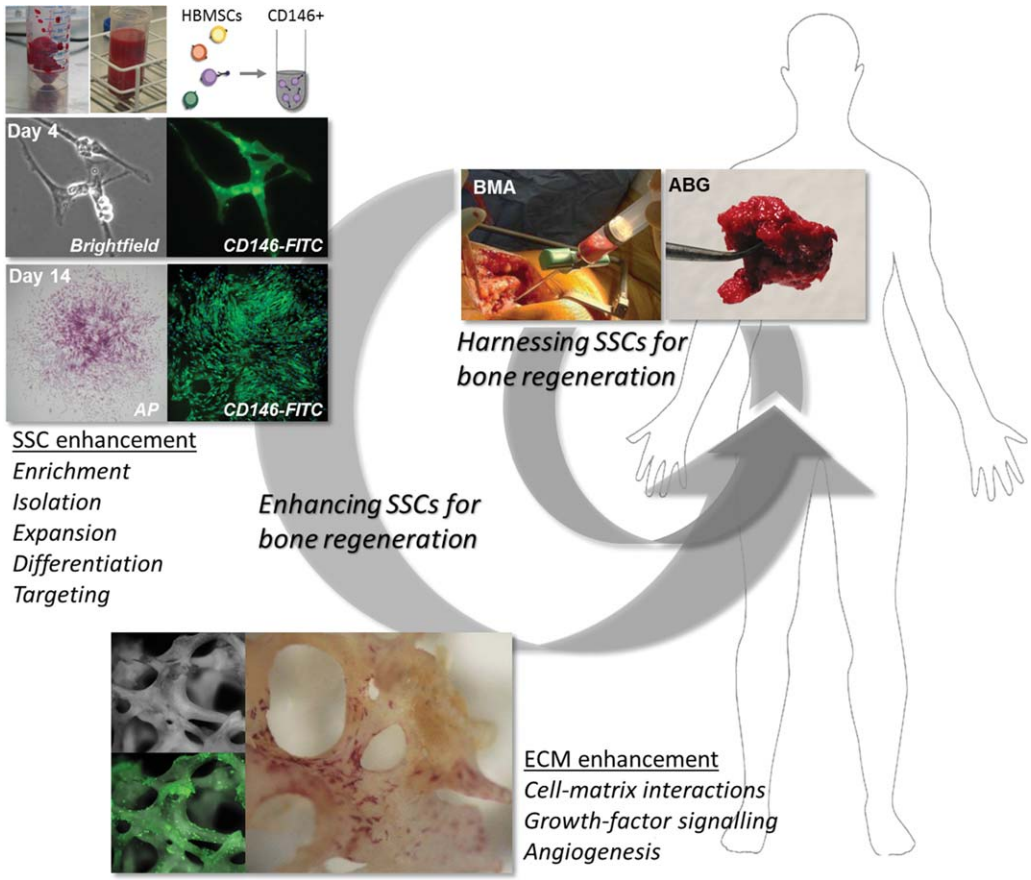

Figure 2. Harnessing and enhancing SSCs for bone regeneration. Regenerative medicine strategies seek to harness the developmental potential of SSCs to replace tissue lost or damaged through injury or disease. Autologous bone grafting effectively harnesses SSC biology to repair but is source restricted. BMA is a readily available source of SSCs but requires enhancement for effective regeneration. Potential enhancement strategies include SSC enrichment or isolation, ex vivo expansion, differentiation and targeting, and the provision of a suitable ECM environment in situ that promotes cell matrix interactions, growth factor signaling, and angiogenesis. Abbreviations: BMA, bone marrow aspirate; ECM, extracellular matrix; SSC, skeletal stem cell.

200 μm observed in, for example, static cultures of large tissue constructs (reviewed in [27] and references therein). Spinner flasks, rotating wall vessel, and perfusion bioreactors (with hollow fiber or nonwoven fiber packed bioreactors as well as automated dual systems with micro carrier-based wave systems) have all been proposed to aid cell expansion and growth in large grafts. Grayson et al. [28] demonstrated the potential to engineer using a perfusion bioreactor system, clinically relevant and sized human bone constructs although issues of cell seeding, automation and noninvasive online process monitoring, evaluation, and control remain central issues to clinical delivery. In the coming decade, the need to develop simple, safe, and efficacious protocols of cell expansion that meet stringent regulatory body criteria for clinical application will be key to the use of SSCs in bone regeneration.

SSC Differentiation

Current protocols for directing the differentiation of human SSCs into osteoblast cells are, typically, based on the application of hormones such as calcitriol, growth factor cocktails composed of bone morphogenetic proteins (BMPs), or transforming growth factor-β family members and supplements in the form of dexamethasone, vitamin C, and organic phosphate donors. However, the efficiency of differentiation is highly variable necessitating the development of clinically tailored reproducible protocols for osteoblast derivation. There is an emerging interest in developing a number of in vitro markers predictive of the in vivo behavior of the bone cell population upon transplantation to monitor the efficiency of differentiation [29].

An alternative approach to generating bone in vivo termed developmental engineering has also been explored with some promise. Recognizing the importance of the endochondral mode of bone formation in development Scotti et al. investigated the potential to generate ectopic bone through chondrogenic differentiation of BMSCs and observed a marked improvement in bone formation in the late-hypertrophic compared with early hypertrophic and prechondrogenic implanted tissue constructs [30].

SSC Targeting for Bone Regeneration

A further challenge facing the successful application of isolated and expanded populations is to ensure their efficient engraftment upon transplantation. This is especially important when SSCs are to be administered systemically for treatment of metabolic bone diseases caused by a broad spectrum of disorders. To address this challenge, a recent study describes a ligand-based approach to directing transfused BMSCs to bone surfaces [31]. Building on the

Table 1. Ongoing clinical trials employing skeletal stem cell containing populations for bone regeneration[a]

Study title	Conditions	Intervention	Cell preparation	Estimated enrollment	Study design	Status
Percutaneous autologous bone-marrow grafting for open tibial shaft fracture (IMOCA) (NCT00512434)	Open tibial fractures	Standard of care, with percutaneous injection, 1 month after fracture, of autologous concentrated bone-marrow to defect site	Concentrated BMA	85	Randomized, parallel assignment, open label	Ongoing
Mesenchymal stem cell for osteonecrosis of the femoral head (NCT00813267)	Osteo-necrosis of femoral head	Infusion of BMSC into the femoral artery	Ex vivo culture	15	Single group	Ongoing
Distraction osteogenesis in limb-length discrepancy with mesenchymal cell transplantation (NCT01210950)	Leg length inequality	Injection of BMSC with plasma-rich protein into callus	Not specified	6	Single group	Recruiting
Mesenchymal stem cells; donor and role in management and reconstruction of nonunion fracture (NCT01626625)	Nonunion fracture	Transplantation of autologous BMSC seeded upon a hydroxyapatite scaffold	Ex vivo culture	10	Parallel assignment, double blind	Recruiting
Clinical trial based on the use of mesenchymal stem cells from autologous bone marrow in patients with lumbar intervertebral degenerative disc disease (NCT01513694)	Intervertebral disc disease	Instrumented posterolateral fusion with autologous BMSC on a phosphate ceramic	Ex vivo culture	15	Single group	Ongoing
Treatment of maxillary bone cysts with autologous bone mesenchymal stem cells (NCT01389661)	Maxillary cyst	Transplantation of autologous BMSC seeded upon an autologous plasma protein matrix into cyst cavity	Seeding on scaffold, ex vivo culture	10	Single group	Recruiting
Safety study of mesenchymal stem cells and spinal fusion (NCT01552707)	Lumbar spondylolisthesis involving L4-L5	Instrumented spinal fusion combined with autologous BMSC on allogeneic bone graft	Ex vivo culture	62	Randomized, parallel assignment, open label	Recruiting
Mesenchymal stem cells in osteonecrosis of the femoral head (NCT01605383)	Avascular necrosis of femur head	Core decompression combined with implantation of autologous BMSC on allogeneic bone graft in lesion	Ex vivo culture	24	Randomized, parallel assignment, open label	Recruiting
Treatment of osteonecrosis of the femoral head by the administration of autologous mesenchymal stem cells (NCT01700920)	Osteonecrosis of the femoral head	Intraosseous injection of autologous BMSC with trocar in the femoral head	Ex vivo culture	10	Single group	Recruiting

Table 1. *Continued*

Study title	Conditions	Intervention	Cell preparation	Estimated enrollment	Study design	Status
The efficacy of mesenchymal stem cells for stimulate the union in treatment of non-united tibial and femoral fractures in Shahid Kamyab Hospital (NCT01788059)	Nonunion Fracture	Percutaneous injection of autologous bone marrow mononuclear cells into defect	BMA mononuclear fraction	18	Single group	Recruiting
Evaluation of efficacy and safety of autologous MSCs combined to biomaterials to enhance bone healing (NCT01842477)	Delayed union after fracture of humerus, tibial or femur	Implantation surgery of a synthetic bone substitute associated with autologous BMSC	Ex vivo culture, osteogenic differentiation, seeding on TCP scaffolds	30	Single group	Recruiting
Treatment of atrophic nonunion fractures by autologous mesenchymal stem cell percutaneous grafting (NCT01429012)	Nonunion Fracture	Percutaneous injection of BM into the nonunion space	Not specified	40	Parallel assignment, double blind	Not yet open
Mononucleotide autologous stem cells and demineralized bone matrix in the treatment of nonunion/ delayed fractures (NCT01435434)	Nonunion fracture	Transplantation of autologous bone marrow mononuclear cells with demineralized bone matrix	BMA mononuclear fraction	Not stated	Single group assignment	Not yet open

[a]Exclusions—treatments based on systemic infusion; treatments using non bone marrow-derived cells.
Abbreviations: BMA, bone marrow aspirate; BMSC, bone marrow stromal cell; MSC, mesenchymal stem cell; TCP, tissue culture plastic.

observation that ectopic overexpression of a certain integrin ($\alpha4\beta1$) increases the homing of osteogenic BMSCs to bone [32], Guan et al. coupled a peptidomimetic ligand specific to activated $\alpha4\beta1$ to a "bone-seeking" bisphosphonate molecule. By preincubating culture-expanded human BMSCs with the coupled ligand prior to intravenous injection into immunocompromised mice, engraftment and consequent increase in bone formation were achieved at both the endosteal and periosteal bone surfaces. Interestingly, intravenous injection of the coupled ligand alone into ovariectomized mice (a model of systemic bone loss) also served to increase osteoblast numbers and bone formation, suggesting the possibility of using this approach to recruit endogenous circulating SSCs [33] for bone regeneration. A similar approach to enhancing SSCs while bypassing harvesting and transplantation steps seeks to stimulate the proliferation and mobilization of endogenous SSCs through the use of a pharmacological ligand. For example, CXCR4 antagonist ADMD3100 combined with insulin-like growth factor-1 was shown to enhance bone healing of a segmental bone defect in mice [34].

Safety of SSC Transplantation

There is some evidence that human MSC BMSC populations are immune-privileged as these cells express intermediate levels of HLA major histocompatibility complex class I molecules, low levels of HLA class II molecules, and do not express costimulatory molecules (CD40, CD40L, CD80, or CD86 [35]). Furthermore, human BMSC populations have been shown to possess immunosuppressive properties demonstrated by inhibition of T-cell alloreactivity induced in mixed lymphocyte cultures or by nonspecific factors in in vitro assays, thereby suggesting the clinical potential of allogeneic human BMSC transplantation [36]. The attraction of allogeneic SSC transplantation lies in the ready development of "off-the-shelf" allogeneic cells from an industrial/commercialization perspective for products ready for use in therapy and for toxicity screening.

Effects of long-term in vitro culture prior to transplantation have led to concerns regarding culture-induced genetic changes that may lead to tumor formation upon clinical transplantation. However, the safety record of human BMSCs remains excellent and, to date, no reported cases of in vivo tumor formation have been linked with their clinical transplantation. Indeed there have been no reported incidences of in vitro spontaneous transformation of culture expanded BMSC populations, although as detailed above culture expansion can result in replicative senescence and thus growth arrest (though see discussion in [37]). However, Lepperdinger et al. have urged caution and further study, following the suggestion that systemically administered populations could promote growth

of a latent tumor due to recruitment of SSCs to the tumor stroma and their ability to support tumor growth as evidenced in certain experimental cancer models [38]. While there is no clinical evidence to support this hypothesis, additional clinical experience will be necessary to resolve this issue.

Cell Matrices for Bone Regeneration

In addition to approaches that seek to enhance the regenerative capacities of SSCs directly, administration of SSCs for local bone regeneration typically relies on efficacy of supportive osteoinductive matrices (or scaffolds) at the site of repair. A variety of materials have been used for bone regeneration together with SSCs including ceramics or materials based on hydroxyapatite, ECM derivatives as well as natural and synthetic polymeric materials. Traditionally, within the field, the tissue engineering scaffold has been assigned a "conductive" role in distinction to an "inductive" role, as played by growth factors for example. However, significant developments in the field of biomaterials over the last decade have made such demarcations increasingly hard to maintain. While the traditional role the scaffold fulfils as cell delivery vehicle and three-dimensional support structure is still vital, the dynamic significance of the scaffold in controlling the spatio-temporal distribution of biochemical signals [39], transmitting mechanical signals [40], influencing cellular metabolism [41], and directing cell function in other, previously unforeseen, ways [42] emphasize the important role biomaterial strategies are likely to play in the success of SSC-based strategies for bone repair. The importance of the extracellular microenvironment at the repair site has been underlined by a recent study that observed significantly improved recruitment of injected BMSCs into a bone defect by codelivering ECM generated by cultured BMSCs under osteogenic induction [43].

In Vivo Models of SSC Regeneration

In vivo models provide the requisite dynamic complex environments and blood supply to enhance our understanding of skeletal growth and fracture repair and, the potential of SSCs which, currently, cannot be achieved through examination of in vitro models alone. Classically, BMSCs seeded on biodegradable scaffolds or within hydrogels have been used to determine their regenerative efficacy in bone defects in vivo. The craniotomy defect model has been used to good effect to measure, in a standard defect site, the regenerative capacity of skeletal progenitor cells including periodontal ligament progenitor cells [44], adipose-derived, periosteum-derived, and bone marrow-derived mesenchymal stem/progenitor cells [45]. Moreover, implanting combinations of vascular cells and BMSCs have demonstrated improved repair of critical-sized calvarial defects [46]. Critically, surrounding cells and tissue play an important role in evaluating stem cell strategies for bone repair. Liu et al. [47] established that proinflammatory T cells reduce the effects of exogenously added BMSCs to facilitate bone repair. Additionally, implantation within a rabbit osteochondral joint of a bilayered scaffold consisting of gelatin, chondroitin sulfate, sodium hyaluronate, and chondrocytes in one layer together with a further layer of gelatin, ceramic bone, and bone marrow cells resulted in the repair of the defect [48].

Load bearing nonunion critical-sized bone defect models have also been used to assess the efficacy of SSC therapy and tissue regeneration. Studies have demonstrated that growth factor release from biodegradable scaffolds can augment bone repair [49] and that the addition of skeletal progenitors within these systems has led to excellent repair of the bone defects [50]. The combined implantation of different cell phenotypes such as endothelial progenitor cells (EPCs) and BMSC into the sites of bone defects has demonstrated not only the healing of the bone but also improved early vascularization of the defect site [51]. Modulation of BMSCs to express vascular and osteogenic factors synergistically improves bone regeneration [52], while the combination effect of dual release vascular and osteogenic stimulating growth factors from implanted scaffolds with seeded BMSCs has enhanced the repair capacity in a segmental bone defect [53]. Platelet rich plasma-based membranes have been administered for enhancing concomitantly angiogenesis and osteogenesis to stimulate bone repair [54]. Furthermore, Wang et al. [55] demonstrated that a prevascularized tissue engineered bone graft could significantly stimulate angiogenesis and bone regeneration compared to non-prevascularized bone grafts.

Preclinical Models of Bone Regeneration

ABG is conventionally the gold standard for the repair of bone defects in large in vivo models and in the clinic. Implantation of BMSCs to ovine hip hemi-arthroplasty impaction and long bone critical-sized defects was shown to improve the regeneration of bone [56, 57]. However, in a comparative study, BMSC loading onto scaffolds did not induce the levels of bone repair compared to that of the autograft or administered rhBMP-7 groups [58]. Knothe et al. [59] demonstrated that isolated periosteum cells and other periosteal factors were able to aid in bone regeneration and that the implantation of blood-derived EPCs was efficacious in bridging critical size defects in the sheep tibia [60].

As previously reviewed [61], neovascularization of the critical-sized bone defects is vital for the integration, survival of the construct/cells, and the successful union and repair of the bone defect. Regeneration of large complex bone defects where the induction of a functional vasculature is lacking is still a major challenge to orthopedics and regenerative medicine as a whole. The above studies indicate the efficacy of a population containing SSCs and supporting cell populations/vasculature but more importantly emphasize the need for homogenous stem cell populations to drive reparative studies forward.

Toward Clinical Translation

The ready accessibility of BMSCs from bone marrow and their ability to differentiate into bone-forming osteoblasts when implanted in vivo have driven application of SSCs in the clinic. However, beyond individual patient-tailored and specific clinical application, successful use of SSCs in therapy necessitates reproducible and well-defined methods to modulate cell growth and lineage specific differentiation.

Clinical Applications of SSCs for Bone Repair and Regeneration

As discussed above, the simplest cell-based strategy is the direct transplantation of autologous BMSC populations to the site of injury. To date, much of the reported literature, encompassing a

small number of cases or poorly controlled clinical trials for applications from osteonecrosis to fracture nonunions has demonstrated the necessity for both a high number and concentration of osteoprogenitors that typically exceed those present in fresh iliac crest marrow aspirates. In large bone defects as observed following tumor resection, the cellular demand is further increased necessitating cell enrichment and/or ex vivo culture expansion to yield sufficient cells for robust bone regeneration. Ex vivo culture expansion of SSCs or BMSCs requires access to good manufacture practice facilities and appropriate regulatory approval/licenses, thereby limiting their use in clinical protocols.

To date, the majority of studies have used autologous bone marrow mononuclear cells (BM-MNC) isolated from iliac crest aspirates harvested in theater during the orthopedic procedure, while a limited number of trials have been conducted using autologous ex vivo culture expanded BMSCs (reviewed in [62]). The BM-MNCs were either used alone or a combination of osteoconductive scaffold [62]. It is important to note that BM-MNC transplants represent a heterogeneous cell population containing BMSC, endothelial cells as well as other hematopoietic cells complicating any interpretation as to the cell type mediating the observed positive effects. The efficacy of BM-MNC cell-based therapy has been reported in the treatment of tibial nonunion fracture [63] and osteonecrosis of head of femur [64] and positive results were also reported using cultured expanded autologous BMSC in the treatment of femoral and tibial osteotomies [65]. Mesimäki et al. have reported on the creation of a vascularized bone graft for reconstruction of a mandible of a 65-year patient with hemimaxillectomy due to a recurrent keratocyst [66].

Translational studies using SSC-containing populations are ongoing. Table 1 details 13 trials currently underway as recorded on the website clinicaltrials.gov maintained by the National Institutes of Health. It is thus apparent that randomized clinical trials using defined and characterized skeletal cell populations are needed to evaluate the efficacy of SSC-based therapy as well as the magnitude of the clinical effects compared to standard therapies.

Summary

The existence of a renewable population of SSCs holds exciting possibilities in regenerative medicine for cell-based tissue engineering approaches to bone regeneration and repair. In comparison to other cell sources, including pluripotent embryonic stem cells and multipotent adult stem cell populations from a range of connective tissues, the relative accessibility of an autologous osteoprogenitor population has fuelled significant progress in the potential application of SSC therapy in the clinic. However, to date, limited understanding of the SSC fate, immuno-phenotype, and selection criteria has proved to be a limiting factor in the widespread clinical application of these cells. New areas of research will also focus on analysis of the phenotypic fingerprint of a SSC at a single-cell resolution, together with the derivation of skeletal cells from pluripotent stem cell sources. Ultimately, approaches will include the development and integration of immuno-privileged constructs containing an appropriate scaffold/growth factor(s) composition for autologous and potentially allogeneic skeletal populations. A multidisciplinary approach harnessing clinicians and life scientists will aid our understanding of the continuum of skeletal cell development, developmental paradigms, skeletal niche, and skeletal cell plasticity. As the world population passes 7 billion and people live longer, robust clinical translation of SSC science holds great potential to improve the quality of life of individuals in a society with an increasing ageing population.

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Author contributions

J.D., J.K., and R. T.: collection and assembly of data, data analysis and interpretation, manuscript writing, and final approval of manuscript; M.K.: financial support, collection and assembly of data, data analysis and interpretation, manuscript writing, and final approval of manuscript; R.O.: conception and design, financial support, collection and assembly of data, data analysis and interpretation, manuscript writing, and final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors have no potential conflicts of interest.

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