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REVIEW

ACTA PHYSIOLOGICA

Platelet biology in regenerative medicine of skeletal muscle

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Abstract

Platelet-based applications such as platelet-rich plasma (PRP) and platelet releasate have gained unprecedented attention in regenerative medicine across a variety of tissues as of late. The rationale behind utilizing PRP originates in the delivery of key cytokines and growth factors from α-granules to the targeted area, which in turn act as cell cycle regulators and promote the healing process across a variety of tissues. The aim of the present review is to assimilate current experimental evidence on the role of platelets as biomaterials in tissue regeneration, particularly in skeletal muscle, by integrating findings from human, animal and cell studies. This review is composed of 3 parts: firstly, we review key aspects of platelet biology that precede the preparation and use of platelet-related applications for tissue regeneration. Secondly, we critically discuss relevant evidence on platelet-mediated regeneration in skeletal muscle focusing on findings from (i) clinical trials, (ii) experimental animal studies and (iii) cell culture studies; and thirdly, we discuss the application of platelets in the regeneration of several other tissues including tendon, bone, liver, vessels and nerve. Finally, we review key technical variations in platelet preparation that may account for the large discrepancy in outcomes from different studies. This review provides an up-to-date reference tool for biomedical and clinical scientists involved in platelet-mediated tissue regenerative applications.

KEYWORDS

growth factors, platelet releasate, platelet-rich plasma, regeneration, skeletal muscle

1 | INTRODUCTION

Platelets, also called thrombocytes, are produced from megakaryocyte projections into micro-vessels in mammalian bone marrow. Freely circulating platelets are the first cellular response following damage to vascular or tissue integrity and play a crucial role in haemostasis, innate immunity, angiogenesis and wound healing.[1] The latter aspect is receiving increased attention as the wound healing effects suggest a regenerative ability for maintaining whole-body integrity and homeostasis.[2-4] Until recently, platelet-rich plasma (PRP; defined as a biologically active, autologous concentration of platelets resuspended in plasma) was extensively used in the medical fields of connective tissue regeneration and thrombosis research, while the study of the regenerative potential of PRP in a clinical context attracted less attention. Although our understanding on the mechanisms linking platelet biology to tissue regeneration is still evolving, at this stage, many aspects remain to be established due to inconsistent and conflicting scientific evidence.[5]

Data from clinical studies on the effectiveness of PRP applications appear to be conflicting or limited to outcomes such as improvement of quality of life, reduction of post-operative pain, improved healing or absence of any beneficial effect. This has been attributed to possible methodological differences of preparation, PRP composition, medical condition of the patients, anatomical location of the lesions and type of injured tissue and has been discussed in relevant reviews of clinical interest.[6-8] Growth factors and cytokines have a crucial role in the healing process with regard to early inflammation in tissue regeneration.[9] Therefore, the rationale for utilizing autologous PRP originates in the easy availability of numerous cytokines and growth factors to the targeted area, acting as biomaterials to promote regeneration (Figure 1).[10-14] These factors in turn upregulate proliferation, differentiation and migration of necessary cells in the area of regenerating tissue.[4] Over the past decade, there have been amounting articles contributing to the knowledge surrounding the mechanisms of growth factors in the regeneration of wounded or dysfunctional tissue.[2,15-17] Due to increasing understanding in cell signalling and growth factor biology, research and clinical attention has been drawn to the use of autologous PRP as a novel means of delivering growth factors to injured tissue such as liver, bone and skeletal muscle (See Table 1: muscle tissue regeneration**,** Table 2: other tissue regeneration and Table 3: in vitro cell studies).

Skeletal muscle is a highly plastic tissue with remarkable capacity to regenerate in response to injury and trauma. The early acceleration of muscle regeneration, specifically within the first week, is a crucial time point to implement a clinical intervention due to the early inflammatory response as well as the regeneration phase taking place.[2,9,18] Therefore, understanding the molecular and physiological mechanisms that link platelet biology

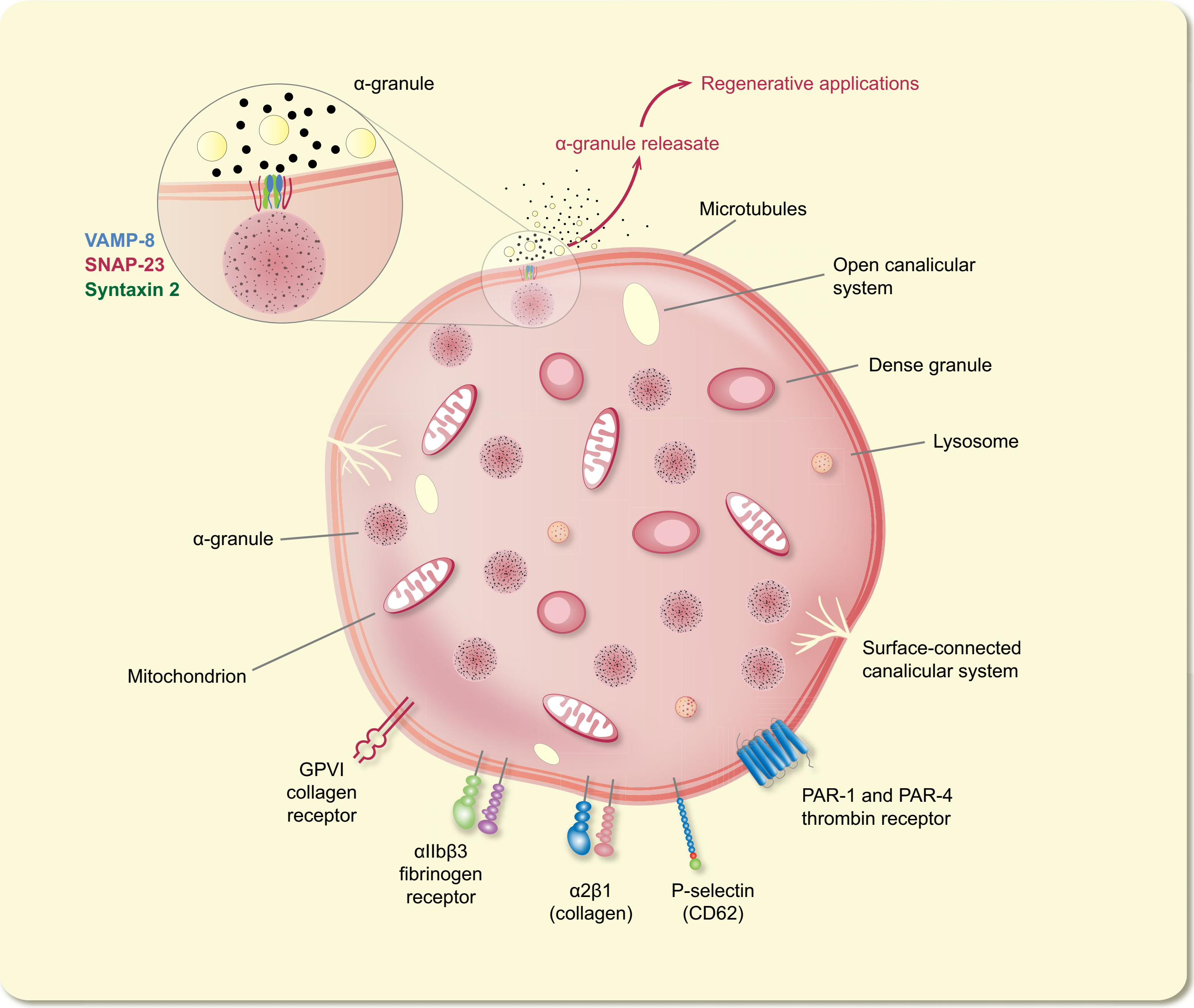


FIGURE 1 Schematic of a platelet together with organelles, highlighting key surface receptors, aggregation factors and an overview of known α-granule releasate factors. These contain adhesive proteins, clotting factors and their inhibitors, fibrinolytic factors and their inhibitors, proteases and antiproteases, growth and mitogenic factors, chemokines, cytokines, membrane glycoproteins and antimicrobial proteins. The platelet releasate may be further used as a biomaterial in numerous applications of regenerative medicine

TABLE 1 Platelets and skeletal muscle regeneration

Reference	Species	Intervention	Findings
9	Rat	PRP on a flexor sublimis lesion	↑ Leucocyte infiltration; ↑ early inflammatory response post-muscle injury
2	Rat	PRP on flexor sublimis incision	↑ mRNA of pro-inflammatory cytokines, MRFs & IGF-1Eb; ↓ myo-miR-133a
70	Rat	PRP on tibialis anterior under muscle strains	↑ Myogenesis ↓ Time-to-recovery after a muscle strain
16	Rat	PRP on gastrocnemius muscle injury	↓ Pain/claudication score
15	Rat	PRP in gastrocnemius contusion	↓ Oxidative stress and ↑ enzymatic antioxidants in injured skeletal muscle
82	Rat	PRP-derived growth factors on rat muscle satellite cells	↑ Proliferation and osteogenic differentiation ability of satellite cells from rat masticatory muscle
54	Rat	Rat releasate on rat gastrocnemius muscle cells in vitro	↑ Proliferation; ↑ cyclin A2, B1, cdk1, cdk2 and PCNA of protein expression (dose-dependently)
17	Rat	TGF-β1 neutralization in PRP on a cardiotoxin-induced muscle injury model	↑ Muscle regeneration; ↓ fibrosis; ↑ angiogenesis; prolonged satellite cell activation; ↑ M2 macrophages to the injury site
78	C2C12 myoblasts and Rat	(i) Human releasate on C2C12 murine myoblasts; (ii) Rat PRP on rat rotator cuff tear	↑ Proliferation; inhibited myogenic differentiation; ↓ expression of adipogenic genes and lipid droplet formation in vivo
69	Mouse	Muscle contusion injury and PRP at different time points	PRP injection 7 d after injury ↑ exercise time; ↓ fibrotic tissue; PRP at 1 and 4 d after injury ↓ exercise time; ↑ fibrotic tissue
94	Mouse	Gelatin hydrogel with platelet releasate in wound healing	↑ Levels of angiogenesis ↑ Wound healing rate
34	Mouse	Human releasate on muscle-derived progenitor cells	↑ Proliferation of hMDPCs; PDGF further increases the proliferative effects of PRP
80	Rabbit	Rabbit PRP with ASC extracts on rabbit myogenic progenitors and human fibroblast culture	ASCs extracts had a stronger effect on proliferation of MPCs than PRP
61	Human athletes	PRP in grade II muscle lesions	↓ Pain in all patients and improved muscle function in 85% of patients after first injection ↓ VAS 2 wk post-treatment. 100% return to sport activities after 35 d (non-controlled study)
62	Human athletes	PRP in acute muscle injury	93% ↓ pain after 28 d vs 80% in control; ↑ range of motion and strength
63	Human patients	PRP in proximal hamstring injuries	↓ VAS and NPRS scores
96	C2C12 myoblasts	Human PRP lysate on C2C12 murine myoblasts	↑ C2C12 proliferation up to 20% PL but mildly cytotoxic at 100%; ↑ C2C12 scratch wound closure
48	Human (*ex vivo*)	(i) PRP (ii) releasate with depleted TGF-β1 and myostatin (iii) PPP; in human skeletal muscle myoblasts	PPP and releasate with depleted TGF-β1 and myostatin induced myoblast differentiation; ↑ myoblast proliferation with PRP

(Continues)

to tissue regeneration has the potential to identify novel opportunities in regenerative medicine in the near future.

Readers interested in the clinical aspects of platelet-based applications for orthopaedic regeneration, in muscle, ligaments and tendons, are directed to recent relevant reviews.[6,8,19,20] In the present review, we assimilate current experimental evidence on the role of platelets as biomaterials in tissue regeneration, particularly in skeletal muscle. Firstly, we review key aspects of platelet biology that precede the preparation and use of platelet-related applications for tissue regeneration. Secondly, we provide a critique of the evidence for platelet-mediated regeneration in skeletal muscle focusing on findings from (i) clinical trials, (ii) experimental animal studies and (iii) cell culture studies. Thirdly, we discuss the application of platelets in

TABLE 1 (Continued)

Reference	Species	Intervention	Findings
47	C2C12 myoblasts and murine satellite cells	PRP + BM-MSC	↑ Proliferation and differentiation

%, percentage; °C, degree Celsius; ↑, an increase; ↓, a decrease; A-PRF, advanced platelet-rich fibrin; ACD, anticoagulant citrate dextrose solution; Akt, protein kinase B; ASC, adipose-derived stem cell; BC, bone cell; bFGF, basic fibroblast growth factor; BMSCs, bone marrow stromal cells; C2C12, mouse myoblast cell line; C57bl6/J, C57 black 6 mouse; CAL-72, human osteoblast cell line; CAM model, chicken chorioallantoic membrane model; cbfa1, core binding factor-alpha 1; CCl4, chemokine (C-C motif) ligand 4; cdk1,2, cyclin-dependent kinase 1,2; CGF, concentrated growth factors; COX-2, prostaglandin-endoperoxide synthase 2; CXCR4, C-X-C chemokine receptor type 4; DMEM, Dulbecco's modified Eagle's medium; DMEM/F-12, Dulbecco's modified Eagle's medium: nutrient mixture F-12; EGF, epidermal growth factor; EGM-2 SingleQuots complete medium, endothelial cell growth medium; EGM2-MV, endothelial cell growth medium; ELISA, enzyme-linked immunosorbent assay; EPC, endothelial progenitor cell; ERK1/2, extracellular signal-regulated kinases; FBS, foetal bovine serum; FCS, foetal calf serum; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; hFOB, transformed human osteoblasts; HGF, hepatocyte growth factor; hMDPCs, human muscle-derived progenitor cells; HMEC-1, human micro-vascular endothelial cell line; HOS, human osteosarcoma cells; HSMM, human skeletal muscle myoblast; HSP, heat shock protein; HUVECs, human umbilical vein endothelial cells; Ibpva55, normal human articular chondrocytes; IGF-1Eb, insulin-like growth factor-1 isoform-Eb; IL-4, interleukin 4; IL-6, interleukin 6; IU, international unit; L-PRP, leucocyte- and platelet-rich plasma; MCF-7, breast cancer cell line; mL, millilitres; mmol L^{-1}, millimolar; MMP-13, matrix metalloproteinase-13; MMP-3, matrix metalloproteinase-3; Mod-PRP, modified PRP; MPCs, muscle progenitor cells; MRFs, myogenic regulatory factors; mRNA, messenger ribonucleic acid; MSTN, myostatin; Myf5, myogenic factor 5; MyoD1, myogenic differentiation 1; myomiRNAs, myo-micro ribonucleic acids; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; NIH-3T3, mouse fibroblast cell line; NPRS, Nirschl Phase Rating Scale; P-PRP, pure platelet-rich plasma; PAR1, thrombin protease-activated receptor 1; PAR1-PR, PAR1-protein releasate; PAR4, thrombin protease-activated receptor 4; PAR4-PR, PAR4-protein releasate; Pax7, paired box protein 7; PBS, phosphate-buffered saline; PCL, poly(ε-caprolactone); PCNA, proliferating cell nuclear antigen; PDGF, platelet-derived growth factor; PL, platelet lysate; PMC, platelet mediator concentrate; PPP, platelet-poor plasma; PRGF, plasma rich in growth factors; PRGF, platelet rich in growth factors; PRP, platelet-rich plasma; PRP-Exos, platelet-rich plasma-exosomes; PRS, platelet-released supernatant; rMSCs, rat muscle stem cells; rpm, rotations per minute; RPMI-1640, Roswell Park Memorial Institute 1640 medium; SaOS-2, sarcoma osteogenic cell line; SC, sodium citrate; SkBM-2, skeletal muscle growth basal medium 2; TGF-β, transforming growth factor beta; TGF-β1, transforming growth factor-beta1; TNF-α, tumour necrosis factor-alpha; TSP-1, thrombospondin 1; TXA2, thromboxane A2; U937 cells, myeloid lineage cell line; μL, microlitres; VAS, Visual Analogue Scale; VEGF, vascular endothelial growth factor; αMEM, α-modification of minimum essential medium; μg, micrograms.

the regeneration of several other tissues including tendon, bone, liver, vessels and nerve.

2 | OVERVIEW OF PLATELET BIOLOGY

The use of autologous PRP in clinical research has grown exponentially over recent years due to the gradually increasing understanding in the role of PRP's growth factors in tissue regeneration[5] (Figure 2). The first publication on PRP was issued in 1954.[21] Ten years later, the first study of PRP being utilized in a therapeutic scenario was published.[22] This increasingly attractive therapeutic tool has made considerable advancements in many areas of regenerative medicine, particularly in the wound healing and skin regeneration, dentistry, plastic and cosmetic surgery, minor wounds, fat grafting, bone regeneration, tendinopathies, ophthalmology, hepatocyte recovery, aesthetic surgery, orthopaedics, veterinary, spinal fusion, treatment of soft tissue ulcers, heart bypass surgery and at last but not least in skeletal muscle injuries.[2,17,23-34] Before we embark into the discussion of current evidence on the role of platelets in tissue regeneration, we briefly review key aspects of platelet biology such as platelet formation, activation and aggregation that precede the release of growth factors and the preparation of PRP.

2.1 | Platelet formation and activation

Hematopoietic stem cells in the red bone marrow give rise to common myeloid progenitor cells which further differentiate to megakaryocytes.[35] Platelets are anucleated products formed from long extensions into vascular sinusoids after migration of the megakaryocytes to the vascular niche.[35,36] Vascular injury leads to exposure of prothrombotic extracellular matrix proteins, which facilitate platelet adhesion and activation. In addition to minimizing blood loss, a major function of platelets is to promote healing of the damaged tissue. This is achieved through the release of cytokines, chemokines and growth factors from platelet granules. There are 3 major types of secretory granules in platelets including the following: (i) α-granules, containing many growth factors and cytokines; (ii) dense γ-granules, which release calcium, serotonin, polyphosphates, pyrophosphates, adenosine diphosphate (ADP) and adenosine triphosphate; and (iii) lysosomes, which contain a number of hydrolytic enzymes.[13]

In particular, there are approx. 50-80 α-granules per platelet with a typical diameter of 200-500 nm that can be released intracellularly or extracellularly.[1,37] Alpha-granule contents secreted by activated platelets release growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-β), insulin-like growth factor (IGF), epithelial growth factor (EGF), endothelial cell growth

TABLE 2 Platelets and other tissue regeneration

Reference	Intervention	Tissue type	Findings
29	PMC	Tendon human and murine tenocytes	↑ Proliferation
28	Thrombin and platelet gel	Tendon rat	↑ Tendon repair (↑ in force at failure and ultimate stress)
18	PRP	Tendon equine flexor digitorum superficialis tenocytes	↑ TGF-β1 and PDGF-BB; ↑ expression of matrix molecules in 100% PRP; no effect on catabolic molecules (MMP-3 and MMP-13)
25	PRP suspended on collagen	Adipose (fat grafting)	Chronic lower-extremity ulcers: 100% re-epithelization, vs 40%-60% of controls
86	Human PMC	Osteoblast (CAL-72) & fibroblast (NIH-3T3)	↑ Proliferation both cell line; osteoblast secretion of IL-6; ↑ differentiation of fibroblasts
26	Calcium & thrombintreated PRP	Osteoblast and endothelial cells	↑ Proliferation
27	PRP	Osteoblast cell line SaOS-2	↑ Chemotaxis and proliferation dose-dependently; PDGF from PRP involved in stimulating cell migration; TGF-β from PRP inhibited cell proliferation
31	PRP	Osteoblast HOS & SaOS-2 cell lines	↑ mRNA: procollagen type I, osteoprotegerin, osteopontin and cbfa1; ↑ bone regeneration
87	Platelet concentrates	Human trabecular bone cells	↑ Proliferation of bone cells independent of cell-to-cell contact
74	Goat, rat and human PRP-coated wells	Rat bone marrow cells	↑ Initial cell growth; human PRP had the most growth factors per platelet; TGF-β1 was the highest growth factor in all PRPs
97	PRP & BMSCs	Bone marrow stromal cells in rat femoral defect	↑ BMSC proliferation; a concentration of platelets at 100×10^4 μL^{-1} with BMSCs in a collagen mixture: ↑ newly formed bone
98	Platelet lysates and platelet exosomes	Bone marrow stromal cells	↑ Proliferation and migration dose-dependently
99	PRP in a polylactic-glycolic acid	Osteochondral articular cartilage defects in rabbits	↑ Osteochondral formation
38	PRP	Chondrocyte lbpva55 cells	↓ Activity of NF-kB, regulating the inflammatory process; ↓ COX-2 and CXCR4 target genes; ↑ HGF, IL-4 and TNF-α
55	Leucocyte and (L-PRP) and pure (P-PRP) PRP	Chondrogenesis of rabbit bone marrow mesenchymal stem cells	P-PRP ↑ both proliferation and differentiation over L-PRP group
88	(Review)	Hepatocyte proliferation	PRP stimulates hepatocyte proliferation by activating the Akt and ERK1/2 signalling pathways in hepatocytes
30	PRP	Hepatocyte (rat hepatic injury)	Hepatoprotective effects of PRP counteract the effects of CCl_4 on liver fibrosis
89	Platelet releasate	Hepatocyte (post-operative patients)	Patients with high TSP-1 and a low VEGF release profile have ↓ liver regenerative capacity
90	PPP and fibrinogen, thrombin or $CaCl_2$	Sciatic nerve rabbit model	Presence of functional nerve regenerates when fibrinogen, in high concentrations, plus factor XIII were used
32	PRP	Peripheral nerve (rat)	↑ Number of regenerating nerve fibres
91	PRP and fibrin sealant	Facial rat nerve regeneration	PRP with a suture established an increase in axon counts and neurotrophic effects
92	Human PRP	Brain reperfusion	Decreases brain injury after focal ischaemia; significantly reduces infarct volume
100	PRP	Nerve-grafted defects (rat)	↑ Nerve gap reconstruction with a 1-cm-long nerve graft

(Continues)

TABLE 2 (Continued)

Reference	Intervention	Tissue type	Findings
57	PRP-PCL scaffold	Angiogenesis in a CAM model	↑ Mesenchymal stem cell attachment and proliferation on scaffold; ↓ differentiation
93	PRP (human patients)	Oral mucosal wound healing	↑ Capillary regeneration in mucosal wound healing
23	PRP (human)	Cutaneous wound healing	↑ Rate of wound healing
94	Gelatin hydrogel and releasate	Cutaneous murine wound healing	↑ Wound area epithelialization rate ↑ Capillary formation
95	Preparation PRGF	Cutaneous ulcers	↑ Healed surface area in PRGF group
53	PRP-Exos (rat)	Cutaneous wound healing, endothelial and fibroblast cell	↑ Proliferation and migration of endothelial cells and fibroblasts ↑ Cutaneous wound healing
42	1- to 21-day-old human platelets	Fibroblast	↑ Proliferation; retention of proliferative activity with old platelets
56	PAR1-PR and PAR4-PR	Endothelial progenitor cells	No effect on EPC proliferation; both ↑ cell migration; PAR1-PR ↑ vasculogenesis

See Table 1 for abbreviations.

factor (ECGF) and fibroblast growth factor (FGF).[38] Upon platelet activation, degranulation follows and release of trophic factors occurs[39] (Figure 1). It has been suggested that interaction of these factors with the hampered tissue structures causes an ameliorated and accelerated healing response.[9]

2.2 | Platelet aggregation

It is important to understand the mechanisms that govern platelet aggregation, as variations in aggregation strength will cause deviations in growth factor release.[40] Differing aggregation procedures in clinical trials may be one of the reasons for conflicting results. Damaged cells or injured tissue release soluble platelet agonists such as ADP and thrombin, which act as platelet-activating factors. These signalling events then allow processes such as platelet spreading, consistent adhesion, granule secretion and clot retraction to occur.[34] To exocytose contents, α-granule's vesicle-associated membrane protein 8 (VAMP-8), synaptosomal-associated protein 23 (SNAP-23) and syntaxin 2 (a Q-SNARE proteins participating in exocytosis) are involved (Figure 1). After an agonist binds to its receptor, platelet shape-change occurs, followed by aggregation and granule content release.

2.3 | The biological role of platelet releasate

Several studies have focused on releasate, which is a refined, centrifuged and purified sample of growth factors released from aggregated platelets when the supernatant is collected, and cellular debris removed.[14,41,42] Soluble bioactive molecules in the releasate secreted by the α-granules of platelets are known to enhance matrix synthesis (eg, TGF-β), upregulate chemo-attraction and proliferation in several cell types (eg, PDGF, VEGF, IGF-I and IGF-II, EGF and ECGF) (Figure 3) and angiogenesis (eg, VEGF, FGF and ECGF).[4,43] It is interesting to note that proteins released by the α-granules, such as platelet factor 4, are inhibitors of angiogenesis, additionally; endostatins are inhibitors of endothelial cell migration. These bioactive molecules may be involved in negative feedback mechanisms to fine-tune the main growth factors such as VEGF which upregulates angiogenesis, or adhesive proteins such as fibronectin that promotes cell migration and differentiation.[40] This opposing effect is essential to create the ability for homeostasis within the injured area capable to react to the surrounding environment.

Growth factors found in releasate are involved in promoting tissue regeneration, such as EGF which causes cell growth, recruitment and differentiation as well as cytokine exocytosis and secretion. Similarly, the growth factor PDGF-BB (homodimers PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD and the heterodimer PDGF-AB) has a physiological effect such that causes significant cell growth, cell migration, blood vessel growth, granulation, growth factor secretion and matrix formation with bone morphogenetic proteins (BMPs).[43] The role of PDGF-BB following muscle damage is still yet to be determined.[44] It has been shown, however, that PDGF-BB upregulates the proliferation of satellite cells (ie, skeletal muscle stem cells) and may affect differentiation negatively.[45,46] Conversely, PRP application on satellite cells was shown to improve differentiation, indicating that the role of PRP on muscle progenitor cell differentiation has yet to be elucidated.[47,48] Additionally, the VEGF and PDGF pathways both interact

TABLE 3 Platelet-based applications on cell culture studies

Reference	Cell type	Findings (proliferation/differentiation)	Platelet preparation method	Sera (eg, FBS%) in culture conditions
74	Rat bone marrow cells	↑ Proliferation between days 0 and 4; ↑ differentiation between days 8 and 12	3.8% SC at 800 rpm for 15 min at 25°C activated with thrombin (300 IU) and 10% $CaCl_2$	10% FCS 10 000 cells/well (24-well) precoated with a PRP gel
97	Rat bone marrow stromal cells (BMSCs)	Higher PRP concentration ↑ cell proliferation; ↑ newly formed bone with 100×10^4 platelets μL^{-1} and BMSCs in a collagen mixture at 8 wk	Whole blood at 600 *g* for 10 min, then 2840 *g* for 15 min; activated with 2% $CaCl_2$ and thrombin	α-MEM with 15% FBS; cells were seeded on a 100-mm dish
11	Human alveolar bone-derived periosteal cells	A-PRF and CGF extracts ↑ proliferation; PRP at 2.5% showed the most proliferative properties with ↓ at higher doses	PRP: whole blood and ACD at 1800 *g* for 4 min then stored at −80°C; PRGF: whole blood and SC at 580 *g* for 8 min; A-PRF and CGF clots: whole blood without anticoagulants at 198 *g* and 692 *g* respectively, frozen, minced, homogenized at 885 *g* for 10 min	Human periodontal tissues in 10% FBS for single cells; cells were seeded at 1×10^4 in 6-well plates for 24 h in 1% FBS; PRP, PRGF, A-PRF extract or CGF extract and the cells were further incubated for 48 h
86	NIH-3T3 cells and CAL-72 cells	PMC ↑ proliferation of murine fibroblasts and human osteoblasts; ↑ NIH-3T3 angiogenesis	PMC using an ATR system kit: human whole blood and anticoagulant and sedimentation accelerator and aggregator	NIH-3T3 culture: DMEM with 10% FCS; CAL-72 culture: 10% FCS over-night, then 2% FCS for 24 h followed by 10% PMC or 10% FCS
98	Bone mesenchymal stem cells	Bone marrow stromal cells treated with platelet exosome concentrations ↑ proliferation and migration; ↑ bFGF, VEGF, PDGF-BB and TGF-β1 in platelet exosomes than in PL	Whole blood and heparin at 1000 rpm for 5 min, then 1900 rpm for 15 min; human PL: PRP was frozen and thawed then at 2600 *g* for 30 min; and heparin for exosome isolation; PL at 2000 *g* for 10 min and heparin; exosome isolation: PL at 500 *g* for a series of spins then at 30 000 rpm for 1 h with repeats	αMEM and 10% FBS; 4 days of culture; adherent mesenchymal stem cells in αMEM and 10% FBS
87	Human trabecular bone cells	↑ Mitogenic activity of BC, independent of cell-to-cell contact	Whole blood and ACD spun to concentrate platelets (3×10^9); then leuco-depleted by a pall filter; washed in Tyrode's buffer, at 1400 *g* for 10 min and resuspended in serum-free medium; activated with thrombin; at 1400 *g*; supernatant at 100 000 *g*	DMEM/F12 with 10% FCS
55	Rabbit bone marrow mesenchymal stem cells (rBMSC)	P-PRP ↓ concentrations of leucocytes and pro-inflammatory cytokines, ↑ proliferation and chondrogenesis of rBMSCs when compared to L-PRP	Whole blood and ACD-A; L-PRP: 250 *g* for 10 min then at 1000 *g* for 10 min, resuspended PPP; P-PRP: 160 *g* for 10 min then 250 *g* for 15 min resuspended in PPP	α-MEM 10% FBS; 96-well plates, 4000 cells/well, then 10% of FBS, L-PRP, or P-PRP
31	Human osteosarcoma cells and SaOS-2	↑ Viability of HOS and SaOS-2 cells dose-dependently; ↑ levels of procollagen type I, osteopontin, osteoprotegerin and core binding factor-alpha 1 (cbfa1) mRNA	Human whole blood and SC. Centrifuged at 2000 rpm for 5 min. PRP Centrifuged again at 2000 rpm for 20 min	SaOS-2 were cultured in RPMI-1640 medium 10% FCS

(Continues)

TABLE 3 (Continued)

Reference	Cell type	Findings (proliferation/differentiation)	Platelet preparation method	Sera (eg, FBS%) in culture conditions
26	HUVECs hFOB	Growth factor concentration variations between individuals. ↑ osteoblast and endothelial cell divisions	PRP and thrombin and $CaCl_2 \cdot 2H_2O$ ×1, ×5 and ×25; at 4000 *g* for 10 min	10% FBS with endothelial cell growth supplement; hFOB cells in DMEM/F12 with 10% FBS, glutamine, and G-418
27	SaOS-2 (sarcoma osteogenic)	PRP ↑ chemotaxis and cell proliferation; PDGF ↑ cell migration and TGF-β ↓ proliferation	Whole blood and Citrate phosphate dextrose at 180 *g* for 20 min, then 580 *g* for 15 min; resuspended in PPP; jellified with calcium gluconate/batroxobine; then at 1400 *g* for 10 min	DMEM with 10% FCS
38	Ibpva55 and U937 cells	PRP in chondrocytes ↓ activity of NF-kB and ↓ expression of COX-2 and CXCR4 target genes; ↑ HGF, IL4 and TNF-a; PRP in U937-monocytic cells ↓ chemokine transactivation and CXCR4-receptor expression	Whole-blood prepared using the platelet concentrate collector system GPS II; activated with thrombin and $CaCl_2 \cdot 2H_2O$; then centrifuged for 10 min at 4000 *g*	DMEM and 20% FBS for Ibpva55 cells; RPMI-1640 medium and 10% FBS for U937 cells
56	EPCs	No benefit on proliferation; ↑ EPC migration; ↑ vasculogenesis	Whole blood at 190 *g* for 20 min; then at 900 *g* for 10 min and PGE_1; resuspended in Tyrode's HEPES buffer at 2×10^9 mL^{-1}; PAR1-AP or PAR4-AP for 10 min; at 15 000 *g* for 10 min	EGM-2 SingleQuots complete medium 10% FBS
101	MCF-7 breast cancer cells and HUVECs	Platelet releasates activated with (i) ADP: ↑ migration and formation of capillary structures by HUVECs; (ii) TXA_2: inhibited migration and formation of capillary structures	Human platelets at 2×10^8 mL^{-1} activated by ADP, thromboxane A_2, PAR4, or exposure to MCF-7 cells at 3×10^6 mL^{-1}	HUVECs (1×10^4 $mL^{-1)}$ in serum-free media on 0.5% gelatin pre-coated/transwell plate with 2×10^8 mL^{-1} platelets in the bottom chamber
57	Human adipose-derived MSCs	MSC seeded on the PRP-PCL nanofibres showed an increased adhesion and proliferation compared to pristine PCL fibres	The buffy coat was centrifuged at 400 *g* for 15 min; 3×10^6 platelets μL^{-1} activated by freeze-thaw cycles; at 12 000 *g* for 10 min	Human MSCs (2×10^4 cells) were seeded in 24-well plates with 1 mL of MSC medium or 1 mL of PRP-rich medium
25	Adipose tissue-derived stem cells	PRP accelerates chronic skin ulcer re-epithelization; ↑ proliferation	SC as an anticoagulant with a 1100 *g* for 10 min spin; Ca^{2+} for activation	DMEM with 10% FBS at 2500-5000 cell cm^{-2}
80	Rabbit myogenic progenitors and ASCs and human fibroblasts	ASCs had an anabolic paracrine effect on proliferation of MPCs; PRP ↑ proliferation of MPCs; ASC extracts ↑ proliferation more than PRP	Rabbit whole blood and SC; at 400 *g* for 10 min; then PRP at 800 *g* for 10 min	Rabbit MPCs and ASCs cultured in EGM-2MV containing 5% FBS; human fibroblasts in DMEM with 10% FBS
54	Rat gastrocnemius muscle cells	Releasate ↑ proliferation of skeletal muscle cells by transitioning cells from G1 phase to S phase and G2/M phases	Whole blood and ACD at 800 *g* for 30 min then at 3000 *g* for 20 min; 10% thrombin with $CaCl_2$; Then at 5500 *g* for 15 min and filtered	DMEM with 10% FBS and 5% chick embryo extract
78	Murine C2C12 myoblasts	PRP ↑ proliferation and inhibited both myogenic and adipogenic differentiation	Whole blood and SC at 2400 rpm for 10 min; then at 3600 rpm for 15 min; activated by freeze-thawing then at 10 000 rpm for 10 min	DMEM with 10% FBS; for myogenesis, DMEM without FBS was used at 5.0×10^4 cells/well

(Continues)

TABLE 3 (Continued)

Reference	Cell type	Findings (proliferation/differentiation)	Platelet preparation method	Sera (eg, FBS%) in culture conditions
81	Murine C2C12 myoblasts	PRP ↑ both myogenic proliferation and differentiation	PRGF: human whole blood in SmartPRePW 2 centrifugation system; Then a freeze-thaw-freeze process to lyse platelets and release their granule contents	High-glucose DMEM with 10% FBS for proliferation; high-glucose DMEM with 2% horse serum for differentiation
96	Murine C2C12 myoblasts	PL ↑ C2C12 proliferation and motility	Platelet lysates; centrifuged, washed, repeatedly frozen and thawed and centrifuged to eliminate debris	DMEM with 10% FBS
48	Human myoblasts	↑ in proliferation; both PPP and MSTN and TGF-β1 depletion in PRP ↑ myoblast differentiation	(i) Pure PRP kit; (ii) PPP; (iii) PRP; (iv) Mod-PRP with TGF-β1 and MSTN depletion; (v) PRP second spin 550 *g*, 5 min	10 000 cells cm^{-2}; 2% horse serum for differentiation; 10% FBS for proliferation
82	Rat MSCs (muscle satellite cells)	PRP-derived growth factors ↑ proliferation on rMSCs and ↑ osteogenic differentiation potential with scaffolds subcutaneously in nude mice	Rat whole blood platelet pellet; resuspended in plasma snap-frozen and thawed then repeated; plasma was separately spun at 3000 *g* for 15 min after clotting	rMSCs cultured in (i) DMEM with 10% FBS, (ii) serum or (iii) PRP-derived growth factors with NHA/PLGA scaffolds in 10% FBS; osteogenic differentiation: DMEM with 10% FBS, b-glyceraldehyde-3-phosphate, L-ascorbic acid and dexamethasone
34	hMDPC;myo-endothelial cells; pericytes	PRP ↑ proliferation; antibody neutralization of PDGF ↓ proliferative effects of PRP and maintained differentiation of hMDPCs	PRP at 3000 *g* for 10 min resuspended in PPP; activated with thrombin then at 3000 *g* spin for 30 min and filtered	hMDPCs, myo-endothelial cells and pericytes: 20% FBS
53	HMEC-1 and primary dermal fibroblasts	↑ Proliferation and migration of endothelial cells and fibroblasts	Whole blood in ACD-A; a series of centrifuges from 160 *g* for 10 min; to 100 000 *g* for 70 min to pellet the exosomes; with washes in PBS	HMEC-1 cells and primary dermal fibroblasts were cultured with 10% FBS
42	Human dermal fibroblasts	21-day-old platelets were as stimulatory as 2-day-old platelets on fibroblast proliferation; total protein, PDGF and TGF concentrations	Platelet activation using zeolite (1 g 10 mL^{-1}) and 1 mL of a 10% calcium chloride; spun at 48 000 *g*	DMEM with 10% FBS; 1×10^4 cells in a 24 well plate; adhere for 6 h; incubating in 10% platelet extract in media without FBS
29	Human and murine Achilles tenocytes	PMC ↑ human tendon cell growth and viability in a dose-dependent manner	An ATR kit was used to prepare PMC in which collected the platelets and activated them; then frozen at −80°C	Human tenocytes—DMEM + 10% FCS; murine tenocytes—50% FCS; reduced to 10% after 1 wk

See Table 1 for abbreviations.

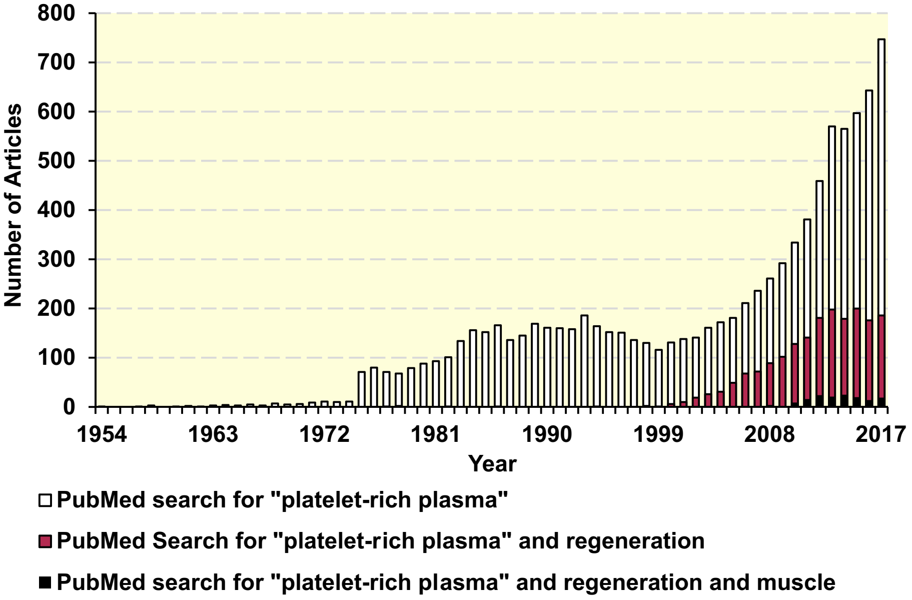


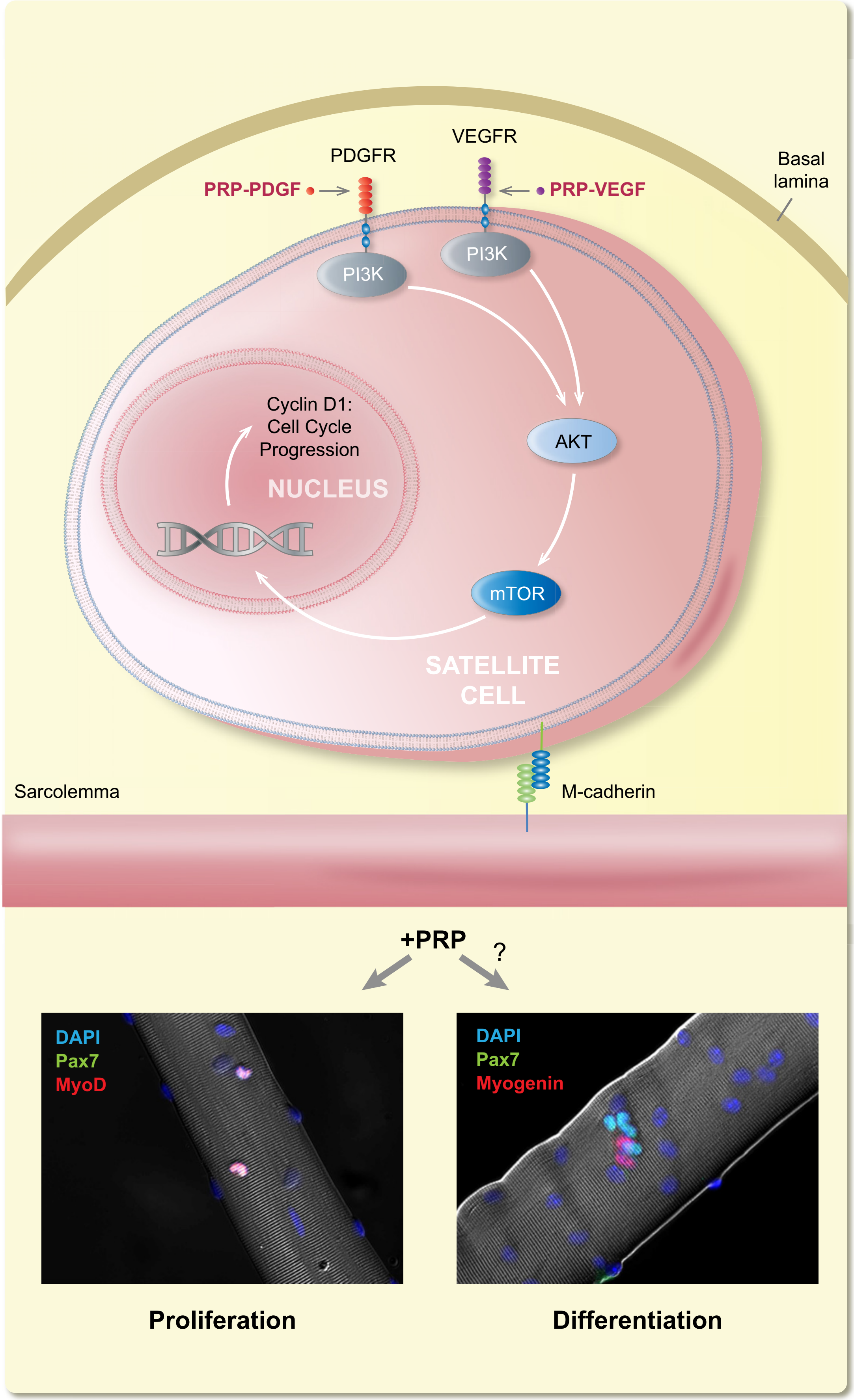
FIGURE 2 PubMed search for publications on (i) “platelet-rich plasma”; (ii) “platelet-rich plasma” AND regeneration; and (iii) “platelet-rich plasma” AND regeneration AND muscle between 1954 and 2017. The diagram reveals that the publication of articles on platelet-rich plasma (PRP) have increased exponentially in the last 2 decades (white bars), while concomitantly scientific interest emerged for exploiting PRP in regenerative applications (red bars). Additional interest to use PRP for skeletal muscle regeneration has developed over the last decade (black bars)

through the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin pathway to induce proliferation of satellite cells (Figure 3).[45,49-51] Another growth factor associated with releasate is TGF-β1, this factor is known to stimulate collagen synthesis, growth inhibition, apoptosis, differentiation and activation.[27] TGF-β1 acts in both an autocrine and paracrine fashion, inhibiting macrophage and lymphocyte proliferation, stimulating mesenchymal stem cell proliferation, while also regulating endothelial fibroblastic and osteoblastic cell mutagenesis, collagenase secretion and collagen synthesis. Additionally, releasate contains IGF-I and IGF-II, which is commonly known to cause cell growth, differentiation, recruitment and collagen synthesis when recruited with PDGF. VEGF and ECGF both target endothelial cells to cause cell growth, migration, new blood vessel growth and antinecrotic properties. Finally, FGF in releasate has been shown to cause cell growth of blood vessels, smooth muscle, fibroblasts and endothelial cells as well as cell migration and blood vessel growth.[3,4,40] The combination of these growth factors has been shown to be beneficial for many types of tissue regeneration (Table 2). However, there is lacking evidence surrounding the role of these growth factors from platelet-based applications in muscle regeneration.[8]

It has been previously argued that discrepancies in releasate content may be accounted for by the varying methods used for activating platelets. In this context, 4 main types of platelet activation (ie, 10% of either collagen type I, $CaCl_2$, autologous thrombin or a mixture of $CaCl_2$ + thrombin) may have an impact in the amount of growth factors and cytokines (eg, TGF-β1, TNF-α, IL-1β, PDGF-AB and VEGF) released by activated platelets, when collecting releasate specifically for regenerative applications.[40] Other methods such as freeze-thaw for activation of platelets have provided insights regarding the shelf-life of platelet products. It has been reported that human platelet releasate can be stored for 21 days and retain its proliferative properties in fibroblasts as much as 2-day-old platelets.[42] Standardization of platelet activation is a crucial step to optimize the various growth factors and cytokines released and implemented in experimental procedures. Different protocols for different tissue or cell types have been outlined (Table 3). Other experimental variables such as platelet count and centrifugation speed have also been recognized and are schematically represented in Figure 4.[52] In terms of platelet count, the variation is donor-specific, which is why resuspending platelets to a standardized concentration is important. Moreover, the release of PDGF and TGF-β from platelet concentrates is pH-dependent, and therefore, standardization of platelet preparation is critical, given that TGF-β1 is one of the growth factors involved in deterring stimulation of differentiation of myoblasts.[48]

Current preparation methods of platelet-based applications are outlined in Table 3. There seems to be

FIGURE 3 A schematic diagram showing a skeletal muscle stem cell’s (ie, satellite cell) possible response to growth factors, based on current published evidence.[2,17,34,47,48,51,54,83] Located between the basal lamina and the sarcolemma of the muscle fibre, the satellite cell may come into contact with hundreds of growth factors and cytokines in response to platelet-rich plasma (PRP) administration. For simplicity, we are presenting platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), which are known to be contained in PRP. PDGF and VEGF interact with tyrosine kinase receptors and induce the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway to drive cell proliferation through transcription factors such as cyclin D1.[45,49-51,102,103] The impact of PRP on muscle progenitor cell differentiation is currently debated and remains to be established


VEGFR
PDGFR
Basal lamina
PRP-PDGF
PRP-VEGF
PI3K
PI3K
Cyclin D1: Cell Cycle Progression
NUCLEUS
AKT
mTOR
SATELLITE CELL
Sarcolemma
M-cadherin
+PRP
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DAPI
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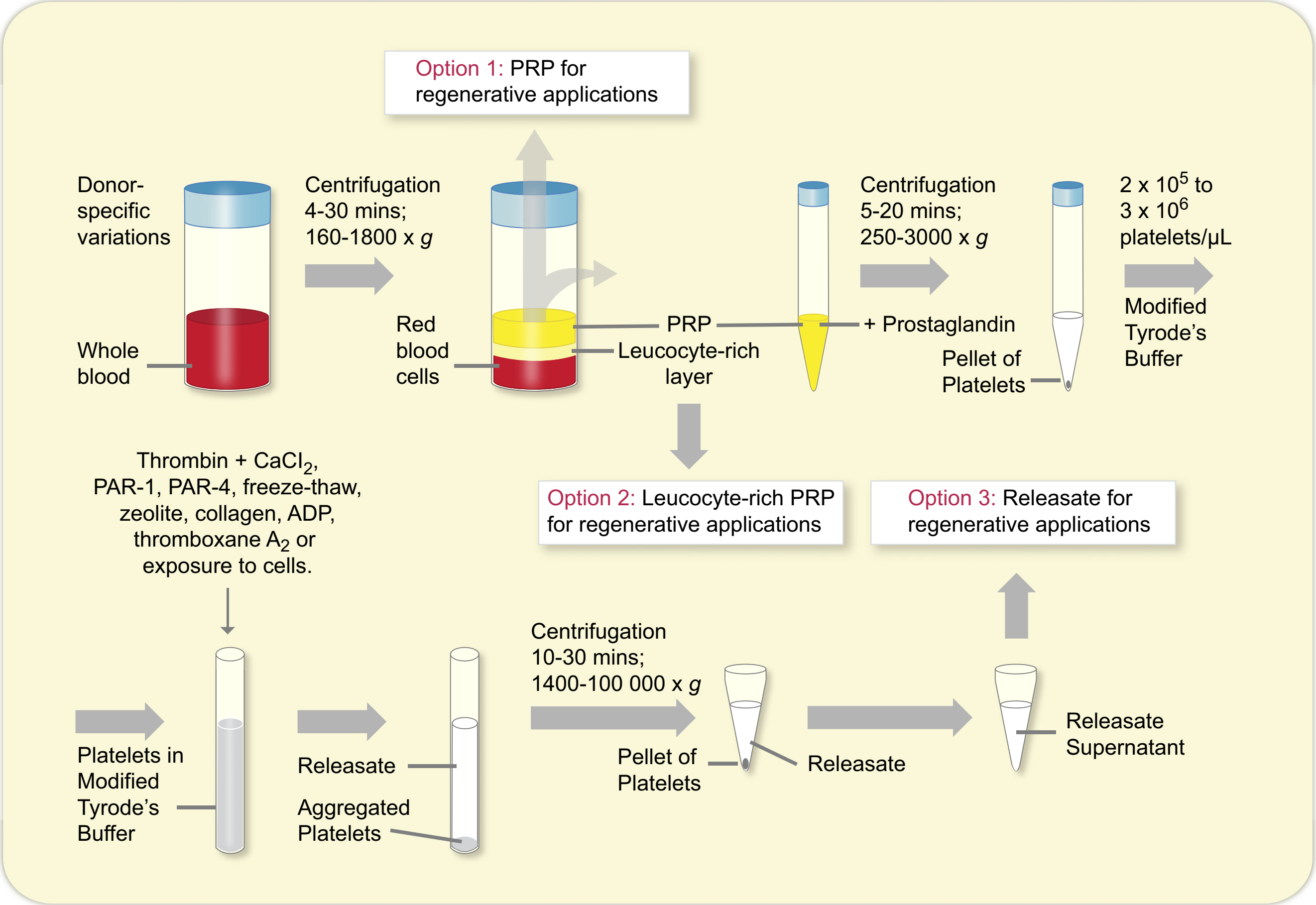

FIGURE 4 A schematic diagram showing the different stages in preparation of platelet-based applications, highlighting possible steps for experimental variations based on published evidence (Table 3). Variations may include donor-specific variability, variability in centrifugations, use of different platelet-based applications (eg, PRP or leucocyte-rich PRP or platelet releasate), platelet concentration, buffer of resuspension, platelet agonists used for activation and the storage conditions

significant variations between preparation methods from different laboratories, even when producing the same product such as PRP. Typically, either the whole blood is inhibited with an anticoagulant such as citrate dextrose solution or sodium citrate and then either centrifuged or processed using a kit. The first centrifugation step can be processed at a speed between 160 and 1800 *g* and between 4 and 30 minutes.[11,53,54] The resultant PRP is then either isolated and used as it is, or inhibited with further anticoagulants such as prostaglandin or prostacyclin and centrifuged between 5 and 20 minutes at 250-3000 *g* in order to pellet the platelets.[48,54,55] Pelleted platelets are then resuspended in either a buffer or platelet-poor plasma (PPP) to a standardized concentration, varying between physiological levels (2×10^5 platelets μL^{-1}) and 3×10^6 platelets μL^{-1}. This resuspension is then aggregated using many methods including thrombin and calcium chloride, thrombin protease-activated receptor (PAR)-1, PAR-4, freeze-thaw cycles, zeolite, calcium, calcium gluconate/batroxobine, ADP, thromboxane A$_2$ or exposure to cells.[25-27,42,56,57] Thrombin is very frequently used in PRP preparations; however, 1 characteristic of thrombin is that it is a protease and may cleave or damage proteins in the releasate sample. For this reason, using a thrombin protease-activated receptor-1 (PAR-1) or PAR-4 peptide may be an appropriate method for releasate preparation, activating the thrombin receptors without the potential of damaging the sample's contents enzymatically.[56] Finally, the releasate can be spun down to remove cellular debris. Expectedly, there is a large variation between publications for this final centrifugation step. In fact, the final centrifugation, excluding platelet exosome isolation, varies from 1400 to 100 000 *g* between 10 and 30 minutes. Such discrepancies in the preparation methods are illustrated in Figure 4. At present, it cannot be ruled out that technical inconsistencies such the ones mentioned above may possibly account for the variable outcomes reported in several studies.

2.4 | PRP alternatives

It is essential to characterize the current forms of platelet-based applications that are derived from PRP methods in order to cope with the current methodological limitations (Table 3). One alternative to PRP that is noteworthy to mention is platelet mediator concentrate (PMC). PMC contains similar factors as PRP such as PDGF-BB, TGF-β1, VEGF, BMP-2, BMP-4, TNF-α, BMP-7 and IL-6.[29] However, it has been reported that low levels of TNF-α and IL-6 in PMC are suitable for tendon healing and reduced scar formation.[29] Platelet-rich fibrin (PRF; a fibrin clot in which contains the platelet cellular debris with their cytokines) also contains many of the above-mentioned growth factors such as PDGF, TGF-β, VEGF, EGF and IGF-1.[58,59] These alternatives may be particularly useful in patient-dependent cases where local injection may not be applicable. Several alternatives to PRP could also be implemented such as advanced platelet-rich fibrin (A-PRF) as well as a concentrated growth factor (CGF) which have higher levels of TGF-β1, PDGF-BB and VEGF as well as higher platelet counts.[11] Leucocyte-rich PRP and pure PRP (leucocyte-poor PRP) are emerging new delineation in platelet-based applications in which describes the hampering effects of having white blood cell contamination in PRP preparations.[55] Other novel platelet therapy methods could be implemented if PRP continues to show promising results in terms of skeletal muscle regeneration. One may speculate that delivering higher concentrations of these factors to the localized injury site may cause further recovery; however, an undesirable ratio of growth factors and cytokines may cause an imbalance in homeostasis which may be detrimental. Attempting to get this ratio correct results in many variations and alternatives in the platelet preparation methods. Novel platelet-based applications such as CGF or A-PRF would have to be a localized implantation.[58] These physical manipulations, as well as leaving the injury site open for minor surgery, may cause dissimilar factor releasate from the platelets, resulting in non-comparable results to PRP.[40,60] It is important to outline the role of platelet aggregation when we are denoting the ratio of the release of growth factors that are crucial in relation to tissue regeneration and choosing the correct method of platelet-based application.

3 | PLATELET-MEDIATED SKELETAL MUSCLE REGENERATION

Current evidence on the role of platelet-based applications in skeletal muscle regeneration derives mainly from (i) human clinical trials, (ii) experimental animal studies and (iii) in vitro cell studies.

3.1 | Clinical trials with platelet-based applications targeting skeletal muscle

Given the recent development of platelet-based applications, the number of studies in this area has increased exponentially, with more than 1000 articles being published in the last 2 years (Figure 2). The studies reviewed in this article were identified through a PubMed search using combinations of the following keywords: platelet-rich plasma, regeneration, skeletal muscle or any other tissue as discussed. Relevant references were reviewed to identify further original research on platelet-based applications and regeneration, focusing on cellular, animal and human studies. There have been several clinical trials examining the use of PRP for muscle regeneration (Table 1). Human athletes with muscle lesions (partially torn) were injected locally with autologous PRP every 7 days for 21 days. Despite not having a control group, this study determined that the autologous PRP injections remained a safe and effective treatment for varying muscle lesions.[61] Ultrasound-guided injections of PRP in professional athletes had an augmented pain relief score as well as increased pain on resisted flexion, strength and range of motion after only 7 days vs conventional treatment.[62] Similarly, pre- and post-treatment of proximal hamstring injuries with PRP was carried out using the Nirschl Phase Rating Scale and Visual Analogue Scale for pain rating.[63] The results of this clinical study showed that pain reduction was augmented in the PRP group over the conventional treatments. A recent study analysed hamstring injuries in professional football players over a 31-month period.[64] This study conversely reported that lesions showed a non-significant healing rate over patients treated with actovegin; however, it reported the safety in use of PRP in human patients.

It has been previously argued that the conclusion of several trials against the use of PRP in sport injuries may be attributed to technical inconsistencies and methodological limitations of the studies.[8] Such limitations are poor sample sizes, non-blinded studies, lack of control studies, inconsistency in PRP preparation methods, platelet concentration and growth factor levels are inconsistent, selection bias in clinical studies and a specific demographic used, such as healthy and fit male athletes as opposed to the general population or patients.[8,61,63,65] One of the major flaws in PRP studies for muscle healing in human subjects is the lack of physiological data and mechanistic insights as an outcome measure; rather, pain scores and return to respective sporting fields have been used.

There have been many speculations made in recent scientific reviews upon the beneficial use of platelet-based applications in musculoskeletal injuries.[6-8,19,20,66-68] These previous reviews of clinical trials using PRP in orthopaedic injuries seem to consistently indicate insufficient results while they recognize that platelet-based applications may hold promise in future applications. A systematic review covering the effects of PRP on muscle lesions in both humans and horses showed that PRP has positive results in 46.7% of the clinical studies.[68] Further to this, Sanchez et al delineate a common protocol used for PRP in muscle injuries in clinical settings as well as the post-infiltration protocol for follow-up potential complications.[7] The protocol here for human patients seems thought out, thorough and effective in follow-up treatment. In fact, this may be a promising approach to go forward for optimization in clinical trials. With the gathered evidence over recent years, one may surmise that there is an effect between platelet-based applications and in the early stages of inflammation with and increased skeletal muscle healing rate.[2,9]

Experimental evidence from animal and cell studies remains to be applied to clinical practice. Optimization of platelet preparation is essential to be standardized or tailored for individual patients, such as depletion of deleterious cytokines.[17] Additionally, the timing of PRP application is pertinent. For example, addition of PRP on days 1 and 4 post-injury hinder skeletal muscle regeneration, but on day 7 has been shown to be beneficial in a rat model.[69] Of note, inconsistencies between clinical and experimental data need to be narrowed, given the current diverse methodologies in platelet preparation among different laboratories as listed in Table 3.

3.2 | Animal studies with platelet-based applications targeting skeletal muscle

Despite the limitations in human trials, with respect to PRP, there have been numerous progressions in the field of skeletal muscle regeneration in rodent models.[2,9,16,70] Accumulating data from animal studies on the role of PRP on skeletal muscle recovery after varying types of injury has emerged (Table 1). Dimauro et al[2] showed promising data on the delivery of PRP on cell proliferation and differentiation as well as satellite cell recruitment that resulted in improved skeletal muscle regeneration. They propose that PRP downregulated myo-miR-133 and increased Pax7 and other myogenic regulatory factors involved in both myoblast proliferation and differentiation in vivo. Myo-miR-133 upregulates myoblast proliferation through the suppression of serum response factor and impede myotube differentiation.[71,72] Conversely, myotube differentiation was upregulated with the application of 20 nmol L^{-1} of double-stranded miRNA for myo-miR-1, myo-miR-133 and myo-miR-206.[73] The conflicting data suggest that the role of miRNAs on muscle regeneration remains to be fully elucidated. It has been reported that the concentrations of the specific growth factors TGF-β1, PDGF-AA, PDGF-AB and PDGF-BB in human PRP are highly elevated over goat and rat growth factors per platelet.[74] Interestingly, TGF-β1 was found to be the highest growth factor concentration across all 3 species' PRP. Both b-FGF and IGF-1 have been found in the α-granules of platelets; these factors are known to independently promote regeneration in vivo in a murine model. For example, gastrocnemius muscles of mice injected with (100 ng mL^{-1}) IGF-1 or with (100 ng) b-FGF on days 1, 3 and 5 showed faster muscle healing and tetanic strength recovery.[75] An influential study addressing the effect of TGF-β1 neutralization in PRP on a rat muscle injury reported that modified PRP with depleted TGF-β1 boosted myofibre regeneration and decreased fibrosis.[17] The study also reported an increase in angiogenesis and greater M2 macrophage localization in the injury site, which are known to have an anti-inflammatory function and regulate wound healing. Additionally, satellite cell number was increased in response to TGF-β1-depleted PRP 2 weeks post-injury. This finding indicates that PRP composition may be modified to optimize benefits towards skeletal muscle regeneration. In a previous mouse study from the same group, it was shown that human muscle-derived progenitor cells cultured in either PRP or foetal bovine serum had the same capacity to regenerate myofibres in vivo upon transplantation into injured gastrocnemius muscle.[34]

Interestingly, local delivery of PRP can shorten recovery time after a muscle strain or multiple muscle strain injuries in rat models leading to faster functional recovery of the tibialis anterior muscle.[70] Additional evidence from a mouse injury model suggests that the optimal time point for a platelet-pure PRP (ie, a leucocyte-poor PRP) injection was 7 days post-injury, leading to reduced fibrosis and better exercise tolerance. However, addition of PRP on 1 or 4 days post-injury that coincide with the period of myoblast fusion and commitment to differentiation causes fibrosis and shortens exercise tolerance.[69] The mechanistic insights of this finding remain to be determined. One possibility is that platelet releasate has a more potent effect on myoblast proliferation, while its use during the early phases of regeneration or cell differentiation may be compromised by inflammatory pathways.[9,17] This notion is supported by in vitro data, where PRP releasate upregulated myoblast proliferation but inhibited myoblast fusion.[48]

Two recent articles relate the role of reactive oxygen species (ROS) and muscle regenerative capacity.[15,76] The role of ROS in myogenic differentiation is multifaceted as cellular responses alter acutely to minute changes in ROS stress levels. Martins et al[15] showed that PRP is capable of modulating the oxidative impairment determined by muscle contusion, defined as a section of damaged tissue where capillaries have been ruptured. The prevalence of contusions is very common both in the general population and in sporting athletes affecting the function of the musculoskeletal system.[8] Contusion, by dropping a 200 g mass directly onto the gastrocnemius muscle, was shown to increase the levels of oxidative stress markers (ie, thiobarbituric acid and oxidized dichlorofluorescein) in both muscle tissue and in erythrocyte preparations.[15] Application of PRP was able to attenuate oxidative stress and increase enzymatic antioxidant defence in injured skeletal muscle. These data suggest that the beneficial effects of PRP on muscle regeneration may, at least in part, be brought about by lower levels of oxidative stress.

It is evident that both animal and human studies have revealed largely dissimilar results when analysing the impact of PRP in various tissues and treatments. The supportive evidence on platelet-based applications from experimental animal studies remains to be validated and extrapolated into human studies with a more thorough experimental design and biological or functional end-point measures. Identification and in-depth assimilation of the mechanisms behind the effect of platelet releasate on these tissues are crucial to design and conduct better human trials.

3.3 | Cell studies with platelet-based applications

Studies now emerging are finding beneficial results with platelet releasate in muscle regeneration similar to PRP treatments.[34,54,77,78] One crucial and recent study added platelet releasate to a primary culture of rat gastrocnemius muscle cells with the aim of investigating the impact of releasate on cell proliferation.[54] It was revealed that releasate increases the proliferative potential of the cells in a dose-dependent manner. This finding was attributed to a continuation of the cell cycle from the G1 phase to the S phase, driving progression through expressions of cyclin and cyclin-dependent kinase (cdk) protein.[54]

One recent study examined the role of releasate on both myogenesis and adipogenesis in rats as well as in a C2C12 in vitro cell line.[78] C2C12 cells are a mouse myoblast cell line capable of differentiation. The localized subacromial injection of PRP proved significantly effective in reducing the instance of adipogenic gene expression as well as suppressing adipogenic differentiation. In the C2C12 cells, there was substantial proliferation when PRP was administered as well as inhibiting muscle and adipocyte differentiation. This finding mirrors the effect of PRP on myoblasts, namely an upregulation in proliferation but presumably an inhibitory role in differentiation.[48] Thrombin-activated PRP has been reported to be detrimental in both SaOS-2 cells (sarcoma osteogenic cell line) and marrow stromal cells when activated with thrombin in terms of cell viability through a 48-hour MTT assay.[79] However, a recent study has shown that co-cultures of adipose-derived stem cells (ASCs) or PRP with myogenic progenitor cells had an augmented effect on myogenic proliferation.[80] Notably, this study reported that the ASCs promoted both myogenic progenitor and C2C12 cell proliferation with PRP. An interesting tissue engineering study looked at C2C12 cells in a PRP treatment embedded in fibres of polydioxanone and polycaprolactone which were electrospun.[81] This study showed proliferative benefits using the electrospun scaffold in myoblasts. Co-culturing myoblasts with a micro-environmental niche such as with ASCs or with a fibrous scaffold may be a more accurate representation of myogenesis in vivo than single cell culture. Conclusively, myoblast cell lines proliferate in response to platelet releasate; however, the role of releasate in cell differentiation is still being discussed.

To determine whether there is any merit to using platelet-based applications as an effective form of regulating cell proliferation and differentiation with an ultimate goal to support regeneration, key cell culture studies were analysed as outlined in Table 3. With the exception of endothelial progenitor cells in 1 study,[56] platelet preparations seemed to produce a positive proliferative effect on various cell types across species. Some of the cell types in Table 3 include myogenic progenitor cells, bone-derived periosteal cells, osteosarcoma, endothelial, trabecular bone cells, human adipose-derived mesenchymal stem cells, fibroblasts, tenocytes, myo-endothelial cells, pericytes, C2C12 cells, adipose-derived stem cells and muscle satellite cells. Variable levels of differentiation have been reported for different cell types, for example increased differentiation for rat bone marrow cells, human skeletal muscle myoblasts, rat muscle satellite cells, rabbit bone marrow mesenchymal stem cells and C2C12 myoblasts or maintained effect with hMDPCs, myo-endothelial cells and pericytes compared to control conditions.[34,47,48,55,74,81-84] Conversely, some studies reported that platelets inhibited differentiation of C2C12 myoblasts.[48,78] This discrepancy in the current literature may provide the basis for a thorough consideration of the technical aspects in platelet applications that may affect the final outcome in a study. For example, despite the majority of studies applying 10% PRP, a recent study used higher concentrations of platelets in smaller volumes (ie, 1%-2%) to induce differentiation, assuming that the concentration of growth factors was not altered.[47] Finally, an interesting study carried out by Miroshnychenko et al[48] has led to a new insight in PRP and PPP effects in vitro on human skeletal muscle myoblast cells. This study looked at PRP, PRP with depleted TGF-β1 and myostatin and PPP in culture with the myoblasts. TGF-β1 and myostatin were depleted due to their detrimental effects on muscle regeneration.[2] The study has reported that PPP and leucocyte-poor PRP with a second centrifugation to remove whole platelets induced myoblast differentiation; however, unmodified leucocyte-poor PRP increased myoblast proliferation.[48] An interesting aspect of this study is that PRP did not seem to induce muscle differentiation; rather, it was more inclined to induce a proliferative property. This study is pertinent due to the method of removing unwanted growth factors from the PRP in which resulted in altered biological properties on the myoblast cell line used. Further studies to eliminate additional releasate factors could be implemented to optimize skeletal muscle regeneration and possibly expand in a clinical application in the near future.

4 | CURRENT EVIDENCE ON PLATELET-MEDIATED REGENERATION IN OTHER TISSUES

There has been an intense interest in determining the effect of platelets and platelet-related application on the regeneration of several other tissues such as tendon, adipose tissue, bone, liver, nerve, vascular tissue and wound healing.

4.1 | Tendon regeneration

One recent study analysed PMC, a centrifugation-free method of preparing human platelet releasate, co-cultured with Achilles tenocytes in vitro from both human and murine tendons.[29] This study reported that PMC concentrations caused an elevation for important growth factors and markers for cell viability in tenocytes, suggesting that autologous PMC may be a future useful therapy in tendon recovery. However, a cross-comparison with PRP or releasate would be useful in determining which treatment at what dose would be optimal for tendon recovery in vivo. An in vivo study looked at platelet gel; a resuspended pellet of platelets activated with thrombin and calcium, in relation to the effect on the transected Achilles tendon of female rats after 14 days.[28] The results, when compared to a saline injection, showed a 42% increase in the force at tendon failure when subject to being pulled at a consistent speed of 1 mm s^{-1}. There was also a 61% increase in ultimate stress (MPa) observed in the tendons when compared to the saline injections, suggesting an increased tendon recovery time. Notably, the platelet gel seemed to have a significantly lower force at failure score than the platelet gel 24% and 42%, respectively. This suggests that the activated PRP with thrombin and calcium is required for optimized tendon recovery. Currently, leucocyte-poor PRP progresses tendon healing and is considered a more viable option for the clinical treatment of tendinopathy after a comparative use in a rabbit model.[85] In general, the current consensus on platelet-based applications on tendon regeneration is a positive one with tenocyte proliferation reported in vivo, as well as structural optimization of the tissue being upregulated.

4.2 | Adipose and endothelial tissue regeneration

A clinical and in vitro study analysing the application of PRP in terms of tissue engineering, specifically fat grafting, for the purpose of plastic surgery, reports an accelerated chronic skin ulcer re-epithelization.[25] Co-culture of adipose tissue-derived stem cells (ADSCs) with PRP shows a proliferative effect. The clinical application of fat grafting with PRP showed consistently higher re-epithelization from 3 weeks until 18 months over a control group. Fat grafts were maintained with PRP up to 69% restoration rate when compared to a 31% control. In an earlier study, activated PRP co-cultured with human umbilical vein endothelial cells or with transformed human osteoblasts showed increased endothelial proliferation.[26] Interestingly, the non-activated PRP group in the osteoblast cells was more proliferative than in the activated group, suggesting that PRP secretions gave no supplementary benefit on osteoblast proliferation over the 3 groups. However, when compared to the minimal medium group, there was a substantial increase in all PRP conditions, showing that platelet extracellular growth factors were expressed in adequate quantities to induce substantial proliferation. These results indicate towards an increased proliferative rate of ADSCs with platelet-based applications, with a possible benefit for cosmetic utilization.

4.3 | Osteoblast and chondrocytic tissue regeneration

Many studies now see the potential mechanisms of PRP acting on osteoblast proliferation and migration.[26,27,31,86] Using the SaOS-2 osteoblast line, both TGF-β and PDGF were analysed for both cell proliferation and cell migration. Notably, TGF-β appeared to have an inhibitory effect on proliferation, while PDGF was reported to upregulate migration. Similarly, Kanno et al[31] have shown the link between a PRP treatment and osteogenesis in vitro in a dose-dependent method. This study suggests that growth factors in PRP, such as TGF-β, prompt pre-osteoblasts to undergo division increasing their quantities through chemotaxis, stimulating differentiation into mature osteoblasts. In connection to osteogenesis, a study makes the connection to bone and cartilage regeneration.[38] In this study, the mechanisms connecting PRP to chondrocyte differentiation and regeneration were assessed by the means of regulating local inflammation in cartilage through decreasing chemotaxis of anti-inflammatory agents such as hepatocyte growth factor (HGF). Releasate from the PRP was found to be accountable for the inhibition of NF-kB-transactivating activity due to the upregulation of HGF.[38] It is still unclear whether the stimulatory effects of PRP, in osteoblast proliferation for example, are connected to the growth factors present or to other factors present in the cytoplasm or cell membranous structures from activated platelets.[87] This study also stated that cell-to-cell contact was not reportedly required for upregulated osteoblastic proliferative effects of platelets. In summary, recent studies indicate that there is a positive proliferative and differentiative effect of platelet-based applications in osteogenesis. PRP also promoted growth and proliferation in chondrogenesis and may be beneficially applicable in cartilage repair.

4.4 | Hepatocyte tissue regeneration

A study evaluated the in vivo effect of PRP on carbon tetrachloride-induced hepatotoxicity in male rats. Animals received PRP treatment twice a week for 8 weeks.[30] After the 8 weeks, the rats were bled and their livers were analysed histopathologically, showing a hepatocytic protection of the PRP as well as showing that PRP itself is not toxic for at least a 3-week period. Further to this, a recent review highlighted the factors released by PRP such as VEGF, HGF and IGF-1 to promote hepatocyte proliferation.[88] It has been hypothesized that an unidentified receptor on the liver sinusoidal endothelial cells interacts directly with the platelets in PRP in which stimulate proliferation in the hepatocytes. Similarly, a recent study followed up from this, analysing patients who underwent hepatic resection.[89] It was shown that a rapid accumulation of platelets to the resection was correlated to regeneration of the liver. Interestingly, an unfavourable ratio of growth factors such as an increased TSP-1 level as well as a lower VEGF level displayed hampered regenerative properties. These studies reveal a proliferative effect in hepatocytes when using platelet-based applications.

4.5 | Nerve tissue regeneration

One of the first experimental uses of platelet-based applications was the use of PPP in a rabbit model in 1973.[90] This study analysed a plasma clot welding of nerves with regained myoneural function and no sign of substance rejection. A more recent study performed a bilateral sciatic neurotomy in rats, followed by being promptly re-anastomosed with a cyanoacrylate glue used in order to study the regenerative properties of PRP in relation to nerve regeneration.[32] The biopsies were harvested 12-week post-operation with the aim to see whether the PRP treatment promoted peripheral nerve healing. The article suggests that through distal axon counts, neurotization indexing and density analysis, a PRP-treated group has potential in enhancing peripheral nerve regeneration. In terms of facial nerve regeneration, a study analysing the effect of PRP and fibrin sealant in a rat model was conducted.[91] Male rats were subject to transection in survival and non-survival surgery groups of the left facial nerve and treated with either PPP, PRP or fibrin sealant using the right facial side as the control. Axon counts and facial nerve motor action potentials were analysed resulting in a faster recovery in the PRP group, and the study reported overall that PRP was notably the better option when sutured compared to the other 2 interventions. A more recent study explored the benefit of PRP lysate on an ischaemic stroke in rats.[92] The outcomes were measured by means of analysing neurological deficit score and infarct volume. One of the more interesting points that this article tackles is the use of human PRP lysate in a rat model and how it shows a significant benefit in recovery after an induced stroke. Overall, platelet-based applications show a beneficial effect on nerve regeneration in animal models.

4.6 | Angiogenesis

In a recent study to analyse angiogenesis on a PRP-seeded poly(ε-caprolactone) scaffold, it was reported that this PRP application method may be beneficial for tissue engineering due to the consistent delivery of growth factors without loss of activity.[57] Not only was there an increase in angiogenesis, the chicken chorioallantoic membrane model also increased the hydrophilicity, attachment of mesenchymal stem cells and cell proliferation on the scaffold. PRP seeding is looking like a promising tissue engineering method for integration into a host. The therapeutic value of PRP in angiogenesis can be seen in a study aiming to evaluate the application of platelet-enriched plasma in oral mucosal healing in terms of capillary count and density in a randomized split-mouth design in patients.[93] The results showed that for the initial 2 weeks, capillary density and capillary count were higher in the PRP treatment over the placebo treatment administered to the contralateral side. A gelatin hydrogel was used with releasate in a recent study to analyse the aspect of angiogenesis in wound healing.[94] This study used male mice to demonstrate that capillary formation was enhanced after 2 weeks in the gelatin hydrogel with PRP group, supporting angiogenesis when compared to a control saline group and a single PRP injection group. The article also reported that augmented wound healing through wound area analysis and angiogenesis using anti-vWF immunohistochemical staining was significantly higher in the treatment group. This study suggests a more specified application of PRP through a hydrogel with releasate can steadily release the growth factors over a period of time being more beneficial than a single PRP injection. It would be interesting to see whether the beneficial effects of platelet-based applications on angiogenesis were directly due to the concentration of VEGF released from the α-granules in platelets, or due to the ratio of growth factors released. Taking these studies into consideration, platelet-based applications are seen to increase angiogenesis.

4.7 | Cutaneous wound healing

PRP has been shown to be increasingly used in wounds that are difficult to heal such as tissue injuries. To address whether PRP was beneficial for acute cutaneous trauma wounds such as open and closed fractures as well as epithelial necrosis and friction injuries, a study looked at patients receiving a local injection of PRP.[23] With conventional treatments given to patients as a control group, the PRP group showed a faster rate of recovery in comparison; measured by the time taken for the wound to heal to such a degree that plastic surgery is applicable. This trend of a faster regenerative rate can also be seen in chronic cutaneous ulcers. A study has shown that a localized 100-200 μL injection of autologous platelet-enriched plasma in patients proved that the percentage area of healed cutaneous ulcer over a standard-care group between 4 and 8 weeks was highly significant.[95] This study suggests that topical application of platelet-enriched plasma is cheap and effective treatment at tackling chronic ulcers in modern health care.

5 | CONCLUSIONS

In conclusion, there is mounting evidence on the use of platelet-based applications in tissue regeneration. Inevitably, there is currently a large discrepancy in the effectiveness of platelet-based applications in the scientific literature, especially between human and experimental animal studies. This may be attributed to methodological differences in platelet preparation and platelet releasate composition among different research groups. At present, there is an intense interest in the field worldwide with tremendous possibilities for exploitation in regenerative medicine. The current consensus with the use of PRP and especially modified PRP (where individual factors are depleted) in skeletal muscle regeneration remains promising, despite an incomplete understanding of mechanistic insights in both knowledge of platelet-satellite cell interactions, as well as PRP preparation optimization. Most importantly, the molecular mechanisms linking platelet biology to skeletal muscle, or other tissue regeneration, have just begun to unravel and are expected to transform our understanding in using platelets as a biomaterial for tissue healing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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