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Operative Techniques in

Sports Medicine

Biologics for Skeletal Muscle Healing: The Role of Senescence and Platelet-Based Treatment Modalities

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Skeletal muscle is a highly interactive connective tissue that makes up a large portion of an adult's body mass. Muscles are integrated with tendons as well as other specialized structures to support physiologic and homeostatic functions. In the state of injury, muscles have the ability to innately repair themselves through various phases of tightly regulated healing that can result in fibrotic tissue development. However, during aging, these homeostatic processes are disrupted leading to sarcopenia and reduced muscle regeneration capacity. Several cell-based therapies and biologic therapies have been investigated to regenerate skeletal muscle tissue and reduce fibrosis following injury or during aging. These include platelet-rich plasma (PRP) and an acellular portion of blood known as platelet-poor plasma (PPP). However, current clinical practice recommendations for the utilization of different PRP preparations vs PPP are unclear. Recent efforts have strove to improve the understanding of the role of senescent cells and profiles in the presence of early to late stage skeletal muscle injury and fibrosis, yet targeted interventions to remove senescent cells and attenuate the secretory environment to improve muscle regeneration are still forthcoming. Therefore, the purpose of this article is to review the basic principles of skeletal muscle repair, the role of senescence in attenuated muscle regeneration, and discuss current standards and literature supporting PRP and PPP treatment for skeletal muscle repair. This review concludes with future directions to improve biologic therapies and ongoing initiatives to customize PRP and PPP preparations using Food and Drug Administration-approved medications.
Oper Tech Sports Med 28:150754 © 2020 Published by Elsevier Inc.

KEYWORDS skeletal muscle, platelet-rich plasma (PRP), platelet-poor plasma (PPP), cytokines, chemokines, senescence, senescence associated secretory phenotype (SASP)

Introduction

Skeletal muscle is a highly interactive connective tissue that makes up 30%-40% of an adult's body mass.[1,2] Muscles are integrated with tendons as well as other specialized structures to support physiologic and homeostatic functions.[2,3] In the state of injury, muscles naturally heal through different phases that can result in fibrotic tissue development, especially in pathologic states or during aging.[3,4] Although, the course of age-related muscle adaptations has shown to hinder recovery, activation of muscle stem cells, and repair mechanisms.[4,5] The increased burden of senescent cells and the senescence-associated secretory phenotype (SASP) has been shown to increase with age and play a significant role in muscle atrophy and reduced repair capacity.[6-9] Recent efforts have strove to improve the understanding of the role of senescent cells and profiles in the presence of early to late stage skeletal muscle injury and fibrosis,[6-8] yet targeted interventions to remove senescent cells

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Conflicts of Interest: The authors have disclosed the following conflicts of interest that are unrelated to the submission of this review manuscript: J. H. receives royalties from Cook Myosite Inc.; T.A.E. has financial interests in Vail Valley Surgery Centers and ProofPoint Biologics. V.O., H.L., W.S.H., K.E.W. have no conflicts to disclose.

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https://doi.org/10.1016/j.otsm.2020.150754
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and attenuate SASP to improve muscle regeneration are forthcoming. Meanwhile, several cell-based therapies and biologic therapies have been investigated to regenerate skeletal muscle, including platelet-rich plasma (PRP) and an acellular portion of blood known as platelet-poor plasma (PPP).[10-17] However, current clinical practice recommendations for the utilization of different PRP preparations vs PPP remain unclear. The purpose of this article is to review the basic principles of skeletal muscle repair, the role of senescence in attenuated muscle regeneration, and discuss current standards and literature supporting PRP and PPP treatment for skeletal muscle repair. This review concludes with future directions to improve biologic therapies and ongoing initiatives to customize PRP and PPP preparations using Food and Drug Administration-approved medications.

Skeletal Muscle Healing and Regeneration

Skeletal muscle has an intrinsic ability to regenerate damaged tissue following injury.[18] In response to muscular injury in the form of strain, contusion or laceration, a specific biological response commences consisting of muscle degeneration, inflammation, regeneration, and fibrotic scar formation.[19] The phases of skeletal muscle healing are summarized below.

Muscle Degeneration: Muscle degeneration near the site of an injury may be induced following a direct tear or compressive force. This is characteristic of contusion or laceration injuries that can induce muscle degeneration near the injury site, while the tensile force of a muscle strain can cause a rupture, resulting in the induction of muscular degeneration near the musculotendon integration point.[20] Though mechanical injury such as a contusion, laceration, or strain can disrupt the entire length of a muscle fiber, the extent of injury and resulting necrosis is contained by cytoskeleton contraction bands that are distributed throughout each muscle cell.[21] Following injury, plasma membrane damage and a subsequent influx of extracellular calcium cause muscle protein degradation, typically reconciling necrosis within a matter of hours. Blood vessel damage precedes swelling and hematoma formation and further promotes muscle degeneration.[21]

Inflammation: The inflammatory cascade is initiated by the release of chemoattractants, such as cytokines, chemokines and growth factors from damaged cells within injured tissue. It is hypothesized that the release of said chemoattractants cause chemotaxis of circulating inflammatory cells to the site of injury via damaged blood vessels and the release of growth factors from the disrupted extracellular matrix.[22,23] Additional growth factors, cytokines, and chemokines are produced by macrophages and fibroblasts in response to injury and serve to further promote the inflammatory response.[24] Neutrophils are the predominant immune cell during the initial pro-inflammatory phase, followed by an influx of monocytes within 2 days of the injury.[25] These monocytes give rise to macrophages that degrade and remove the necrotic tissue prior to skeletal tissue regeneration.[25]

Regeneration: Satellite cells are a major contributor to muscle regeneration, which occurs approximately 1-4 weeks after the injury.[20] Though muscle fibers are terminally differentiated, skeletal muscle contains undifferentiated satellite cells that are capable of forming new muscle tissue.[26] These cells are found underneath the basal lamina of myofibers and are stimulated by growth factors secreted during the immune response.[27] These cells become myoblasts, capable of fusing with the injured myofibers. Additional progenitor cells found in muscle tissue, blood vessels, bone marrow, connective tissue, and mesenchymal tissues are also critical contributors to muscle regeneration.[28]

Fibrosis: Fibrosis, the formation of a connective tissue scar, begins with the cross-linking of fibrin and fibronectin within a hematoma to create early granulation tissue.[29] Fibroblasts contribute to fibrosis by anchoring to the granulation tissue and synthesize extracellular matrix components. Several days later, fibroblasts produce type I collagen, characteristic of mature scar tissue.[2] Though this fibrotic tissue increases muscular tensile strength, the scar-myofiber interface is susceptible to rupture and is therefore often implicated in skeletal muscle reinjury.[30]

The Role of Cellular Senescence in Skeletal Muscle Healing

Somatic stem cells reside in an undifferentiated state in several adult tissues. In muscle, satellite cells play a pivotal role in muscle regeneration in a coordinated effort with immune cells and nascent muscle cells.[5,31-33] Upon activation driven by immune cell signals, satellite cells proliferate and fuse to form new muscle fibers to regenerate muscle fibers, followed by a return to a quiescent state for downstream regeneration.[34] However, aging disrupts this process leading to significant reduction in regeneration and growth, resulting in conditions such as frailty and sarcopenia. First, there is evidence of an age associated decline in satellite cell number.[35-37] In addition, there is a noticeable attrition in satellite cell function with age[38-40] including loss of self-renewal capacity, loss of homeostatic signaling ability which leads to impaired regeneration capacity and functional muscle loss.[5,32,37,41-44] Aging is also associated with an increased burden of senescent cells,[37,45] which is thought to contribute to stem cell dysfunction through the production of senescence associated pro-inflammatory and antiregenerative factors.[46,47] Thus, age-related decline in muscle regeneration involves cell autonomous mechanisms (loss of satellite cell function) and noncell autonomous mechanisms (senescent cells).

Immunosenescence is a major noncell autonomous driver of age-related musculoskeletal demise. Immunosenescence is an age-associated phenomenon whereby immune cells exhibit altered function or *anergy*.[48-53] This includes significant alteration in the secretory profile of immune cells resulting in an increase in circulating pro-inflammatory cytokines like TNF-α, IL-6, and/or IL-1β which leads to a chronic state of low-grade inflammation, called *inflammaging*.[31,47] This significantly impacts muscle regeneration and induces cellular senescence due to the fact that the cross-talk between immune cells and muscle satellite cells is an early and critical stage of muscle repair.[22] Immunosenescence has been well reviewed elsewhere,[48-53] but in the context of muscle regeneration, macrophages play a critical role in reduced repair capacity with age.

Macrophages are critical to signal proliferation of satellite cells and to protect satellite cells from apoptosis[54] but these functions are blunted during the aging process.[55] In murine studies, it has been shown that the number of activated MyoD expressing satellite cells co-expressing pro-apoptotic markers like Bax is higher in aged animals.[55] In addition, isolated satellite cells from aged animals exhibit reduced expression of the antiapoptotic marker Bcl-2, associated with an increased sensitivity to TNF-α induced pro-apoptotic caspase activation.[56] In human studies, it has been found that aged adults (71+) have fewer macrophages than those of younger individuals and that macrophages of aged patients exhibited higher expression levels of both pro-inflammatory cytokines IL-1β and anti-inflammatory cytokines IL-1RA and IL-10 indicating a general disruption of muscle homeostasis at the macrophage level with aging.[57] Accordingly, it was also found that macrophage response following exercise was reduced, in regards to their pro-inflammatory and anti-inflammatory expression profiles.[57,58]

Muscle stem cells are also directly affected by age in a cell autonomous manner via the induction of cellular senescence which is thought to directly lead to dysfunction and loss of proliferative ability and response to immunomodulation during muscle regeneration. With aging, muscle stem cells display a senescent phenotype typified by expression of the cell cycle inhibitor $p16^{Ink4a}$. This leads to depression of Rb/E2F target genes[5] and in addition to $p16^{Ink4a}$, p21Cip1 expression is increased leading to cell cycle arrest. This is most clearly demonstrated using the BubR1 hypomorphic mouse model of progeria whereby the expression of $p16^{Ink4a}$ in muscle stem cells has been shown to directly contribute to muscle wasting and repair dysfunction[5,45,59,60] and that conditional ablation of $p16^{Ink4a}$ expressing muscle stem cells improves muscle performance and attenuates wasting[59] Thus, cell autonomous alterations due to senescence induction in muscle stem cells significantly limits the self-renewal capacity of the tissue[5,41,42,44] and targeting senescence in aged muscle tissue may improve muscle repair[42] but can also be used to delay sarcopenia.[61]

Of note, while senescence in muscle stem cells and infiltrating immune cells likely disrupts muscle homeostasis with age, emerging evidence suggests a positive role for acute cellular senescence during muscle regeneration. It was recently found that senescence of fibro-adipogenic progenitors (FAPs), in response to exercise-induced muscle damage, is required to maintain levels of important pro-inflammatory regenerative factors to support optimal muscle regeneration.[62] FAPs are muscle resident platelet-derived growth factor receptor-α-positive (PDGFRα+) mesenchymal progenitors that are essential regulators of inflammation during the regeneration process via promotion of satellite cell differentiation.[63,64] When FAPs become dysfunctional, due to aging or disease, they play a role in chronic inflammation and fibrosis.[65-67] During the chronic inflammation setting, like in muscle aging, FAPs have been found to take on a senescent phenotype that is anti-apoptotic resulting in lack of FAP clearance and increased fibrosis.[62,64] Thus, the transition of FAPs to a senescent phenotype seems to have positive effects on muscle regeneration and the prevention of fibrosis and should thus be considered when targeting senescence in muscle as an intervention to improve muscle regeneration/repair. The notion that senescence can play an important role in tissue homeostasis, outside the pathologic setting, is not foreign. Indeed, senescent cells have been found to be important for tissue remodeling during embryonic development,[68,69] tissue repair,[70,71] and tumor suppression.[72]

Overall, targeting senescent cells in muscle may have potential therapeutic benefits for tissue repair via restoration of stem cell function and SASP factor reduction. However, it remains to be determined whether senescence should be targeted at a particular phase in the muscle healing process, especially considering the protective role of FAP senescence in early stages of muscle regeneration. More studies are necessary to uncover these answers using novel tools that target senescent cells and SASP, such as transgenic murine systems and senolytic agents that selectively eliminate senescent cells. There will always be a delicate balance between preservation of stem cell benefits and removal of deleterious effect of cellular senescence.

Challenges in Current Biological Approaches to Skeletal Muscle Healing

Recent biological approaches to improve muscle healing after injury have focused on enhancing muscle regeneration and reducing muscle fibrosis.[10,19,29,63,73] These alternative approaches include, gene therapy, exercise, neuromuscular electrical stimulation, blood flow restriction, and massage therapy; however, additional research is necessary to determine their efficacy in enhancing skeletal muscle repair.[10,19] Traditionally, rest, ice, compression, and elevation, or RICE protocols, in conjunction with nonsteroidal anti-inflammatory drugs (NSAIDs), have been recommended in the treatment of acute musculoskeletal injury.[3] Though immobilization immediately after injury is beneficial in reducing bleeding, swelling, and fibrotic scar size, after several days, immobilization becomes detrimental to muscle regeneration.[74] Prolonged immobilization can lead to muscle atrophy and decreased strength, increased connective tissue deposition, as well as decreased histologic and functional healing, as demonstrated in a mouse laceration model.[75] Similarly, NSAID administration was investigated to determine the effect of cyclooxygenase-2-specific inhibitors on muscle atrophy in vitro and in vivo.[76] These experiments demonstrated delayed musculoskeletal healing and increased fibrotic deposition after treatment with NSAIDs.[76]

Biologic augmentation with stimulatory growth factors, gene therapies, cell therapies, and antifibrotic agents aim to improve muscle regeneration and repair. Despite the safety and accessibility of recombinant growth factor injections, this treatment is often only efficacious with a high concentration of recombinant factors and consecutive injections.[77] Preclinical investigations of gene therapy suggest that this novel approach may be useful for delivering beneficial factors while targeting specific deleterious factors, though its application is currently encumbered by its immune response and cytotoxicity side effects.[78] Allogenic stem cell therapies have shown

promise in pre-clinical studies, as results have shown that injection of muscle-derived stem cells following surgical repair can increase angiogenesis and reduce scar tissue.[79] Additionally, intraperitoneal injection of muscle-derived stem cells has been found to increase lifespan and reduce fibrosis in progeroid animals.[80]

Platelet-Rich Plasma and Plasma Treatment Background

PRP is a biological treatment strategy that is thought to promote tissue regeneration and healing.[27] This promising autologous augmentation option has prompted increased scientific interest and has become widely utilized in the field of orthopaedics over the last 15 years.[81] Such widespread application is validated by strong market projections, with the global market for PRP predicted to reach $590 million by 2025, at a Compound Annual Growth Rate of 10.9%.[82]

High concentrations of blood cells, growth factors, cytokines, chemokines, and other biologically active factors in PRP are thought to direct tissue formation during the acute healing phase and as such, have been widely investigated for musculoskeletal repair and regeneration.[83] Though there is clinical evidence to suggest that return to activity can be achieved earlier with PRP treatment at the site of injury, the effect has been found to be short term.[84,85] Furthermore, although PRP therapy for skeletal muscle repair has demonstrated functional improvement in preclinical and limited clinical trials,[84-88] there remains a lack of standardization, clinical uniformity, and robust scientific evidence needed to affirm its clinical utility in treating skeletal muscle strain injuries.

Despite the increased prevalence of orthobiologic utility, evaluating the clinical efficacy of PRP remains a significant challenge due to widespread variability and inadequate clinical reporting standards. Insufficient reporting of preparation techniques, composition and other variables that may influence clinical outcomes precludes interpretation, reproducibility and cross-study comparison.[89] In response to clear evidence of such defective reporting methodologies, the American Academy of Orthopaedic Surgeons (AAOS) established minimum reporting requirements for clinical studies evaluating the efficacy of PRP. The Minimum Information for Studies Evaluating Biologics in Orthopaedics (MIBO) guidelines for PRP were created to promote increased transparency, reproducibility, and enhanced clinical evaluation capabilities.[89] Though these enhanced reporting standards will likely provide clarity on the utility of autologous blood concentrates in musculoskeletal treatment, a more novel, diverging theory may provide evidence instead for the use of PPP in facilitating muscle regeneration.

It is known that PRP is comprised of various biologically active factors including PDGF, VEGF, TGF-β1, EGF, and IGF-1.[90,91] The concentrations of these factors vary with the preparation of PRP, as leukocyte-poor PRP (LP-PRP), leukocyte-rich PRP (LR-PRP), and PPP all contain different compositions of bioactive molecules, as previously described

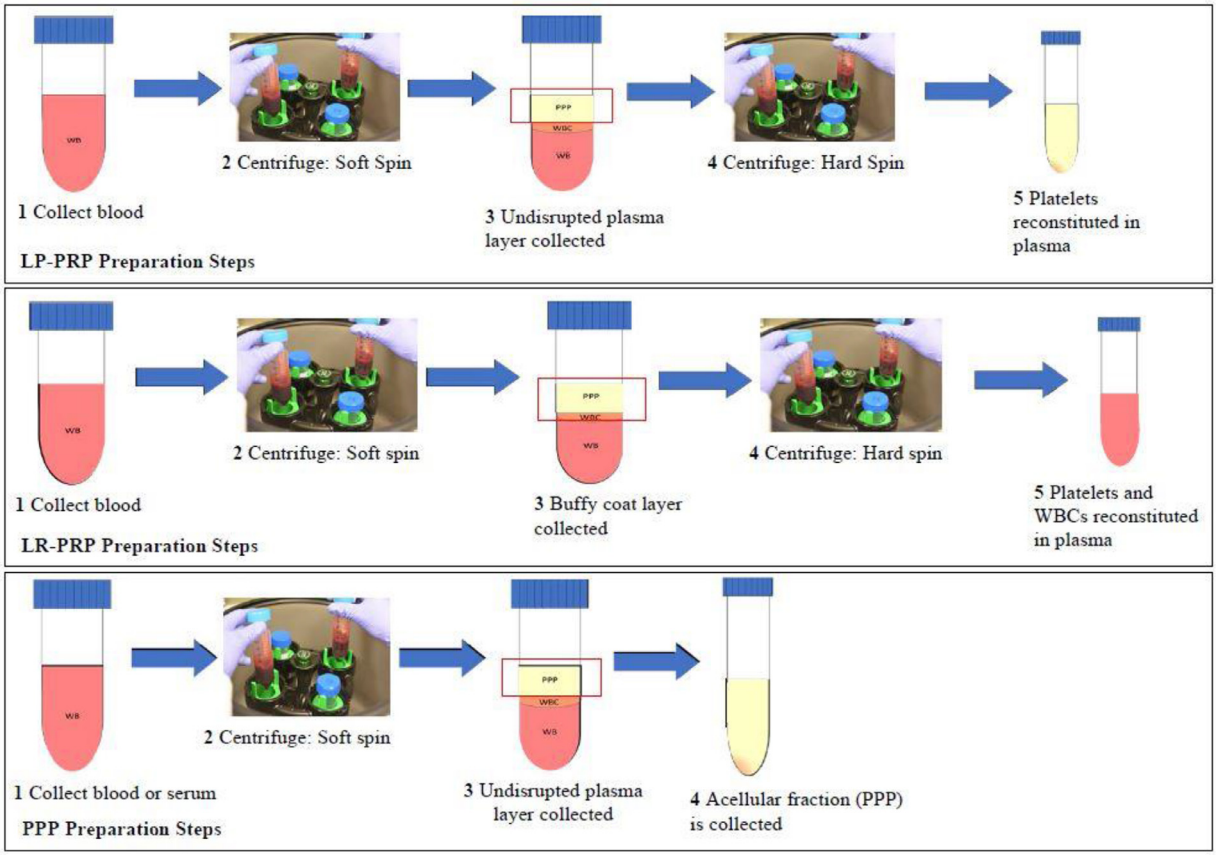

Figure Preparation differences between platelet-rich plasma (PRP) and platelet-poor plasma (PPP).

(Figure).[92-94] PPP is a modified preparation of whole blood or PRP by means of a second centrifugal spin to remove blood cells, and it has also been shown to contain biologically active factors.[95] PPP contains smaller concentrations of bioactive molecules including PDGF and IGF-1, which are theorized to be responsible for muscle repair.[96] In a recent laboratory study, blood from 7 human donors was processed to produce PPP and LP-PRP preparations that were subsequently subjected to a second spin to remove the platelets.[14] In vitro analysis of these biologic products revealed that these acellular platelet-poor preparations led to the stimulation of myoblast differentiation necessary for skeletal muscle regeneration, while unmodified LP-PRP led to myoblast proliferation.[14] Though further clinical studies are required to further elucidate the effect of these biologics, these preliminary data suggest that PPP may in fact be a more optimal treatment for muscle regeneration than traditionally formulated PRP.[14]

A literature search was performed in an attempt to find studies pertaining to PRP (different preparations) or PPP and their effect on aspects of skeletal muscle healing and regeneration. The following summarizes the general findings of the literature search, which includes both preclinical and clinical studies.

Platelet-Rich Plasma and Plasma Treatment: Clinical Studies

A search for ongoing and completed clinical trials investigating the use of PPP and PRP on skeletal muscle healing revealed a total of nine studies (Table 1), 3 of which are active or currently recruiting (Table 2). All but 2 of the identified studies investigated the effects of PRP in its application as an interventional treatment for skeletal muscle (NCT01440725, NCT03676205, NCT02607462, NCT02726464, and NCT03371888),[86,97] the remaining 2 studies compare the effects of PPP and PRP as an investigational treatment for skeletal muscle healing (NCT03618979 and NCT01812564). Of the 9 studies, results were only available in 4 (NCT03371888 and NCT01812564).[86,97]

Outcome measures were available for all studies with a large amount of overlap as many studies examined: time to recovery (NCT01440725),[86,97] time to return to play (NCT03676205 and NCT01812564), and pain intensity (NCT01440725, NCT03676205, NCT02726464, NCT03371888, and NCT03618979) between treatment and placebo groups.[86,97] All of the aforementioned studies reported on visual analog scale for pain between baseline to postintervention; results on this outcome were only available for studies that used PRP as a treatment (NCT03371888).[86,97] A statistically significant short-term improvement in pain intensity from baseline to various time points post-PRP injection was reported in all PRP studies that included pain as an outcome (NCT03371888).[86,97]

While available results were sparse, the bulk of the findings were complementary to each other. A study comparing the effects of PPP and PRP injections in the treatment of hamstring strains in athletes found that subjects injected with PRP returned to play significantly faster than those who received PPP injections to the control group (NCT01812564). Similarly, a study examining the effect of PRP injections as a treatment for acute muscle tears found a statistically faster return to play time in subjects receiving a PRP injection, when compared to subjects who received no injection at all.[97] Results from the included clinical studies provide evidence that PRP injections into injured skeletal muscle may have a therapeutic effect in reducing pain as a result of injury (NCT03371888),[86,97] while also reducing return to play time (NCT01812564).[97]

Platelet-Rich Plasma and Plasma Treatment: In Vitro Studies

Five studies investigated the use of PPP and PRP on myogenesis in vitro (Table 3). Four of the 5 studies use only PRP as the investigational treatment for skeletal muscle cells[98-101]; the 1 remaining study used several autologous blood products, including PRP and PPP, to compare the effects of investigational treatments on skeletal muscle cells.[14]

The type of cell used in vitro varied from study-to-study, including C2C12 myoblasts (murine), human CD56 positive myoblast cell line (hMC), human skeletal muscle myoblast, and skeletal muscle cells (intrinsic to Sprague-Dawley rats).[98] While no 2 studies had identical outcome measures, each study analyzed the effects of the respective treatment on cellular proliferation and differentiation in the in vitro model(s).[14,98-101] Interestingly, all 5 studies using PRP as either the main investigational treatment or as a comparative treatment saw a statistically significant increase in myogenic proliferation.[14,98-101] On the contrary, 1 study reported inhibition of myogenic differentiation,[14] while the rest of the studies reported no significant changes in myogenic differentiation from PRP treatment alone.[98-101] A significant increase in myogenic differentiation was reported in 2 studies: 1 study used PPP and platelet-depleted PRP as separate investigational treatments,[14] while the other study used a treatment composed of a combination of PRP and Decorin, a TGF-β inhibitor.[99]

Platelet-Rich Plasma and Plasma Treatment: Preclinical Studies

A search for ongoing and completed preclinical trials investigating the use of PPP and PRP on skeletal muscle healing gave rise to 10 studies (Table 4). While all of the studies investigated the in vivo effects of PRP as a skeletal muscle treatment in animal subjects, none of the included studies used PPP as an investigational treatment. PRP was the primary investigative treatment used in the majority of the studies, though several studies investigated the use of PRP in concomitance with other treatments, such as exercise training,[102] cold-water immersion,[103] swim training,[104] and low-level laser therapy.[105]

The lack of studies investigating PPP as a treatment in animal models makes it near impossible to compare the therapeutic effects of PRP and PPP treatment on skeletal muscle in preclinical applications. Further, due to each study manipulating different variables (ie, treatments and outcomes), no concise conclusion on the effect of PRP treatment in skeletal muscle could be deduced from the above studies. Nonetheless, following application of PRP, many studies found evidence of

Table 1 Completed and Ongoing Clinical Trials Investigating the Use of PPP and PRP on Skeletal Muscle Healing

Study Title	Outcome	Results	Intervention
Efficacy of platelet-rich plasma for treatment of muscle rupture with hematoma (NCT01440725)	Time to recovery of lesions (secondary; pain)	No results	PRP
Platelet-rich plasma in acute muscle injuries (NCT03676205)	Return to play, pain intensity	No results	PRP
PRP therapy to m. gluteus medius during THA (NCT02607462)	The decrease of T2-weighted signaling between PRP and placebo group	Recruiting	PRP
Testing the characteristics of platelet-rich plasma in sports medicine (NCT02726464)	Pain score	Ongoing	PRP
The platelet-rich plasma in the therapy of temporomandibular disorders (NCT03371888)	Pain intensity	Significant improvement in pain per the VAS	PRP
Ultrasound-guided injections of platelet-rich plasma for muscle injury in professional athletes. Comparative study[86]	Pain relief, recovery, regeneration	Short-term significant improvement in pain per the VAS and strength and range of motion	PRP
Does platelet-rich plasma decrease time to return to sports in acute muscle tear? A randomized controlled trial[97]	Pain, recovery time, return to play	Significant improvement in pain, return to play and full recovery. No statistical significance at 2-year follow-up	PRP
A trial comparing three orthobiologic therapies on atrophied multifidus muscles in patients with low back pain (NCT03618979)	Changes in atrophy of muscle, (secondary; pain)	Recruiting	PPP + extracellular matrix, PRP, PRP + platelet lysate
Use of platelet-rich plasma in the management of acute hamstring muscle strain injury (NCT01812564)	Return to play	Significant difference in return to play for the PRP vs PPP group (quicker for PRP)	PRP, PPP

Table 2 Ongoing/Active Clinical Trials Investigating the Effects of platelet-rich plasma (PRP) or platelet-poor plasma (PPP) on Skeletal Muscle, Per clinicaltrials.gov

Study Title	ClinicalTrials.gov Identifier	Status	Location
A trial comparing three orthobiologic therapies on atrophied multifidus muscles in patients with low back pain	NCT03618979	Recruiting	Centeno-Schultz Clinic, Broomfield, Colorado, United States|Centeno-Schultz Clinic, Lone Tree, Colorado, United States
PRP therapy to m. gluteus medius during total hip arthroplasty	NCT02607462	Recruiting	Satakunta Central Hospital, Pori, Finland
Testing the characteristics of platelet-rich plasma in sports medicine	NCT02726464	Active—not recruiting	Allan McGavin Sports Medicine Centre, Vancouver, British Columbia, Canada

enhanced skeletal muscle healing and regeneration[102,104-109] and a decrease in oxidative damage at the site of the injury.[103,110] However, the effect on skeletal muscle healing depends on the concentration of PRP used, the time frame post-injury that the PRP was injected and whether the PRP was used as a solo treatment or in combination with other treatments.

Future Directions and Conclusion

The application of PRP for the treatment of skeletal muscle has been widely investigated, while very limited data exist on PPP treatment for skeletal muscle repair and regeneration. PRP and PPP also contain deleterious cytokines, such as TGF-β1, that can cause fibrosis and inhibit native skeletal muscle regeneration.[15,111] Recent efforts have focused on the customization of PRP and PPP to inhibit/reduce biological factors associated with SASP and fibrosis for local administration.[12,15,16] When investigating the effect of TGF-β1 neutralization, an increase in regenerative myofibers and satellite cells was observed in PRP and customized PRP (inhibition of TGF-β1), though there was a notable decrease in fibrotic deposition in the customized PRP.[16] This suggests that neutralizing TGF-β1 within PRP can promote muscle regeneration while significantly reducing fibrosis.[12,16,112,113] Various antifibrotic agents that block the effects of TGF-β1 are being investigated, including the administration of Food and Drug Administration-approved antihypertensive medication, losartan (Cozaar). By blocking the stimulation of TGF-β1 production, losartan (Cozaar) has been shown to significantly reduce fibrosis and enhance muscle fiber regeneration in mice, prompting further investigation and promising preliminary outcomes in clinical case studies.[12]

An intriguing option to improve PRP and PPP treatments for skeletal muscle regeneration is the use of senolytic agents. As previously discussed, cellular senescence leads to satellite cell dysfunction through cell autonomous pathways, senescent transition of muscle stem cells themselves, or through noncell autonomous means through exposure to senescence associate secretory factors in neighboring muscle and immune cells.[5,38-43] Thus, it stands to reason that targeting senescence may improve biologics efficacy either through the reduction of local SASP factors prior to treatment, or via removal of senescent cells from the biologic itself. In the case of plasma-based biologic therapies, this would likely be through pretreatment in the patient given the regenerative properties of plasma are likely related soluble factors and not progenitor cells themselves.[114] Indeed, pro-fibrotic factors have been found in the secretome of senescent fibroblasts, including ACTA2, encoding α-SMA, COL1A1, COL1A2, TGF-β, and fibronectin 1 (FN1). These factors activate macrophages and myofibroblasts, decrease FAP apoptosis, and increase local inflammation which when dysregulated will lead to excessive extracellular matrix (ECM) production and the formation of a permanent fibrotic scar.[46,63,73,115,116] Aberrant muscle fibrosis is certainly present in pathologic settings (ie, muscle dystrophy) but also during aging given the chronic inflammation and immunosenescence known to occur during the aging process.[51,52,73] Indeed, several murine studies demonstrate that senescent cells and SASP factors are known to accumulate in aged and dystrophic muscles[73] highlighting senescence as a therapeutic target. Accordingly, there is also some evidence to support a beneficial role for senolytics to improve muscle regeneration through the modulation of SASP production and reduction of fibrosis. It has been shown in humans and mice that cellular senescence mediates idiopathic pulmonary fibrosis, and senescent cell ablation with senolytic drugs (dasatinib plus quercetin) in the idiopathic pulmonary fibrosis setting improves pulmonary function through reduction in fibrosis.[37,45,46,51,52,59,60,73,117] Thus, senolytic treatment prior to autologous PPP treatments may improve the regenerative potential of the biologic through reduction in fibrosis, especially in aged or sarcopenic patients.

In summary, our understanding of PRP/PPP and their mechanism of action continues to expand while further innovative strategies are developing to optimize the therapeutic efficacy. There is recent evidence that a synergistic effect on muscle healing has been demonstrated using a combinatorial approach of antifibrotic agents (ie, losartan [Cozaar]) and PRP.[12] However, further clinical research is warranted to support these findings prior to routine clinical utility. Furthermore, there is a paucity of in vivo studies and clinical trials supporting the use of an acellular blood fraction (PPP) for the treatment of injured skeletal muscle. The future direction of PRP or PPP treatment for skeletal muscle repair will be based on the customization of biological factors by targeting disease-specific markers (ie, SASP or individual factors such as TGF-β1) and senescent cells that attribute to the development of fibrosis.

Table 3 Completed In Vitro Trials Investigating the Use of PPP and PRP on Skeletal Muscle Healing

Study Title	Outcome	Results	Intervention	Cell Model
Platelet-rich plasma promotes skeletal muscle cell migration in association with upregulation of FAK, paxillin, and F-Actin formation[98]	Cell migration, proliferation, differentiation (or fusion), regenerative effect	PRP Group: Statistically significant change in cell migration (dose dependent), wound healing, cell spreading, and increased F-actin	PRP	Skeletal muscle cells (rats)
Platelet-rich plasma, especially when combined with a TGF-Œ ≤ inhibitor promotes proliferation, viability and myogenic differentiation of myoblasts in vitro[99]	Proliferation, metabolic activity, cytokine profile, expression of myogenic regulatory factors	PRP Groups: Statistically significant: increase in proliferation, downregulation of TGF-β expression PRP + Decorin group: Downregulation of MSTN levels, myogenic differentiation	PRP and PRP + Decorin (TGF-β inhibitor)	Human myogenic progenitors (hMC)
The influence of platelet-rich plasma on myogenic differentiation[100]	Differentiation and proliferation	PRP groups: Statistically significant: increase in cell proliferation, change in MRFs (differentiation marker)	PRP at different concentrations	Myoblasts (mice, C2C12)
Effect of platelet-rich plasma on degeneration change of rotator cuff muscles: In vitro and in vivo evaluations[101]	Degenerative changes	PRP: Stimulated proliferation and inhibited myogenic and adipose differentiation	10% PRP	Myoblasts (mice, C2C12)
The use of platelet-rich and platelet-poor plasma to enhance differentiation of skeletal myoblasts: Implications for the use of autologous blood products for muscle regeneration[14]	Differentiation and proliferation	Platelet depleted (PPP, ssPRP and ssMod-PRP) groups: Increase in myoblast differentiation and decrease in proliferation PRP Group: Increase in myoblast proliferation	PRP, PPP and Mod-PRP (TGF-b1 and MSTN depleted)	Human skeletal muscle myoblasts

Table 4 Completed Preclinical Trials Investigating the Use of PPP and PRP on Skeletal Muscle Healing

Study Title	Outcome	Results	Intervention	Model
Combined platelet-rich plasma and cold water immersion treatment minimize the damage following a skeletal muscle stretch injury in rats[103]	Oxidative damage (via oxidative stress marker levels)	PRP, CWI and PRP + CWI groups: Significant reduction in oxidative markers	PRP, cold water immersion (CWI), PRP + CWI	Rat—gastrocnemius
Platelet-rich plasma does not reduce skeletal muscle fibrosis after distraction osteogenesis[118]	Fibrosis	No significant difference in fibrotic area between PRP and control groups	PRP	Wild-type mice—gastrocnemius
Does the injection of platelet-rich plasma induce changes in the gene expression and morphology of intact Thoroughbred skeletal muscle?[106]	Expression of marker genes related to muscle regeneration, satellite cell activity, pro-inflammatory and pro-myogenic cytokine levels	PRP Group: Significantly higher levels of MHC-e gene expression (day 2), MHC-I gene expression (day 7) and HGF (7 days)	PRP	Thoroughbreds—gluteus medius
Postinjury exercise and platelet-rich plasma therapies improve skeletal muscle healing in rats but are not synergistic when combined.[102]	Skeletal muscle healing	Exer, PRP and PRP-Exer groups: Statistically significant increase in muscle force myofiber CSA and area density of collagen I. Greatest improvements seen w/ individual treatments (PRP, Exer)	PRP, exercise (Exer), PRP + Exer (PRP-Exer)	Wistar rat—gastrocnemius
Analysis of photobiomodulation associated or not with platelet-rich plasma on repair of muscle tissue by Raman spectroscopy[105]	Photobiomodulation	PRP group: Presence of regeneration cells LLtP group—greatest presence of cells in regeneration, lower area of injury, healthy-appearing muscle fibers	PRP, low-level laser therapy (LLt), PRP + low-level laser therapy (LLtP)	Wistar rat—gastrocnemius
Platelet-rich plasma reduces the oxidative damage determined by a skeletal muscle contusion in rats[110]	Oxidative damage (via oxidative stress marker levels)	PRP group: Statistically significant reduction in oxidative markers	PRP	Wistar rat—gastrocnemius
Effect of platelet-rich plasma concentration on skeletal muscle regeneration: An experimental study[107]	Muscular regeneration, neurovascularization, fibrosis and inflammation	PRP Groups: Enhanced muscle regeneration, neurovascularization and slight reduction in fibrosis	Two concentrations of PRP	Wistar rat—longissimus dorsi
Effect of platelet-rich plasma therapy associated with exercise training in musculoskeletal healing in rats[104]	Regenerative effect in musculoskeletal healing	SWP group: Both a statistically significant decrease in Type I collagen fibers and an increase in Type III collagen fibers at	Sedentary + PRP (SPRP), swim trained (ST), swim trained + PRP (SWP)	Wistar rat—vastus lateralis

Table 4 (Continued)

Study Title	Outcome	Results	Intervention	Model
		injury site. SWT and SWP: Statistically significant decrease in lactate levels after training		
Platelet-rich plasma and skeletal muscle healing: A molecular analysis of the early phases of the regeneration process in an experimental animal model[108]	Molecular events involved in early stages of skeletal muscle regeneration	PRP: statistically significant increase in IL-1b, and TGF-b1, leading to an increase in several myogenic regulatory factors	PRP	Wistar rat—flexor sublimus
Platelet-rich plasma in a murine model: Leukocytes, growth factors, Flt-1, and muscle healing[109]	Muscle healing (fibrosis), exercise performance	PPRP1d: Significantly larger fibrotic area (%) than control. PPRP 4d: Significantly larger fibrotic area (%) than control and PPRP7d. PPRP7d: Significantly better exercise test results than all other PRP groups	Pure platelet-rich plasma (PPRP) injected at 1 day (PPRP1d), 4 days (PPRP4d) or 7 days (PPRP7d) postinjury	Wild-type mice—tibialis anterior

Acknowledgments

The authors would like to acknowledge the Steadman Philippon Research Institute for supporting the co-authors involved in the development of this review paper.

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