Injectable Biologics

What Is the Evidence?

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Abstract: Injectable biologics have attracted considerable interest in the field of musculoskeletal medicine. Biologics encompass a broad and diverse group of human tissue–derived therapeutics. The most commonly reported biologics for use in musculoskeletal conditions include platelet-rich plasma, bone marrow aspirate concentrate, mesenchymal stem cells, microfragmented fat, stromal vascular fraction, amniotic membrane–based products, and autologous conditioned serum. The benefits of biologics in tissue healing and regeneration are thought to be derived from their trophic, paracrine, and immunomodulatory functions. The purpose of this review is to define commonly used injectable biologics and to appraise current evidence on its efficacy in the treatment of musculoskeletal disease.

Key Words: Platelet-Rich Plasma, Mesenchymal Stem Cells, Biological Products, Injectable, Osteoarthritis, Tendinopathy

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Degenerative diseases of the musculoskeletal system are a growing epidemic and include ailments such as osteoarthritis (OA), degenerative disc disease, and tendinopathy.[1] Pharmacotherapy aims to slow or reverse cellular destruction and loss of tissue function. The development of stem cell–based biologics may also reduce pain and is proposed to promote a structural and functional reparative process and may defer surgical intervention.

In general, biologics are products derived from human, animal tissue, or microorganisms that include products derived from whole blood, cells, gene therapy, or recombinant proteins.[2] The US Food and Drug Administration (FDA) addressed the development and use of human cells, tissues, and cellular and tissue-based products (HCT/Ps).[3] Per the FDA, HCT/Ps are defined as "articles containing or consisting of human cells or tissues that are intended for implantation, transplantation, infusion, or transfer into a human recipient."[4]

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There are a variety of biologics that are used mainly in the treatment of musculoskeletal conditions, including platelet-rich plasma (PRP), bone marrow aspirate concentrate (BMAC), microfragmented fat (MF) preparations, stromal vascular fraction (SVF), mesenchymal stem cells (MSCs), amniotic membrane product (AM), and immunomodulatory molecules such as autologous conditioned serum (ACS). Mechanistically, these products are thought to confer benefits through immunomodulation and trophic activity. Furthermore, attenuation of inflammation within the injured tissue and release of growth factors and other regulatory proteins can improve tissue preservation and repair.[5,6] This clinical review defines commonly used injectable biologics and appraises current clinical evidence on their efficacy in the treatment of musculoskeletal disease along with brief descriptions of mechanism of action.

MATERIALS AND METHODS

We broadly searched all articles identified from PubMed (1966–July 2019) and Google Scholar (2004–July 2019) databases. Broad MeSH terms and Boolean operators were selected for each database search, including terms and synonyms for the following: MSCs, adipose-derived MSCs, bone marrow–derived MSCs, BMAC, PRP, ACS, MF, SVF, and amniotic membrane. The previously mentioned terms were further combined with the most common musculoskeletal conditions and included OA, tendinopathy, cartilage lesions, degenerative disc disease, and back pain.

In this study, we chose articles with the highest evidence level for each injectable biologic treatment and its application to musculoskeletal conditions. If available, meta-analyses and randomized controlled trials (RCTs) were used to appraise evidence for each type of biological treatment. If the biologic agent was not studied in any RCT, articles with a lower evidence level (e.g., prospective observational study and retrospective observational study) were reported in each category.

RESULTS

Platelet-Rich Plasma

Platelet-rich plasma is autologous platelet concentrate suspended in plasma.[5] Platelet-rich plasma stimulates recruitment, proliferation, and differentiation of regenerative cells via release of growth factors.[7] These proteins function as adhesive proteins, clotting factors, fibrinolytic factors, proteases, cytokines, antimicrobial proteins, and membrane glycoproteins.[8] Once the platelets are injected and activated by clotting, 70% of stored growth factors are released after 10 mins.[5]

Although initial studies with PRP were directed toward treatment of chronic tendinopathies, recent studies of PRP

reveal novel applications to treating ligament injuries, muscle tears, OA, discogenic back pain, and other spine-related pathology.[9] However, a recent meta-analysis evaluating the evidence of PRP injection in nonsurgical orthopedic procedures reported only marginal effectiveness in terms of pain and functional scores in the short term (up to 3 mos) and intermediate term (4–6 mos).[10] Hereinafter, we present findings from recent meta-analyses of PRP injection for musculoskeletal conditions.

Platelet-Rich Plasma in Tendinopathies

Most studies investigating PRP use in tendinopathies are focused on lateral epicondylosis/epicondylitis, plantar fasciitis, and rotator cuff tendinopathies. A systematic review of overlapping meta-analyses on PRP use in lateral epicondylitis revealed nine meta-analyses including 8656 patients.[11] Seven of these meta-analyses found that PRP significantly improved pain and elbow function in the intermediate term (12–26 wks), whereas four studies found that corticosteroid injections relieved pain and improved elbow function in the short term (<12 wks). The highest quality RCT by Arirachakaran et al.[12] (per Quality of Reporting of Meta-analyses (QUOROM) and Oxyman-Guyatt scores) found that PRP is most effective in the intermediate term, whereas corticosteroid injection improves pain and functional outcomes in the short term.

Similar findings are demonstrated for rotator cuff tendinopathies and plantar fasciitis. In patients with rotator cuff tendinopathy, a meta-analysis revealed that corticosteroid injection plays a role in pain reduction and functional improvement in the short term (3–6 wks), but not in the long term (over 24 wks); in contrast, PRP injection yields better outcomes in pain and function in the long term (over 24 wks).[13] Another meta-analysis on plantar fasciitis revealed that PRP is superior to steroid injections for treatment of long-term pain relief (after 24 wks), but no differences were observed between short (4 wks) and intermediate terms (12 wks).[14]

Limited evidence is present for other tendinopathies including Achilles and patellar tendinopathies. A meta-analysis of five RCTs comparing PRP with placebo (saline or eccentric loading) in Achilles tendinopathy revealed no differences in Victorian Institute of Sports Assessment-Achilles (VISA-A) scores. Visual analog scale (VAS) pain scores did not differ at 6 and 24 wks but were lower in the PRP group at 12 wks. This meta-analysis concludes that limited evidence supports that PRP injection is superior to placebo treatment.[15] For patellar tendinopathies, two RCTs are reported. A meta-analysis investigating the use of PRP in patellar tendinopathies reported that eccentric exercise therapies were associated with the best result at short term (<6 mos); however, PRP injection demonstrated superior results at long-term follow-up (>6 mos; Table 1).[16] In terms of dosing, studies investigating PRP use in tendinopathies reported injectate volumes ranging from 1.5 to 6 ml per injection. There is limited evidence for the preferred platelet concentration factor, which was variable among studies and inconsistently reported. Some reviews reported a platelet concentration factor of 1.5- to 8-fold increase in platelet content.[22–24]

Platelet-Rich Plasma in Joint and Cartilage Damage

Platelet-rich plasma use in knee OA has been extensively studied in 15 RCTs comprising 1314 patients. A meta-analysis reported that PRP injections reduced pain more effectively than hyaluronic acid (HA) injections at 6 and 12 mos of follow-up. Better functional improvement was demonstrated in the PRP cohort at 3, 6, and 12 mos of follow-up (Table 1).

The shock-absorbing and load-bearing lumbar and cervical facet joints can also degenerate from mechanical stress and arthritis. Trials suggest that PRP may offer chondroprotection, anti-inflammatory effects, cell-phenotype modulation, and joint pain attenuation.[25] A recent prospective randomized trial compared the efficacy and safety of autologous PRP *vs.* local anesthetic/corticosteroid intra-articular injection for management of lumbar facet joint syndrome.[18] This study revealed improvement in VAS scores in both groups at multiple time points up to 6 mos compared with pretreatment; however, the VAS scores were lower in the autologous PRP group compared with control (Table 1).

Platelet-Rich Plasma in Discogenic Back Pain

Three unique pathologies of the intervertebral disc include internal disc disruption, degenerative disc disease, and disc herniation.[26] Traditional interventions include physical therapy, percutaneous epidural steroid injection, discectomy, surgical decompression, arthrodesis, and arthroplasty.[26] There is limited evidence on injection of biologics. The first RCT compared intradiscal PRP *vs.* intradiscal contrast injection in chronic lumbar discogenic pain.[17] There were improvements in physical functioning, pain, and satisfaction in the PRP group at 8 wks. The association of better physical function scores was sustained for at least 1 yr (Table 1).

Bone Marrow Aspirate Concentrate

Bone marrow aspirate concentrate is prepared by centrifugation of bone marrow and extraction of the layer containing concentrated mononuclear cells. It is composed of bone marrow cells, small tissue fragments, and blood from venous sinuses.[27] The nucleated cells in BMAC may deliver cytokines and growth factors at the delivery site, including Platelet-derived growth factor (PDGF), Transforming growth factor β (TGF-β), and bone morphogenetic protein (BMP)-2 and BMP-7, which possess both anabolic and anti-inflammatory effects.[28] The MSCs in BMAC may offer a source for repair.[27] However, MSC only comprise 0.001%–0.01% of mononuclear cells in BMAC.[29] Bone marrow aspirate concentrate has been used in many musculoskeletal conditions including knee and talar osteochondral lesions, osteonecrosis of the femoral head, OA, fracture repair, acetabular, and tibial bone defect.[30]

Bone Marrow Aspirate Concentrate in Osteochondral Lesions and Osteoarthritis

Only one study with level II evidence has investigated BMAC use in osteochondral lesions, which demonstrated that HA scaffold with BMAC provides better clinical outcomes and more durable cartilage repair compared with microfracture in patients with grade IV knee cartilage injury (Table 1).[20] Although both groups of patients appreciated clinically significant outcomes, 100% of the BMAC-treated group maintained improvement after 5 yrs, whereas less than one-third of the microfracture-treated group maintained improvement. Only one study with level II evidence has investigated the efficacy of BMAC in knee OA without an adjunctive procedure. The

TABLE 1. Studies assessing platelet-rich-plasma and bone marrow aspirate concentrate

Authors, Year	Study Type	No. Studies/ Patients	Condition	Treatment	Volume/Dose	Outcomes
Houck et al., 2019[11]	Systematic review of meta-analyses	9 studies	Lateral epicondylitis	PRP	X	PRP significantly improved pain and elbow function in the intermediate term (12–26 wks), steroid injections relieved pain, and improved elbow function in the short term (<12 wks)
Arirachakaran et al., 2016[12]	Meta-analysis	10 studies	Lateral epicondylitis	PRP	X	PRP injection improved pain and decreased risk of complications, disability scores, and pain but had a higher risk of complications
Lin et al., 2019[13]	Systematic review and meta-analysis	18 studies	Rotator cuff tendinopathy, plantar fasciitis	PRP	X	Steroids reduced pain and improved function in the short term (3–6 wks), but not in the long term (over 24 wks); in contrast, PRP injection yields better outcomes in pain and function in the long term (over 24 wks).
Yang et al., 2017[14]	Meta-analysis	9 studies	Plantar fasciitis	PRP	X	PRP was superior to steroid for treatment of long-term pain relief (after 24 wks), but no significant differences were observed between short (4 wks) and intermediate terms (12 wks).
Liu et al., 2019[15]	Meta-analysis	5 studies	Tendinopathies	PRP	X	No differences between PRP and placebo in VISA-A scores after 12 wks, 24 wks, and 1 yr. VAS lower in PRP group at 12 wks.
Andriolo et al., 2019[16]	Meta-analysis	22 studies	Patellar tendinopathies	PRP	X	Eccentric exercise showed the best result at short term (<6 mos); however, PRP demonstrated the best results at long term (>6 mos)
Tuakli-Wosornu et al., 2016[17]	RCT	47 pts	Lumbar discogenic pain	PRP	30 ml conc. into 3–4 ml of PRP, 1–2 ml injected	PRP showed significant improvements in patient satisfaction scores (FRI, NRS, NASS) over 8 wks vs controls. PRP group maintained significant improvements in FRI scores to 1 yr.
Wu et al., 2017[18]	RCT	46 pts	Facet joint arthropathy	PRP	0.5 ml, conc. of 100–300 bil./ml	PRP *vs.* local anesthetic/steroid intra-articularly showed that both are effective and safe; however, PRP has better long-term efficacy
Gianakos et al., 2017[19]	Systematic review	9 studies	Nonunion or delayed union	BMAC (alone or with combination treatment)	X	Studies reported lower or similar complication rates postoperatively in BMAC compared with placebo.
Gobbi et al., 2016[20]	RCT	50 pts	O/CH lesions	BMAC	6× higher conc. from baseline	HA scaffold with BMAC provides better clinical outcomes and more durable cartilage repair compared with microfracture in patients with grade 4 knee cartilage injury
Shapiro et al., 2017[21]	RCT	25 pts	Knee OA	BMAC	5 ml of BMAC in 10 ml of PPP	Demonstrated improved clinical outcomes at 1 wk and 3 mos in the BMAC group, but no difference after 6 mos.

bil., billion; conc., concentration; FRI, Functional Rating Index; NASS, North American Spine Society Outcome Questionnaire; NRS, Numerical Rating Scale; O/CH, osteochondral; PPP, platelet-poor plasma; pts, patients.

authors demonstrated improved clinical outcomes at 1 wk and 3 mos in the BMAC group, but no difference after 6 mos (Table 1).[21]

Bone Marrow Aspirate Concentrate in Nonunion or Fractures

A systematic review reported that nine studies evaluated the use of BMAC in bone healing, of which eight studies looked at nonunion or delayed union. All studies reported lower or similar complication rates postoperatively in the BMAC group compared with placebo. Bone consolidation and time-to-bone-union were also better in the BMAC group compared with the autograft group. The studies used BMAC alone or in conjunction with freeze-dried allograft, bone chips, or demineralized bone matrix with recombinant human BMP-2.[19] (Table 1).

Fat Tissue–Derived Biologics

Microfragmented Fat

Microfragmented fat is an adipose-tissue derivative prepared via mechanical breakdown of fat tissue into tiny particles to release cells from the extracellular matrix. No collagenase treatment or culture expansion is involved during its preparation process. The resulting product is rich in MSCs, preadipocytes, fibroblasts, and macrophages.[31] Despite its wide point-of-care use in a number of countries, the literature does not provide satisfactory evidence of efficacy.

We found four nonrandomized trials for the treatment of OA and cartilage defects that were either prospective or retrospective (level of evidence III–IV).[32–35]

Microfragmented Fat in Knee Osteoarthritis and Cartilage Defects

A prospective open label study demonstrated that MF injection into osteoarthritic knees induced an increase in cartilage glycosaminoglycan content and improved VAS scores at 3, 6, and 12 mos of follow-ups.[33] Two other studies demonstrated significant improvement in clinical scores that lasted 12 mos after injection.[32,35] One of them applied MF in a combination with chondral shaving (Table 2).[35]

A retrospective study evaluating patients with degenerative chondral defects grade I–IV showed improvement in subjective subscore of International Knee Documentation Committee questionnaire, in total Knee injury and OA Outcome Score (KOOS), and in VAS scores at 12 mos. In this case, MF was applied during arthroscopy alone in 20% of patients and in combination with ligament reconstruction, high tibial osteotomy, or meniscectomy in 80% of studies (Table 2).[34]

Microfragmented Fat in Shoulder Osteoarthritis and Rotator Cuff Tear

Effectiveness of MF injections in the treatment of shoulder OA and rotator cuff tear was demonstrated in one prospective nonrandomized study. As the treatment was targeting pain of multifactorial origin, each patient received injections to different locations. The American and Shoulder Elbow Surgeons score and Numerical Pain Rating Scale score improved significantly at all follow-up evaluations and were maintained for at least 12 mos after injection (Table 2).[36]

Stromal Vascular Fraction

Stromal vascular fraction is a term used for a product consisting of a heterogeneous mixture of cells. The product is obtained from adipose tissue that is digested with collagenase. Adipocytes and free fat are then removed after centrifugation. Mechanical agitation of the harvested tissue may be used instead to avoid controversial enzymatic separation.[42,43] The population of cells in SVF includes MSCs along with macrophages, red blood cells, T-cells, preadipocytes, pericytes, fibroblasts, and endothelial cells.[44] Different methods to prepare SVF have been found to yield inconsistent final product in terms of cell type, proportions, and total cell counts.[45] Stromal vascular fraction is most commonly used in the treatment of OA of the knee and hip, cartilage lesions, and Achilles tendinopathy.

Stromal Vascular Fraction in Osteoarthritis, Osteochondral, and Chondral Defects

Three articles discussing SVF use in cartilage damage were identified.[40,41,46] A self-controlled study treated patients with knee OA.[40] One knee was injected with SVF and the other with HA after an arthroscopic debridement. The SVF group showed improvement in VAS, Western Ontario and McMaster Universities OA Index (WOMAC), and radiological scores compared with the knees treated with HA and baseline scores (Table 2). A retrospective study evaluated SVF with arthroscopic bone marrow stimulation in the treatment of osteochondral lesions *vs.* bone marrow stimulation alone. Patients underwent this treatment during second-look arthroscopy after supramalleolar osteotomy combined with bone marrow stimulation. Stromal vascular fraction was found to be superior compared with bone marrow stimulation alone in VAS scores, American Orthopedic Foot and Ankle Score (AOFAS), and Tegner scores (Table 2).[41] Another study evaluated SVF in conjunction with microfracture for treatment of cartilage defects compared with microfracture alone. Pain scores improved and persisted at least 18 mos in the SVF and microfracture group.[46] In some other studies, PRP was added to SVF under the hypothesis that PRP has supportive growth factors[46–50]; however, no comparative study has been reported on the outcome.

Stromal Vascular Fraction in Achilles Tendinopathy

Three articles reported results of an RCT for Achilles tendinopathy comparing SVF with PRP.[37–39] They presented clinical outcomes and correlation between magnetic resonance and ultrasound findings. There were higher VAS, AOFAS, and VISA-A scores in groups treated with SVF injections at 15 and 30 days; however, later follow-ups did not show significant lasting benefits. According to the study, tendon thickness measurements by magnetic resonance showed correlation with ultrasound results. The thickness increased by 1 mm as measured by both modalities (Table 2).

Mesenchymal Stem Cells

Mesenchymal stem cells are cells derived from mesenchymal tissues and arise from pericytes.[51] They were first described in 1991 as cells of multilineage potential for their ability to differentiate into osteocytes, adipocytes, chondrocytes, tendons, and other cell types.[52] Since then, they have been isolated from different human adult tissues, which are bone marrow, adipose tissue, umbilical cord, and amniotic membrane.[53] Mesenchymal

TABLE 2. Studies assessing microfragmented fat and stromal vascular fraction

Authors, Year	Study Type	No. Patients (Joints)	Condition	Treatment	Volume/Dose	Outcomes
Russo et al., 2017[34]	Retrospective review	30 pts	Knee OA	MF	10–15 ml	VAS decreased by 24 points, Tegner Lysholm Knee by 31 at 12 mos of follow-up, IKDC-subjective and KOOS improved by 20 points; 1 patient had recurrent effusions in the first months.
Cattaneo et al., 2018[35]	Retrospective review	38 pts	Knee OA ± meniscal injury	Chondral shaving ± menisectomy and MF	10 ml	Improvement noted in all clinical scales, KOOS subscales and WOMAC at all time points for chondral shaving group only, mild decrease of effect in menisectomy group from 6 to 12 mos.
Hudetz et al., 2017[33]	Prospective open label	17 pts	Knee OA	MF	4–15 ml	Increase in GAG in cartilage (dGEMRIC), improved VAS score at 3, 6, and 12 mos
Panchal et al., 2018[32]	Prospective open label	17 pts (26 knees)	Knee OA, grade III–IV	MF	Not known	Significant improvements were noted in the mean values of NPRS, FXN, and LEAS at 6 wks, 6 mos, and 12 mos. The KSS significantly improved at 6 wks and 12 mos. No serious adverse events were reported.
Striano et al., 2018[36]	Prospective open label	18 pts	Shoulder OA	MF	4 ml intra-articular, 1–2 ml in other perilesional locations	NPRS and ASES improved at all time points, scores changed from 7.94 to 3.7 (NPS) and 33 to 66 (ASES) at 12 mos.
de Girolamo et al., 2016[37]	RCT	56 pts	Achilles tendinopathy	SVF vs PRP	Not known	Significantly better VAS, AOFAS, and VISA-A scores in SVF group starting at 15 d. Improvement in both groups on MRI, US at 6 mos. No AEs.
Albano et al., 2017[38]	RCT	43 pts	Achilles tendinopathy	SVF vs PRP	4 ml	Significant decrease of VAS from pretreatment (6.4 ± 1.4) to 6 mos (1.8 ± 1.7). Tendon thickness increased on MRI and US and power Doppler signal in SVF group.
Usuelli et al., 2018[39]	RCT	44 pts	Achilles tendinopathy	SVF vs PRP	4 ml	VAS, AOFAS, and VISA-A improved at 15 and 30 d in SVF compared with PRP group. No correlation between clinical and radiological findings was found.
Hong et al., 2019[40]	Randomized self- controlled trial	16 pts	OA K-L II or III	SVF vs HA in each patient	4 ml	SVF-treated knees improved in VAS, WOMAC scores, and ROM at 12 mos of follow-up. In control group, all scores worsened from baseline to the last follow-up visit. WORMS and MOCART revealed cartilage repair in SVF-treated knees.
Kim et al., 2014[41]	Cohort study	49 pts (50 ankles)	Osteochondral lesion talus	Bone marrow stimulation + SVF (24) or bone marrow stimulation alone (26)	3.94 mil. cells	The mean VAS, AOFAS, Tegner, and MOCART scores improved significantly in the MSC group compared with the conventional group. Significant correlations of the MOCART score with clinical outcomes were found in both groups.
Nguyen et al., 2016[46]	Prospective cohort study	30 pts	OA K-L III or IV	Microfracture + SVF + PRP or microfracture alone	5 ml, 10 mil. SVF cells/ml	Significantly reduced pain and improved WOMAC, Lysholm, and VAS scores compared with the placebo group maintained for at least 18 mos.

AE, adverse event; ASES, The American Shoulder and Elbow Surgeons Shoulder Score; dGEMRIC, delayed gadolinium-enhanced MRI of cartilage; FXN, function score; GAG, glycosaminoglycans; IKDC, International Knee Documentation; K-L, Kellgren-Lawrence score; KSS, Knee Society Score; LEAS, Levels of Emotional Awareness Scale; mil., million; MOCART, Magnetic Resonance Imaging Osteoarthritis Knee Score; NPRS, Numeric Pain Rating Score; pts, patients; SVF, stromal vascular fraction; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

stem cells were initially thought to be used mainly in reconstitution of injured tissues. However, according to latest literature, the effects of MSCs have been reconsidered. The immunomodulatory and trophic functions are believed to be crucial for their role in regeneration.[53] Defining characteristics distinguishing MSCs are surface markers CD90, CD105, CD73, CD44, and their adherence to plastic surface of the cell culture flask.[54,55] Culture expansion with the use of human platelet lysate may produce high yields of zoonotic free MSCs.[54] All studies examining MSCs for the indications below showed preliminary efficacy and safety with only minor adverse events such as self-limited mild joint swelling. This was true for adipose-derived MSCs (AMSCs) and bone marrow–derived stem cells (BMSCs) including autologous as well as allogeneic stem cells that were injected into soft tissues such as intervertebral discs, joints, muscles, and tendons.

Mesenchymal Stem Cells in Osteoarthritis

We have identified five systematic reviews and/or meta-analyses appraising effects of MSCs in OA and cartilage repair.[56–60] These studies included all types of MSCs containing treatments and included MSCs extracted from all cell source types. One systematic review and meta-analysis evaluating MSCs use in OA showed significant improvement in pain, function, and cartilage quality. However, the evidence for these outcomes was considered low to very low.[56] Another systematic review for knee OA evaluated one observational study and five RCTs with a high level of bias and showed efficacy in patient-reported outcomes at final follow-up at 24–48 mos and improvements in radiology assessments.[57] Generally, patients with additional debridement, lower OA grade, and use of activation agents had better outcomes.[58] The dosing and volumes varied in reviewed articles (Table 3). Mesenchymal stem cells containing products were commonly applied in volumes appropriate to the injected tissue. For example, as most studies for OA injected knees, volume was usually from 0.5 to 15 ml. Doses of stem cells varied from 10 to 150 million cells divided into one to two doses. In our review, we present original studies to evaluate potential differences between types of MSCs.

Adipose-Derived MSCs

Adipose-derived MSCs and BMSCs are currently used in many clinical trials and are the most commonly used types of MSCs in both clinical and research applications. Adipose-derived MSCs and BMSCs were found to be used in clinical studies examining OA and degenerative disc disease at about the same frequency. One benefit of AMSCs is that they are obtained through a relatively painless procedure. In addition, adipose tissue biopsy yields higher amounts of cells than bone marrow biopsy.[69]

Adipose-Derived MSCs in Osteoarthritis and Cartilage Defects

The benefits of AMSCs in OA have been demonstrated in three RCTs examining knee OA.[61,62,70] A study using culture-expanded AMSCs showed 55% improvement in total WOMAC score in the AMSC group compared with placebo at 6 mos. There were no signs of worsening of the cartilage defect in the AMSC group, but defects' sizes increased in the control group (Table 3).[62] Another RCT using two different doses of autologous culture-expanded AMSCs showed improvement in pain and function at 12 mos. In addition, radiological analysis of the defects indicated modification of disease progression. The benefits were maintained longer in the two-injection group when patients received 100 million cells at baseline and at 6 mos.[61] Microfracture, a commonly used procedure for cartilage repair, was reported to be combined with AMSCs and fibrin glue in a nonblinded study. In this case, it was not completely clear whether only AMSCs or SVF were applied. The combination was shown to be superior to microfracture alone in terms of improvement in pain and KOOS symptom subscore. At 24 mos of follow up, lesions were smaller in the AMSC group compared with the microfracture only group (Table 3).[70]

Bone Marrow–Derived MSCs

The first isolated and in vitro tested MSCs were derived from bone marrow. Bone marrow–derived stem cells closely resemble AMSCs in terms of biological properties and immunomodulatory functions[69,71] although minor differences have been noted in their differentiation and expansion capacity. Bone marrow–derived stem cells demonstrate increased differentiation potential into osteocytes and chondrocytes compared with AMSCs.[72]

Bone Marrow–Derived Stem Cells in Osteoarthritis and Cartilage Defects

We identified five RCTs using injection of BMSCs for knee OA. A phase 1/2 study using three different doses of autologous MSCs reported significant improvements in WOMAC and VAS scores at 12 mos, with the best results in the highest dose group. Furthermore, there was radiographic evidence showing no decrease in joint space.[63] Bone marrow–derived stem cells were also applied as an adjunctive therapy to surgical procedures, e.g., in addition to microfracture and high tibial osteotomy. After surgery and BMSCs application, The Tegner, Lysholm, International Knee Documentation, and Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scores improved significantly when BMSCs were combined with HA vs in HA alone.[67]

Transplantation of allogeneic BMSCs to osteoarthritic knees significantly improved VAS and WOMAC pain subscores at 6 and 12 mos after injection[64]; however, another study using allogeneic cells reported only nonsignificant improvements in clinical scores.[65] A double-blinded RCT used BMSCs in conjunction with HA *vs.* HA alone and showed increase in meniscal volume after partial meniscectomy in the BMSC groups (Table 3).[66]

Bone Marrow–Derived Stem Cells in Degenerative Disc Disease

One RCT using BMSCs for intervertebral disk disease was identified.[68] This RCT reported that intradiscal injection of BMSCs led to decreased pain scores by 3 mos, which were maintained at 6 and 12 mos of follow-ups. Disc height decline was lower in the MSC group compared with controls (Table 3). A recent systematic review summarized work on evidence of stem cell use in degenerative disc disease. They identified one RCT and case series showing either no difference in symptoms between the stem cell and control cohort or relative improvement in symptoms without clear clinical significance.[73]

TABLE 3. Studies assessing mesenchymal stem cells

Author, Year	Study Type	No. Studies/ Patients	Condition	Treatment	Volume/Dose	Outcomes
Iijima et al., 2018[56]	Meta-analysis	35 studies	Knee OA	MSCs	X	Significant improvement in pain, function and cartilage quality. However, the evidence for these outcomes was considered low to very low.
Pas et al., 2017[57]	Systematic review	5 RCTs and 1 non-RCT study	Knee OA	MSCs	X	All RCTs showed benefits in patient-reported outcomes and on imaging studies compared with control at 24–48 mos. 2 trials reported better histological or arthroscopic evaluations.
Freitag et al., 2019[61]	RCT	30 pts	Knee OA	Autologous AMSC cultured (1 or 2 doses) vs standard of care treatment	100 mil. 1 or 2 injections, 3 ml of isotonic fluid	Significant pain and functional improvement at 12 mos. Radiological analysis using MOCART indicated modification of disease progression.
Lee et al., 2019[62]	RCT	12 pts	Knee OA	Autologous AMSC cultured	100 mil. cells, 3 ml	WOMAC score was significantly improved at 6 mos in the AMSC group but not in the control group. The defect was stable in AMSC group but increased in the control group.
Koh et al., 2015[70]	RCT	44 pts	Knee cartilage defect	Microfracture + AMSCs or microfracture alone	Cell-thrombin-fibrinogen solution	In AMSCs group, the defect was healed compared with microfracture alone. The mean KOOS pain and symptom subscores were greater at follow-up in AMSCs than in microfracture. Second-look arthroscopy showed some tissue repair that was not significant.
Lamo-Espinoza et al., 2016[63]	RCT	30 pts	Knee OA	HA or autolog. BMSCs (2 groups for 2 doses 10 M or 100 M)	10 or 100 mil, in 1.5 ml or 3 ml Ringers Lactate followed by 4 ml of HA	VAS decreased in all follow-ups and at 12 mos. WOMAC improved at 6 mos in lower dose and was maintained to 12 mos in higher dose. X-ray showed reduction in knee joint space in controls only. ROM improved at 12 mos in BMSCs group only.
Gupta et al., 2016[64]	RCT	60 pts	Knee OA	Allogeneic BMSCs (Stempeucel), 25 M, 50 M, 75 M, 150 M	25, 50, 75, 150 mil. cells, in 2 or 4 ml or PLASMA-LYTE	Improvement in 25-M-cell dose group in VAS, ICOAP, and WOMAC scores (not statistically significant). AEs predominant in 50, 75, and 150 M cells, WORMS did not reveal any difference from baseline
Vega et al., 2015[65]	RCT	30 pts	Knee OA	Allogeneic BMSCs	40 mil. cells in 8 ml	MSC-treated patients displayed significant improvement in algofunctional indices vs controls. Quantification of cartilage by T2 relaxation measurements showed cartilage quality improvements in MSC patients.
Vangsness et al., 2014[66]	RCT	55 pts	Knee OA and meniscal regeneration	Allogeneic BMSC cultured (2 doses vs sodium hyaluronate)	50 or 150 mil. cells in HA, human serum albumin and PlasmLyte, 5 ml total	Pain relief in VAS score and significant improvement in 50 M dose (24% increase) vs in 150 M (6% increase) in meniscal volume.
Wong et al., 2013[67]	RCT	56 pts	Knee OA and genu varum	Microfracture + BMSCs with HA or + HA only	14 mil. in 0.5–1 ml	Age-adjusted assessments showed improvement in Lysholm, Tegner, and IKDC scores in favor of BMSCs.
Noriega et al., 2017[68]	RCT	24 pts	Degenerative disc disease	Allogeneic BMSCs	25 mil. cells	Responders (40% of the cohort) displayed improvement in algofunctional scores vs controls. Pfirrmann improved in the MSC-treated patients and worsened in the controls.

AMSCs, adipose-derived mesenchymal stem cells; HA, hyaluronic acid; ICOAP, Measure of Intermittent and Constant Osteoarthritis Pain; M, million; mil., million; pts, patients; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

Amniotic Membrane Product

Amniotic membrane is a thin and flexible placenta-derived membrane composed of an epithelial layer, basement membrane, and avascular mesenchymal tissue.[74] Biologically active amniotic membrane compounds include MSCs, cytokines, HA, extracellular matrix proteins, growth factors, and other proteins. It has been documented that amniotic membrane–derived MSCs have the ability to differentiate into several lineages.[75] Various types of products using amniotic membrane preparations have been used in clinical studies. Some studies used cryopreserved amniotic membrane product that included MSCs, keratinocytes and fibroblasts, and amniotic fluid.[76] Other studies used a cell-free dehydrated amnion/chorion membrane allograft.[77,78] Amniotic cells have been shown to be immunomodulatory in vitro.[79]

Amniotic Membrane in Tendinopathies

Two RCTs have been conducted using the micronized dehydrated human amnion/chorion membrane and cryopreserved amniotic membrane for treatment of refractory plantar fasciitis.[77,78] A blinded feasibility study showed significant improvements in AOFAS score compared with placebo at 1 wk and during final follow-up at 8 wks.[77] The second double-blinded study did not show any significant improvements when compared with steroids injections. Only the Foot Health Status Questionnaire score was improved in the group receiving two injections of micronized dehydrated human amnion/chorion membrane at 18 wks (Table 4).[78]

Autologous Conditioned Serum

Autologous condition serum is defined as autologous serum enriched in products released by platelets and leukocytes.[83] It is an acellular treatment and is produced by incubating venous blood in a specialized ACS-processing syringe.[84] When the blood is exposed to the internal surface of the specialized syringe, the blood cells produce anti-inflammatory cytokines including interleukin (IL)-1 receptor antagonist (IL-1Ra), IL-4, and IL-10.[85] In contrast to PRP, ACS does not contain additives (e.g., anticoagulants) and involves a single withdrawal of patient blood.[84] Importantly, ACS has a different cytokine profile, containing high concentrations of IL-1Ra, which contributes to its anti-inflammatory profile.[84] Autologous condition serum preparation should be avoided in patients with elevated C-reactive protein as any preexisting systemic inflammation at the time of blood sampling may shift the cytokine levels in favor of pro-inflammatory factors.[86] This may enhance the inflammatory response after ACS injection.

Autologous Conditioned Serum in Osteoarthritis

Two RCTs have been reported on ACS for treatment of knee OA. One of the studies randomized patients with knee OA to receive ACS, HA, or intra-articular saline injection. Compared with HA and saline injections, ACS was found to be more efficacious as assessed by the WOMAC index, global patient assessment, and VAS scores at 7, 13, and 26 wks of follow-up, and these improvements were clinically meaningful.[87]

TABLE 4. Studies assessing amniotic membrane product and autologous conditioned serum

Author, Year	Study Type	No. Studies/ Patients	Condition	Treatment	Volume/Dose	Outcomes
Zelen et al., 2013[77]	RCT	45 pts	Plantar fasciitis	Micronized dehydrated human amniotic/ chorionic membrane	0.5 or 1.25 and 2 ml of Marcaine 0.5% plain	Significant improvement in function, pain, and well-being in patients receiving 0.5 or 1.25 ml μDHACM *vs.* controls throughout the study period.
Hanselman et al., 2015[78]	RCT	37 pts	Plantar fasciitis	Cryopreserved human amniotic membrane	2.5 ml	Cryopreserved hAM had greater FHSQ foot pain improvement at 18 wks in the 2-injection cohort.
Auw Yang et al., 2008[80]	RCT	167 pts	Knee OA	ACS	2 ml	ACS injection relative to saline injection was associated with improvements in KOOS symptom and sport parameters, although there was no difference in the primary endpoint (WOMAC scores).
Becker et al., 2007[81]	RCT	32 pts	Lumbar radiculopathy	ACS	Not known	ACS injections were compared with triamcinolone, reductions in pain and disability in both groups were observed, ACS group was superior only at week 22.
Goni et al., 2015[82]	RCT	40 pts	Neck pain	ACS	2.5–3 ml	ACS injection and MP injections both led to improvement in VAS, neck disability index, and SF-12, the improvement in the ACS group was more gradual and sustained over time while the MP pts deteriorated.

μDHACM, micronized dehydrated human amniotic/chorionic membrane; c-AM, cryopreserved amniotic membrane; FHSQ, Foot Health Status Questionnaire; hAM, human amniotic membrane; mil., million; MP, methylprednisolone; pts, patients; SF-12, 12-item Short-Form Health Survey.

These associations were also supported by another RCT that demonstrated ACS injection relative to saline injection was associated with improvements in KOOS symptom and sport parameters, although there was no difference in the primary endpoint of WOMAC scores (Table 4).[80]

Autologous Conditioned Serum in Other Musculoskeletal Conditions

It is well-known that lumbar radiculopathy is mediated by inflammatory cytokines such as IL-1. Thus, studies theorized that because ACS is enriched in IL-1Ra and other anti-inflammatory cytokines, injection at the site of compression and inflammation may relieve lumbar radiculopathy.[81] An RCT comparing ACS injections with triamcinolone injections in lumbar radiculopathy revealed reductions in pain and disability in both ACS and glucocorticoid injections, although significant superiority in the ACS group was seen at week 22.[81] Similarly, another RCT revealed that ACS injection and methylprednisone injections both led to improvement in pain scores and neck disability index, although improvement in the ACS group was more gradual and sustained over time, whereas the methylprednisone group experienced deterioration over time (Table 4).[82]

DISCUSSION

Injectable biologics for musculoskeletal disease consist of a heterogeneous group of stem cell derivatives. Although no direct comparisons of efficacy, functional scores, and pain scores between biologic treatments were conducted, categorization of biologics may provide a helpful heuristic schema by which to summarize potential benefits and limitations. A framework for categorizing stem cell products has been described and organizes MSCs according to the "five generations" model based on MSC preparation strategy.[88] Bone marrow aspirate concentrate and MF prepared with minimum manipulation and for homologous use are considered to contain the first generation of MSCs. Other types of MSCs being studied across the translational spectrum include culture-expanded stem cells (second generation), lineage-directed stem cells (third generation), genetically modified stem cells (fourth generation), and induced pluripotent stem cells (fifth generation).

In general, the most widely used MSCs are minimally manipulated (first generation) or culture-expanded (second generation) MSCs. The first-generation MSCs such as those present in BMAC or MF were investigated in studies with low- to moderate-level evidence evaluating osteochondral defects, bone fractures, and OA. Bone marrow aspirate concentrate was beneficial in the treatment of OA and osteochondral defects if used in combination with an HA scaffold. Microfragmented fat was generally beneficial for OA, cartilage defects, and rotator cuff tears.

Stromal vascular fraction is not considered minimally manipulated because it is processed using collagenase[4] and thus not considered exempt from FDA regulation as a drug. Studies with moderate evidence suggested that SVF may be associated with higher functional scores and lower pain scores in OA, osteochondral and chondral defects, and Achilles tendinopathy. Common cartilage repair procedures such as bone marrow stimulation and microfracture combined with SVF also significantly improved chondral and osteochondral defects.

Injection of culture-expanded AMSC and BMSC (second generation) also showed improvement in pain and functional scores in the treatment of OA and cartilage defects, with radiographic evidence of reduction in disease progression. The evidence of MSCs is limited for other indications with only one RCT found for disc degeneration. We did not appreciate a difference among the spectrum of indications or in effectiveness of different types of MSCs. In studies using AMSCs, autologous cells were more commonly applied. Bone marrow–derived stem cells were more often introduced as allogeneic.

When comparing the three adipose-derived biologics (AMSCs, SVF, MF), it remains unclear whether one is more beneficial than the other for any specific application. The included studies differ in terms of clinical indications and used variable methodology. Stromal vascular fraction use is most commonly investigated in treatment of tendinopathies, whereas AMSCs and MF were generally used in treatment of OA and cartilage defects. From our review, higher-level evidence was present for SVF and culture-expanded AMSC. There were no RCTs reported for MF, which interestingly is the most convenient and the least manipulated adipose-derived biologic. In addition, SVF and MF were more often used in combination with surgical procedures.

In terms of the persistence of benefits, AMSCs and BMSCs may be associated with long-term pain and functional improvements maintained at study follow-up periods between 12 and 48 mos in OA and cartilage defects and at 12 mos in degenerative disc disease. Use of ACS and MF may be associated with symptom relief at 26 wks and 12 mos, respectively, in OA. However, there is a paucity of high-level evidence to support any recommendation suggesting use of a specific injectable biologic for long-lasting effects.

Platelet-rich plasma remains the best-studied biologic with the strongest evidence available regarding its safety and efficacy in a variety of musculoskeletal conditions. Its effects have been shown to be most beneficial in the treatment of lateral epicondylitis, plantar fasciitis, and rotator cuff tendinopathy in several meta-analyses with results suggesting superiority to corticosteroid and placebo treatment over the intermediate to long-term periods, generally over 3–6 mos. Injectate volumes were not always reported and varied from 1 to 10 ml.[10] Overall, we suggest that further studies are necessary to investigate optimal PRP dosage for various targeted structures as the current dose heterogeneity may have impacted the results of the previously mentioned outcomes.

Cryopreserved amniotic membrane injections were investigated in plantar fasciitis in a relatively smaller sample size (42 patients) and showed relief of symptoms for up to 3 mos. The effects were comparable with steroids and did not show any adverse effects demonstrating potential in efficacy and opportunity to further explore its use in other indications. One of the benefits of using amniotic membrane product might be its potential use after dehydration and/or freezing, which makes it easier to store and use whenever needed. However, amniotic membrane product characteristics were different in both studies, and although both complied with the 361 of the Public Health Service Act, the safety and efficacy of amniotic membrane should be further investigated.

Finally, acellular ACS has demonstrated benefits compared with placebo and steroids in treatment of OA, radiculopathy,

and neck pain in four randomized trials. Its effects may last up to 8.5 mos. As these trials were published between 2007 and 2015, it is possible that newer studies focus on some of the specific molecules included in ACS such as IL-1Ra.

Injectable biologic use in the research and clinical setting is dependent on regulatory body approval. The FDA has published documents on the use of HCT/Ps.[3] Some HCT/Ps such as BMAC do not require premarket approval by the FDA and are regulated solely under section 361 of the Public Health Service Act and 21 CFR Part 1271 if they meet specific criteria. These criteria include having undergone only minimal manipulation, intended for homologous use only, not combined with another agent, and either not expected to have any systemic effect or autologous, allogeneic from a first- or second-degree blood relative, or for reproductive use. An institution removing and subsequently administering HCT/P without any intervening manufacture or steps beyond rinsing, sizing, or shaping may qualify for the surgical procedure exemption from regulation.[4] All HCT/Ps prepared with more than minimum manipulation are considered drugs. The FDA approval for Investigational New Drug license is required for any human application in clinical trial settings. Currently, only use of autologous PRP, MF, and BMAC may be considered as manufactured under the minimal manipulation definition. Culture-expanded or further modified stem cells are considered drugs and have to undergo the FDA approval to be used clinically.

There are several limitations in the assessment of biologics for musculoskeletal conditions. Although the number of published studies is dramatically increasing, there is a paucity of high-quality RCTs to support the efficacy of biologics in functional and pain scores and their regenerative capability. There are inconsistencies in methodology from study design to product preparation to therapeutic target. Heterogeneity in dosing and variable combination with an adjunct drug or procedure also make reproducibility and generalizability challenging.

CONCLUSIONS

It is unclear what likelihood the injectable biologics will play in the role of musculoskeletal medicine. Currently, there is only relatively short-term data (12 mos) on the efficacy of injectable biologics in improving pain, functional scores, and structural degeneration reducing properties with largely low- to moderate-level quality of evidence. For this to have a more likely role in the future of care, there will need to be high-quality, well-powered clinical trials, which show not only efficacy in pain reduction and functional improvement but also cost-effectiveness in reducing the need for future treatments.

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