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Regenerating musculoskeletal tissues: possibilities for rheumatoid diseases

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Rheumatoid arthritis is a chronic inflammatory systemic autoimmune disease that destroys cartilage and peri-articular bone. Recent therapeutic advances for this disease have yielded promising results, the most notable of which have been pharmacologic agents that block tumor necrosis factor (TNF)-α. Despite these advances, the search for new therapies continues, amongst which stem cells are being developed for potential applications in cartilage- and bone-tissue engineering. Given the large clinical demand for such stem-cell applications, muscle-derived stem cells are being heavily investigated due to their ease of isolation and ability to differentiate into multiple lineages, including osteogenic and chondrogenic lineages. Furthermore, when genetically modified *ex vivo* to express growth factors, these cells can repair bone and cartilage in animal models. Accordingly, regenerative therapies and tissue engineering that are based on muscle-derived stem cells are emerging with promising experimental results thus far for treating various types of bone and cartilage injuries, including those caused by rheumatoid arthritis.

Keywords: cartilage tissue engineering, gene therapy, muscle, rheumatoid arthritis, stem cell

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Current treatments for rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by pain, swelling and the subsequent destruction of joints. This disease involves the synovial membrane, which becomes inflamed and exposed to inflammatory cytokines that progressively destroy bone and cartilage and portents patients to functional disability, substantial morbidity and even accelerated mortality [1].

For many years, RA has been treated with disease-modifying anti-rheumatic drugs (DMARDs), albeit with limited effects on the radiological progression of the disease that now has restricted use of these agents primarily to methotrexate and sulfasalazine, all of which have had better clinical outcomes compared with other DMARDs [2]. Efforts to seek alternative therapies and recent progress in biotechnology have led to our enhanced understanding of the immunopathogenesis for RA, consequently facilitating the development of novel therapies that target specific dysregulated components of the immune system. Such therapies focus on targeting pro-inflammatory cytokines that play a crucial role in the pathogenesis of this disease, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1 and -6 [3–5]. For some time now, TNF-α inhibitors in particular have yielded dramatic therapeutic improvements and revolutionized treatment paradigms for RA [6–8]. Despite improvements seen with these agents – most of which displayed virtually no serious adverse effects on initial studies – the expanded use of TNF antagonists has begun to unmask complications that include hematologic abnormalities such as aplastic anemia and lymphoma, as well as other cancers, lupus-like autoimmune disease and multiple sclerosis-like demyelinating disorders, severe allergy, infection, aseptic meningitis, vasculitis and liver disease [9–16,201]. Whereas these side effects are quite rare, researchers now pursue new strategies for treating RA that may reduce such limitations and side effects or replace these drugs.

Bone- & cartilage-tissue engineering using stem/progenitor cells

Bone-tissue engineering

A recently evolving strategy for treating various diseases, including RA, has been to develop the use of stem cells for regenerative medicine and tissue engineering. Stem cells display multipotency toward various lineages of organ-specific precursors and progenitor cells that enable them to repopulate and differentiate into multiple types of tissues. Adult stem and progenitor cells of the mesenchymal lineages in particular are the focus of intense research as they are readily accessible from various tissues and organs such as bone marrow (BM) [17–19], peripheral blood or blood vessels [20–22], adipose tissue [23,24], synovium [25,26], umbilical cord blood [27,28] and skeletal muscle [29,30].

A specific focus in regenerative medicine has been to use BM-derived mesenchymal stem cells (BMMSCs) to regenerate large segmental bone defects that result from trauma and tumor resection, as well as joint destruction that results from

metabolic and autoimmune diseases (AD) such as RA. While segmental defects are presently treated with bone auto- and allografts, there are only limited quantities of bone that can be harvested from a single donor, making tissue engineering with these and other highly proliferating stem cells an attractive therapeutic alternative that has been successful thus far in animal models [31–36]. Based on the preclinical success of this cell-based therapy for addressing bone injuries [37] and even addressing osteogenesis imperfecta [38], the transplantation of whole BM cells or BMMSCs for bone regeneration is now entering clinical trials [39].

As with BMMSCs, human peripheral blood endothelial progenitor cells (EPCs) [40–42] are being investigated to specifically address the problem of delayed and atrophic non-unions in fracture healing, which has a significantly high (5–10%) annual incidence amongst all long bone fractures and result from an inadequate local blood supply around the zone of injury [43,44]. Because securing an adequate blood supply to this area is crucial for bone healing to occur [45,46], as would be evidenced radiographically by the formation of bridging callus along a former fracture gap, an emerging focus in regenerative medicine is to develop EPCs to promote neoangiogenesis. EPCs are appealing for this task in large part because the link between angiogenesis and the development of native bone on a larger scale has led to the discovery on a cellular level that there exists a developmental reciprocity between endothelial cells and osteoblasts [47]. EPCs are also appealing for this task because a more traditional approach for enhancing the local vascularity along a non-union or delayed union has been to perform vascular bone grafting, which requires painstaking microvascular surgical skills [43].

The osteogenic potential of human peripheral blood EPCs has been discovered to occur both directly through osteogenic differentiation [48–51] as well as indirectly via local induction of osteogenesis and/or angiogenesis [51–53]. With regards to the former, 20% of human circulating $CD34^+$ cells co-express the osteoblast-specific marker, osteocalcin, as detected by single-cell reverse transcriptase (RT)-PCR [51]. In addition to these peripheral blood endothelial progenitors, circulating skeletal progenitor cells have also been isolated [20,21], with recent reports demonstrating that 37% of osteocalcin-sorted osteoprogenitor cells co-expressed the CD34 cell-surface marker, thus suggesting that these skeletal progenitor cells somehow overlap developmentally with EPCs [54]. As EPCs are highly and readily accessible within the peripheral circulation and comprise a population of cells with high osteogenic and endothelial potential, they represent an important cell population for up-and-coming strategies for overcoming the problem of large segmental bone defects, as well as delayed and non-unions.

Cartilage-tissue engineering

Cartilage is often subject to full-thickness injuries and osteochondral defects that are caused by diseases such as RA and osteoarthritis (OA). To complicate matters, this tissue has a poor vascular, nerve and lymphatic supply, all of which makes it difficult to regenerate this tissue and render patients with a poor prognosis for healing after damage. Over time, unabated cartilage damage lead to advanced osteoarthritis, which often requires substantial surgery such as total knee arthroplasty. Accordingly, there is a great demand for advances in the field of cartilage-tissue engineering.

In the past decade, autologous chondrocyte implantation (ACI) has emerged as a novel therapy for cartilage regeneration, in which autologous chondrocytes are isolated from a cartilage biopsy, expanded *in vitro*, and seeded a periosteal flap for implantation onto the site of an osteochondral defect. While this procedure has been received with much excitement, its efficacy has been the focus of numerous clinical investigations through which its inherent limitations have been exposed [55–62]. Among these limitations are: the low cell density of each mature donor cartilage harvest; the concern for a potential leakage of the cells from the acceptor-site defect; and an uneven distribution of remaining cells below the periosteal flap, all of which confer a substantial risk for uneven surface, hypertrophy and ossification. In order to address these problems, chondrocyte-seeded collagen type I/III membranes have substituted the periosteal seal as a way to secure the implanted cells to the defect area [63]. This biomaterial thus far seems clinically promising [64], and a similar chondrocyte-seeded hyaluronan-based biodegradable polymer scaffold has also yielded good short-term results [65,66]. Presently, however, the long-term utility of chondrocyte transplantation remains unclear, in part because of reports on this cell-type's dedifferentiation and loss of reparative ability over time [67–69].

More recently, implantation of stem cells for cartilage regeneration has been the subject of much interest in regenerative medicine, in large

part because these cells display a superior proliferative capacity and tolerance for stress when compared with aged chondrocytes. BMMSCs in particular can undergo *in vitro* chondrogenesis when exposed to TGF-β and incubated in a 3D culture environment (e.g., cell pellets and micromasses). These cells can also upregulate the *in vivo* expression of type II collagen and aggrecan, as well as the *in vivo* synthesis of cartilage matrix for up to 4 weeks after being lipofected and injected into a sheep model [70]. To date, several reports on BMMSCs indicate that these cells have great potential for cartilage regeneration and repair in experimental cartilage injury models [71–75], with studies on autologous stem cell-based tissue engineering now entering clinical phases for cartilage repair and regeneration [76,77].

In addition to BMMSCs, stem cells isolated from the synovium are being investigated for their chondrogenic potential. Following the first report on synovium-derived stem cells by De Bari *et al.* [25], Sakaguchi *et al.* have reported that compared with BM-, periosteum-, adipose-, and muscle-derived stem cells, these cells have the best potential for chondrogenesis *in vitro* [26,78]. Subsequent *in vivo* experiments have confirmed that synovium-derived stem cells do contribute to cartilage regeneration [79].

Muscle derived cells – a putative source of stem cells

A population of regenerative cells that has recently been heralded for its remarkable potential in the field of tissue engineering and regenerative medicine is that of muscle-derived cells (MDCs). These cells are rapidly gaining popularity because they can be safely obtained in a minimally-invasive manner through a skeletal muscle biopsy, subsequently tolerate *ex vivo* manipulation very well, and are thereby easily transduced with a variety of viral vectors. Because of this, they have been used in several clinical trials [80–84].

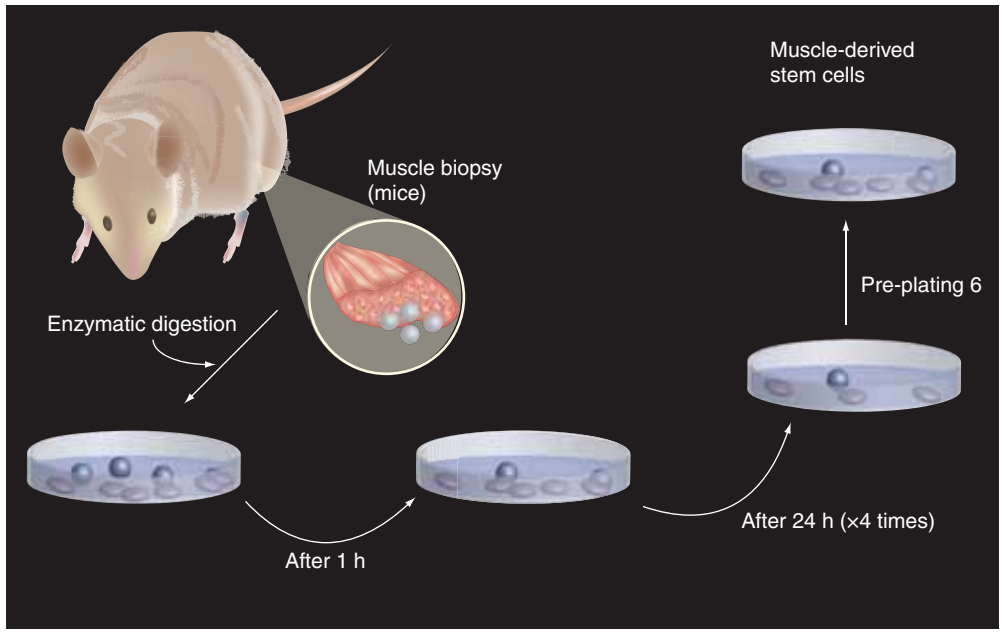
MDCs consist of a heterogeneous group of cells that predominantly consists of two broad populations, including satellite cells and a subset of multipotent adult muscle-derived stem cells (MDSCs) [85–87]. The satellite cells are located beneath the basal lamina of mature skeletal muscle fibers and have long been considered to only give rise to cells of the myogenic lineage, whereas MDSCs to date have been isolated from skeletal muscle of postnatal mice by using the pre-plate technique (Figure 1) and are being recognized for their multipotency [88–92]. While there are also side-population cells, mesoangioblasts and pericytes that are starting to become considered as other categories of skeletal muscle cells with regenerative potential [30,86,93], the origin of these cells and their relationship to satellite cells or MDSCs remains unclear.

As noted, MDSCs can be isolated from skeletal muscle through the preplate technique in a highly purified fashion. These cells exhibit the capacity for long-term proliferation, immune-privileged behavior and multilineage differentiation both *in vitro* and *in vivo* [30,94], all of which are important features for regenerative therapies. MDSCs are isolated in low ratios of 1:100,000 from murine skeletal muscle, yet they maintain great fidelity to their cellular characteristics by the time they are sizeably populated, much as is seen with BMMSCs [92]. Accordingly, only a small muscle biopsy is sufficient for large-yield therapeutic gains. The therapeutic application of MDSCs has already been demonstrated in a mouse model for Duchenne muscular dystrophy with great success, where, unlike with satellite cells and myoblasts, MDSCs have significantly improved the efficiency of muscle regeneration and the delivery of dystrophin to dystrophic muscle.

While the isolation of MDSCs is currently limited to the murine model, our group has recently isolated a population of myoendothelial cells from adult human skeletal muscle, which is a newly discovered type of MDC. These cells differ from satellite and endothelial cells that are isolated from the same source in that they uniquely co-express myogenic and endothelial cell markers. These myoendothelial cells demonstrate a very good capacity for regenerating injured skeletal muscle and undergoing myogenic, chondrogenic and osteogenic differentiation *in vitro* [95]. A similar type of regenerative cell that we have isolated from humans is the well-known pericyte, which is isolated from microvascular walls. Using flow cytometry, our group and those of others have isolated human pericytes that are myogenic precursors distinct from satellite cells, and may be a promising candidate for upcoming cell-therapy endeavors [22,95,96].

As we will highlight, skeletal MDCs or MDSCs are being used to improve musculoskeletal healing after injury in bone and cartilage, similar to BMMSCs and EPCs. These cells may also be used for the healing of muscle, ligament and meniscus, although more research in this area is necessary. In any case, MDSCs are able to effectively deliver growth factors and cytokines through gene therapies for musculoskeletal diseases (e.g., Duchenne muscular dystrophy) and beyond (e.g., hemophilia B and diabetes),

Figure 1. Isolation of muscle derived stem cells using the pre-plate technique.



Muscle-derived cells are enzymatically dissociated from mouse skeletal muscle. Myogenic cells are seeded – thus, the population of nonadhering cells is re-plated for 1 h to obtain a second pre-plate population. This procedure is then repeated in 24 h intervals until the sixth pre-plate population, in order to further purify the myogenic cells. The sixth pre-plate population takes an additional 24–72 h to attach to collagen-coated dishes, and this adhesive population is used as muscle-derived stem cells.

making these cells excellent candidates for the development of therapies for bone and cartilage injuries secondary to RA.

Bone- and cartilage-tissue engineering using muscle-derived cells

Bone-tissue engineering

Our initial work on bone-tissue engineering involved experiments with severe combined immunodeficiency (SCID) mice in which their osteogenic potential is achieved by exposing them to bone morphogenetic proteins (BMPs), or also as genetically modified MDSCs to express BMP-2. In each case, MDSCs formed ectopic bone along the hindlimb muscle and elicited complete closure of critical-sized skull defects of the recipient mice [97,98]. Through this research, we confirmed that MDSCs do indeed differentiate towards the osteogenic lineage by identifying, amongst MDSCs containing a *LacZ* marker gene, a portion of cells that co-express β-galactosidase and the osteogenic differentiation marker, osteocalcin [99,100]. Additionally, from ectopic bone and rat calvarial defect regenerates formed from genetically engineered MDCs and MDSCs, our group clonally isolated MDSCs and found that 95% of these cells exhibited osteogenic differentiation [88].

In a similar fashion, we performed experiments on immunocompetent rats in which we transduced MDSCs with a *BMP-4*-eoncoding retrovirus and subsequently formed *de novo* bone where we transplanted these cells. While these mice did generate a local immune reaction, this did not interfere with osteogenesis [101]. It therefore appears that MDSCs have a lower immunogenicity, which thereby permits them to persist longer at the sites of transplantation, perhaps making them better cellular vehicles than primary MDCs for bone formation through *ex vivo* gene therapy. Several studies on genetically engineered primary MDCs have confirmed that these cells can induce ectopic ossification and heal rat calvarial defects [102–104].

As fracture healing relies heavily on the local blood supply, we transduced MDSC–BMP-2 and MDSC–BMP-4 with *VEGF* to determine whether this would impact bone-tissue engineering. We implanted these cells into the muscle pockets of mouse calvarial defects, and noted that in the early phase of endochondral ossification, VEGF did not significantly impact chondrogenesis in the BMP-2 group, but did so for the BMP-4 group, and by the end of this process, there was a larger amount of bone

formed in the latter compared with the former [105,106]. Overall, recipients of the *VEGF–BMP* constructs displayed greater amounts of bone formation compared with mice receiving MDSCs expressing BMP but not VEGF. In citing this work, it is important to note that increasing the local vascular supply may, in addition to providing tissues with homeostatic nutrients, provide a portal by which other stem cells that are present in the circulation can be chemo-attracted at the site of injury.

It is likely that by enhancing the local vascularity, VEGF enhances the oxygenation of local tissues and cells. This is worth mentioning because it appears that oxygen tension provides an environmental stimulus that drives stem cells to differentiate into either osteogenic, chondrogenic or fibroblastic lineages. In a pioneering study by Urist *et al.* in which BMP was discovered, the mixture of connective tissue cells and BMP formed cartilage when placed in an avascular environment, and bone within a vascular environment [107]. Additionally, Bassett and Herrmann formed bone and cartilage when MDCs were exposed to low oxygen tensions with compaction, and fibroblasts when these cells were exposed to high oxygen concentrations with mechanical tension [108]. Several studies since then have confirmed that low oxygen tension steers mesenchymal stem cells (MSCs) to differentiate into the chondrocyte lineage, in part by upregulating a program of chondrocyte-specific gene expression under the control of hypoxia-inducible factor 1 (HIF-1) [109–112]. Additionally, numerous clinical reports implicate hypoxia in the pathogenesis of heterotopic ossification, by which MSCs pathologically form bone along the soft tissues [113–116]. Not surprisingly, our research indicates that bone formation is influenced by the ratio of VEGF to BMP, where bone healing occurs with low VEGF:BMP-4 ratios [105].

While it is important to devise ways in which stem cells can produce bone, it is equally important to have them do so in a controlled fashion. In order to accomplish this, we engineered a self-inactivating tet-on retroviral vector to modulate BMP-4 expression *in vitro* and regulate bone formation *in vivo* [117]. After implanting MDSCs transduced with this vector into critical-sized calvarial defects, we initially noticed residual bone formation without induction and bony overgrowth after induction, even after reducing the number of implanted cells. We then co-implanted MDSCs expressing BMP-4 with those expressing Noggin, a BMP antagonist, into the hindlimbs of mice and critical sized calvarial defects, and were subsequently able to inhibit the amount of bone formation in a dose-dependent manner. This permits us to obtain a tighter control of osteogenesis with gene therapy [118,119]. Remarkably, the bone that we have generated through these experiments is anatomically and histologically similar to native bone.

Finally, BMP-4-expressing MDSCs have therapeutic applications for orthopedic patients with large segmental bone defects secondary to the resection of tumors or infected and noninfected non-unions, as well as to acutely comminuted open fractures. While allografts and autografts are traditionally used to occupy defects that are void of bone, allografts have a limited healing capacity and autografts are limited by their low availability. By contrast, stem cells can be combined with various scaffolds to promote bone healing. Most scaffolds possess osteoconductive properties and must be infused with osteoinductive agents, including growth factors or cells engineered to secrete BMPs to induce *de novo* bone formation. Our group has used collagen and gelatin sponge scaffolds carrying BMP-4-expressing MDCs to regenerate mouse calvarial defects, albeit with bony overgrowth. While spongeous materials are also available for such use, gel scaffolds have the distinct advantage that they can be applied to a defect through an injection rather than through an open surgical wound.

Cartilage-tissue engineering

Several groups including ours have successfully repaired full-thickness cartilage defects in the knees of rats and rabbits by combining stem-cell therapy with *ex vivo* gene therapy. In our studies, we adenovirally transduced skeletal MDCs with either insulin growth factor-I (IGF-I) or BMP-4, and seeded these cells into collagen gel or fibrin sealant matrices for implantation [120]. These defects healed remarkably well when compared with untreated rabbits, without any evidence that our delivery device adversely affected the *in vivo* viability, proliferation or differentiation of our MDCs. Using the same animal model, Adachi *et al.* transduced purified MDCs with *LacZ*, cultured these cells *in vitro* for 3 weeks, and seeded them into bovine type I collagen gels for delivery into injured knees [121]. This group compared the healing of osteochondral defects of these animals to that of rabbits receiving chondrocyte transplantations, and showed that autologous MDCs healed defects with better integration and more expression of type II collagen for up to 24 weeks.

Using immunodeficient rats, Kuroda *et al.* [122] obtained similar results using MDSCs transduced with *BMP-4* and, additionally, detected *LacZ* transgene expression in repaired tissues at 12 weeks post-transplantation, as well as a persistent repair of the osteochondral defects in histological grading up to 24 weeks after surgery. These studies suggest that MDCs serve as both a gene-delivery vehicle and a population of stem cells that differentiate into chondrocytes capable of repairing cartilage defects.

As previously noted, there is emerging evidence that cartilage-tissue engineering can be augmented by inhibiting the expression of and antagonizing VEGF. A characteristic of chondrogenesis is that, in its terminal stages, there are high levels of VEGF expression and angiogenesis. This vascularity lead to endochondral ossification [123,124], making it important to control VEGF signaling during the chondrogenic differentiation of stem cells in order to steer these cells toward the formation of articular cartilage rather than bone. Using MDSCs retrovirally transduced with chondrogenic genes such as *BMP-4*, our group has suppressed VEGF expression and used the VEGF antagonist, s-Flt1, to block angiogenesis. As a result, we increased the expression of these genes by MDSC, ultimately improving the regeneration of articular cartilage (Matsumoto T, Stem Cell Research Center, Children's Hospital of Pittsburgh and the Department of Orthopedic Surgery, University of Pittsburgh Medical Center, PA, USA. Unpublished Data). In the study, *sFlt1* gene therapy improved BMP4-induced chondrogenic gene expression of MDSCs *in vitro*, and improved the persistence of regenerated articular cartilage by preventing vascularization and bone invasion into the regenerated articular cartilage (Matsumoto T. Unpublished Data). These phenomena were confirmed not only in a full-thickness cartilage defect model, but also in a model for osteoarthritis (OA) in immunodeficient rats (Matsumoto T. Unpublished Data). When delivered via intracapsular injection into these rats, *BMP-4*-transduced MDSCs differentiated into chondrocytes and displayed an increase in chondrogenesis compared with nontransduced MDSCs via BMP-4 in an autocrine/paracrine manner, while s-Flt1-transduced MDSCs blocked VEGF to provide an environment in which chondrocytes underwent proliferation rather than apoptosis. By combining both cells, there was ultimately substantial cartilage regeneration and healing. It is interesting to note that these data are consistent with our discussion above on bone regeneration and prior data on VEGF, and the likely role of oxygen tension on steering stem-cell differentiation toward various different lineages.

Therapeutic potential of stem cells for rheumatoid arthritis

Joint destruction in RA results from a systemic autoimmune process in which therapy has accordingly focused on the use of anti-inflammatory and immunosuppressant drugs. Much as in other AD, such as multiple sclerosis, systemic sclerosis, juvenile idiopathic arthritis and systemic lupus erythematosus, RA is caused by an immunologic imbalance and a loss of immunologic tolerance in which the immune system ultimately approaches major histocompatibility complex (MHC)-II antigens along host tissues as foreign bodies, rather than native proteins, and thereby attacks various organs that specifically express these antigens. This process can be initially mediated by immune complexes, circulating autoantibodies or autoreactive T lymphocytes.

While conventional AD therapies are effective in most patients, resistance to anti-inflammatory and immunosuppressant agents is not uncommon. Furthermore, some patients are capable of responding only to high doses of such medications, placing them at risk for serious adverse effects such as infection, cancer and poor tissue healing, amongst other ill effects. In such cases, stem cells may provide an important clinical strategy for treating these diseases either alone or with the combination of anti-inflammatory and immunosuppressive drugs. Accordingly, the therapeutic potential of stem cells for treating RA is currently being developed through animal research on bone- and cartilage-tissue engineering with various multipotent cells, including hematopoietic, mesenchymal and muscle-derived stem cells. These emerging therapeutic avenues will be discussed below.

Hematopoietic stem cells

While the immune system and mesenchymal tissue are comprised of cells with different functional roles, there is mounting evidence that hematopoietic stem cells (HSCs) are cellular precursors of the immune system and can interact with osteoblasts to regulate this system [125–128]. This is an important discovery, as patients with AD are often immunosuppressed by drastic methods such as immunoablation, and subsequently require BM reconstitution with HSCs. According to studies using experimental animal models of AD [129], as well as clinical reports on AD patients, HSC

transplants (HSCT) conferred autologous tolerance and disease remission, respectively [130,131], making HSCT a promising therapy for severe AD such as RA, multiple sclerosis, systemic sclerosis, juvenile idiopathic arthritis and systemic lupus erythematosus in the past several decades [132–141]. In addition to supplementing high-dose immunosuppression with HSCT, HSC mobilization with granulocyte-colony stimulating factor has also become a therapeutic approach for many immune-mediated diseases. Of note, HSCT has been shown in JIA patients to restore $CD4^+/CD25^+$ T cells, which are the principal regulators of the immune system [142].

Despite these encouraging findings, there are limitations to combining immunoablation with HSCT. Specifically with RA, some patients enter relapses in which analyses of synovial-infiltrating lymphocytes suggests that the initial ablation was incomplete, as local or lesional T cells were found to be derivatives of the pre-treatment BM [143]. This suggests that even after BM reconstitution with HSCT, some level of immunosuppression is still required to be therapeutically desired.

Mesenchymal stem cells

The combination of HSCT with just the right amount of immunosuppression may be obtained by engrafting HSCs with MDSCs at the time of BM reconstitution. These cells are not only multipotent, but also confer anti-proliferative and immunomodulatory effects on the recipient immune system, thereby reducing the risk for transplant rejection, and perhaps even disease recurrence from T cells that persist from before immunoablation. Accordingly, while HSCT provides a way to reconstitute BM and thereby address aplasia, it is the MSCs that may actually be responsible for the direct therapeutic effects of immunosuppression.

In support of the immunosuppressive role that MDSCs can play, several researchers have reported that T- and B-lymphocyte proliferation, either occurring in mixed lymphocyte cultures or induced *in vitro* by mitogens and antibodies, can be suppressed by these cells in a dose-dependent and MHC-independent fashion [144–151]. This suppression persists in human cell cultures even after separating MSCs from lymphocytes in transwell assays, indicating that cell-to-cell contact is not required [144,148,152]. From an *in vivo* standpoint, an immunosuppressive effect of MSC was first suggested in a baboon model, where infusion of *ex vivo*-expanded donor or third-party MSC delayed the time to rejection of histoincompatible skin grafts [149]. Based on these findings, researchers employed MSCs to successfully treat experimental T-cell-mediated autoimmune encephalomyelitis in an animal model [153,154].

While the immunosuppressive effects of MSCs are appealing in many regards, this effect also warrants caution. Fortunately, clinical trials in which *ex vivo*-expanded MSCs have been intravenously infused have thus far been free of any adverse events during and after infusion [155–159]. While low levels of engrafted MSCs have been detected in several tissues, durable stromal cell chimerism has been difficult to identify [156,159,160]. In light of this, it is worth highlighting a recent case report in which the systemic infusion of MSCs suppressed a grade IV graft-versus-host disease in a 9-year-old child who had previously received a BM transplant [161]. Therefore, thus far MSCs represent promising avenues through which to direct the local paracrine production of therapeutic growth factors and provide a form of immunosuppressive therapy that shows no evidence, to date, of adverse effects that accompany more traditional forms of immunosuppression and immunomodulation.

As mentioned, a potential therapeutic approach for AD may be to combine more traditional immunosuppressive modalities, such as anti-inflammatory drugs or steroids, with stem-cell therapy [162]. When considering such combination therapies, however, it is important to recognize that the proliferation and differentiation potential of at least some types of stem cells may be compromised by the use of steroids, in particular. In one study, Cui *et al.* demonstrated that pluripotential BM stromal cells become increasingly adipogenic and less chondrogenic over time when exposed to dexamethasone in a dose-dependent fashion [163]. While this work consisted of *in vitro* experiments, it is conceivable that in an *in vivo* AD model in which BM reconstitution is being performed with stem cells, steroids may compromise the pluripotency of implanted stem cells and perhaps induce fatty infiltration of the marrow. Interestingly, this same group found that this adipogenesis can be inhibited with lovastatin both *in vitro* and in a chicken model for osteonecrosis of the femoral head [164]. In contrast to these results, Kastrinaki *et al.* found no difference in the clonogenic and proliferative potentials of MSCs of RA patients untreated and treated with antirheumatic agents such as methotrexate, corticosteroids or anti-inflammatory agents [165]. However, they did find a difference between RA and healthy patients when comparing these parameters in isolated

stem cells. This may suggest that BMMSCs from healthy patients may be therapeutically beneficial when transplanted into RA patients.

Muscle-derived stem cells

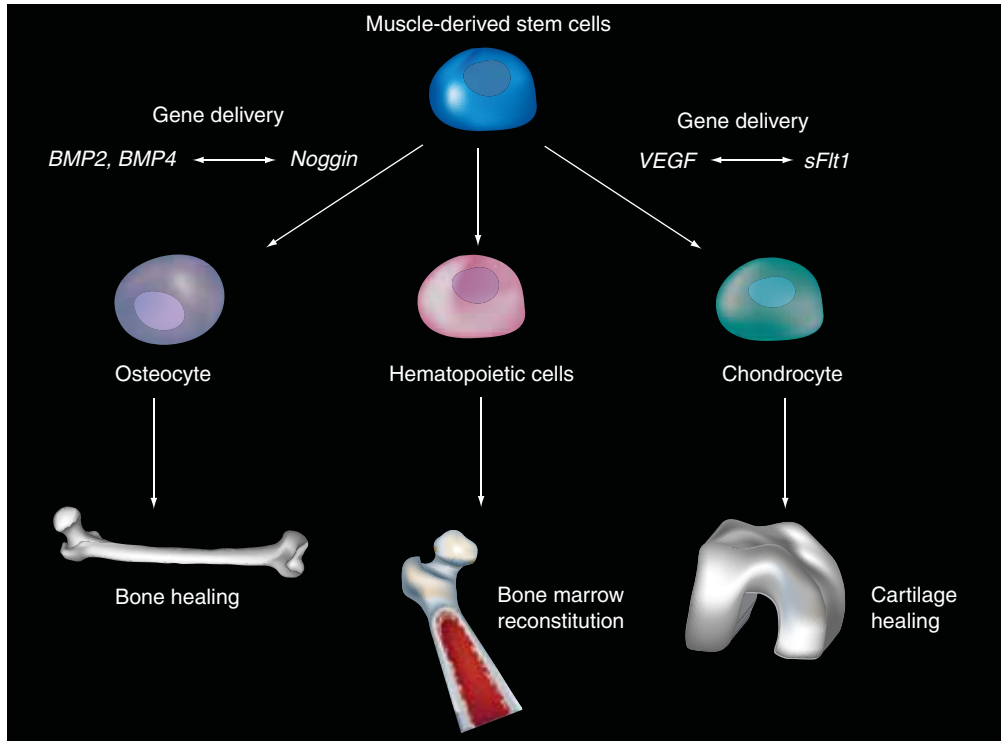
As MDSCs are a novel population of highly proliferative, self-renewing and multipotent muscle stem cells that display an immune-privileged behavior, these cells have tremendous potential for bone and cartilage regeneration in RA patients. Full-thickness articular cartilage defects in our experimental models have already been very promising to this end, as described above [122]. Recently, we found gender differences in the treatment efficacy of this model, in which the transduction of male MDSCs with BMP-4 displays greater proliferation and better chondrogenic potential *in vitro*, as well as cartilage regeneration *in vivo* when compared with female MDSCs (Matsumoto T. Unpublished Data). While the prevalence of RA is higher in females than in males, it is unclear how these findings will, if at all, impact the treatment of different genders. Specifically, it remains to be seen whether joint repair in females through the use of MDSCs would be more efficacious with autologous cells or with allogeneic cells obtained from male donors. If the latter proves to be the case, then this gender difference may provide a clinical strategy for the allogeneic use of MDSCs to repair the joints of RA.

Finally, purified MDSCs are capable of differentiating into hematopoietic lineages from which the immune system develops [88,166–176]. In fact, MDSC express the hematopoietic stem cell markers CD34 and Sca-1, suggesting that these cells contain intrinsic characteristics of HSCs that may make them capable of not only regenerating bone and cartilage, but also of maintaining BM homeostasis and possibly even reconstituting immunoablated BM (Figure 2). While limited evidence suggests that these cells are not too immunosuppressive, it remains to be seen whether their suppressive effects are sufficient to render RA patients with an effective therapeutic modality, and thereby give these cells an advantage over the other stem cells for treating this and other similar AD.

Conclusions & future perspective

Stem cells are capable of multilineage differentiation toward bone and cartilage, reconstituting the

Figure 2. Therapeutic potential of muscle-derived stem cells for rheumatoid arthritis.



Muscle-derived stem cells have capacities for multilineage differentiation, especially toward bone and cartilage, suggesting the possibility in therapeutic application for joint destruction of rheumatoid arthritis. The potential of muscle-derived stem cells for hematopoietic differentiation also provides a useful strategy and widens clinical application for rheumatoid arthritis.

BM, and inducing anti-inflammatory and immunomodulatory effects in activated target cells, all of which are emerging criteria for an effective clinical treatment strategy for RA. MDSC-based regenerative therapy and tissue engineering using *ex vivo* gene therapy provide promising approaches for treating various types of bone and cartilage injuries, including those caused by RA.

We believe that stem cell-based therapy and tissue engineering may one day provide the solution for patients suffering from RA. Before we can employ the routine use of stem cells in clinical settings, further *in vitro* and *in vivo* investigations are required to better delineate their mechanisms and better define each clinical concept that is necessary for effective therapies. Toward this end, some have recently reported on the anti-inflammatory and immunomodulatory effects of BMMSCs with promising clinical results for treating RA. As these cells share many characteristics with MDSCs, it is likely that MDSCs may be the focus of such studies targeting RA and other AD in the future.

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Executive summary

Bone- & cartilage-tissue engineering using stem cells

- Sources of stem cells: bone marrow (BM), peripheral blood or blood vessels, adipose tissue, synovium, umbilical cord blood and skeletal muscle.
- Bone-tissue engineering: BM-derived mesenchymal stem cells (BMMSCs), circulating skeletal progenitor cells and circulating $CD34^+$ cells (endothelial progenitor cells), amongst others.
- Cartilage-tissue engineering: autologous chondrocyte implantation (ACI), second-generation of ACI, BMMSCs and synovium-derived stem cells combined with various scaffolds, amongst others.

Muscle-derived stem cells (MDSCs)

- Capacity for differentiation toward the myogenic lineage and mesenchymal multilineage.
- Long-term proliferation ability and the capacity for self-renewal and immune-privileged behavior.
- Human-muscle-derived cells: myoendothelial cells that co-express myogenic and endothelial cell markers with a superior capacity to regenerate injured skeletal muscle and multipotent differentiation toward myogenic, chondrogenic and osteogenic lineage, when compared with other muscle cells.

Bone & cartilage tissue engineering using MDSCs

- Bone-tissue engineering: MDSC-based *ex vivo* gene therapy with a retrovirus encoding bone morphogenic protein (BMP)-2 or -4, and MDSC-based *ex vivo* gene therapy with a retrovirus encoding BMP-2 or -4, as well as VEGF.
- Cartilage-tissue engineering: muscle-derived cell (MDC)-based *ex vivo* gene therapy, MDSC-based *ex vivo BMP-4* gene therapy, and MDSC-based *ex vivo BMP-4* and *sFlt1* (VEGF antagonist) gene therapy combined with collagen gel or fibrin sealant matrices.

Therapeutic potential of stem cells for rheumatoid arthritis

- Stem cells including MDSCs with a high potential for bone- and cartilage-tissue engineering in rheumatoid arthritis (RA).
- Hematopoietic stem cells (HSCs) with the capacity to reconstitute BM for maintaining homeostasis.
- Mesenchymal stem cells (MSCs) with multipotent differentiation, supporting cells for HSC engraftment and anti-proliferative and immunomodulating cells.
- MDSCs with the potential for hematopoietic as well as osteogenic and chondrogenic-lineage differentiation.

Conclusion & future perspective

- Stem cell (including MDSCs) based regenerative therapy and tissue engineering using *ex vivo* gene therapy have capacities for multi-lineage differentiation toward bone and cartilage, reconstituting BM and inducing anti-inflammatory and immunomodulatory effects in activated target cells, providing the clinical strategy for the treatment of RA.
- Allogeneic use of stem cells in the clinical setting of RA needs further *in vitro* and *in vivo* investigations for identification of the mechanism of each concept.

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