Engineering Stem and Stromal Cell Therapies for Musculoskeletal Tissue Repair

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Stem cells and tissue-derived stromal cells stimulate the repair of degenerated and injured tissues, motivating a growing number of cell-based interventions in the musculoskeletal field. Recent investigations have indicated that these cells are critical for their trophic and immunomodulatory role in controlling endogenous cells. This Review presents recent clinical advances where stem cells and stromal cells have been used to stimulate musculoskeletal tissue repair, including delivery strategies to improve cell viability and retention. Emerging bioengineering strategies are highlighted, particularly toward the development of biomaterials for capturing aspects of the native tissue environment, altering the healing niche, and recruiting endogenous cells.

Musculoskeletal tissue injuries and degeneration are common and debilitating for a high number of patients (Brooks, 2006). Unfortunately, endogenous musculoskeletal tissue regeneration is limited in many cases and may be affected by inflammation and the degree of damage. For example, most fractures of long bones heal spontaneously, whereas large segmental defects fail to heal. Additionally, although articular cartilage has almost no intrinsic reparative potential, tendons and ligaments may heal, but often with inferior properties. The high prevalence of these injuries has led to significant investment in the development of new therapies to enhance healing and augment current surgical interventions. Often the goal is to mimic and recapitulate the natural healing cascade and developmental process by transplantation of tissue-specific stromal and progenitor cells or by endogenous manipulation to enhance the native repair capacity of cells.

There has been a continuing increase in the number and type of stem and stromal cells being pursued in human clinical trials for treatment of musculoskeletal injuries (Steinert et al., 2012). Most approaches in this area are based on *ex vivo*-expanded mesenchymal stromal cells (MSCs) derived from bone marrow (BM). Originally identified and characterized by their multilineage differentiation potential *in vitro*, multipotent capabilities of MSCs *in vivo* have not been clearly demonstrated to date, particularly because of the lack of methods to identify and define differentiated populations (Nombela-Arrieta et al., 2011). Central to recent progress in the field has been the understanding that stem and progenitor functions of MSCs may not be the key attribute that mediates tissue repair. In addition, there is outstanding controversy over the terminology of exogenously supplied MSCs as stromal cells, and various terms, including medicinal signaling cells, have been proposed to more accurately reflect their therapeutic function *in vivo* (Caplan, 2017). Nevertheless, the therapeutic benefit of these cells has been largely explored. Significant advances have been made in developing strategies that deliver, protect, and recruit stem cells, and the bioengineering field is evolving to improve current surgical techniques.

This review first describes current treatments and reports the recent progress in clinical investigations of stem and stromal cell-based therapies for musculoskeletal repair with a particular focus on bone and fibrocartilaginous tissues. The current understanding of appropriate cell sources and delivery strategies is then illustrated toward endogenous repair of musculoskeletal tissues. Last, emerging therapeutic concepts are highlighted in the context of biomaterials as a particularly attractive tool to control stem and stromal cell behavior both *ex vivo* and *in vivo*, to recruit endogenous stem cells, and to control the local healing environment. Such approaches have great potential for future therapies in musculoskeletal repair.

Current Surgical Interventions and Associated Limitations

Damage from trauma is a major cause for surgical repair of musculoskeletal structures and correlates with the increasing prevalence of post-traumatic and degenerative pathologies. Detailed descriptions of the indications, clinical applications, and outcomes of current surgical procedures have been provided in several excellent reviews (Makris et al., 2015; Grayson et al., 2015; Sakai and Andersson, 2015). A brief understanding of these surgical principles is important because many cell-based interventions have been developed that aim to improve, not substitute, surgical repair (Table 1).

Bone Repair

The intrinsic repair of bone defects mirrors many events of embryonic development and makes fracture healing one of the rare postnatal processes that are regenerative and can ultimately restore damaged tissue to its pre-injury structure, composition, and biomechanical function (Figure 1). In spite of the unique capacity of bone to heal, a number of clinical indications remain where therapeutic intervention is required. In the case of complex trauma with multiple fractures, infections, and tumor-associated and endocrine diseases (e.g., diabetes, osteoporosis), the body's natural healing response is impaired, and non-union can occur in up to 15% of cases (Grayson et al., 2015). Another debilitating disorder is non-traumatic avascular osteonecrosis, which can lead to collapse of the femoral head and accounts for 10,000–20,000 total hip replacement surgeries in the United States per year (Figure 1; Moya-Angeler et al.,

Table 1. Musculoskeletal Repair: Surgical Techniques and Limitations

Condition	Surgical Technique/Treatment	Limitation
Long bone defects	autologous bone graft	donor site morbidity, graft size, bone quality
	allogeneic bone graft	slower healing compared with autograft, risk of rejection
	bone substitutes	mechanically inferior to bone grafts
Osteonecrosis of femoral head	decompression and autologous bone graft	palliative treatment
	joint replacement	limited implant lifetime, requiring replacement, particularly for young patients
Articular cartilage defects	microfracture	formation of fibrocartilage with inferior mechanical properties, formation of subchondral bone cysts
	autologous chondrocyte implantation	Long healing process, *ex vivo* expansion and de-differentiation of chondrocytes, limited to focal cartilage defects, OA is contraindication
	joint replacement	risk of complications, including aseptic loosening, dislocation, and infection
Meniscal tears	meniscal suture (peripheral regions)	limited to small tears
	partial meniscectomy (in central regions)	increased risk of OA
	meniscal allograft/ synthetic substitute	do not match mechanical complexity
Volumetric muscle loss	scar tissue debridement	functional deficiency often remains
	autologous innervated muscle tissue transfer	donor site morbidity, complex surgical procedure
Rotator cuff injuries	subacromial decompression and tendon debridement	creates more space but does not treat the tear
	suture and re-attachment of the tendon to the bone	risk of re-tear, scar tissue and fibrosis may cause impingement
IVD degeneration	resection of protrusions	often causes imbalance of adjacent segments
	segmental fusion	limited motion, increased risk of adjacent segment degeneration
	total disc arthroplasty	Increased risk of adjacent segment degeneration

IVD, intervertebral disc; OA, osteoarthritis.

2015). Autologous bone grafting represents the gold standard for management of bone defects and non-unions, and union rates of more than 90% have been reported using iliac crest bone. However, considerable donor site morbidity and limited volumes must be taken into consideration. Additionally, allogeneic or synthetic bone substitutes, such as ceramics, corals, or polymer-based materials, have not reached the biological and mechanical properties equivalent to autologous bone (Table 1).

Skeletal Muscle
In addition to direct traumatic injury, complex damage of bone tissue (e.g., open fractures, tumor ablations) often results in concomitant soft tissue injury, including adjacent muscles. Although skeletal muscle has the inherent ability to regenerate after injuries, the regenerative capacity fails when a large volume of muscle is lost (i.e., volumetric loss). Such severe injuries may lead to fibrosis, atrophy, and ischemia when left untreated, accounting for significant socioeconomic costs ($18.5 billion in healthcare costs are associated with sarcopenia alone) (Janssen et al., 2004). Therapeutic treatment options are limited to physical therapy, scar tissue debridement, and transfer of healthy, innervated, and vascularized autologous muscle tissue. However, the outcomes of surgical reconstructions often remain aesthetically and functionally deficient (Grogan et al., 2011; Table 1).

Articular Cartilage and Meniscus
In contrast to bone and skeletal muscle tissue, the poor intrinsic healing capacity of articular cartilage and meniscus tissue presents a major challenge in clinics. Lesions from injuries or degeneration often result in gradual tissue erosion, leading to impaired function of the affected joint and degenerative osteoarthritis (OA) (Figure 1). Patients with post-traumatic OA account for more than 10% of the 27 million adults in the United States that have a clinical diagnosis of OA (Johnson and Hunter, 2014). Commonly, the first-line treatment of articular injuries includes arthroscopic lavage, partial meniscectomy, and BM stimulation techniques to penetrate subchondral bone (Table 1). Microfracture has been considered the gold standard for stimulating endogenous repair; however, it often results in the formation of inferior fibrocartilaginous repair tissue. This cartilaginous tissue is vulnerable due to altered biomechanics of the subchondral bone, which raises concerns about the long-term efficacy of microfracture (Solheim et al., 2016). Therefore, secondary and more complex procedures strive to restore the hyaline cartilage, such as osteochondral autografting from the less weight-bearing periphery (mosaicplasty) and autologous chondrocyte implantation (ACI). ACI represents one of the first clinical applications of tissue engineering where a biopsy from a low-weight-bearing region is performed, and *ex vivo*-expanded chondrocytes are implanted in a second operation. The de-differentiation of
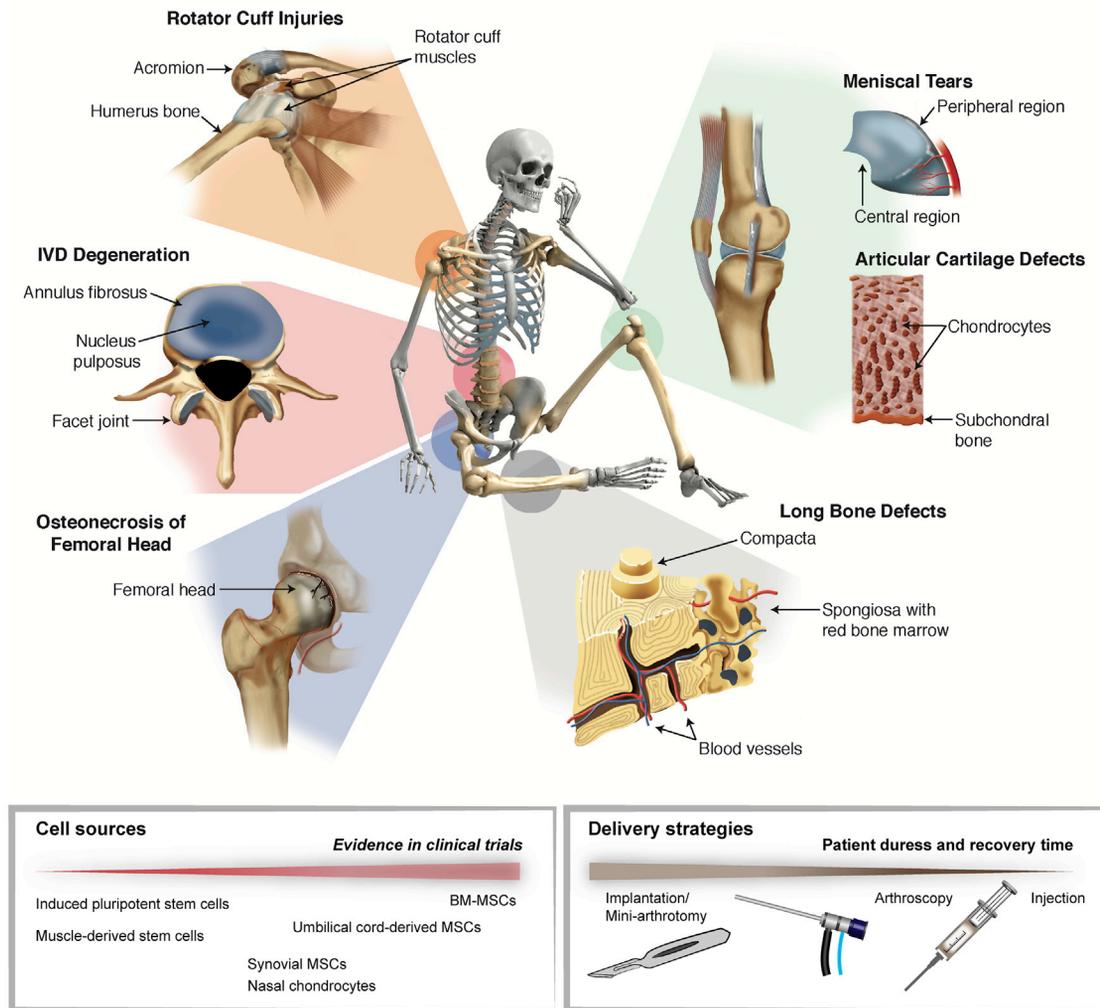
Figure 1. Musculoskeletal Tissues with a High Incidence of Injuries and Degeneration
The skeleton, joints, cartilage, intervertebral discs (IVDs), tendons, ligaments, and muscles are part of the musculoskeletal system, which provides stability and motion. Musculoskeletal diseases because of injuries and degeneration are one of the major causes of pain and disability. Cell therapies for musculoskeletal tissue repair are at different levels of evidence in clinical trials. For implantation of these cells, various delivery approaches are being used to optimize viability and minimize patient duress.

monolayer expanded chondrocytes and potential of recovery when implanted has been a matter of debate, and matrix-based ACI techniques have been developed that use absorbable scaffolds (e.g., porcine collagen) to support the implanted cells (Makris et al., 2015). An important limitation of these techniques is the long recovery time (6–12 months) to ensure neotissue formation. The choice of articular injury treatment depends on several factors, including localization and size of the lesion, the level of activity, and the degree of associated damage of menisci and ligaments.

Tears of the fibrocartilaginous menisci require surgical intervention for nearly 1 million patients in the United States annually (Vrancken et al., 2013). For lesions located in the peripheral vascularized region of the meniscus, repair strategies such as sutures and anchors allow preservation of the meniscal tissue. However, meniscal lesions often appear in the avascular central regions, which makes them less suitable for healing and usually

requires partial or (sub)total meniscectomy (Figure 1; Table 1). In some cases, further treatment with a meniscal substitute, such as an allograft or a synthetic implant, is indicated to limit OA (Vrancken et al., 2013).

Other Fibrous Musculoskeletal Tissues

Another large proportion of musculoskeletal injuries in the clinic is represented by other damaged fibrous structures, including tendons, ligaments, and the annulus fibrosus (AF). Often, degenerative pathology precedes acute trauma, and, like articular cartilage, these tissues have a limited healing capacity. One of the most common tendon injuries presented clinically is tearing of one or more of the interdigitating tendons of the rotator cuff (Figure 1). Failure of initial physical therapy or acute trauma in young patients motivates surgical repair using open or arthroscopic approaches for subacromial decompression, tendon debridement, and suture or anchor supplementation (Table 1). Still, repair is limited, particularly

Table 2. Recent Clinical Interventions in Musculoskeletal Repair: Cell Source and Delivery Strategies

Cells	Trauma/ Disease	Carrier	Delivery	Reference	Comments
BM-MSCs	cartilage OA	HA solution	injection	(Wong et al., 2013)	microfracture and HTO
		saline	injection	(Vega et al., 2015)	allogeneic
		HA solution	injection	(Vangsness et al., 2014)	allogeneic
	cartilage defect	HA solution	injection	(Gupta et al., 2016)	allogeneic, placebo-controlled (HA only)
	osteonecrosis	saline	injection (through decompression canal)	(Zhao et al., 2012)	BM subtrochanteric from femoral head
	IVD	saline	injection	(Noriega et al., 2017)	allogeneic
		HA solution	injection	NCT01290367	allogeneic
Synovial MSCs	cartilage defect	collagen membrane (Chondro-Gide)	mini-arthrotomy	(Akgun et al., 2015)	cultured for two additional days on collagen membrane
		none	synovial brush	NCT02696876	microfracture
Umbilical cord-derived MSCs	cartilage defect	HA solution (Cartistem)	arthroscopy	(Park et al., 2017), NCT01626677, NCT01733186	allogeneic, microfracture
	ACL defect	HA solution (Cartistem)	arthroscopy	NCT02755376	allogeneic
Nasal chondrocytes	cartilage defect	collagen membrane (Chondro-Gide)	mini-arthrotomy	(Pelttari et al., 2014), NCT01605201, NCT02673905	cultured on collagen membrane, autologous serum

ACL, anterior cruciate ligament; BM, bone marrow; HA, hyaluronic acid; HTO, high-tibial osteotomy; IVD, intervertebral disc; MSC, mesenchymal stromal cells; OA, osteoarthritis

within the complex anatomic arrangement forming the shoulder cuff. The formation of fibrovascular scar tissue frequently leads to significant morbidity, re-ruptures, and difficulties in treatment choice.

The intervertebral discs (IVDs) are composed of the nucleus pulposus (NP), a hydrophilic proteoglycan-rich gelatinous core, surrounded by a dense fibrocartilage ring—the AF (Figure 1). The gradual progression of IVD degeneration and the extrusion of the NP through defects in the AF is a major cause for lower back pain, a leading cause of global disability (Sakai and Andersson, 2015). Available treatments are mostly symptomatic, and surgical treatments often resect the structural obstruction resulting from herniation or fuse motion segments (Table 1). However, the complex structural features of IVDs surrounded by neural elements and inflammation frequently cause a homeostatic imbalance favoring a catabolic response governed by the loss of the IVD structure, which is often followed by facet joint arthritis and vertebra deformation, canal stenosis, and even deformations. Most importantly, disc replacement with synthetic implants or fusion of the motion segment does not cure the underlying pathology of IVD degeneration (Sakai and Andersson, 2015).

Cell-Based Interventions in the Clinic

Cell therapy approaches have evolved to face challenges associated with restoring tissue homeostasis and to direct endogenous healing of musculoskeletal tissues (Figure 1; Table 2). To date, BM-derived MSCs are the most frequently used cell source for these applications. This section focuses primarily on recent developments in the use of BM-MSCs and highlights some newer stem cell sources, which will likely be important in the

development of future therapies. There has been extensive clinical activity in examining the benefit of adipose tissue-derived stromal cells (ASCs) and unprocessed stromal vascular fractions (SVFs) in cartilage repair (Pak et al., 2017). Concerns have been raised regarding whether these cell populations have been clearly defined and characterized prior clinical application (Keating, 2012). There are also discrepancies among clinical trials regarding isolation and expansion conditions and parameters such as cell dose and preparation of ASCs and SVFs. Thus, care must be taken with ASCs and SVFs to evaluate their use as a cell product, and randomized trials are needed to validate the clinical benefit.

Tissue-Derived Cells

BM-MSCs. The harvest of autologous BM is a minimally invasive procedure of percutaneous aspiration of the iliac crest. BM-MSCs are then isolated using density centrifugation to separate the mononuclear fraction from the other marrow constituents and plating onto tissue culture plastic to separate the MSCs from the non-adherent hematopoietic cells using good manufacturing practices (GMPs). Given the long expansion time required for autologous BM-MSC expansion (2–3 weeks), off-the-shelf allogeneic BM-MSCs are increasingly being investigated in the field of skeletal repair. These MSCs are generally considered immune-suppressive by virtue of their expression of cytokines and growth factors, and their trophic and anti-inflammatory properties render them potentially useful for clinical application.

Clinical trials exploring allogeneic BM-MSC therapy have largely been sponsored by companies with MSC products, such as Mesoblast Ltd and Stempeutics. Their cell products are often subjected to extensive culture expansion to achieve

the desired quantities, which may lead to reduced potency (Ankrum et al., 2014). Nevertheless, randomized controlled trials have shown benefits of both autologously and allogeneically derived BM-MSCs. For implantation, these cells are often suspended in saline and directly delivered into the targeted musculoskeletal tissue via a syringe or arthroscopic port (Figure 1). However, limited cell engraftment has motivated the use of biomaterial carriers (e.g., hyaluronic acid [HA], fibrin, collagen, and platelet-rich plasma [PRP]) that help to retain injected cells and provide a microenvironment that supports cell function (Burdick et al., 2016; Table 2). This section is not meant to be comprehensive but, rather, highlights recent examples of clinical interventions that represent the field of BM-MSC therapy for musculoskeletal tissue repair.

Cell therapies for cartilage repair have predominantly been performed with autologous chondrocytes, such as in ACI, where a small biopsy of cartilage provides a chondrocyte population that is expanded *in vitro* and implanted into the cartilage defect in a second operation. Functional benefits have been reported in clinical trials and long-term case series, but many surgeons are still concerned about the clinical efficacy, particularly given the complexity of the procedure and long recovery time (Makris et al., 2015). Thus, to avoid the limitations associated with ACI techniques, methods have been developed using BM-MSCs. For example, OA of the knee was improved in clinical parameters in response to microfracture of the cartilage lesion and injection of autologous BM-MSCs, as assessed by pain, knee functionality, and disability (Wong et al., 2013). Injected MSCs with HA as a cell carrier significantly improved cartilage coverage and integration in 9 of 28 patients (32%) relative to HA injections alone (0%), as evaluated by MRI scans after a 1-year follow-up. These are encouraging data, and long-term follow-up results will be critical to evaluate the clinical relevance of autologous BM-MSC injections in cartilage repair. However, the unblinded study design (BM harvest from the iliac crest) and potential placebo effects may make it difficult to show efficacy.

BM-MSCs from allogeneic sources have also been explored in OA therapy of the knee. In a multicenter study (15 patients per arm), the pain-reducing effect of allogeneic BM-MSCs (40 million cells/knee) relative to HA alone was observed in patients with primary idiopathic OA of the knee (Vega et al., 2015). Although intra-articular injection of MSCs had a minor beneficial effect on cartilage quality after 6- and 12-month follow-up (evaluated by MRI), the analgesic effect was remarkable, with 38%–40% improvement in pain compared with 10%–14% in control groups. This study suggested that there may be anti-inflammatory effects of allogeneic MSC therapy in OA, and sustained benefits now need to be demonstrated in trials involving larger numbers of patients. It is of interest to note that the majority of patients (50%–60% of both groups) reported local pain in the injected knee and that co-administration of anti-inflammatory agents (ibuprofen) may be a cofounding factor. In another example, a symptom- and pain-reducing effect of allogeneic BM-MSCs (50 million cells) was observed in osteoarthritic knees with subtotal meniscectomy (Vangsness et al., 2014). Although this study also reported indications of meniscus repair in 14% of the patients (evaluated by MRI), the contribution of allogeneic MSCs to tissue repair is unlikely to be the major effect and may arise from a number of confounding factors, including inconsis-

tent MRI scans and the small number of patients (20 per arm). In this study, administration of higher cell numbers (150 million cells/knee) had no additional benefit, and a greater incidence of adverse events was reported in a similar randomized trial for Stempeucel, an allogeneic pooled BM-MSC source (Gupta et al., 2016). Importantly, it has yet to be shown clinically that higher numbers or improved *in vivo* survival of delivered MSCs leads to enhanced therapeutic efficacy.

A number of safety and feasibility studies have also been undertaken for chronic lower back pain, using intradiscal injection of allogeneic and autologous BM-MSCs (reviewed in Sakai and Andersson, 2015). These have shown favorable trends in pain reduction that encourage further randomized and controlled trials to evaluate the contribution of BM-MSCs to pain relief. For example, in a study with 24 patients with lumbar pain, sustained improvement in daily life activities was reported after intradiscal injection of 25 million allogeneic BM-MSCs (1-year follow-up) compared with impaired disability in the control group, whose patients received only the local anesthesia injections (Noriega et al., 2017). Of note, all patients reported improvements in pain, and the large placebo effect makes it difficult to evaluate the clinical efficacy of intradiscal MSC injections. A randomized, controlled multicenter study is now evaluating the sustained benefit of intradiscal injection of allogeneic BM-MSCs (6 or 18 million) with HA as a carrier and HA or saline as a control (25 patients per arm) (NCT01290367). Preliminary results have been reported by the company Mesoblast Ltd regarding improved pain relief in patients after 24 months compared with patients who received saline control injections (Trounson and McDonald, 2015).

Although early case reports and studies of small patient numbers have suggested that BM-MSCs have the potential to enhance healing of bone non-unions (reviewed in Steinert et al., 2012), a clinical benefit from BM-MSCs in controlled large trials remains elusive. In the treatment of early-stage osteonecrosis, injection of BM-MSCs has been investigated in a randomized clinical trial with 93 patients (50 patients in the BM-MSC group and 43 patients in the control group). Comparison of core decompression with and without 2 million BM-MSCs/hip obtained from the subtrochanteric region showed significant protection of autologous BM-MSC-treated hips from collapse (Zhao et al., 2012). Progression to advanced stages of osteonecrosis was reported for 2 of the 53 hips (4%) compared with 10 of the 44 hips (23%) in the control group that required autologous bone grafting or hip arthroplasty. These data suggest a clinical benefit of BM-MSCs and core decompression in delaying the need for total hip replacement in early-stage osteonecrosis (Zhao et al., 2012).

It is important to note here that approaches using autologous BM or freshly isolated mononuclear cells, processed in the operating room, are being explored in a large numbers of clinical trials (Chahla et al., 2016). Particularly the availability of BM and whole blood concentration devices has motivated many surgeons to use cell therapy without the need of GMP facilities. Each of these cell sources is highly heterogeneous between patients and within cell populations, and interpretation of its clinical value necessitates a better mechanistic insight.

Synovium-Derived Stromal Cells. Based on studies reporting a resident population of MSCs within the synovia (Karystinou et al.,

2009), clinical trials have investigated intra-articular implantation of autologous synovial MSCs for cartilage repair. Randomized comparison of chondrocytes and synovial MSCs for matrix-induced implantation (collagen membrane) into chondral lesions showed little differences at early time points (synovial MSCs improved pain and disability), and good cartilage quality and integration were reported for both chondrocyte and synovial MSC implantation (Akgun et al., 2015). The better clinical outcomes are believed to be due to the anti-inflammatory effects of synovial MSCs. Another approach targets the stimulation of endogenous MSCs through synovium brushing during microfracture, and a proof-of-concept study is currently recruiting patients (NCT02696876). It is apparent that more information regarding the mechanism and further clinical trials are needed to determine the benefits of synovial MSCs.

Nasal Septum-Derived Chondrocytes. An alternative approach has been taken to improve the efficacy of ACI. Compared with the mesodermal origin of articular cartilage, chondrocytes from the nasal septum are derived from the neuroectoderm, reflecting a higher self-renewal capacity (Pelttari et al., 2014). Studies in a goat articular cartilage defect model indicated environmental plasticity of nasal chondrocytes, suggesting their contribution to the repair process, similar to what has been described for skeletal progenitor cells. Preliminary results have been reported in a clinical safety study for the implantation of *ex vivo*-engineered constructs for cartilage repair, with no adverse events in seven patients (Pelttari et al., 2014; NCT01605201). A multi-center phase II clinical trial is now underway to compare the efficacy of such nasal chondrocyte-based tissues and nasal chondrocyte-based cell therapy (14 days versus 2 days *ex vivo* culture on a collagen I/III membrane) in cartilage defect repair (NCT02673905).

Umbilical Cord Blood-Derived Cells. MSCs isolated from allogeneic umbilical cord have also been considered for treatment of cartilage defects and associated OA. In 2011, a clinical safety trial with allogeneic umbilical cord blood-derived MSCs in combination with HA (Cartistem) has received regulatory approval. Early-phase studies showed no severe adverse events in seven patients treated with 10–20 million cells/knee (Park et al., 2017). Randomized clinical trials are ongoing for the evaluation of the long-term benefit in comparison with microfracture (NCT01626677 and NCT01733186). Another controlled trial (30 patients) is currently investigating the benefit of Cartistem for enhancing the healing response of anterior cruciate ligament (ACL) reconstructions (NCT02755376).

Allogeneic versus Autologous. Taken together, *ex vivo*-expanded MSCs have shown promising results in randomized trials, and general trends toward allogeneic sources are apparent. However, no definitive clinical advantage of allogeneic MSCs over autologous MSCs has been demonstrated to date, and a better understanding of the particular MSC mechanisms that contribute to the therapeutic effect is needed. In particular, recent preclinical and clinical observations of MSC immune responses have raised concerns regarding the general assumption that allogeneic MSCs are immune-privileged and can represent an off-the-shelf clinical product (Ankrum et al., 2014; Griffin et al., 2013). Although a comprehensive understanding of the therapeutic function of allogeneic MSCs remains elusive, autologous MSCs are not without limitations. For example, generating

therapeutic doses generally requires several weeks, and the risk of epigenetic changes and senescence is higher when cells are obtained from diseased donors (Alt et al., 2012; Stenderup et al., 2003). Given the significant variations in the trophic and immunomodulatory potency of individual donors, variability between patients is likely to lead to highly variable outcomes. Importantly, the spectrum of regulatory and trophic factors secreted by MSCs and the mechanism of how they affect musculoskeletal repair are beginning to be elucidated (Hofer and Tuan, 2016; Malda et al., 2016). Identifying and characterizing the factors that can promote tissue repair or activate endogenous cells seems essential for clinical interventions. Ultimately, to realize the potential of MSC therapy, the field is challenged with the validation of potency assays and/or biomarkers that predict whether a patient is responsive to treatment (Ankrum et al., 2014).

Clinically Emerging Stem Cell Populations

Human Pluripotent Stem Cells. There has been considerable progress in the use of pluripotent stem cells (embryonic stem cells [ESCs] and induced pluripotent stem cells [iPSCs]) for a variety of applications (Jung et al., 2012). Human ESCs have the potential to differentiate into all types of adult human tissues and possess an unlimited capacity to self-renew. Despite optimism for their therapeutic potential, ethical controversy (use of human embryos) and safety concerns (rejection of cells and tissues derived from allogeneic ESCs) have limited their clinical translation. Given these concerns, human iPSCs, somatic cells reprogrammed to become pluripotent cells, have demonstrated great promise to overcome these limitations and to resemble human ESCs. Particularly MSCs derived from iPSCs can be generated *in vitro* and may exert greater repair potential because of less senescence compared with BM-MSCs (Lian et al., 2010). Human iPSCs may offer optimism for autologous and allogeneic stem cell therapies in the musculoskeletal field (Craft et al., 2015; Kanke et al., 2014; Chal et al., 2015); however, important safety issues, including the possibility to form tumors and genomic aberrations in the reprogrammed cells, need to be addressed before clinical application (Jung et al., 2012).

Muscle-Derived Stem and Progenitor Cells. Muscle satellite cells (MuSCs), the resident stem cells of skeletal muscle, have the capacity to self-renew and generate large numbers of myogenic progenitor cells in response to injury. There has been enthusiasm in using MuSCs as a transplantable cell population to restore the stem cell pool in aged and diseased muscle. In fact, the delivery of freshly isolated MuSCs into the intramuscular space in mice enhanced regenerative outcomes following injury, as shown by new muscle fiber generation (Sacco et al., 2008) and the repopulation of the satellite niche to contribute to future muscle repair (Sacco et al., 2008; Cerletti et al., 2008). Although the results are promising in rodents, self-renewal capacity in large animals and humans has yet to be demonstrated. A major obstacle to this approach is that MuSCs are very rare and that removal of these cells from their endogenous niche (for *in vitro* expansion) rapidly alters their cellular state and ultimate functional capacity. Thus, new culture systems that more closely mimic the *in vivo* niche environment are essential to yield sufficient numbers of functional MuSCs (Cosgrove et al., 2014; Gilbert et al., 2010). As a therapy for

volumetric muscle loss, MuSCs are not currently being investigated in clinical trials.

Biomaterials to Improve Retention of Delivered Cells

In most clinical interventions, cells are injected directly into the targeted tissue via a syringe or through an arthroscopic port. However, biomaterials may play a role to enhance the viability and engraftment of cells by retaining them at the injection site as well as to provide tissue-specific microenvironmental niches that play a particular role in encouraging endogenous repair (Wagers, 2012). Natural and synthetic materials have been employed in clinical practice, mostly with materials that have a long history of clinical use (Table 2). Given the diversity of musculoskeletal tissues, the scaffold design depends on the delivery mode and properties of the targeted tissue (Jeon and Elisseeff, 2016). For example, the lack of a supportive structure may account for the variable outcomes/deterioration of ACI; thus, a second generation of ACI addressed this limitation by implanting the cells seeded onto a collagen scaffold.

One of the most established materials in clinical practice has been the injection of HA for treatment of OA. HA is a polysaccharide present in body tissue, and when high-molecular-weight HA is combined with water, it forms a highly viscous solution. Intraarticular injection of HA is well tolerated as a therapeutic modality for the treatment of OA of the knee joint, particularly for patients who are at risk for orthopedic surgery. The effects of HA are suggested to be initially biomechanical, with the viscoelastic solution providing lubrication and shock absorption. Physiologically, HA has been demonstrated to be chondroprotective, analgetic, and anti-inflammatory. Such properties may prove to be relevant for cell-based interventions in cartilage defects as well. Thus, several clinical trials have used HA as a cell carrier, which may be an effective strategy for increasing the viscosity of the cell solution and enhancing the retention and efficacy of injected cells (Vangsness et al., 2014; Gupta et al., 2016; Park et al., 2017; Wong et al., 2013). However, at the same time, the persistence of HA after injection depends on several parameters, such as inflammation and activity, which may reflect the heterogeneity of outcomes (Campbell et al., 2015). Collagen is another clinically available biomaterial that has been used as an implantable construct to deliver cells. One of the commercial products available for orthopedic implantations is Chondro-Gide, a collagen type I/III membrane obtained from pig collagen. Lately, Chondro-Gide matrices have been used for implantation of synovial MSCs (Akgun et al., 2015) and nasal septum-derived chondrocytes (Pelttari et al., 2014) to treat cartilage defects (Table 2). The safety of such matrix-associated therapies encourages further controlled clinical trials with other cell sources, such as culture-expanded BM-MSCs.

Strategies to Enhance Endogenous Repair

Beyond only cell delivery, musculoskeletal tissue repair may benefit from enhancing the recruitment of endogenous stem cells to the damaged tissue to harness their repair response (Ivkovic et al., 2011). Early evidence of stem cell niche therapies, presented by microfracture and tissue debridement, support the concept that cells and matrix factors derived from BM and blood are candidates for endogenous tissue healing. Given the role of blood platelets in wound healing and immune response, PRP has taken a prominent place in medical practice and for different areas of musculoskeletal repair (Padilla et al., 2017). PRP con-

tains a cocktail of growth factors released from platelets and endogenous fibrin and, as a minimally manipulated product, may be prepared intra-operatively from patients' whole blood using centrifugation devices. The presence of autologous fibrinogen in PRP results in platelet gel formation upon thrombin or calcium activation. Such niche-directed interventions have been employed to boost recruitment of endogenous cells after arthroscopic microfracture. Given the poor long-term outcomes of this technique, augmentation strategies following microfracture have been developed to improve the quality of the repair tissue (Strauss et al., 2010). For example, injections of autologous PRP following marrow stimulation of articular cartilage defects improved the clinical parameters of pain and knee function (Mancò et al., 2016). At the same time, 2-year follow-up data showed no benefits in cartilage coverage and quality. Still, improvement of pain is an important outcome for the patient and may indicate that recruitment of stem cells may alter the inflammatory response in OA and could aid cartilage repair (Centeno et al., 2014). In addition to PRP-augmented marrow stimulation, clinical approaches have also included strategies that use PRP gels as a cell carrier to further enhance homing of endogenous stem cells to the damaged tissue (Liebergall et al., 2013; Koh et al., 2014). However, the definitions and characterization of PRP vary considerably among studies, and the differences in content may account for conflicting clinical results.

An alternate strategy is the use of tissue-specific extracellular matrix (ECM), which represents a source of various sequestration sites for growth factors and cytokines that can serve as signals to recruit endogenous stem cells. Decellularized ECM materials are fabricated by cell removal and washing out their remnants with various treatments that conserve tissue-specific ECM, a mixture of proteins and proteoglycans, which then can be processed into implants or hydrogels. This ECM closely mimics the native tissue from which the tissue is derived and may provide biological signals important for cellular functions. Such approaches, if successful, could generate ECM-based materials designed to activate endogenous repair processes (Monibi and Cook, 2017). Clinically, decellularized allografts have been used as an injectable paste to augment microfracture (Biocartilage; Hirahara and Mueller, 2015) and as osteochondral scaffolds for full-thickness cartilage defects (Chondrofix; Long et al., 2016) but generally with minor benefits and high failure rates (Monibi and Cook, 2017). Promising clinical data showed restoration of vascularized and innervated muscle tissue formation in five patients with volumetric muscle loss upon implantation of acellular pig bladder matrix (Sicari et al., 2014). Although the connection to the physiological repair response is less clear, similar results were demonstrated in eight patients upon implantation of ECM-derived scaffolds derived from other tissues (porcine dermal, small intestinal submucosa), which suggests the presence of similar signaling mechanism within non-tissue specific ECM-derived materials (Dziki et al., 2016).

Progress and Concerns Regarding Stem and Stromal Cell Therapy

The recent progress of stem and stromal cell clinical trials in the field has been encouraging, and published results have demonstrated strikingly positive therapeutic effects of these cells in the musculoskeletal field. However, most clinical reports have dealt with small numbers of patients, and there have been few

controlled prospective trials, as highlighted in this review. At this point, clinical applications of stromal cells and heterogeneous cell populations, including unprocessed BM and SVFs, have often been without significant preclinical evidence or a thorough understanding of the mechanism of action (Prockop et al., 2014). This may arise in part from the different perspectives of scientists investigating the basic biology of stem and stromal cells and clinicians facing patients who may benefit from new therapies even prior to the establishment of rigorous scientific evidence. Major challenges for successful clinical trials are the characterization of multipotency and regulatory properties of heterogeneous cell populations and an understanding of their *in vivo* role during tissue repair. This includes quantitative assays for labeling and monitoring MSCs after implantation in humans to study their persistence and long-term therapeutic effect. Another aspect is the standardization of isolation and culture protocols to reduce variability and to compare MSCs across laboratories (Keating, 2012; Prockop et al., 2014).

With respect to the well-characterized culture-expanded BM-MSCs, the field would greatly benefit from larger placebo-controlled clinical trials that can evaluate the efficacy of these therapies and detect rare side events. One challenge is that there is often a heavy emphasis on subjective outcome measures (e.g., daily activities and pain scales) that are vulnerable to placebo effects and do not consistently correlate with physiological outcomes (e.g., cartilage thickness and disc height) (Mundi et al., 2014). A second issue is the difficulty of blinding surgeons and patients in a surgical intervention, such as with autologous BM aspiration, which is critical for determining whether isolated cells can be effective in treating tissue injuries or degeneration. Notably, allogeneic bone marrow-, adipose tissue-, and umbilical cord-derived MSCs are in the largest number of blinded clinical trials currently registered. Because these are often industry-sponsored, negative outcomes may rarely be published and require careful evaluation.

As indicated, there are now numerous biomaterials in clinical practice. These materials in combination with MSCs or other cell sources may improve the clinical benefits of delivered cells. Presently, there are limited clinical observations regarding cell engraftment and therapeutic outcomes in humans, but there are encouraging preclinical data and current developments that suggest the benefit of supportive biomaterials.

Advanced Bioengineering Concepts

Although stem cell therapies are advancing toward the clinic, there is no standard protocol for the optimal number of cells to be implanted for maximal effect, and cell survival has often been observed to be less than 26% (Quintavalla et al., 2002; Emans et al., 2006; Marquardt and Heilshorn, 2016). Given the low engraftment and survival rates of delivered cells, large numbers have been implanted into patients. As such, there is significant effort toward the use of bioengineering principles to further improve these therapies. As described above, biomaterials have been used in the clinical application of stem and stromal cells, such as collagen and HA materials; however, they have mainly been applied as cell carriers without specific focus on their properties and ability to regulate cell behavior. Thus, beyond initial retention, advanced biomaterials are being designed to capture some of the critical biochemical and biophys-

ical ECM signals found in native connective tissues to direct the formation of new functional tissue (Figure 2A). Likewise, biomaterials are being developed to deliver biological factors that can play a role in recruiting stem cells for repair, controlling cell behavior, or for immunomodulation to alter the healing environment (Figure 2). Although it is not meant to be comprehensive, this section will highlight exemplary advances in this area toward the repair of musculoskeletal tissues.

Control and Regulation of Stem and Stromal Cell Behavior

Biochemical Signals. The spatially and temporally complex interactions of cells directly with their ECM environment and with each other are profound in their ability to regulate stem cell fate and function (Wagers, 2012). With a focus on their biochemical composition, biomaterial fabrication has evolved to present defined adhesive molecules (e.g., laminin, fibronectin) or signaling ligands (e.g., transforming growth factor β [TGF-β], bone-morphogenetic protein [BMP]) to modify the structural environment of cells (Figure 2B). A growing body of data indicates that such microenvironmental cues can be engineered into biomaterials to guide cellular behaviors (Guvendiren and Burdick, 2013). For example, hydrogel-based presentation of specific integrin binding domains, which are cell-adhesive ligands found in the ECM, improved MSC attachment and bone repair upon implantation (Kisiel et al., 2013, Shekaran et al., 2014). Features of native myofiber ECM (e.g., integrins, laminins) can also be engineered into hydrogels to preserve MuSC quiescence and enhance subsequent engraftment *in vivo* (Quarta et al., 2016). As another example, both TGF-β and BMP are implicated in MSC differentiation, and biomaterials that present these biological cues have shown controlled differentiation of stem cells toward chondrogenic and osteogenic lineages (Re'em et al., 2012).

Biophysical Signals. The composition and organization of musculoskeletal tissues that give rise to their biophysical properties also influence the function of resident cells (Figure 2A). Extensive studies have probed these critical mechanical signals in different biomaterial systems (Discher et al., 2009). In bone formation, for example, matrix elasticity is directly related to mechanically induced osteogenesis and biomaterials that allow cells to generate traction forces and reorganize their microenvironment support osteogenic lineages (Figure 2B; Khetan et al., 2013). In this regard, hydrogels that can decouple matrix elasticity of the bulk hydrogel from cell confinement (for example, by incorporating hydrolytically degrading sacrificial gel porogens) have enabled MSCs to spread and improve bone regeneration in rat cranial defects (Huebsch et al., 2015). Such bioengineered materials are useful to improve the survival and differentiation of transplanted cells, which then serve as a source of osteogenic signals and recruitment of endogenous cells for new tissue formation. The importance of matrix reorganization has also been shown in non-covalently crosslinked alginate hydrogels, where the rate of visco-elasticity and stress relaxation determined the degree of new bone tissue formation (Darnell et al., 2017). Thus, biomaterials that facilitate cells to invade and remodel these niches may be effective in inducing functional tissue repair.

Scaffolds can also be fabricated to recreate the architecture of native ECM tissue on a micro- and nanoscale. For example, the nanofibrous and anisotropic structure of musculoskeletal tissue can be mimicked by controlled alignment of polymer fibers using
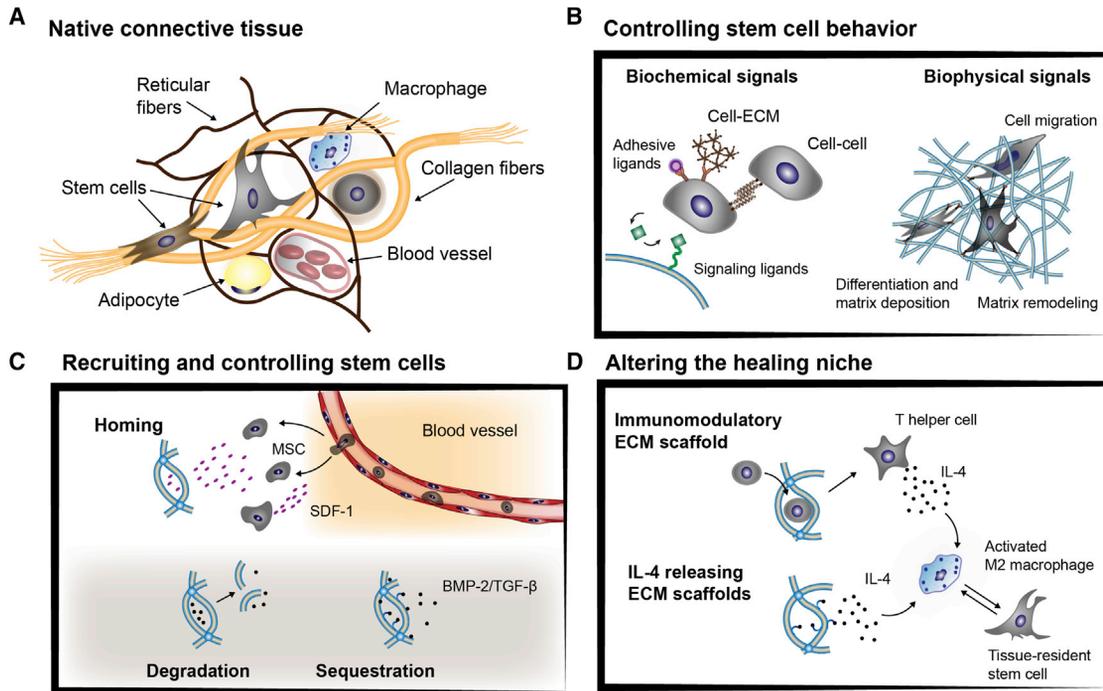
Figure 2. Advanced Bioengineering Concepts using Biomaterials to Control Cell Behavior
(A) The ECM of native connective tissue is highly dynamic and supports resident cells through presentation of biological and biophysical cues.
(B) Biomaterials can recreate aspects of the tissue-specific microenvironment with biochemical signals to mimic cell-ECM and cell-cell interactions or to allow encapsulated cells to actively interact and integrate with their matrix environment.
(C) Biomaterials can also be engineered to release chemo-attractive cytokines (e.g., stromal cell-derived factor 1α [SDF- 1α]) that enable migration of resident cells (e.g., mesenchymal stromal cells [MSCs]) or direct cell behavior by controlled release of encapsulated biological factors (e.g., bone-morphogenetic protein [BMP], transforming growth factor-β [TGF-β]).
(D) Scaffold microenvironments are further being developed to alter the healing niche; for example, by inducing a specific anti-inflammatory immune response or by releasing cytokines (e.g., interleukin 4 [IL-4]) that activate M2 macrophages and promote tissue repair.

electrospinning (Figure 2B). Toward tissue repair applications, it is important that these fibrous scaffolds recapitulate the dense and organized matrix structure but not impede endogenous cell migration and tissue formation. For example, the hierarchical structure has been fine-tuned in nanofibrous scaffolds by multi-layers of aligned fibers to improve ASC migration and tenogenic matrix deposition (Orr et al., 2015). More recently, implantation of polymer fibers mimicking the structural organization of the rotator cuff tendon, together with delivered MSCs, induced repair similar to intact tendon tissue (Peach et al., 2017). In addition to methods like electrospinning, self-assembled peptide hydrogels are an emergent means of recapitulating the microstructural organization of the ECM. The use of such self-assembled nanofibers led to greater engraftment of freshly isolated MuSCs upon injection in mice, which was partially attributed to improved cell alignment and proliferation (Sleep et al., 2017; Figure 3A). Such studies highlight that biomimetic scaffolds may be critical for MuSC engraftment and to instruct MSCs to orchestrate stem cell mobilization and tissue-specific repair. The mimicry of tissue biophysical properties can further be combined with the presentation of biochemical factors and has been tuned for specific applications, such as the conjugation of connective tissue growth factor (CTGF) for ligament repair (Pauly et al., 2017) and the release of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) for myofiber repair (Sleep et al., 2017).

Recruitment of Endogenous Stem and Progenitor Cells
Advances in our understanding of the fundamentals of healing and the inherent repair capacity of many musculoskeletal tissues has led to the design of biomaterials that specifically recruit endogenous cells by delivering soluble signals (Figure 2C). As an example, it has been described that stromal cell-derived factor 1α (SDF-1) plays a critical role in regulating stem progenitor cell recruitment and engraftment at the injury site. For example, SDF-1 acts as a chemoattractant for CXCR4-presenting cells (e.g., MSCs and endothelial progenitor cells) from the BM, where it regulates cell mobilization into the targeted tissue. Studies in a rabbit model of calvarial defects indicated that electrospun polycaprolactone (PCL)/gelatin fiber scaffolds improve bone formation when SDF-1 is physically adsorbed, in part by providing a proangiogenic environment in the defect area through recruiting hematopoietic stem cells (Ji et al., 2013). SDF-1 has also been suggested to decrease the effective BMP-2 dose for calvarial bone repair when co-delivered from proteolytically degradable HA hydrogels (Holloway et al., 2015). Similarly, SDF-1-containing collagen-hydroxyapatite gels enhanced osteoinductive properties of decellularized bone scaffolds in a rabbit large bone defect model (Chen and Lv, 2017). Combinations of SDF-1 with collagen (Chen et al., 2015) and fibrin (Yu et al., 2015) scaffolds have been optimized to treat osteochondral and full cartilage defects, aiming to enhance the migration of stem and chondroprogenitor cells from the underlying subchondral BM. Targeting
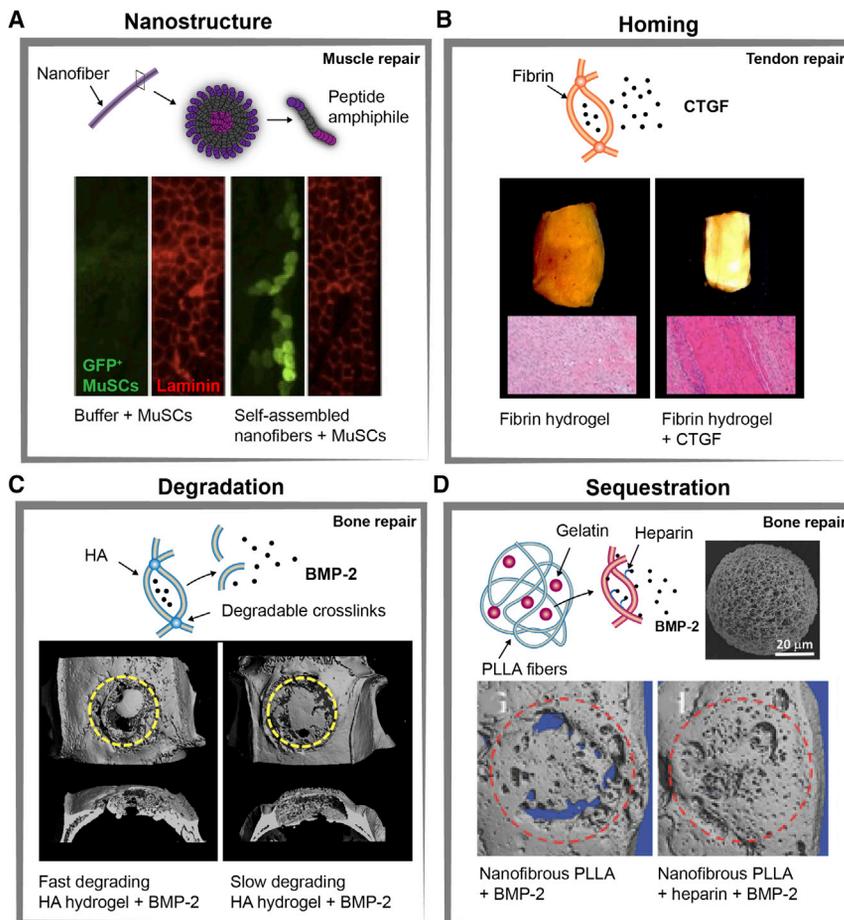
Figure 3. Examples of Biomaterials Engineered to Recruit and Control Endogenous Stem and Stromal Cell Behavior *In Vivo*

(A) Implantation of muscle satellite cells (MuSCs) in self-assembled nanofibers enhanced donor cell-mediated repair of myofibers. Representative immunostaining of muscle tissue sections 5 weeks after implantation shows enhanced engraftment of GFP[+] MuSCs compared with cells injected in buffer only.

(B) Connective tissue growth factor (CTGF) released from fibrin hydrogels improved the repair of transected rat patellar tendons. Gross images and representative histological images 4 weeks after implantation showed dense alignment of collagen fibers for fibrin gels with CTGF by stimulating the proliferation and differentiation of endogenous tendon progenitor cells.

(C) BMP-2 incorporated into matrix metalloproteinases (MMP)-degradable hyaluronic acid (HA) hydrogels sustained release of BMP-2 through cell-mediated hydrogel degradation. Representative micro-computed tomography (μ-CT) images of rat calvarial defects 6 weeks after implantation demonstrate increased bone volume for faster-degrading HA hydrogels.

(D) New bone tissue formation can also be increased through implantation of nanofibrous poly(l-lactic acid) (PLLA) microspheres that contain gelatin-heparin/BMP-2 nanoparticles (scanning electron microscope image of a typical nanofibrous PLLA microsphere). Representative μ-CT images of rat calvarial defects 6 weeks after implantation show improved bone regeneration for PLLA microspheres with heparin-conjugated gelatin because of sustained release of BMP-2.

Adapted with permission from the following: (A) (Sleep et al., 2017). (B) American Society for Clinical Investigation; Lee et al. (2015). (C) Elsevier; Holloway et al. (2014). (D) Wiley; Ma et al. (2015).

SDF-1-mediated recruitment of MSCs from the surrounding cartilage and synovia, particularly in partial defects, may also be an effective strategy for repairing cartilage (Zhang et al., 2013). Finally, SDF-1 release from hydrogels has potential as an effective strategy for reactivating endogenous repair of fibrocartilaginous tissue (Shen et al., 2010; Pereira et al., 2014). Given the relatively low number of SDF-1-responsive MSCs (often less than 5% express CXCR4 on the cell membrane), increasing receptor expression with specific cytokines may present a potential strategy to augment these bioengineering approaches (Shi et al., 2007, Wynn et al., 2004). Tissue-specific growth factors may also be employed to boost endogenous stem cells and orchestrate healing. For example, preclinical data in a rat tendon model suggest that activation of tendon-resident stem/progenitor cells by CTGF, encapsulated in a fibrin gel, enhances homing, proliferation, and tenogenic differentiation of this rare stem cell population (Figure 3B; Lee et al., 2015). Targeting such endogenous niches, particularly in poorly healing tissues, may be an effective strategy for circumventing *ex vivo* manipulation of transplanted stem and stromal cells.

Control of Endogenous Stem Cell Behavior

In addition to recruiting endogenous cells to damaged tissues, advances in biomaterial design can further be tuned toward the delivery of appropriate cues so that they allow spatiotemporal control of the microenvironment of these cells (Figure 2C). The controlled delivery of biological factors is one approach where biomaterials can alter cell behavior and fate. The release profiles of these factors can be actively controlled through biomaterial degradation and their affinity or binding to the released molecules. The bioengineering strategies are diverse in how they bind the molecules, ranging from covalent conjugation to electrostatic interactions and hydrophobic associations (Li and Mooney, 2016). One of the most known clinical applications of biomaterial carriers in musculoskeletal repair has been the INFUSE bone graft device, which consists of a recombinant BMP-2-soaked collagen sponge. The INFUSE system provides affinity- and diffusion-controlled BMP-2 release for use in lumbar spinal fusion and open tibial fracture (Carragee et al., 2011). However, the collagen carrier does not provide tight control over the release, and high doses of BMP are implanted, causing initially supraphysiological drug levels.

To better control the release of biological factors, one approach is the use of biomaterials that respond to environmental stimuli, such as the enzymatic and proteolytic activity of migrating tissue-resident cells. For example, biomaterials have been engineered to degrade via proteases, such as matrix metalloproteinases (MMPs) that are associated with cellular migration and have a key role in ECM remodeling and angiogenesis during bone regeneration. This mode of degradation allows

the release of entrapped biomolecules during cell-mediated material remodeling (Lutolf et al., 2003). With this, hydrogels are tuned for BMP-2 release, supporting new bone tissue formation in rat calvarial defects (Holloway et al., 2014; Shekaran et al., 2014; Figure 3C). Although these examples have focused on cell-mediated material remodeling, hydrolytic degradation can also be used to control molecule delivery and, thus, cell behavior (Patterson et al., 2010). When hydrolysis governs the delivery of biomolecules, the release profiles depend on the biomaterial degradation kinetics, influenced by factors such as crosslinking density, hydrophobicity of the polymers, as well as molecular weight and concentration.

Complementary to sustained diffusion-based release, the interaction between biomaterials and molecules can mediate their sequestration, presentation, and release behavior (Figure 2C). Previous studies have reported successful incorporation of heparin and heparin mimetics to exploit the natural affinity between native ECM and heparin binding domains on growth factors. For example, BMP-4 and TGF-β have been physically entrapped during the crosslinking of two-layer alginate hydrogels containing sulfate groups as heparin mimetics (Re'em et al., 2012). Prolonged presentation of BMP-4 and TGF-β upon implantation in osteochondral defects in rabbits resulted in cartilaginous tissue formation with subchondral bone underneath. Such effects demonstrate the complex interplay between growth factors (i.e., TGF-β and BMPs) and ECM proteins in the differentiation of migrated cells (Martino et al., 2011; Wagers, 2012).

To more closely mimic the native ECM structure, nanofibers can be engineered to mimic the function of growth factor-sequestering microfibrils. For example, nanofibers self-assemble upon mixing of heparin-binding amphiphiles composed of a self-assembling domain and a bioactive TGF-β binding domain. These nanofibers specifically sequester and enhance the activity of supplemented and endogenous TGF-β, resulting in improved cartilage repair in a rabbit model following microfracture (Shah et al., 2010). For bone regeneration, nanofibers have also been engineered to sequester and enhance the activity of BMP-2 through a heparin binding domain (Lee et al., 2013). When these nanofibers were incorporated into a collagen scaffold and implanted into rat femoral defects, a reduced concentration of supplemented BMP-2 was needed to elicit the therapeutic effect. Similarly, BMP-2 cooperates synergistically with the integrin-binding regions of fibronectin or engineered recombinant proteins (Martino et al., 2011), improving cell migration and bone formation when incorporated into covalently crosslinked HA hydrogels (Kisiel et al., 2013).

Although these methods allow mimicking of the fibrous ECM, they are often limited toward minimally invasive filling of complex 3D shaped skeletal tissue defects. To address this, complementary microscale hydrogel constructs have been engineered to deliver bioactive molecules as injectable carriers (Tai et al., 2013; Liang et al., 2013) or encapsulated within hydrogel scaffolds (Spiller et al., 2012; Bian et al., 2013) to add functionality that stimulated the differentiation of delivered and migrated cells. These microspheres can be modified in similar ways to macroscale hydrogels to alter growth factor interactions. By using covalently crosslinked heparin microspheres, BMP-2 signaling can be enhanced by sequestering encapsulated BMP-2 and

cell-secreted growth factors (Hettiaratchi et al., 2014). To combine fibrous structures with the advantages of microspheres, BMP-2-loaded heparin-gelatin nanoparticles can also be encapsulated into nanofibrous poly(l-lactic acid) (PLLA) microspheres (Ma et al., 2015; Figure 3D). Because these PLLA microspheres have a high porosity, they enable greater ECM deposition and, in combination with sustained BMP-2 release, improved bone regeneration in a rat calvarial bone defect model. Many of the principles here are also used for the delivery of genes that can act on delivered and recruited cells (Evans and Huard, 2015). Similarly, biomaterials can be useful for effective and controlled delivery of extracellular vesicles; for example, for restoration of joint homeostasis and repair (Malda et al., 2016).

Altering the Healing Niche: Immunomodulatory Considerations

Although providing the biochemical and biophysical signals of native ECM in a biomaterial is important for stem and stromal cell delivery and homing, variability in patient response is likely to also extend to heterogeneity in patient physiology. This is reflected by an increased understanding that the body's immune response is critical in tissue repair (Sadtler et al., 2016). The close association of many stem cell types and immune cells within the tissue-specific stem cell niche allows for modulation of stem cell responses by actively triggering the immune response. Specifically, macrophages as a heterogeneous population of the innate immune system exhibit multiple phenotypes in response to the external environment, with a spectrum ranging from classically inflammatory M1-like to less inflammatory M2-like phenotypes. These effects can partially be explained by an array of soluble mediators that induce macrophage polarization, some of which induce a specific macrophage subtype; for example, interleukin (IL) 1β, 4, 10 and 13; interferon-gamma (IFN-γ), and TGF-β. The plasticity of these cells and their diverse roles in tissue repair therapies have been reviewed elsewhere (Spiller and Koh, 2017). Therefore, bioengineering strategies that harness the regenerative potential of residual immune cells, either by controlling macrophage polarization through designing microenvironmental cues or controlling the release of anti- or pro-inflammatory cytokines, may activate and increase the repair potential of endogenous stem/progenitor cells (Sridharan et al., 2015; Figure 2D). Indeed, such an immunomodulatory approach has been effectively employed in tissue regeneration, where muscle injuries in a mouse were treated with decellularized ECM scaffolds (Sadtler et al., 2016). In this example, the immune response induced by the scaffold microenvironmental niche involved IL-4 releasing T helper cells that released anti-inflammatory IL-4 and activated macrophages toward an M2-like phenotype, which supported the healing response. These data indicate that inflammatory signals may be essential for initiating the crosstalk between macrophages and endogenous stem cells and the transition into tissue repair.

This conceptual strategy is particularly attractive for the complex but highly regulated sequence of inflammatory and anti-inflammatory signals in bone healing. More specifically, decellularized bone scaffolds have been designed to physically and covalently bind cytokines for rapid release of IFN-γ, an inflammatory cytokine to promote inflammation, and sustained release

of IL-4 to promote vascularization (Spiller et al., 2015). This immunomodulatory bone scaffold resulted in sequential actions of M1 and M2-like macrophages *in vitro* and exemplified that specific immunological cues can be embedded into biomaterials. These recent advances merit further research toward harnessing the complex immunomodulatory properties of macrophages. However, at the same time, non-specific protein adsorption and the accompanying foreign body response, when implanted, present major hurdles toward clinical translation. Therefore, a mechanistic understanding of how biomaterials interact with the immune system both locally and systematically will help develop materials that can actively alter the physiological healing niche.

Conclusion and Future Outlook

Within the field of musculoskeletal tissue repair and regeneration, particularly regarding tissues such as bone, cartilage, intervertebral disc, tendons, ligaments, and skeletal muscle, there is a large amount of preclinical and clinical data that support cell-based interventions. The majority of clinical trials to date have used BM-MSCs with success in contributing to tissue repair and reduction of pain. There is notable heterogeneity in cells described as MSCs and the isolation methods being used, which are likely to affect *in vivo* function and therapeutic potential. Although such problems can probably be addressed by using animal models in which donor and endogenous cell response can be better quantified—e.g., implantation of GFP⁺ cells in bone defects (Zeitouni et al., 2012) and for muscle repair (Sleep et al., 2017)—there has been limited availability of valid assays to elucidate the effects on inflammation and pain reduction that are thought to support the clinical observations. In addition, these functions are regulated in the context of host tissue physiology and the nature of repair, and animal models need to be developed to identify the role of exogenous MSCs. At the same time, the establishment of MSC-based therapies in the musculoskeletal field requires evidence-based clinical trials with appropriate follow-up of clinical parameters that may also investigate the mechanism behind the therapeutic benefit. A limitation is the current recognition of MSCs as a human cell product that requires approval by the United States Food and Drug Administration (FDA), and cells must be cultured under defined GMP conditions, which have continued to increase the cost and slow the development of therapies. Further success of the field will provide avenues for clinicians to work closely with scientists to improve stem and stromal cell-based therapies. Bioengineering principles, such as in the development of engineered biomaterials, will play a role in these advances. Targeting the complexity of physiological healing requires a fundamental understanding of the cellular and biological signals that constitute the healing niche under degenerative and repair conditions and the design of therapies with this in mind. Our knowledge is continuously increasing, and *ex vivo* models are being developed to better understand stem and stromal cell responses in musculoskeletal tissues. Likewise, the capabilities of biomaterials presenting specific chemical, biological, and physical cues is expanding and will define new avenues to provide instructional microenvironments for stem and stromal cell-induced tissue repair. An important aspect of future cell-based therapies in musculoskeletal tissue repair is a better understanding of distinct healing mechanisms, whether through recapitulating developmental processes or by providing signals for endogenous repair.

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