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REVIEW

# Current perspectives on biological approaches for osteoarthritis

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**Musculoskeletal injuries that disrupt the structure and function of diarthrodial joints can cause permanent biomechanical alterations and lead to a more severe, chronic condition. Despite advancements that have been made to restore tissue function and delay the need for joint replacement, there are currently no disease-modifying therapies for osteoarthritis (OA). To reduce the risk of OA, innovative preventive medicine approaches have been developed over the last decade to treat the underlying pathology. Several biological approaches are promising treatment modalities for various stages of OA owing to their minimally invasive nature and actively dynamic physiological mechanisms that attenuate tissue degradation and inflammatory responses. Individualized growth factor and cytokine therapies, tissue-engineered biomaterials, and cell-based therapies have revolutionary potential for orthopedic applications; however, the paucity of standardization and categorization of biological components and their counterparts has made it difficult to determine their clinical and biological efficacy. Cell-based therapies and tissue-engineered biologics have become lucrative in sports medicine and orthopedics; nonetheless, there is a continued effort to produce a biological treatment modality tailored to target intra-articular structures that recapitulates tissue function. Advanced development of these biological treatment modalities will potentially optimize tissue healing, regeneration, and joint preservation strategies. Therefore, the purpose of this paper is to review current concepts on several biological treatment approaches for OA.**

**Keywords:** stem cell therapy; growth factor and cytokine therapy; tissue engineering

## Introduction

Several biological products have been developed over the last decade owing to the increasing prevalence of osteoarthritis (OA) in young and adult populations. Aside from the younger population, the natural course of aging is a growing concern. Currently, one of two young adult Americans will report an musculoskeletal (MSK) condition,[1] and 67 million (25% of the adult population) will be diagnosed with some form of arthritis by 2030.[2] With the early enthusiasm for the use of cutting-edge biologics in the medical field, these types of patient-centered issues have generated increased pressure on clinicians to treat patients with uninsured biological procedures that have limited proven clinical benefit in order to maintain patient satisfaction. Even though the scientific community's level of regenerative research faces many challenges, significant advancements have been made.

Biomaterial innovations for injection therapy, tissue grafting, and surgical augmentation have grown exponentially in the field of sports medicine and orthopedics.[3] Biologics are intended to improve tissue healing in both acute and chronic conditions by stimulating healing processes to restore native tissue and minimizing risks for treatment failure.[4–6] The primary function of biological therapeutics is to replicate complex tissue structures to augment surgical intervention and other noninvasive approaches as an analgesic agent for arthritic tissue.

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Certain autologous biologic modalities are currently practiced for the treatment of OA (e.g., platelet-rich plasma (PRP), bone marrow concentrate (BMC), and lipoaspirates). However, there is little consensus on the application of these heterogeneous cell products, which has led to inconsistencies in clinical and basic science documentation, methodologies, and results.[7–9] Given the inconsistencies in clinical efficacy and lack of randomized controlled trials (RCTs) and standard biologic preparation and application,[10] it is difficult to establish a clinical standard, and it remains unclear whether tissue healing or regeneration can be attributed to these autologous cell-based treatments.[11,12] Mechanics and loading variations have also been reported as barriers to successfully adhering tissue-engineered and autologous biologics to damaged tissue, regenerating homogeneous tissue, revascularizing tissue sufficiently and in a timely manner, and preventing hypertrophy.[13–15] However, many forms of autologous and allogenic cell-based therapies and engineered biomaterials, such as scaffolds, nanomaterials, rapid prototyping, and three-dimensional (3D) bioprinting, can be modified to retain biological factors and cells that are naturally receptive to environmental factors and signaling pathways.[16,17] With advanced microtechnology and nanotechnology, biological expansion and engineering can be used to inhibit specific factors, cells, and defect fillers and provide adherence to damaged bone and cartilage tissue.[14,18,19] Contemporary literature suggests that biological applications in sports medicine and orthopedics are promising approaches and in some cases have proven to be effective. Moreover, there are several conventional and unconventional regenerative forefronts that are effectively being used for the treatment of OA. Here, we review the innovative and clinically available biological treatments and the future directions of these biological approaches for the treatment of OA.

## Growth factor therapies and platelet-rich plasma

### *Individual growth factor and cytokine therapies*

**Background.** Growth factors and cytokines have many diverse functions, including stimulating multiple biological changes through cell signaling via binding specific receptors. There is a wide range of individual growth factors and cytokines that have been directed toward cartilage and bone regeneration, while other individual therapies have been targeted to mitigate inflammation. Several methods are used to produce synthetic and naturally occurring biological factors to target arthritic tissue. Biodegradable scaffolds, hydrogels, and other biologically engineered platforms can be used to deliver individual growth factors, cytokines, or other adjuncts to the site of injury. Moreover, individual growth factor and cytokine therapies are relatively safe and useful treatment approaches for cartilage and bone healing and regeneration. Given the wide variety of targeted growth factor and cytokine treatment approaches, this section will review a few common individual growth factors and cytokines that are used to treat arthritic tissue and subsequent inflammation (Table 1).

**Transforming growth factor-β.** The transforming growth factor-β (TGF-β) cytokine family has contrasting roles that are modulated by other locally acting cytokines, chemokines, and cells. TGF-β is known for regulating mesenchymal stem cell (MSC) proliferation and differentiation[20,21] but has also been identified as a key growth factor involved in the development of fibrosis.[22–24] TGF-β can be stimulated and locally administered using a scaffold or synthetic biomaterials; however, synthetic microspheres can also be used to deliver isolated human recombinant TGF-β.[25] Human recombinant TGF-β has been shown to enhance collagen type II and aggrecan synthesis.[25] Several lines of fundamental research have reported the therapeutic effects of TGF-β using different delivery methods, yet there is much discrepancy in its use for OA applications. One such discrepancy is that most studies fail to document the TGF-β isoform that is being studied.[26] TGF-β has three isoforms that exhibit separate functions, in that TGF-β1 and TGF-β2 promote fibrotic tissue formation (profibrotic),[22] and TGF-β3 promotes scar-free healing.[26,27] Current efforts have focused on the safety and quality of human recombinant TGF-β3 (avotermin) for wound healing.[26,28–30] With the emerging field of agents that block deleterious biological factors, new treatment strategies that utilize TGF-β3 to form hyaline cartilage may be advantageous.

**Bone morphogenetic protein.** Bone morphogenetic protein (BMP), a macromolecule that belongs to the TGF-β family, has a vital role in the development of bone and cartilage, as well

**Table 1. Advantages and disadvantages of individual growth factor, cytokine, and PRP therapies**

| | Advantages | Disadvantages |
|---|---|---|
| **Individual growth factor and cytokine therapies** | • Several diverse functions on cells<br>• Deliverable to damaged tissue sites<br>• Direct and indirect effects on cell- and protein-binding sites | • Potential immunologic response to treatment<br>• Difficult to prepare and deliver<br>• Limited clinical use<br>• Synthetically derived products<br>• Treatment is costly<br>• Risk of rejection |
| **PRP** | • Blood extraction is minimal risk to the patient; clinical procedure<br>• Blood is minimally manipulated; low risk of infection<br>• Can be combined with other therapies, such as hyaluronic acid<br>• Concentration of platelets can be modified to adapt the injection for different pathologies<br>• Inactivated and activated PRP is a highly biocompatible alternative therapeutic agent that can be prepared as an injectable or fibrin clot for surgical augmentation | • Potential inflammatory response to high platelet concentrations<br>• Few standardized studies for intra-articular applications<br>• No optimal preparation method<br>• Potential detrimental effect of RBCs when used in intra-articular environments<br>• Heterogeneous solution that may indirectly affect other intra-articular tissues<br>• The fibrin clot can only be used to target specific intra-articular tissues<br>• Variable growth factor and cytokine quantities |

as in tissue homeostasis.[31] BMP subgroups have several diverse functions in chondrogenesis and osteogenesis, but are mainly involved in cell recruitment and stimulating MSC differentiation.[25,32] BMPs have been largely studied in the tendon–bone junction,[33] subchondral and osteochondral defects,[34] nonunions,[35–37] fracture repair,[38,39] and spinal fusions.[38,40,41] Viral and nonviral gene products, as well as scaffold composites, have been used to deliver individual BMPs or a combination of BMPs, such as BMP-2,[42–44] -6,[45] -7,[46] and -9,[47] for osteochondral defects in preclinical models. Recombinant human (rh) BMP-2 is a potent osteoinductive factor that has been used as an off-label biological grafting agent to promote bone formation in posterior, lateral, and transforaminal lumbar fusions.[48–50] Structural and functional improvements have also been reported for tendon–bone insertion healing using BMP-2, as well as BMP-4, -7, -12, and -14.[51–55] Although these results suggest that osteoinductivity of BMPs may be a favorable adjunct in bone repair and formation, there is a high rate of postoperative adverse events.[56,57] Despite research advancements for individual BMP treatment approaches, robust clinical studies that address adequate dosing and applications of these fundamental prerequisites that are currently lacking are necessary in order to validate its clinical efficacy.

**Interleukin inhibitors.** Inflammation is a subsequent response after joint trauma or injury. Elevated concentrations of proinflammatory cytokines (e.g., interleukins (ILs)) and chemokines are present at the injury site for months after injury.[58] Even though the pathophysiological mechanisms are not fully understood, several observations suggest that the presence of upregulated immune processes, and specifically inflammatory factors, may predispose the articular structures to OA.[59] IL-1 is one such proinflammatory cytokine that is responsible for stimulating catabolic factors and, ultimately, predisposing intracapsular structures to degenerative processes.[60]

One first-line pharmacological treatment modality that targets IL-1 is a recombinant form of its receptor IL-1Rα (i.e., Anakinra), and it has been shown to be clinically safe; however, an RCT for the treatment of knee OA found little to no difference in outcomes between treated and placebo groups, in spite of effective IL-1 neutralization.[61] These results reinforce the notion that OA is a multifactorial disease and that there may not be a single

factor or pathway propagating OA-induced inflammation and degradation. In fact, Nasi *et al.* recently demonstrated that the two forms of IL-1 (IL-1β and IL-1α) were not key mediators in the progression of OA.[60] These results highlight the potential gaps in our understanding of the etiology of OA. Despite these major challenges, several other agents that target inflammatory diseases are clinically available and have demonstrated positive clinical results for the treatment of cardiovascular disease (IL-1β monoclonal antibody, Canakinumab),[62] psoriasis (IL-17 antibody, Ixekizumab),[63] and psoriatic arthritis (IL-17, Secukinumab),[64–66] but longitudinal observations to elucidate their clinical potential for the treatment of OA-related inflammation are necessary.

**Vascular endothelial growth factor.** Vascular endothelial growth factor (VEGF) is a key component that has been shown to have a profound effect on new blood vessel formation and regenerating vascularity in skeletal muscle[67,68] and bone.[69–74] VEGF has a profound effect of reestablishing nutrient flow in both *in vivo* and *in vitro* models; however, a recent study found that VEGF receptor 1 overexpression is linked to age and may be associated with clinical implications.[75] VEGF is inherently proanabolic and has detrimental pathophysiological effects on articular cartilage. In fact, it has been demonstrated that OA tissue has increased vascularity and expresses a significantly higher concentration of VEGF. This finding may indicate that higher concentrations of VEGF could cause detrimental effects, depending on the specificity of donor demographics. Conversely, TGF-β is an activating factor in chondrogenesis and can induce fibrotic activity.[70] This in turn can create deleterious scar matrices that impede skeletal muscle healing.[23] Eliminating detrimental factors to promote chondrogenesis or angiogenesis for indicated pathology and developing a slow-release delivery method to provide growth factor and cytokine sustainability are imperative for future individual therapy applications. Further analyses on growth factor and cytokine effects *in vivo* and *in vitro* are necessary to fully understand the longevity of VEGF's implications on microenvironmental factors in various arthritic conditions.

**Fibroblast growth factor.** Fibroblast growth factor-18 (FGF18) is an essential growth factor in cartilage and bone development and cartilage homeostasis.[76,77] The importance of FGF18 in cartilage damage is that it secretes anabolic factors to promote chondrogenic differentiation while inhibiting cell proliferation.[76–78] Its role in cartilage repair has been demonstrated using a preclinical rat model,[79] but several pharmacologic recombinant forms of FGF18 (Sprifermin) are still in phase II/III clinical trials. FGF18 may be an innovative approach to treat OA tissue, but its clinical efficacy will depend on the outcomes of the ongoing clinical trials.

### *Platelet-rich plasma*

**Background.** Thrombocytes, also known as platelets, have critical roles in maintaining tissue homeostasis and initiating immunological responses and coagulation in the body.[80–82] Peripheral venous blood can be minimally manipulated into concentrated blood fractions comprising a small volume of plasma and a rich source of platelets that is separated from erythrocyte and leukocyte blood layers. A volume of concentrated platelets composites bioactive proteins and molecules that have demonstrated several direct and indirect roles for various cellular processes.[83–86] Automated commercial systems and manual processing methods are used to minimally manipulate desired blood fractions to concentrate leukocyte-rich PRP (LR-PRP) and leukocyte-poor PRP (LP-PRP) but have been found to produce product variations in blood cell and growth factor concentrations.[63,84,87–89] Activating platelets within PRP via endogenous or exogenous coagulation triggers degranulation and subsequent secretion of hundreds to thousands of platelet-derived growth factors (e.g., growth factors, cytokines, and chemokines).[88,90] Inactivated and activated PRP is a highly biocompatible alternative therapeutic agent that can be prepared as an injectable or fibrin clot for surgical augmentation to initiate tissue healing and regeneration. However, further research is warranted to elucidate the biological mechanisms of action, which are still largely unknown.[91]

**Relevant applications.** PRP is widely applicable for the treatment of OA conditions that lack the ability to stimulate tissue healing and regeneration on their own. Despite broad application of PRP, there is insufficient evidence on the dosing, frequency, and type of PRP, such as LR-PRP, LP-PRP, or PRP releasate, for intra-articular applications.[7] Given that LR-PRP is composed of

high concentrations of both leukocytes (e.g., granulocytes, monocytes, and lymphocytes) and platelets that simultaneously secrete proinflammatory factors that have been found to be beneficial for soft tissue healing,[92–94] there is still great controversy regarding the optimal quantity of platelets and leukocytes that defines its biological composition for intra-articular applications.[95,96] Leukocytes and platelets can create an imbalance within the microenvironment by emanating infiltration of degradative tissue growth factors (e.g., matrix metalloproteinases) that can lead to the activation of unregulated signaling pathways that cause negative phenotypical changes to the tissue. In contrast, LP-PRP therapy is used for its analgesic effects that ameliorate pain and improve joint function when applied to osteochondral defects and osteoarthritic joints.[97] However, these beneficial effects are short-lived,[98] and there are several ambiguous reports that do not clearly define the observed PRP type (e.g., LP-PRP versus LR-PRP), its site of application, the baseline or concentrated blood cell concentrations, or the volume administered.[7,8] Currently, there is no consensus on the recommended dosage, frequency of treatment, or type of PRP that should be used for hard or soft tissue treatment. Two classification systems have enforced the need to form a classification system to record specific criteria that describe the biological profiles and application of PRP products uniformly,[7,8] but these two systems have not yet been adopted.

The literature collectively suggests that cellular and molecular variations are directly associated with processing methodology to obtain desired PRP types.[63,88,89,96,99,100] In this regard, it should also be considered that variations in platelet concentrations and other platelet-derived constituents are influenced by harvest, patient demographics, and severity of pathology.[101] It is probable that biological profiles that do not contain high concentrations of key factors that are involved with tissue healing (e.g., growth factors) may also interfere with subjective patient-reported outcomes and objective outcomes following PRP treatment. In conclusion, PRP is deemed clinically safe and is a useful alternative therapeutic option to reduce pain and improve quality of life. However, in order to validate its clinical efficacy, robust randomized clinical studies and clinically relevant basic science analyses are necessary.

## Stem cell therapy, bone marrow concentrate, lipoaspirate, and adipose-derived stem cells

### *Sources of stem cells and their therapeutic potential*

The diversification of stem cell isolation techniques and applications for hard tissue restoration has had a significant impact on the development of novel technologies and strategies. While tissue-engineering and regenerative technologies continue to progress, current autologous and allogeneic approaches have been shown to regrow tissues that are otherwise challenging to heal with or without intervention. Although stem cells have better survival rates after transplantation than differentiated cells, strategies that regulate apoptotic and cell-senescent signaling to improve cell survival remain under investigation.[102] Stem cell transplantation from the same source (autologous) versus a different source but the same species (allogeneic) has been a controversial subject that continues to be investigated. Historically, autologous transplantation has been the gold standard for cell therapies to avoid an adverse immune response or even donor rejection. There are several reports of clinical improvements using autologous and allogeneic MSCs and chondrocytes for subchondral bone and cartilage repair.[103–108] Moreover, allogeneic stem cells are promising, readily available products that could revolutionize interventions for the treatment of early-onset OA. However, it is also important to remember that clinical judgment, using autologous or allogeneic stem cells, should be based on evidence. At this time, it is critical for quality and safety of these cell-based therapies to be validated before these treatments are used routinely in clinical practice.

MSCs are among the most studied stem cells because of their multipotent nature, immunoregulatory function, and ease of isolation from different tissue sources. MSCs are derived from adult stem cells (ASCs) that exhibit different features when obtained from different tissue sources.[109] The International Society for Cellular Therapy has established common characteristics that define an MSC, such as plastic adherence; $CD90^+$, $CD105^-$, $CD73^+$, $CD34^-$, $CD45^-$, and $CD14^-$ expression of surface markers; and the ability to differentiate into osteoblasts, adipocytes, and chondroblasts.[110] In OA tissue, there is evidence that the hemostatic role

and proliferative capacity of MSCs may be permanently altered in these conditions.[111] As a result, a myriad of MSC delivery strategies have been developed over the last decade to enhance articular cartilage repair and, ultimately, to delay OA. Our understanding of MSCs for the treatment of OA has improved on the basis of preclinical animal models that have demonstrated reparative effects of MSCs for meniscus, cartilage, and bone repair.[112,113] The therapeutic application of MSCs is in the early stage of clinical translation; however, for the field to develop further, standardized protocols for MSC banking and expansion need to be carefully considered before clinical translation.[114] In addition, low quantities of MSCs are isolated from bone marrow (BM MSCs), a common tissue source, and controlled *in vitro* expansion is necessary. Long-term cultivation has been shown to reduce the differentiation potential and cause permanent morphological changes.[115,116] Despite these limitations, there are a growing number of clinical trials testing MSCs for OA using different delivery approaches.[109,112,114,117] The results generated from these studies may fulfill regulatory needs and demonstrate their safety and effectiveness.[112]

Induced pluripotent stem cells (iPSCs) are adult cells that have been reprogrammed using embryonic transcription factors to become pluripotent.[118] Like ASCs, iPSCs circumvent the ethical issues of embryonic stem cells (ESCs), and their pluripotency makes them a promising candidate for cartilage and bone repair.[119–122] The first human clinical trials using iPSCs are currently underway to treat macular degeneration.[123] Moreover, iPSCs are the focus of research for other conditions, such as Alzheimer's,[124] Parkinson's,[125] and cardiovascular disease.[126] Once preliminary clinical trials demonstrate safety and efficacy in humans, iPSCs may prove to be a valuable tool in regenerative MSK medicine.

ESCs offer the greatest plasticity with the ability to differentiate into a wide variety of tissues; however, MSCs are among the most studied stem cells owing to their multipotency and ability to differentiate into several different types of tissues.[127] The given ethical concerns of harvesting ESCs, in conjunction with the potential for rejection or tumor formation, make the use of these cells challenging. Different methods have been developed over the years to reduce the risk of an immunological rejection and tumor formation by isolating MSCs from a variety of adult tissues, such as bone marrow, adipose tissue, muscle, and amniotic fluid. However, the differentiation potential of ASCs is significantly less compared with ESCs, and they require more extensive manipulations to steer them toward different lineages. By regulating the growth environment in a controlled *in vitro* setting, studies have demonstrated that ASCs can become bone[128] and cartilage[129] precursor cells under the right conditions and are able to differentiate into different types of tissue cells. Clinical trials have demonstrated the safety and efficacy of ASCs for treating urinary incontinence,[130] articular cartilage injury,[131] and impaired cardiac function.[132]

### *Bone marrow concentrate*

**Background.** Bone marrow serves many important roles in maintaining hematopoiesis,[133] neutralizing systemic inflammation,[134] regulating lymphatic production and the immune system, and giving rise to blood cell lineages and progenitor stem cells.[135–137] There has been growing interest in a minimally invasive application of BMC harvested from the posterior superior iliac crest.[138] Given the origin from which BMC is derived, clinical applications have been primarily focused on bone formation and regeneration.[139–142] However, bone marrow harbors progenitor stem cells with potent differentiating capacities in the presence of stimulating factors. BMC is a heterogeneous mixture of blood components, such as erythrocytes, leukocytes (e.g., granulocytes, lymphocytes, and monocytes), platelets, plasma, several stromal cell populations, and growth factors.[87,133] To produce BMC, the erythrocyte and leukocyte blood fractions are isolated using a benchtop centrifuge or automated system. Erythrocytes and leukocytes provide beneficial systemic effects to numerous biological processes, but the inclusion of erythrocytes and leukocytes in BMC therapy for cartilage and bone tissue healing and regeneration is controversial. It is known that blood-induced joint damage enables erythrocyte and leukocyte infiltration that subsequently causes chondral and subchondral degeneration and often leads to arthritis.[143–145] Historically, BM MSCs have been of particular interest for isolating owing to their multilineage differentiation capacity capable of generating chondrocytes, osteoblasts, tenocytes, myocytes, adipocytes, and mesodermal cells[146] and

to their responsiveness to surrounding environmental cells, biological factors, and mechanical stresses. For example, BM MSCs prevent neutrophil apoptosis under oxidative stress,[147] and they also express unique cytokine and chemokine receptors that cue cells to undergo proliferation or apoptosis.[148] More importantly, BM MSCs are responsive to certain activated platelet growth factors.[149–151] However, patient demographics, such as age, gender, and comorbidities, have been shown to negatively affect the therapeutic potential of BM MSCs.[152–155] Specifically, in aged individuals, a higher prevalence of cell senescence and a lower production of BM MSCs reduce the regenerative potential to repair damaged tissue.[156,157]

**Relevant applications.** The applications of BMC include surgical injection augmentation, scaffolding augmentation, and conservative injection therapy. In a systematic review, 11 studies reported observation of knee OA ($n = 8$) and focal cartilage defects ($n = 3$) with BMC treatment with good to excellent patient-reported results.[158] Significant results were found when OA was incipient to moderate and when the focal chondral defect was relatively small (less than 2 $cm^2$) or the patient had relatively few chondral lesions.[158] The heterogeneity in indications, processing methods, and outcome measures impedes the ability to draw significant conclusions. Although it is difficult to analyze, the placebo effect cannot be disregarded in the results obtained by these studies. Certainly, larger series of RCTs will bring clarity to the subject, since the current literature lacks methodological consistency. Consistent reporting and nomenclature use will aid in enhancing scientific progress.

BM MSCs play a key role in interactive relationships with surrounding factors. For example, BM MSCs prevent neutrophil apoptosis under oxidative stress.[147] BM MSCs also express unique cytokine, chemokine, and other growth factor receptors that regulate cell growth and apoptosis.[148] More importantly, BM MSCs respond to certain activated platelet growth factors.[149,150] IL-1 receptor antagonist is one such platelet-derived growth factor that can be found at high concentrations within BMC and has been thought to be primarily responsible for mediating inflammatory factors. Although previous studies have suggested that BMC contains a small quantity of BM MSCs (0.001–0.01%), the therapeutic effects of BMC may be regulated by the processing methodology that produces increased concentrations of growth factors that regulate the activity of BM MSCs. Profiling the biological niche of BMC will provide tremendous value and is necessary before conditional specifications of cellular and growth factors can be developed for individualized interventions.

### *Lipoaspirates and adipose-derived stem cells*

**Background.** Fat or adipose tissue is an abundant source of autologous adipose-derived stem cells (ADSCs), which are a type of MSC with the ability to differentiate into multiple cell lineages, including cartilage, bone, muscle, and adipose tissue. ADSCs were first identified as MSCs in adipose tissue by Zuk *et al.*[159,160] Unlike human bone marrow, adipose tissue has been studied as a source of high numbers of progenitor or stem cells that can be isolated from either minimally invasive liposuction aspirate (biological waste from common surgical procedures to remove excess fat) or subcutaneous fat tissue fragments. In addition, adipose tissue has been identified as an excellent scaffold or matrix material for tissue-engineering applications and regenerative medicine. Although ADSCs have been given many different names, such as preadipocytes, adipose-derived stem/stromal cells (ASCs), adipose-derived stromal cells, processed lipoaspirate cells, in 2004, the International Fat Applied Technology Society reached a consensus to term ADSCs as "ASCs" to identify the isolated, multipotent, and plastic-adherent cell population.[161] ASCs secrete a wide variety of growth factors, cytokines, chemokines, and exosomes that have positive effects on surrounding progenitor cells[162] and can affect the microenvironment to trigger repair mechanisms. Since ASCs have multilineage and regenerative potential, their therapeutic applications have been investigated in several *in vitro* and *in vivo* studies to restore and treat bone, tendon, intervertebral disc, and various MSK disorders and in pain management. ASCs have also been investigated in the treatment of cartilage injuries and OA in animal models, and these studies showed evidence of cartilage regeneration upon administration of ASCs.[163,164] Hence, preclinical and clinical studies using ASCs are rapidly expanding, owing to promising results and significant outcomes in the field of orthopedic sports medicine (Table 2).

**Table 2. Advantages and disadvantages of BMC, lipoaspirate, and stem cell therapies**

| | Advantages | Disadvantages |
|---|---|---|
| **BMC** | No culture expansion<br>No risk of allogeneic disease transmission and low risk of infection or immune reactions<br>Minimal resources required to produce BMC<br>May be performed with concomitant procedures | Potential risk of pain during harvest<br>Variable stem cell quantities<br>Potential detrimental effects of RBCs when used in intra-articular environment<br>Few standardized studies for intra-articular applications |
| **Lipoaspirate** | Can harvest adipose tissue from multiple sites; minimally invasive procedure<br>Good source of stromal vascular fraction or stromal cells<br>No culture expansion required<br>No risk of allogeneic disease transmission, infection, or immune reactions<br>Rapid, efficient, and nonenzymatic isolation of fat tissue<br>Minimally manipulated and clinical-grade injectable tissue<br>Natural scaffold and can be admixed with other scaffolds | Potential risk of pain and penetration of surrounding tissue structures during harvest<br>Variable stem cell quantities in stromal vascular fraction isolated from lipoaspirate<br>Unlike ASCs, lipoaspirate may yield fewer cytokines and growth factors<br>Low yield of cells; contains other biological material<br>Adipose harvest (liposuction) may cause pain at the harvest site(s) and is time consuming<br>May contain traces of contaminating cells that cannot be separated |
| **Stem cell therapy** | Allogeneic and autologous cell transplantation for clinical use<br>Can isolate cells from several tissue types<br>Expanded and differentiated in culture under controlled settings<br>May be performed with concomitant procedures | Cells must be expanded *ex vivo*<br>Potential immunologic risk to patients<br>Risk of allogeneic rejection<br>Treatment restrictions set by the U.S. Food and Drug Administration |

Mechanical defragmentation and enzymatic digestion of fat/adipose tissue are common methods that are used to isolate ASCs following liposuction to produce an injectable lipoaspirate and stromal vascular fraction (SVF).[161,165] Liposuction or lipoaspiration of excessive fat tissue is a commonly practiced cosmetic procedure, and the end product, lipoaspirate, is a biological waste that contains large populations of stem cells. Autologous lipoaspirate is often used as an injectable scaffold and the source of ASCs in soft tissue reconstruction procedures. Furthermore, lipoaspirate can be digested with the enzyme collagenase to extract ASCs embedded within adipose tissue, and the enzyme can be washed away by centrifugation to yield a cell pellet, which is considered SVF. SVF is a heterogeneous mixture comprising tiny adipose tissue fragments, macrophages, blood cells, preadipocytes, pericytes, fibroblasts, smooth muscle cells, and endothelial progenitors/mature cells.[166] With the increasing interest in clinical applications of adipose tissue in sports and regenerative medicine, fully automated or semiautomated equipment needs to be developed to employ manual mechanical or enzymatic digestion of adipose tissue to scale up the yield of lipoaspirate, SVF, and ASCs. Currently, most of the devices marketed to obtain SVF from harvested adipose tissue or lipoaspirate require collagenase digestion. Collagenase enzyme is more commonly used in efficient dissociation of adipose tissue or lipoaspirate than mechanical methods to yield higher volumes of SVF and maximum recovery of ASCs. Despite the clinical applications of ASCs in regenerative medicine, there are several challenges to address and overcome. The U.S. Food and Drug Administration permits isolation and administration of autologous lipoaspirate using nonenzymatic minimal manipulation techniques, but the SVF yielded from enzymatically digested whole fat or lipoaspirate is classified as drug and more than minimally manipulated.[167–169] Hence, several other mechanical methods have been developed for

alternative nonenzymatic processing of adipose tissue to isolate SVF by washing and shaking/vibrating lipoaspirate followed by centrifugation to enrich SVF.

**Relevant applications.** Recently, several nonenzymatic fat processing kits have become available that can generate SVF-enriched adipose tissue via shaking and washing methods. Examples include devices that harvest and homogenize adipose tissue containing autologous endothelial cells (Baxter International Inc.), Puregraft® (Bimini Technologies LLC), Fastkit (Fastem) (CORIOS Soc. Coop.), LipiVage™ (Genesis Biosystems, Inc.), Revolve™/GID 700™ (LifeCell Corporation, USA/GID Group, Inc.), Lipogems® (Lipogems International SpA), Lipo-Kit GT (Medikan International Inc.), StromaCell™ (MicroAire Surgical Instruments, LLC), and myStem® (MyStem LLC). However, each method or system has different advantages and disadvantages and is under continuous development and standardization. Further comparative studies, optimized methodologies, and preclinical/clinical outcomes may allow further development of effective therapies in clinical setup.

Autologous, nonenzymatically processed, SVF-enriched lipoaspirate ASCs are currently being used in clinical settings for treating various orthopedic sports injuries. Because of the regenerative potential of ASCs and their healing capability in MSK injuries, SVF-enriched lipoaspirate is being used to treat patients with OA, meniscus tears, and other orthopedic disorders without any serious side effects. Liposuction can be performed on common accessible areas of the body, such as the abdomen, buttocks, arms, or thighs. ASCs from SVF-enriched lipoaspirate are readily available in an outpatient setting and can be accessed by simple liposuction. ASC treatment could potentially provide a safe, less invasive, and nonsurgical treatment for OA; however, there is limited evidence for efficacy of this type of treatment in the literature with reference to safety and efficacy. The harvest site and volume of extracted fat tissue play vital roles in the number of ASCs, but there are variances among patients. For example, the number of ASCs obtained from 1 g of adipose tissue may range from 5000 to 200,000 cells, according to flow cytometry quantification methods. Harvest techniques have historically used a relatively large volume (approximately 100 g) of adipose tissue to yield an increased concentration of ASCs.[170] Recent studies have achieved high concentrations of ASCs by extracting a relatively small volume (approximately 19 g) of adipose tissue.[171] Overall, it can only be estimated that adipose tissue from different regions of different patients' bodies may contain different numbers of ASCs. In theory, 0.5–20 million ASCs may be isolated from 100 g of adipose tissue, and if the number of MSCs in adipose SVF is 5%, approximately 10 million ASCs may be obtained from 100 g of adipose tissue.[172] For a general clinical setup, approximately 5–15 mL of SVF-enriched lipoaspirate is drawn into a 60-mL syringe and minimally processed via low-speed centrifugation or by allowing the layers to settle over several hours. Then, the top oil layer is removed, and 5–10 mL of adipose tissue can be injected into the articular space between the meniscus and overlying collateral ligament to treat OA conditions.[172] However, treatment methods vary from system to system, with subjective evaluation.[173]

In addition to lipoaspirate administration, cultured ASC therapy is an emerging approach for OA treatment. In one recent study, researchers investigated the efficacy of intra-articular injection of $1 \times 10^6$ scaffold-free and culture-expanded allogeneic ASCs for the treatment of OA in an experimental rat model.[174] Interestingly, allogeneic ASCs did not induce any adverse local or systemic reactions.[174] Further preclinical studies are needed to evaluate the safety, efficacy, paracrine effects on chondrocytes, and therapeutic action of culture-expanded allogeneic ASCs for treatment of OA and to monitor the fate of allogeneic-transplanted ASCs. Overall, intra-articular injection of SVF-enriched lipoaspirate with an optimized number of ASCs may be a good alternative treatment method for OA conditions and sports injuries, but safe and efficacious treatment methods need to be evaluated in the clinic.

## Tissue engineering

### *Scaffolds and synthetics*

**Background.** Regenerating tissue via cell transplantation is a compelling strategy to repair otherwise difficult-to-heal tissues, such as cartilage and bone. Advances in tissue engineering have shown that conventional constructs can recapitulate tissue structures using various natural and synthetic materials. The development and

adoption of fabricated scaffold materials, such as polymer, ceramic, and metal, have been shown to be effective for joint repair applications. Cell-based, cell-free, and scaffold-free approaches have been developed to replicate native tissue function. Cell-based and scaffold-free approaches use various autologous, allogeneic, and xenogeneic cell sources to expand and isolate cell populations to seed a sufficient number of cells.[175] While absorbable and permanent cell-free constructs are composed of collagen matrix, hyaluronan- and polymer-based materials require cellular recruitment *in situ*.[176] Each approach shares a common challenge in that it is difficult to grow tissues into their native conformation to replicate the natural structure and function.[132] Scaffolds can provide structural support so that cells grow in the correct shape and location. They also ensure proper interactions between growth factors and progenitor cells to induce proliferation and differentiation of the latter into various cell types. Additionally, scaffolds can provide an efficient stem cell delivery system for effective tissue proliferation and differentiation. The self-organization method is an alternative method to support the cell differentiation process and the creation of cell sheets by growing cells in a monolayer. These monolayers can be manipulated by wrapping or draping them over structures. This technique has been successfully reported with chondrocytes in the repair of microtia,[177] osteochondral defects,[178] and even cardiac defects.[179]

**Relevant applications.** Decellularized, allogeneic, and xenogeneic scaffolds can be used with minimal donor site morbidity after autologous scaffold implantation. Risks associated with transplantation after prolonged culture can be avoided by using a cell-free extracellular matrix, allowing host cells to populate the scaffold without introducing foreign cells.[180] Nonetheless, further bench work is needed to determine whether seeding cells onto the decellularized grafts is necessary. While some studies have shown that scaffolds seeded with cells may have better outcomes initially,[181] it has also been shown that all scaffold donor cells deplete over 30 weeks post-transplantation and are replaced by host cells.[182] The following subsections review current applications and approaches using scaffolds and synthetic material to regenerate structures commonly affected by OA.

*Meniscus.* The meniscus is a complex hypovascular structure that comprises the medial and lateral menisci. The meniscus is a supportive structure that distributes mechanical load and protects the underlying articular cartilage in the knee. Owing to its biological and anatomical complex nature, meniscus repair and regeneration has been by far one of the most difficult challenges to effectively restore. Meniscal damage left untreated can cause mechanical alterations and morphological changes that express detrimental bioactive factors that accelerate the progression of OA.[183,184] Artificial biomaterials, tissue allografts, and bioengineered scaffolds have been tested in joints with severe meniscus damage with arthritic conditions and in partial meniscectomies to preserve joint survivability. However, these implants have been proven to be unsuccessful and have limited applicability on the basis of the severity of pathology. In contrast, scaffolds can be engineered as absorbable templates that consist of a cell population or are cell-free with biological factors to provide adequate cellular infiltration to the matrix synthesis for the damaged meniscal tissue. Type I collagen is a highly advantageous factor that can stimulate several healing processes. Specifically, a collagen meniscus implant, also known as Menaflex® (ReGen Biologics, Inc., Franklin Lakes, NJ), is composed of purified collagen type I fibers.[185,186] Absorbable scaffolds are currently engineered using bovine and synthetic polymers that are prepared with matrices to augment a variety of biomaterials. Superior short-term and long-term clinical improvements have been reported using absorbable collagen implants for meniscal tears that have undergone partial meniscectomies.[187,188] Other synthetically derived meniscus scaffolds, such as Actifit® (Orteq Bioengineering Ltd, London, UK), present an alternative to collagen-based scaffolds and have been shown to be just as effective in the short term.[189] Leroy *et al.*[190] recently reported that failure rates for Actifit® remain relatively high after 5-year follow-up. Despite the advancements in enhancing meniscus healing and regeneration using bioresorbable scaffolds, the challenges to control implant resorption and maintain durability persist. Biomimetic and microsphere scaffolds contain bio-informed materials that are used to stimulate native cells *in vivo* to restore zone-specific meniscal tissue.[191,192] Current efforts focus on stimulating cells and other seeded biomaterials before their

application. Strategies to address and improve these issues are currently under investigation.

*Cartilage.* Cartilage is inherently difficult to heal and regenerate, owing to its avascular nature. Autologous chondrocyte implants (ACIs) have been historically applied to chondral defects.[193,194] Several cell-free and cell-based variations have been developed since ACI constructs were first introduced. Other first-line, clinically available biomaterials include hyaluronan, agarose, chitosan, fibrin glue, alginate, and polylactic acid.[194] Because cartilage is under constant cyclic loading, successful scaffolds must be mechanically similar to native cartilage in order to withstand the constant load and maintain their shape and functionality over long periods of time.[195] Moreover, many synthetic and natural options exist for these purposes, such as hyaluronan-based scaffolds, porous poly(L-lactide) scaffolds, silk fibrin/chitosan blends, and multilayered systems that combine different materials, such as collagen, hyaluronan, and hydroxyapatite.[196] Hydrogel scaffold implantation is a promising approach to closely replicate the highly hydrated extracellular matrix and the overall native tissue.[195,197] These systems may induce cells from the surrounding native tissue to infiltrate the scaffold, be seeded with cells before implantation, or feature a combination of the two.

Scaffold-free methods can be valuable tools because they more closely replicate native tissue development. Self-assembly scaffold-free systems, such as octapeptides, can be good strategies to allow hydrogels to form spontaneously through molecular forces. A major benefit of self-assembly systems is that they closely replicate the natural development process of cartilage. Using custom-shaped molds, it is possible to grow materials to the exact shape and size desired for specific purposes. However, the drawbacks to self-assembly methods include the large number of cells necessary to successfully build a scaffold, as well as survival of the cells during the long assembly period (e.g., up to 196 days for blood vessels).[198] Decellularized cartilage can be used as a scaffold to recruit native chondrocytes. This method is especially successful when combined with cell sheet engineering to introduce chondrocytes to the decellularized scaffold *in vivo*.[199]

*Bone.* Bones constitute a unique environment owing to their ability to self-heal if, in the case of a bone fracture, the anatomical alignment is reset and the biological environment normalizes. However, in certain cases, such as infections or segmental resections due to tumors or nonunions, the bone is unable to heal on its own and requires surgical intervention via bone grafting.[200] Since bone grafting is prone to complications, harvest site morbidity, and prolonged recovery periods, scaffolds are a good alternative in certain cases. Alternatively, there are several design approaches using artificial bone fabrications.[201,202] It is important to note that it is difficult to create a porous structure that will allow adequate cell infiltration from the surrounding tissue. The porous nature of bone has led to the development of alternative materials (i.e., decellularized bone scaffolds) to create a biologically supportive material that can be utilized as a template to retain implanted cells and other bioactive factors to regrow durable bony tissue. It is a major challenge using autologous decellularized bone, which promotes donor site morbidity, while there is an increased risk of disease transmission and immune response using allogenic decellularized bone. Several promising synthetic options also exist, such as glass–ceramic,[203] hydroxyapatite, fibronectin, alginate,[204] and biphasic calcium phosphate, which reduce the risk of an immunological response but may lack material properties and factors native to human bone, which would reduce their efficacy as scaffolds. There is continued effort to design an implantable natural or synthetic scaffold with stem cells or bioactive factors that modulates a subsequent immunological response that consists of native-like biological materials to regenerate bony defects.[205,206]

### *Nanotechnology, 3D bioprinting, and rapid prototyping*

A material with a grain size of less than 100 nm is defined as a nanomaterial. Most biological molecules (e.g., proteins, enzymes, and nucleic acids) have similar dimensions and properties to nanomolecules, and therefore their biological behavior is closely related. On this basis, regenerative medicine applications for nanotechnology have been developed and studied in animal models. Cartilage regeneration is a current research focus, since OA is a particularly common pathology that has significant socioeconomic consequences. Self-assembling peptide amphiphile nanoscaffolds have been shown to potentiate cartilage repair in a microfracture animal model by increasing

the concentration of TGF-β1 in full-thickness chondral defects, which promotes chondrogenic differentiation of human mesenchymal stem cells released after microfracture.[19] Nanomaterials with manipulated surfaces resemble the extracellular matrix of cartilage tissue. Nanocomposite hydrogel systems are promising for improving the mechanical properties of conventional hydrogels and have been used successfully in cartilage regeneration studies.[18] The main advantage of nanoscaffolds when used in regenerative medicine is their small size, which allows them to mimic the natural nanofibrous matrix for cartilage cells and induce intracellular signaling processes more effectively.[207]

Rapid prototyping and 3D bioprinting are evolutionary fields with various applications to tissue engineering and joint arthritis therapy. 3D rapid prototyping models can be used for preoperative planning of advanced procedures, such as joint replacement surgery. This method has been used to aid in preoperative planning for the placement of the acetabular component in patients undergoing total hip arthroplasty.[208] Virtual templates have been created on the basis of cadaveric modeling and translated into physical templates.[181] 3D bioprinting has been successful in producing cartilage implants (polyethylene glycol dimethacrylate with human chondrocytes) to repair defects in osteochondral plugs (3D biopaper) in a layer-by-layer assembly.[209] Bioprinted osteochondral composites have also been produced and tested *in situ* in a rabbit model to restore trochlea cartilage damage, in which PEG/β-TCP composites fabricated by 3D bioprinting induced subchondral bone migration and subsequent cartilage repair.[210] Furthermore, these are only some examples that highlight the potential of nanotechnology, rapid prototyping, and 3D bioprinting technologies to strongly affect the therapeutic approach to degenerative joint disease.

## Current challenges and future directions

We have touched on several biological approaches that are used to enhance tissue healing and regeneration of OA tissues that have limited capabilities or are incapable of self-repair. This line of research has been shown to offer value for orthopedic and sports medicine applications; however, the therapeutic effects and underlying biological processes, functions, and applications of these biological approaches are still in their infancy.

Autologous PRP and BMC are promising sources to remodel cartilaginous and bony tissue. Chemotactic platelet- and plasma-derived growth factors are short-lived constituents that stimulate cell proliferation, multilineage cell differentiation, and other tissue metabolic processes. Minimal resources or closed systems may be used to produce autologous products that reduce the risk of a negative immunological response and are generally biocompatible. However, the scientific community continues to speculate about the clinical efficacy and the long-term effects of these biologic approaches, on the basis of published findings that contradict outcomes and lack of published standard operating procedures and formulations. In addition, the biological heterogeneity and complexity of PRP and BMC make it very difficult to characterize essential biological processes and functions *in vitro* and *in vivo*. Isolating specific growth factors or progenitor stem cells that undergo disparate molecular and cellular processes is imperative to characterize the underlying mechanisms and individual functions that contribute to functional tissue repair. The common denominator of the studies reporting on regenerative products has been positive functional clinical results (with limited objective data, such as imaging or histology) and no major adverse events, with short- to mid-term follow-ups. Additionally, the body of literature is highly heterogeneous (with regard to indications, processing method, number, and form of applications),[211] the improvements reported are usually modest and randomized, and unblinded methodologies do not control for patient- or clinician-related bias. As a result, no strong conclusions can be drawn, suggesting that current methods of cell therapy provide generalizable benefits to patients.[212]

Tissue-engineered scaffolds, synthetics, nanotechnologies, bioprinting, and rapid-prototyping are alternative favorable approaches. However, it is equally difficult to remodel cartilage and bone tissues with implantable scaffolds, synthetics, and nanomolecules, because physical ailments and biomechanical derangements caused by injury, trauma, or wear and tear can impair their function.

Blood-derived therapies and engineered biologics have been well established and are promising adjuncts to surgical intervention and injection therapy. There remains a significant amount of clinical and bench science work to be conducted to fully

understand the physiological mechanisms of, and suitable applications for, these biological derivatives to affect the standard of care.

## Competing interests

The authors declare no competing interests.

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