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THE *IN VITRO* EFFECTS OF PLATELET PRODUCTS ON THE BIOPHYSIOLOGICAL FUNCTIONS OF HUMAN BONE MARROW MESENCHYMAL STROMAL CELLS: A SYSTEMATIC REVIEW

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Abstract

Platelet products (PP) and bone-marrow aspirate are popular sources of osteoinductive signalling molecules and osteogenic bone marrow mesenchymal stromal cells (BM-MSCs) used in the treatment of impaired bone healing. However, the combined use of PP and BM-MSCs in clinical studies has reported mixed results. Understanding the cellular and molecular interactions between PP and BM-MSCs plays the important role of guiding future research and clinical application. This systematic review investigates the effects of PP on the biophysiological functions of BM-MSCs in *in vitro* human studies, including (i) proliferation, (ii) migration, (iii) differentiation, (iv) growth factor/cytokine/protein expression, (v) immunomodulation, (vi) chemotactic effect on haematopoietic stem cells, (vii) response to apoptotic stress, and (viii) gene expression. *In vitro* studies in human have demonstrated the multi-faceted 'priming effect' of PP on the biophysiological functions of BM-MSCs. PP has been shown to improve proliferation, migration, osteogenic differentiation, reaction to apoptotic stress as well as immunomodulatory, pro-angiogenic and pro-inflammatory capacities of BM-MSCs. Several factors are highlighted that restrict the transferability of these findings into clinical practice. Therefore, more collaborative *in vitro* research in humans modelled to reflect clinical practice is required to better understand the effects of PP exposure on the biophysiological function(s) of BM-MSCs in human.

Keywords: Bone marrow mesenchymal stem cell(s), mesenchymal stromal cell(s), platelet-rich plasma, platelet lysate, platelet concentrate, platelet releasate, platelet gel, platelet-rich fibrin, bone healing, non-union.

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List of Abbreviations

αMEM	alpha-minimum essential medium
aPRP	activated PRP
ACAN	aggrecan
ACD-A	acid-citrate dextrose solution A
ADIPOQ	adiponectin, C1Q and collagen domain containing
aFGF	acidic fibroblast growth factor
ALP	alkaline phosphatase
APOE	apolipoprotein E
bFGF	basic fibroblast growth factor
BMA	bone marrow aspirate
BMAC	BMA concentrate
BM-MSCs	bone marrow mesenchymal stromal cells
BMP	bone morphogenetic protein
CEPBA	CCAAT/enhancer-binding protein α
CFU	colony-forming unit
CM	conditioned medium
COL1	collagen type 1
COL1A1	collagen type 1 α1
COL2A1	collagen type 2 α1
COMP	cartilage oligomeric matrix protein
COX-2	cyclooxygenase-2
CPD	citrate phosphate dextrose
DAPI	4',6-diamidino-2-phenylindole
DMEM	Dulbecco's modified Eagle's medium
EDTA	ethylenediamine tetraacetic acid
FABP4	fatty acid binding protein 4
FBS	foetal bovine serum
fPL	filtered PL

FTPL	frozen-thawed PL
G-CSF	granulocyte colony-stimulating factor
GLUT4	glucose transporter type 4
HG-DMEM	high-glucose DMEM
HGF	hepatocyte growth factor
HPLF	human platelet lysate from fresh platelet concentrates
HPLO	human platelet lysate from expired platelet concentrates
HSCs	haematopoietic stem cells
IL	interleukin
IP-10	interferon-γ-inducible protein 10
iNOS	inducible nitric oxide synthase
ITS	insulin-transferrin-selenium
KO-DMEM	knock out-DMEM
L-PRP	leukocyte-rich PRP
L-PDGS	lipocalin-type prostaglandin D synthase
LG-DMEM	low-glucose DMEM
LPL	lipoprotein lipase
LXRα	liver X receptor alpha
MCP-1	monocyte chemoattractant protein-1
MMP	matrix metalloproteinases
MNC	mononuclear cell
MIP	macrophage inflammatory protein
naPPP	non-activated PPP
naPRP	non-activated PRP
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NM	nutrient medium
NO	nitric oxide
OM	osteogenic differentiation medium
PAI-1	plasminogen activator inhibitor-1
PBMC	peripheral mononuclear cells
PD	population doubling
PDGF	platelet-derived growth factor
PG	platelet gel
PG-CM	platelet gel conditioned-medium
PGE2	prostaglandin E2
PL	platelet lysate
PLGF	placental growth factor
PLIN	perlipin
POU5F1	POU class 5 homeobox 1
PP	platelet product
PPARG	peroxisome proliferator-activated receptor gamma
PPP	platelet-poor plasma
P-PRP	leukocyte-poor PRP
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PR	platelet releasate
PRC	platelet-rich concentrate
PRGF	plasma rich in growth factors
PRF	platelet-rich fibrin
PRP	platelet-rich plasma
PRP-CM	PRP-conditioned culture medium
PTGES	prostaglandin E synthase
RANTES	regulated on activation, normal T cell expressed and secreted
RUNX2	runt-related transcription factor 2
SDF-1	stroma-derived factor-1
SEM	scanning electron microscopy
SNPL	PL obtained by sonification
SOX	SRY-box transcription factor
SPARC	secreted protein acidic and cysteine rich
SREBP1	sterol regulatory element-binding transcription factor 1
TERT	telomerase reverse transcriptase
TGF	transforming growth factor
ucPRP	umbilical cord PRP
uPA	urokinase-type plasminogen activator
VEGF	vascular endothelial growth factor receptor

Introduction

Bone healing is a unique biological process leading to bone repair and restoration of bone function to its pre-injury level (Einhorn and Gerstenfeld, 2015). According to the 'diamond concept', bone healing involves the orchestrated interaction between multiple factors at the molecular, physiological and biomechanical level (Giannoudis *et al.*, 2007).

The three fundamental molecular components of bone healing are: (a) osteogenic progenitor cells, such as BM-MSCs; (b) osteoinductive signalling molecules, such as growth factors and cytokines; (c) osteoconductive scaffold/matrix (Calori and Giannoudis, 2011). In addition, other essential factors at the physiological and biomechanical levels crucial to successful bone healing include the host (*i.e.* patient) physiology, mechanical stability and vascularity of the affected local bone environment (Giannoudis *et al.*, 2008).

Although every attempt has been made to optimise the physiological and biomechanical factors during primary surgery, impaired bone healing (*e.g.* delayed union and non-union) remains common, occurring in 5-10 % of all acute fractures (Ekegren *et al.*, 2018; Mills and Simpson, 2013). Impaired bone healing is challenging to treat and poses a significant burden on the patient, socioeconomic and healthcare systems (Bishop *et al.*, 2012; Hak *et al.*, 2014). Multiple operations are often required to achieve treatment success, which exposes patient to further risks of peri-operative complications, prolonged periods of rehabilitation and delay in returning to work. From a socioeconomic perspective, the direct treatment costs have been estimated to be between £7,000 and £79,000 per person depending on complexity (Bishop *et al.*, 2012). In European healthcare systems the indirect costs through productivity losses have been estimated to be 10 times higher (Hak *et al.*, 2014).

In an effort to optimise bone healing, surgeons have recently attempted a 'polytherapy' approach involving the simultaneous application of all the

three fundamental molecular components of bone healing, as mentioned previously (Calori *et al.*, 2011). Autologous sources of these biological components, which guarantees histocompatibility, minimal morbidity and ease of access during the harvesting process, are ultimately desirable. This has made autologous iliac crest bone graft, PPs and BMA/BMAC popular sources of osteoconductive scaffolds, osteoinductive molecules and osteogenic BM-MSCs, respectively (Calori and Giannoudis, 2011; Gianakos *et al.*, 2017; Roffi *et al.*, 2017).

PPs or platelet derivatives are terminologies often used interchangeably to represent haemoderivatives containing platelets and growth factors, cytokines and molecules beneficial for bone healing. PPs, which are commonly used in the clinical and laboratory settings, include PRP, PL, PRC, PR, PG and PRF.

Briefly, when harvested in the presence of anticoagulants, peripheral blood can be centrifuged to produce two distinct fractions: (i) PRP (also referred to as platelet concentrates) and (ii) PPP. On the other hand, centrifugation of blood harvested in the absence of anticoagulants induces coagulation, producing PRF. Finally, when peripheral blood is allowed to naturally coagulate and then is centrifuged, this produces autologous serum.

Inducing the release of growth factors from PRP *ex vivo* could be achieved through two common methods: (i) physical disruption/lysis and (ii) chemical activation. Physical disruption of PRP, such as repeated freeze-thaw cycles and ultrasound sonification, results in platelet lysis and, therefore, acellular PL rich in growth factors. Chemical activation of PRP (commonly with calcium salts or thrombin) leads to the production of two distinct products, due to two distinct processes that occur during activation: (i) platelet degranulation and the release of a plasmatic fraction rich in growth factors, known as platelet releasate or PRGF or platelet released supernatants; (ii) fibrinogenesis leading to formation of a platelet-rich clot, also known as PG (Soares *et al.*, 2020).

Several classification systems for platelet concentrates and PRP have been developed in the last decade (DeLong *et al.*, 2012; Dohan Ehrenfest *et al.*, 2009; Lana *et al.*, 2017; Magalon *et al.*, 2016; Mautner *et al.*, 2015; Mishra *et al.*, 2012). The strengths of these classifications lie in their call for a comprehensive documentation of centrifugation and preparation protocol, cellular constituents (platelet concentration, red blood cell count, white blood cell count) and method of platelet activation. However, the choice of using the term PRP for PP that have undergone exogenous chemical activation (which strictly speaking should be called PG) in these classification systems creates further confusion. Consequently, PRP is often used loosely as a universal term encompassing all forms of platelet-rich preparations with variable preparation protocols and composition. Therefore, there still remains a lack in standardised terminology that allows for the effective, accurate description of these PPs, reflective of its contents and physical properties (*e.g.* liquid, gel, fibrin membrane).

Clinical studies investigating the use of PPs either alone or in combination with BM-MSCs have reported mixed results (Andia and Maffulli, 2019; Bielecki *et al.*, 2008; Calori *et al.*, 2008; Chiang *et al.*, 2007; Etulain, 2018; Galasso *et al.*, 2008; Golos *et al.*, 2014; Malhotra *et al.*, 2015; Mariconda *et al.*, 2008; Roffi *et al.*, 2017; Sanchez *et al.*, 2009; Say *et al.*, 2014; Verboket *et al.*, 2018). The pooling of fractures from different anatomical sites (*e.g.* tibia, femur, humerus) (Bielecki *et al.*, 2008; Calori *et al.*, 2008; Chiang *et al.*, 2007; Galasso *et al.*, 2008; Golos *et al.*, 2014; Malhotra *et al.*, 2015; Mariconda *et al.*, 2008; Roffi *et al.*, 2017; Sanchez *et al.*, 2009; Say *et al.*, 2014), coupled with the heterogeneity in techniques used to harvest and prepare PPs and BM-MSCs (Etulain, 2018; Roffi *et al.*, 2017) made comparison between studies difficult, hindering the in-depth scientific understanding of these biological therapies.

A systematic review on *in vitro* studies in human will provide an improved scientific understanding of how PPs interacts with and affects the biophysiological functions of BM-MSCs at the cellular level. This has far-reaching importance, serving to guide future research and the clinical application of BM-MSCs and PPs when treating patients with or at risk of impaired bone healing.

The main objective of the present systematic review was to investigate the biophysiological functions of BM-MSCs influenced by PPs. Additionally, the different methods to harvest, prepare and culture BM-MSCs and PPs were also evaluated.

Materials and Methods

Protocol

This systematic review was conducted in accordance with the key principles recommended in PRISMA statement (Hutton *et al.*, 2015; Moher *et al.*, 2009).

Information sources and search strategy

A comprehensive systematic bibliographic search was performed in the following databases on the 8th of September 2020: PubMed, Medline (via Ovid), Embase (via Ovid), Scopus, Google Scholar and the Cochrane Library. No restriction was applied to the date of publication. The following search terms were used: 'bone' or 'bone marrow' or 'mesenchymal stem cell(s)' or 'mesenchymal stromal cell(s)' or 'MSC' or 'platelet product' or 'platelet rich plasma' or 'PRP' or 'platelet lysate' or 'platelet rich concentrate' or 'platelet releasate' or 'platelet rich fibrin' or 'platelet gel' or 'platelet released growth factors'. The choice of studies was limited to human studies only. Bibliographies of all identified studies were manually searched for any additional eligible studies.

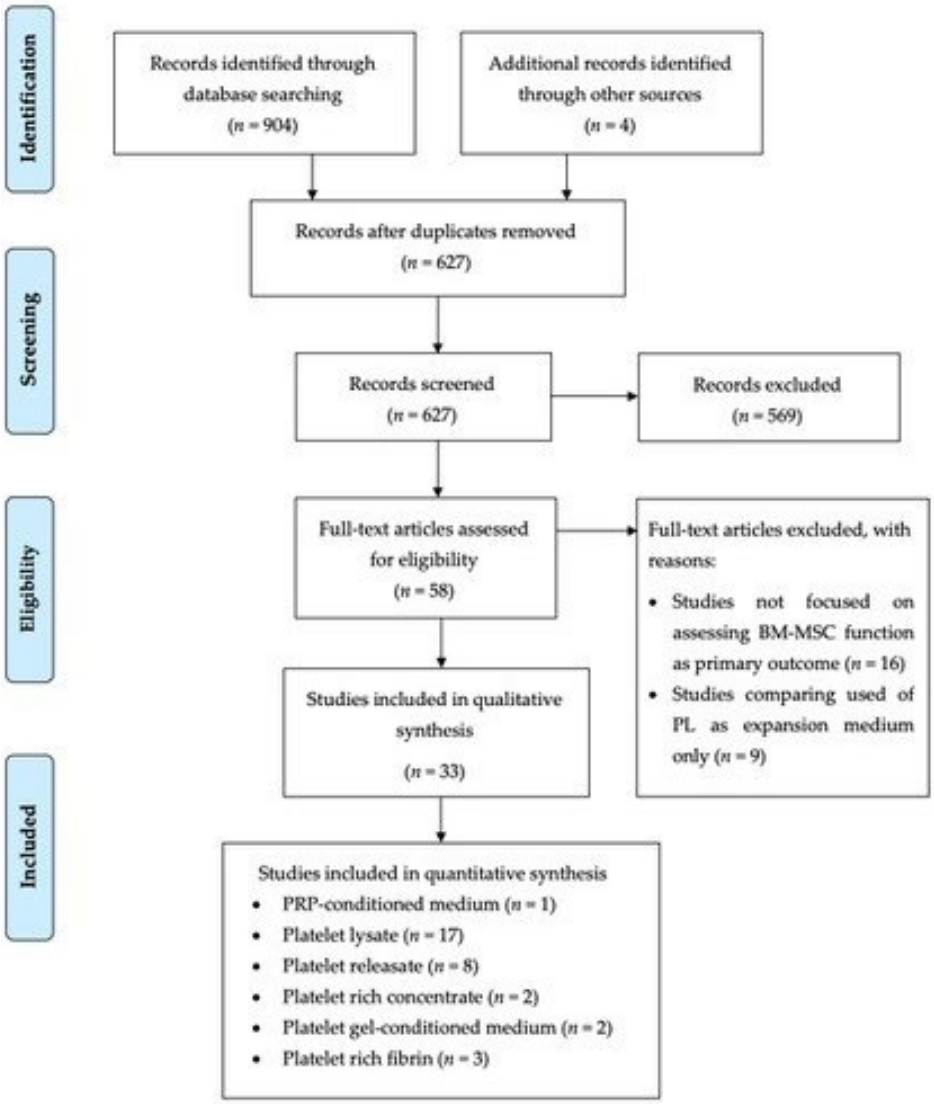

Figure 1. PRISMA flowchart on study selection.

Eligibility criteria

Studies were original research articles that fulfilled the following inclusion criteria: (i) *in vitro* studies in human; (ii) studies that included comparison between BM-MSCs *versus* PPs and BM-MSCs. Studies involving use of scaffold (in addition to PPs and BM-MSCs) were excluded, since the presence of another osteoconductive ± osteoinductive element (*i.e.* the scaffold itself) complicates the interpretation on how PP affects the biophysiological function of BM-MSCs. Furthermore, *in vitro* studies combining a xenogeneic component (PPs or BM-MSCs) with human BM-MSCs or PPs, clinical studies and case reports were also excluded.

Study selection

All retrieved records were first uploaded into EndNote followed by removal of duplicate records. Then, retrieved records were independently screened by two reviewers (JV, MP) in a blinded, standardised manner. Title and abstract sift was conducted first, followed by review of full text. Only studies fulfilling the eligibility criteria were included. Any discrepancies or disagreement between reviewers were resolved by consensus.

Data extraction

Relevant information on author, year of publication, patient demographics, site of harvest, processing methods (*e.g.* centrifugation), tissue characteristics, culture method, incubation period, laboratory assay, biophysiological functions of BM-MSCs were carefully extracted. The biophysiological functions of interest were proliferation, migration, differentiation, cytokine/protein/growth factor expression, gene expression, immunomodulation and reaction to apoptotic stress.

Data analysis

Outcomes of interest were entered into an electronic database. The following were compared across different studies: (i) harvesting process, (ii) centrifugation technique/preparatory steps, (iii) tissue culturing and incubation period of PPs and BM-MSCs, (iv) effect of PP exposure on the biophysiological functions of BM-MSCs.

Results

Study selection

A total of 904 citations were identified during the searches, followed by removal of duplicate records (n = 281). At title and abstract sift, 569 citations not meeting the inclusion criteria were excluded. Full text sifting of the remaining citations (n = 58) excluded a further 25 citations, yielding a total of 33 citations for the final analysis (Fig. 1).

Study characteristics

Table 1 summarises the main study characteristics of the 33 studies included in the analyses and the BM-MSC functions assessed. The choice of PPs used was as follows: PRP-CM (n = 1); PL (n = 17); PR (n = 8) ; PRC (n = 2); PG-CM (n = 2) and PRF (n = 3). The effect of PPs on the proliferation of BM-MSCs was most commonly assessed (n = 27), followed by differentiation (n = 23); gene expression (n = 14); migration (n = 8) ; cytokine/growth factor release (n = 7) and immunomodulation (n = 6). Other functions less commonly assessed were the response of BM-MSCs towards apoptotic stress (n = 1) (Yin *et al.*, 2016) and stimulation of fibrinolysis and plasminogen activity (n = 1) (Agis *et al.*, 2009).

The majority of the studies (21 out of total 33) reported on the sample sizes for both PPs and BM-MSCs. Only 6 of these 21 studies had a sample size of $n \geq 10$ for both components (PPs and BM-MSCs) (Ben Azouna *et al.*, 2012; Jenhani *et al.*, 2011; Lucarelli *et al.*, 2003; Moisley *et al.*, 2019; Verrier *et al.*, 2010; Yin *et al.*, 2016); with the remaining having a small sample size of $n \leq 6$ for one or both components. Discrepancy in sample size between PPs and BM-MSCs within the same study was also observed (Amable *et al.*, 2014; Ben Azouna *et al.*, 2012; Bernardi *et al.*, 2017; Bernardi *et al.*, 2013; Goedecke *et al.*, 2011; Gottipamula *et al.*, 2012; Infante *et al.*, 2017; Jenhani *et al.*, 2011; Kasten *et al.*, 2008; Parsons *et al.*, 2008; Perut *et al.*, 2013; Prins *et al.*, 2009; Vogel *et al.*, 2006; Yin *et al.*, 2016) (Table 2**a**,**b** and 3**a**,**b**).

Harvesting process

With the exception of two studies (Dohan Ehrenfest *et al.*, 2010; Verrier *et al.*, 2010), PPs and BM-MSCs used in most of these *in vitro* studies were harvested from different donors, explaining the discrepancy in sample size between PPs and BM-MSCs seen in some studies (Table 2**a**,**b** and 3**a**,**b**).

PPs

PRP-CM (Yin *et al.*, 2016), PRC (Parsons *et al.*, 2008; Samuel *et al.*, 2016), PG-CM (Perut *et al.*, 2013; Schar *et al.*, 2015) and PRF (Dohan Ehrenfest *et al.*, 2010; Lucarelli *et al.*, 2010; Moradian *et al.*, 2017) were all harvested from peripheral blood. For studies assessing PR, only half (n = 4) used PR processed from peripheral blood; whilst the rest were from platelet apheresis/concentrates. As for studies using PL, with the exception of 3 studies (peripheral blood: n = 2; commercial grade PL: n = 1), all other studies used PL processed using buffy coats or platelet apheresis/concentrate acquired from a blood bank.

Only a small number of studies reported on harvest volume (n = 12). The health (n = 12) and age (n = 8) of volunteers/patients were only reported in some studies (Table 2**a**,**b**).

BM-MSCs

In most of the studies BM-MSCs were harvested from BMA (n = 20) and bone marrow harvested during surgery (n = 5).The remaining studies used either commercially purchased BM-MSCs (n = 4) or cryopreserved BM-MSCs (n = 4) (Table 3**a**,**b**).

The commonest harvest site was iliac crest (n = 11), followed by long bones (n = 3). Other sources of BM-MSCs include femoral head (n = 1) (Agis *et al.*, 2009) and maxilla (n = 1) (Dohan Ehrenfest *et al.*, 2010); whilst 17 studies did not specify harvest site of the BM-MSCs used. The volume of bone marrow harvested was only reported in 6 studies. Likewise, the age (n = 16) and health (n = 19) of volunteers/patients were only reported in some studies.

Preparation and processing of harvested samples

PPs – centrifugation, anticoagulant, platelet activation and concentrations of platelet and leukocytes

As illustrated in Table 4**a**-**e**, the centrifugation protocol was very variable among studies, most of which used a laboratory centrifugation system.

Similarly, the use of anticoagulants was variable and dependant on (i) protocol, (ii) source of platelet used (*e.g.* peripheral blood, platelet apheresis concentrate, commercial PL) and (iii) the choice of PPs intended for the experiment. Anticoagulants, such as ACD-A (n = 8), heparin (n = 3), CPD (n = 3) and sodium citrate (n = 1) were used if the first step involved producing PRP, before subjecting the sample to other processes, such as freeze-thawing or activation.

Activation of platelet to release the alpha-granules (Marx, 2004) was performed in studies assessing PR, PG-CM and PRF. Activation was achieved by exposing PPs to calcium chloride in 5 studies, to thrombin in 4 and to both thrombin and calcium gluconate in 1 study.

Leukocyte and platelet concentrations of PPs were not reported in most studies. Leukocyte concentration was only documented in 4 studies (Infante *et al.*, 2017; Moisley *et al.*, 2019; Perut *et al.*, 2013; Yin *et al.*, 2016), whereas platelet concentration was reported in more studies (n = 15). Only 3 studies compared the effects of leukocyte content on the biophysiological functions of BM-MSCs (Moisley *et al.*, 2019; Perut *et al.*, 2013; Yin *et al.*, 2016).

BM-MSCs – centrifugation isolation method, fresh vs. subcultured cells

Most of the studies used a laboratory system to isolate mononuclear cells rich in BM-MSCs from the

Table 1. Study characteristics. O = osteogenesis; A = adipogenesis; C = chondrogenesis.

Author (year)	Proliferation	Differentiation	Migration	Cytokine/ protein expression	Gene expression	Immunomodulation	Others
PRP-CM							
Yin *et al.*, 2016	✓	O	✓	✓	✓	✓	Cell viability/ apoptosis
PL							
Karadjian *et al.*, 2020	✓	O					
Moisley *et al.*, 2019	✓		✓				
Skific *et al.*, 2018	✓	O and A					
Infante *et al.*, 2017	✓		✓		✓		
Muraglia *et al.*, 2015	✓						
Muraglia *et al.*, 2014 (lyophilised PL)	✓						
Jonsdottir-Buch *et al.*, 2013	✓	O, A and C			✓	✓	
Bernardi *et al.*, 2013	✓	O, A and C				✓	
Murphy *et al.*, 2012	✓		✓				
Lange *et al.*, 2012		A			✓		
Gottipamula *et al.*, 2012	✓	O, A and C		✓	✓	✓	
Ben Azouna *et al.*, 2012	✓	O, A and C		✓	✓		
Jenhani *et al.*, 2011	✓			✓			
Goedecke *et al.*, 2011	✓	O and A	✓	✓			
Verrier *et al.*, 2010		O		✓	✓		
Prins *et al.*, 2009	✓	O, A and C					
Vogel *et al.*, 2006		O, A and C					
PR							
Nguyen *et al.*, 2019	✓		✓				
Liou *et al.*, 2018	✓	C			✓		
Bernardi *et al.*, 2017 (assessed PR and PL)	✓	O, A and C				✓	
Kosmacheva *et al.*, 2014		O			✓		
Amable *et al.*, 2014	✓	O, A and C		✓	✓		
Agis *et al.*, 2009							Stimulation of fibrinolysis and plasminogen activity
Gruber *et al.*, 2004	✓	O	✓		✓		
Lucarelli *et al.*, 2003	✓	O			✓		
PRC							
Samuel *et al.*, 2016	✓	O			✓		
Parsons *et al.*, 2008	✓	O			✓		
PG-CM							
Schar *et al.*, 2015			✓				
Perut *et al.*, 2013	✓	O					
PRF							
Moradian *et al.*, 2017	✓						
Lucarelli *et al.*, 2010	✓						
Dohan Ehrenfest *et al.*, 2010	✓	O					

bone marrow harvested. This was achieved by using a density-gradient centrifugation system (n = 19), and laboratory-grade centrifugation into pellets and manual isolation by nucleated cell counting before seeding (n = 3). 5 studies using clinically harvested bone marrow and 5 studies using commercially/tissue bank acquired BM-MSCs did not describe the method used to isolate mononuclear cells.

With the exception of 2 studies that strictly used freshly aspirated BM-MSCs only in all of their laboratory assays (Muraglia *et al.*, 2014; Perut *et al.*, 2013), the majority either used fresh BM-MSCs in their proliferation assays only (n = 3) or subcultured BM-MSCs (BM-MSCs transferred from previous culture into a new vessel for continued growth in fresh culture medium) (n = 31). Huge variation exists in tissue culture medium used for cultivation/expansion and the passage number of BM-MSCs used for assays (median: passage 3, range: passage 0 to 6) (Table 5**a**-**c**). Cellular detachment of subcultured BM-MSCs was performed by enzymatic methods (porcine trypsin, n = 22; recombinant corn-derived enzyme TrypZean, n = 2).

Incubation of BM-MSCs with PPs

All studies were conducted by exposing BM-MSCs to a continuous dose of PPs. As previously mentioned, except for 2 donor-matched studies (Dohan Ehrenfest *et al.*, 2010; Verrier *et al.*, 2010), BM-MSCs and PPs used in all other *in vitro* studies were not harvested from the same donor(s). Most studies (70.9 %, n = 22 out of total 31) with more than one donor reported pooling PPs in experiments (Table 6**a**-**c**).

Incubation period of BM-MSCs with PPs was variable among studies and dependent on the physiological function of BM-MSCs assessed: (i) migration: 4 to 48 h; (ii) proliferation: 2 h to 14 d; (iii) differentiation: 8 to 21 d; (iv) gene expression: 5 to 21 d; (v) cytokine, protein, growth factor expression: 3 to 14 d; (v) cell viability/apoptosis: 7 d; (vi) fibrin dissolution assay: 24 h (Table 6**a**-**c**). The choices of tissue culture medium and seeding density used for each assay were reported in all studies. Notably, the choices of tissue culture medium, culture dish/well plates and seeding density for assays were very variable among studies (Table 7**a**-**c**).

Effects of PPs on proliferation of BM-MSCs

Table 8**a**-**c** summarises the choice of laboratory assays, PPs used, comparative groups and their findings. The presence of PPs was found to significantly increase proliferation of BM-MSCs in most of the studies, when compared to controls: PRP-CM (n = 1), PL (n = 13), PR (n = 3), PRC (n = 1), PG-CM (n = 1), PRF (n = 2).

The effects of (i) concentration/dose of PPs, (ii) leukocyte content, and (iii) incubation time on proliferation of BM-MSCs have been assessed in some studies. In terms of concentrations of PPs in PP-conditioned medium, variable concentrations have been used, with 5 % to 10 % being the commonest. The increase in proliferation of BM-MSCs demonstrated a positive correlation with PP concentrations up to 10 %. Only 2 studies assessed the effect of PP concentrations over 10 % (Amable *et al.*, 2014; Lucarelli *et al.*, 2010), with opposing findings. Amable *et al.* (2014) demonstrated that PR-CM with PR concentrations over 10 % had an inhibitory effect towards BM-MSC proliferation, whereas Lucarelli *et al.* (2010) demonstrated that PRF-CM at 20 % induced stronger proliferation when compared to 10 % PRF-CM. A dose-dependent increase in proliferation was also observed in a study comparing single and double PRF membranes (Dohan Ehrenfest *et al.*, 2010).

The effects of leukocyte content in PP was only assessed by 3 studies, each using a different PP (Moisley *et al.*, 2019; Perut *et al.*, 2013; Yin *et al.*, 2016). When compared against its leukocyte-poor counterparts, BM-MSCs incubated in leukocyte-rich PG-CM demonstrated greater proliferative capacity (Perut *et al.*, 2013); whereas L-PRP-CM was found to be inferior (Yin *et al.*, 2016). However, leukocyte content in PL was not found to influence proliferation (Moisley *et al.*, 2019). Increasing platelet concentrations in PR was found to result in improved BM-MSC proliferation (Gruber *et al.*, 2004).

The effect of incubation time on PPs was only assessed by 1 study (Parsons *et al.*, 2008), whereby BM-MSCs incubated with 2.5 % PRC showed more proliferation in the first 24 h only, with proliferation equal to that of the control group at 48 h and inferior to control group at 72 h.

Effects of PP on migration of BM-MSCs

The chemotactic effects of PP on the migratory capacity of BM-MSCs was only assessed by 8 studies (Table 9). PRP-CM, PR, PRF-CM and PG-CM demonstrated positive chemotactic effects on the migratory capacity of BM-MSC *in vitro* (Gruber *et al.*, 2004; Nguyen *et al.*, 2019; Schar *et al.*, 2015; Yin *et al.*, 2016). Most PL studies reported on its positive chemotactic effects (Infante *et al.*, 2017; Moisley *et al.*, 2019; Murphy *et al.*, 2012). Migratory capacity of BM-MSCs improved with increasing PP concentration (up to 10 %) in most studies that compared between different PP concentrations [PL: n = 2 (Goedecke *et al.*, 2011; Murphy *et al.*, 2012) and PR: n = 2 (Gruber *et al.*, 2004; Nguyen *et al.*, 2019)]. However, no studies have compared PP concentrations over 10 %. The effects of leukocyte content in PP on the migratory capacity of BM-MSCs was assessed by 2 studies, with different conclusions (Moisley *et al.*, 2019; Yin *et al.*, 2016). Whilst Yin *et al.* (2016) demonstrated that P-PRP-CM performed significantly better than L-PRP-CM, Moisley *et al.* (2019) demonstrated that leukocyte depletion do not affect BM-MSC migration.

Effects of PPs on differentiation of BM-MSCs

Table 10**a**-**c** summarises the effects of PPs on the differentiation potential of BM-MSCs into different mesenchymal lineages. Osteogenesis was most commonly assessed (n = 18), whilst adipogenesis

Table 2a. Harvesting process of PPs.

Author (year)	Sample size (*n*)	Patient's health	Age	Source	Harvest volume
PRP-CM					
Yin *et al.*, 2016	10	Healthy	21-45 years	Peripheral blood	Not stated
PL					
Karadjian *et al.*, 2020	Not stated (commercially purchased human PL)	Not stated	n/a	Commercial human PL (PL BioScience, Aachen, Germany)	n/a
Moisley *et al.*, 2019	11	Healthy	22-58 years	Peripheral blood	Not stated
Skific *et al.*, 2018	Not stated	Not stated	Not stated	Pooled platelet products from blood bank	Not stated
Infante *et al.*, 2017	20	Not stated	Not stated	Peripheral blood	Not stated
Muraglia *et al.*, 2015	Not stated (5-10 buffy coats)	Healthy	Not stated	Buffy coat units from blood bank	Not stated
Muraglia *et al.*, 2014 (lyophilised PL)	Not stated (10-20 buffy coat used)	Healthy	Not stated	Buffy coat units from blood bank	10-20 × buffy coat bags (30-40 mL each bag)
Jonsdottir-Buch *et al.*, 2013	Not stated	Not stated	Not stated	Platelet rich concentrates derived from buffy coat	Not stated
Bernardi *et al.*, 2013	28	Not stated	Not stated	PRP from apheresis units	50 mL of pooled PRP
Murphy *et al.*, 2012	Not stated	Not stated	Not stated	Buffy coat units from blood bank	Not stated
Lange *et al.*, 2012	1	Not stated	Not stated	Buffy coat units from blood bank	7-13 pooled buffy coats
Gottipamula *et al.*, 2012	30	Not stated	Not stated	Platelet concentrate from blood bank	Not stated
Ben Azouna *et al.*, 2012	≥ 10	Not stated	Not stated	Platelet apheresis collections from blood bank	450 ± 45 mL per donor
Jenhani *et al.*, 2011	≥ 10	Not stated	Not stated	Platelet apheresis collections from blood bank	450 ± 45 mL per donor
Goedecke *et al.*, 2011	2	Not stated	Not stated	Platelet apheresis concentrate from blood bank	Not stated
Verrier *et al.*, 2010	16	patient undergoing routine orthopaedic surgery involving iliac crest exposure	28-79 years (average: 49 years)	Whole blood	100 mL
Prins *et al.*, 2009	5	Not stated	Not stated	Pooled platelet products from blood bank	Not stated
Vogel *et al.*, 2006	5	Not stated	Not stated	Buffy coat units from blood bank	Not stated

Table 2b. Harvesting process of PPs.

Author (year)	Sample size (*n*)	Patient's health	Age	Source	Harvest volume
PR					
Nguyen *et al.*, 2019	Not stated	Healthy, non-smokers	20-30 years	Peripheral blood	Not stated
Liou *et al.*, 2018	3	Not stated	Not stated	Whole blood from blood bank	Not stated
Bernardi *et al.*, 2017 Assessed PR and PL	15	Not stated	Not stated	Platelet apheresis (PR: Haemonetics, MA; PL: Trimal Accel separator, Caridian BCT Inc)	Not stated
Kosmacheva *et al.*, 2014	Not stated	Not stated	Not stated	Platelet concentrate (> 5 doses)	Not stated
Amable *et al.*, 2014	18	Not stated	Not stated	Peripheral blood	Not stated
Agis *et al.*, 2009	6	Not stated	Not stated	Platelet apheresis concentrates (MCS+ device, Haemonetics)	Not stated
Gruber *et al.*, 2004	4	Not stated	24-39 years	Platelet apheresis concentrate	Not stated
Lucarelli *et al.*, 2003	10	Healthy	Not stated	Peripheral blood	Not stated
PRC					
Samuel *et al.*, 2016	6	Healthy	Not stated	Peripheral blood	25 mL
Parsons *et al.*, 2008	11	Not stated	Not stated	Peripheral blood	80 mL
PG-CM					
Schar *et al.*, 2015	11	Healthy	27-38 years (mean: 32.6 years)	Peripheral blood	65 mL
Perut *et al.*, 2013	10	Healthy	26-38 years	Peripheral blood	200 mL
PRF					
Moradian *et al.*, 2017	Not stated	Not stated	Not stated	Peripheral blood	Not stated
Lucarelli *et al.*, 2010	3	Healthy	Not stated	Peripheral blood	18 mL
Dohan Ehrenfest *et al.*, 2010	1	Patient undergoing implant placement in maxilla for first molar tooth replacement	54	Peripheral blood	9 mL

Table 3a. Harvesting process of BM-MSCs. ‡: same study cohort and methodology.

Author year	Sample size (*n*)	Patient's health	Age	Source	Harvest site	Volume of aspirate
PRP-CM						
Yin *et al.*, 2016	14	Not stated	25-48 years	BMA	Greater trochanter of femur fracture patients; bone debris filtered before culture	Not stated
PL						
Karadjian *et al.*, 2020	10	Patient undergoing surgery of proximal femur	29-73 (median 46) years	Bone marrow washouts	Proximal femur	Not stated
Moisley *et al.*, 2019	11	Healthy patient undergoing elective orthopaedic surgery	17-69 years	BMA	Anterior iliac crest	4 mL
Skific *et al.*, 2018	13	Healthy donors	8-52 (median 22) years	BMA	Iliac crest	3-5 mL
Infante *et al.*, 2017	3 commercially available	Not stated	Not stated	Commercial Lonza	Not stated	Not stated
Muraglia *et al.*, 2015	Not stated	Healthy donors	Not stated	BMA	Iliac crest	Not stated
Muraglia *et al.*, 2014	Not stated	Not stated	50-78 years	BMA	Long bone not clearly specified	Not stated
Jonsdottir-Buch *et al.*, 2013	3	Not stated	Not stated	Commercial Lonza	Not stated	Not stated
Bernardi *et al.*, 2013	4	Not stated	Not stated	BMA	Not stated	Not stated
Murphy *et al.*, 2012	Not stated	Healthy young volunteers	Not stated	Passage 3-6 cryopreserved BM-MSC prepared from BMA	Not stated	Not stated
Lange *et al.*, 2012	$n = 3$ for differentiation $n = 5$ for gene expression	Healthy donors	Not stated	BMA	Not stated	Not stated
Gottipamula *et al.*, 2012	4	Healthy donors	Not stated	BMA	Iliac crest	Not stated
Ben Azouna *et al.*, 2012 ‡	13	Not stated	16-41 (mean 33 ± 2) years	National Bone marrow Graft centre During orthopaedic surgery	Not stated	Not stated
Jenhani *et al.*, 2011 ‡	13	Not stated	Not stated	National Bone marrow Graft centre	Not stated	Not stated
Goedecke *et al.*, 2011	5	Fit and well	Not stated	Bone marrow samples	Not stated	Not stated
Verrier *et al.*, 2010	16	Patient undergoing routine orthopaedic surgery involving iliac crest exposure	28-79 (average: 49) years	BMA	Iliac crest	60 mL
Prins *et al.*, 2009	9	Patient undergoing hip operations or stem cell harvest procedures	4-74 years	BMA	Iliac crest	Not stated
Vogel *et al.*, 2006	6	Healthy donors undergoing total hip replacement or spinal surgery	12-75 (mean: 40.7 ± 25.5) years	BMA	Not stated	Not stated

Table 3b. Harvesting process of BM-MSCs. ‡: same study cohort and methodology.

Author year	Sample size (*n*)	Patient's health	Age	Source	Harvest site	Volume of aspirate
PR						
Nguyen *et al.*, 2019	Not stated	Not stated	Not stated	Commercially purchased passage 3 human BM-MSCs	Not stated	Not applicable
Liou *et al.*, 2018	3	Patients undergoing arthroplasty	65-68 years	trabecular bone marrow	Not stated	Not stated
Bernardi *et al.*, 2017	3	Not stated	Not stated	Bone marrow	Not stated	Not stated
Kosmacheva *et al.*, 2014	Not stated	Healthy donors	Not stated	Bone marrow biopsy	Not stated	Not stated
Amable *et al.*, 2014	4	Not clearly stated; patients with fracture non-union	Not stated	Bone marrow	Posterior iliac crest	Not stated
Agis *et al.*, 2009	6	Not stated	60-87 years	Trabecular bone marrow	Femoral head	Not stated
Gruber *et al.*, 2004	4	Healthy donors	33-54 years	Bone marrow	Iliac crest	Not stated
Lucarelli *et al.*, 2003	10	Not stated	10-33 (mean: 18.3 ± 6.9) years	BMA	Iliac crest	2 mL
PRC						
Samuel *et al.*, 2016	Not stated	Patients undergoing total knee/ hip arthroplasty	Not stated	BMA	Not stated	Not stated
Parsons *et al.*, 2008	4	Not stated	Not stated	Commercially acquired Lonza group Ltd	Not stated	Not applicable
PG-CM						
Schar *et al.*, 2015	Not stated	Not stated	Not stated	Cell bank	Not stated	Not stated
Perut *et al.*, 2013	3	Not stated	55, 75 and 65 years	Bone marrow samples from discarded material collected during reconstructive surgery	Not stated	Not stated
PRF						
Moradian *et al.*, 2017	Not stated	Healthy donors	19-45 years	BMA	Iliac crest	5 mL
Lucarelli *et al.*, 2010	3	Patients undergoing elective orthopaedic surgery	Not stated	BMA	Iliac crest	10 mL
Dohan Ehrenfest *et al.*, 2010	1	Patient undergoing implant placement in maxilla for first molar tooth replacement	54	Bone marrow/cells harvested from site of bone defect created by drilling for future implant placement at maxilla	Maxilla	Not applicable

($n = 8$) and chondrogenesis ($n = 7$) were less commonly investigated.

Osteogenic differentiation of BM-MSCs following PP exposure was found to be increased in most of the studies (66.7 %, $n = 12$ out of 18 studies) (Amable *et al.*, 2014; Dohan Ehrenfest *et al.*, 2010; Gottipamula *et al.*, 2012; Karadjian *et al.*, 2020; Kosmacheva *et al.*, 2014; Lucarelli *et al.*, 2003; Parsons *et al.*, 2008; Perut *et al.*, 2013; Samuel *et al.*, 2016; Skific *et al.*, 2018; Verrier *et al.*, 2010; Yin *et al.*, 2016). In contrast, PP exposure did not improve the adipogenic potential (87.5 %, $n = 7$ out of 8 studies) (Amable *et al.*, 2014; Ben Azouna *et al.*, 2012; Goedecke *et al.*, 2011; Gottipamula *et al.*, 2012; Lange *et al.*, 2012; Prins *et al.*, 2009; Vogel *et al.*, 2006) and chondrogenic potential (66.7 %, $n = 4$ out of 6 studies) (Ben Azouna *et al.*, 2012; Liou *et al.*, 2018; Prins *et al.*, 2009; Vogel *et al.*, 2006) of BM-MSCs in the majority of the studies.

Only Lucarelli *et al.* (2003) investigated the effect of PP withdrawal on BM-MSC differentiation. They reported that despite PP withdrawal, BM-MSCs previously exposed to PPs retained their improved osteogenic and chondrogenic capability, when compared against controls. Finally, only 2 studies investigated the effect of leukocyte content on osteogenic differentiation of BM-MSCs, with opposing findings (Perut *et al.*, 2013; Yin *et al.*, 2016).

Effects of PPs on growth factor/cytokine/protein expression of BM-MSCs

Table 11**a**,**b** summarises the findings of studies that have investigated the effect of PP on the expression of growth factors, cytokines, proteins and enzymes by BM-MSCs. Overall, PPs were found to have a chemotactic, pro-inflammatory, pro-osteogenic and pro-angiogenic effect on BM-MSCs. Chemotactic factors such as RANTES (Ben Azouna *et al.*, 2012; Jenhani *et al.*, 2011), SDF-1α (Goedecke *et al.*, 2011), eotaxin (Amable *et al.*, 2014), IP-10 (Amable *et al.*, 2014), MIP-1β (Amable *et al.*, 2014) and MCP-1 (Amable *et al.*, 2014) were upregulated in BM-MSCs exposed to PPs.

Pro-inflammatory cytokines (PGE2, NO, IL-2R, IL-6, IL-7, IL-8, IL-12 and IL-15) were universally upregulated by PPs in all *in vitro* studies (Amable *et al.*, 2014; Ben Azouna *et al.*, 2012; Jenhani *et al.*, 2011; Yin *et al.*, 2016). Comparing the effects of leukocyte content in PRP-CM on the release of pro-inflammatory cytokines PGE2 and NO by BM-MSCs, Yin *et al.* (2016) demonstrated that only L-PRP-CM upregulates the production of these pro-inflammatory cytokines significantly when compared to P-PRP-CM and controls; whereas no difference was observed when comparing P-PRP-CM to control (FBS).

Exposure to PPs was found to significantly upregulate most pro-osteogenic proteins by BM-MSCs. These include osteocalcin (Samuel *et al.*, 2016; Yin *et al.*, 2016), RUNX2 (Yin *et al.*, 2016) and BMP2 (Verrier *et al.*, 2010). Comparing the effect of leukocyte concentration, Yin *et al.* (2016) reported stronger upregulation in P-PRP-CM in comparison to L-PRP-CM ($p < 0.001$). With regards to angiogenesis, PR resulted in an increased release of thrombospondin (Amable *et al.*, 2014), PLGF (Amable *et al.*, 2014), aFGF (Amable *et al.*, 2014), VEGF-D (Amable *et al.*, 2014) by BM-MSCs (Amable *et al.*, 2014). However, VEGF release was found to be equivocal [PR: upregulated ($n = 1$) (Amable *et al.*, 2014); PL: less than control ($n = 2$) (Ben Azouna *et al.*, 2012; Jenhani *et al.*, 2011)]. On the other hand, the release of adipogenic molecules L-PGDS (Lange *et al.*, 2012) and leptin (Ben Azouna *et al.*, 2012) by BM-MSCs following PP exposure was found to be weaker than control group.

PPs increased the secretion of catabolic MMPs (MMP1, MMP3, MMP7, MMP8 and MMP13) and extracellular matrix proteins and other molecules (heparan sulphate, elastin, laminin, collagens I and III) (Amable *et al.*, 2014). Amable *et al.* (2014) studied also the release of other growth factors and found that PP increases the secretion of HGF, G-CSF, TGF-β1 and TGF-β2; whereas it downregulates PDGF-BB secretion.

Effects of PPs on other BM-MSC functions

The effect of PPs on the immunomodulatory function of BM-MSCs was assessed by 4 studies (Bernardi *et al.*, 2017; Gottipamula *et al.*, 2012; Jonsdottir-Buch *et al.*, 2013; Yin *et al.*, 2016) (Table 12). Whilst L-PRP-CM was found to induce the activation of the NF-κB signalling pathway, which could activate genes responsible for inflammation and other immune responses (Yin *et al.*, 2016), the immunomodulatory effects of BM-MSCs exposed to PL seemed to be equivocal. Jonsdottir-Buch *et al.* (2013) demonstrated PL to have no effect on the ability of BM-MSCs to reduce mononuclear cell proliferation; whilst 2 other studies demonstrated BM-MSCs cultured with PL to have weaker inhibitory effects on PBMCs (Bernardi *et al.*, 2017) and T-cell proliferation (Gottipamula *et al.*, 2012) when compared to controls.

Other biophysiological function of BM-MSCs assessed were (i) response towards apoptotic stress, (ii) chemotactic effect on HSCs and (iii) capacity to stimulate plasminogen activation. Yin *et al.* (2016) found that both L-PRP- and P-PRP-CM are able to inhibit camptothecin-induced apoptosis of BM-MSCs and, therefore, enhance its viability, with L-PRP-CM having the strongest effect. The chemotactic effect of BM-MSCs in terms of inducing migration of HSCs was only assessed by 1 study, whereby the chemotactic effect of BM-MSCs cultured in 5 % and 10 % PL was demonstrably inferior when compared to control (Goedecke *et al.*, 2011). Agis *et al.* (2009) found that BM-MSCs pre-incubated with PR demonstrated a stronger capacity to stimulate plasminogen activation and fibrinolysis.

Gene expression of BM-MSCs following PP exposure

Table 13**a**-**c** summarises the effect PP has on gene expression of BM-MSCs, based on the biophysiological functions of BM-MSCs. Samples were taken from BM-

Table 4a. Preparation and processing of PPs. ‡: same study cohort and methodology.

Author (year)	Type of PRP used	Leukocyte/platelet concentration	Anticoagulant used at blood collection	Processing (steps)	Activation	Activator used
PRP-CM						
Yin *et al.*, 2016	Both P-PRP and L-PRP	Leukocyte: 34.58 ± 8.48 × 10^9/L (L-PRP) 0.18 ± 0.18 × 10^9/L (P-PRP) Platelet: 1436.70 ± 257.97 × 10^9/L (L-PRP) 1461.80 ± 189.14 × 10^9/L (P-PRP)	ACD-A	L-PRP: • Automated clinical grade centrifuge • (WeGo, Shandong Weigao Group Medical Polymer Products Co., Ltd) P-PRP: • Laboratory-grade centrifuge • 1st centrifugation: 160 ×*g* for 10 min • 2nd centrifugation: on Separated plasma. 250 ×*g* for 15 min • Extraction of supernatant • Precipitated platelets resuspended in residual plasma ≥ P-PRP	Not stated	Not stated
PL						
Karadjian *et al.*, 2020	Not stated	Leukocyte: not stated Platelet: not stated	Not stated	• Not stated	Not stated	Not stated
Moisley *et al.*, 2019	Clinical grade L-PRP (cPRP) Laboratory-grade PRP (PRP) Filtrated Laboratory-grade PRP (fPRP)	Leukocyte: 20.6 × 10^3/μL (cPRP) 1.8 × 10^3/μL (PRP) Platelet: 6.3 × 10^5/μL (cPRP) 15.9 × 10^5/μL (PRP)	ACD-A	cPRP: • Automated clinical-grade centrifuge, BioCUE™ device, Zimmer Biomet • 1,100 ×*g* for 15 min PRP: • Laboratory-grade centrifuge • 1st centrifugation: 400 ×*g* for 10 min • 2nd centrifugation: - extraction of supernatant - 2,700 ×*g* for 10 min - resuspension in 1/5th residual supernatant fPRP: • PRP passed through white blood cell syringe filter (Acrodisc) cPL, PL and fPL: • 3 freeze-thaw cycles of cPRP, PRP and fPRP	Not stated	Not stated
Skific *et al.*, 2018	Platelet apheresis product	Not stated Apheresis product containing 5 × 10^{11} platelets	Heparin (5,000 UI)	• Laboratory-grade centrifuge • Freeze − 80 °C, thawing 37 °C of freshly collected platelet apheresis product • Heparin added to avoid gel formation • 3 centrifugation cycles at 900 ×*g* for 30 min • Pooled as culture supplement	Not applicable	Not applicable
Infante *et al.*, 2017	P-PRP	Leukocyte: no leukocytes Platelet: not stated clearly; "moderate enrichment of platelets (2×)"	Acid citrate dextrose	• Laboratory-grade centrifuge • 570 ×*g* for 7 min • Plasma layer collected • PL: 3 freeze-thaw cycles of P-PRP at − 20 °C/37 °C • Sterile filtration: 0.22 μm filter	Not stated	Not stated

Table 4b. Preparation and processing of PPs. ‡: same study cohort and methodology.

Author (year)	Type of PRP used	Leukocyte/platelet concentration	Anticoagulant used at blood collection	Processing (steps)	Activation	Activator used
PL						
Muraglia *et al.*, 2015	Not stated	Leukocyte: not stated Platelet: 10×10^6 platelets/μL	Not stated	• Laboratory-grade centrifuge • PRP: centrifugation of buffy coat (1,100 rpm for 10 min • 2nd centrifugation of PRP: 2600 rpm for 20 min • PL: 3 freeze-thaw cycles of PRP and centrifugation at high-speed for 20 min, room temperature	Not stated	Not stated
Muraglia *et al.*, 2014	Not stated	Leukocyte: not stated Platelet: 10×10^6 platelets/μL	Not stated	• Laboratory-grade centrifuge • 230 g × 15 min, room temperature • Removal of plasma and buffy layers • Further centrifugation: 1,100 ×*g* for 15 min to produce P-PRP • PL: freeze-thaw cycles of P-PRP	Not stated	Not stated
Jonsdottir-Buch *et al.*, 2013	Not stated	Leukocyte: not stated Platelet: not stated	Not stated	• Laboratory-grade centrifuge • 1st centrifugation: - 4,975 ×*g* for 20 min of frozen/thawed PRC - Supernatant filtration: 40 μm cell strainer - Further filtration: 0.45 μm stericups • 2nd centrifugation: 4,975 ×*g* for 20 min • Filtration and freezing at − 80 °C • Centrifugation at 4,975 ×*g* for 10 min • Supernatants used as culture medium supplements	Not stated	Not stated
Bernardi *et al.*, 2013	Not stated PL produced by sonification of PRP	Leukocyte: not stated Platelet: not stated	Not stated	• Not applicable	Not stated	Not stated
Murphy *et al.*, 2012	Not stated	Leukocyte: not stated Platelet: 10^6 platelets/μL	Not stated	• Laboratory-grade centrifuge • 1st centrifugation: 200-300 ×*g* for 15 min to separate RBC fraction • 2nd centrifugation: 1,600 ×*g* for 10 min • Removal of platelet poor plasma • P-PRP: resuspension of platelet pellet, standardised to 10^6 platelets/μL • PL: freeze-thaw cycles of P-PRP	Not applicable	Not applicable
Lange *et al.*, 2012	Not stated	Leukocyte: not stated Platelet: not stated	Not stated	• PRP: Laboratory-grade centrifugation of pooled thrombocyte concentrates 200 ×*g* for 20 min • PL: Freeze thawing of PRP	Not stated	Not stated
Gottipamula *et al.*, 2012	Not stated	Leukocyte: not stated Platelet: not stated	Not stated	• 5 freeze-thaw cycles of pooled platelet concentrates • Laboratory-grade centrifugation 4,000 ×*g* for 15 min to remove platelet fragments	Not stated	Not stated
Ben Azouna *et al.*, 2012	P-PRP used to produce PL following freeze-thaw cycle	Leukocyte: not stated Platelet: not stated	Heparin	• Buffy coats (free of red blood cells and plasma: automated clinical grade system; Compomat G3) • P-PRP: - Laboratory centrifugation of buffy coat units - soft spin at 341 ×*g* for 6 min at 22 °C - In-line filtration to deplete WBC (PALL autostop), frozen at − 30 °C • PL: - Freeze-thaw cycles of PRP - Laboratory centrifugation: 4,000 ×*g* for 15 min	Not stated	Not stated

Table 4c. Preparation and processing of PPs. ‡: same study cohort and methodology.

Author (year)	Type of PRP used	Leukocyte/platelet concentration	Anticoagulant used at blood collection	Processing (steps)	Activation	Activator used
Jenhani *et al.*, 2011‡	P-PRP used to produce PL following freeze-thaw cycle	Same as Ben Azouna *et al.* (2012)	Same as Ben Azouna *et al.* (2012)	• Same as Ben Azouna *et al.* (2012)	Not stated	Not stated
Goedecke *et al.*, 2011	Not stated	Leukocyte: not stated Platelet: not stated	Not stated	• Freeze-thawing (− 20 °C/56 °C) of platelet apheresis concentrates • Laboratory centrifugation: 5,000 ×*g* for 45 min at 4 °C • Filtration: 40 μm cell filter	Not stated	Not stated
Verrier *et al.*, 2010	Not stated	Leukocyte: not stated Platelet: 10×10^6 platelets/μL	CPDA	• Laboratory-grade centrifugation (2 steps): - 200 ×*g* for 30 min (room temperature) - 2,000 ×*g* for 5 min (room temperature) • All supernatants discarded • Resuspension of platelet pellet in sterile PBS at 1 : 10 • Subsequent freeze-thaw cycles	Not stated	Not stated
Prins *et al.*, 2009	Not stated	Leukocyte: not stated Platelet: 1×10^9 platelets/mL	Not stated	• Freeze (− 80 °C)-thawing of platelet apheresis concentrates • Laboratory-grade centrifugation: 750 ×*g* for 10 min to eliminate platelet fragments	Not stated	Not stated
Vogel *et al.*, 2006	P-PRP	Leukocyte: not stated Platelet: 1.56×10^6/μL	Citrate-phosphate-dextrose	• P-PRP: laboratory-grade centrifugation of buffy coat, 205 ×*g* for 10 min • Leukocyte filtration (Fresenius Bio P Plus) • P-PRP frozen at − 80 °C until use	Not stated	Not stated
PR						
Nguyen *et al.*, 2019	Not stated	Leukocyte: not stated Platelet: not stated	Not stated	• Laboratory-grade centrifugation • 1st centrifugation: 2,000 rpm 20 min • Removal of supernatant • 2nd centrifugation: 3,500 rpm for 5 min • Platelet activation: $CaCL_2$	Yes	$CaCL_2$
Liou *et al.*, 2018	Not stated	Leukocyte: not stated Platelet: 1×10^6/μL	Yes, but choice of anticoagulant not specified	• Laboratory-grade centrifuge 480 ×*g* for 20 min • Platelet activation: $CaCL_2$ • Overnight incubation at 37 °C • Further centrifugation at 2,000 ×*g* for 10 min • Supernatant obtained after discarding fibrin clot = PRP releasate	Yes	$CaCL_2$
Bernardi *et al.*, 2017 Assessed PR and PL	Not stated	Leukocyte: not stated Platelet: not stated	Not stated	• Laboratory-grade centrifuge • PR: - Activated platelet apheresis incubated at 40 °C for 60 min until complete clot formation - Centrifugation at 2,200 ×*g* for 5 min - PR collected and stored at − 80 °C • PL: - 2× freeze (− 80 °C)-thaw cycles and centrifugation of platelet apheresis product - 1,600 ×*g* for 15 min, room temperature - Supernatants pooled and filtered at 70 μm cell strainer - Stored at − 20 °C	PR: Yes PL: no	PR: $CaCl_2$ PL: no

Table 4d. Preparation and processing of PPs. ‡: same study cohort and methodology.

Author (year)	Type of PRP used	Leukocyte/platelet concentration	Anticoagulant used at blood collection	Processing (steps)	Activation	Activator used
PR						
Kosmacheva *et al.*, 2014	Not stated	Leukocyte: not stated Platelet: 5×10^9 cells/mL following first centrifugation	Not stated	• Laboratory-grade centrifugation of pooled platelet concentrate to produce platelet concentrate (5×10^9 cells/mL) • Thrombin activation: 20 min incubation at 20 °C • Clot centrifugation at 3,500 ×*g* for 20 min • Supernatant: PR collected and stored at − 20 °C	Yes	Thrombin
Amable *et al.*, 2014	P-PRP used to produce PR	Leukocyte: not stated Platelet: 10×10^6 platelets/μL	Acid citrate dextrose	• Laboratory-grade centrifugation of peripheral blood: 300 ×*g* for 5 min • Further centrifugation: 700 ×*g* for 17 min • Platelet activation: $CaCL_2$ at 37 °C for 1 h • Overnight incubation at 4 °C • PR: centrifugation of clot at 3,000 ×*g* for 20 min	Yes	$CaCL_2$
Agis *et al.*, 2009	P-PRP used to produce PR	Leukocyte: depleted Platelet: 2×10^8 platelets/mL	Not stated	• Laboratory-grade centrifugation of leukocyte-depleted platelet apheresis concentrate aliquots - 1,400 ×*g* for 10 min - Resuspension of pellets in serum-free α-MEM • PR: - Activation of platelet suspension (human thrombin) - 30 min at room temperature - Centrifugation and collection of supernatants (PR) • PL: repeated freeze-thaw cycles of platelet suspension (in absence of thrombin) • Centrifugation of supernatants from PR and PL were filtered sterile and stored at − 40 °C	PR: yes PL: no	Human thrombin (for PR)
Gruber *et al.*, 2004	P-PRP used to produce PR	Leukocyte: depleted Platelet: 1×10^9 cells/mL	ACD-A	• Laboratory-grade centrifuge • Centrifugation of platelet concentrates: 1,400 ×*g* for 10 min • Human thrombin activation • Further centrifugation: 1,400 ×*g* for 10 min • Sterile filtration of supernatants (PR) • Stored at − 80 °C	Yes	Human thrombin
Lucarelli *et al.*, 2003	Not stated	Leukocyte: not stated Platelet: not stated	Citrate-phosphate-dextrose	• Laboratory-grade centrifugation (2 steps) • 1,000 ×*g* for 15 min at 20 °C to remove red blood cells • 3,000 ×*g* for 10 min at 20 °C to obtain PRP • Thrombin activation leading to PG formation • Filtration and supernatant stored (PR at – 80 °C)	Yes	Thrombin and calcium gluconate
PRC						
Samuel *et al.*, 2016	Not stated	Leukocyte: not stated Platelet: not stated	ACD	• Laboratory-grade centrifuge • 1st centrifugation: 200 ×*g* for 10 min • 2nd centrifugation: 800 ×*g* for 10 min • Resultant platelet pellets isolated, resuspended in sterile PBS ≥ PRC	No	n/a
Parsons *et al.*, 2008	Not stated	Leukocyte: not stated Platelet: not stated	ACD-A	• Centrifuge device not used • Utilised CAPTION device (Smith and Nephew relying on filtration technology)	No	n/a

Table 4e. Preparation and processing of PPs. ‡: same study cohort and methodology.

Author (year)	Type of PRP used	Leukocyte/platelet concentration	Anticoagulant used at blood collection	Processing (steps)	Activation	Activator used
PG-CM						
Schar *et al.*, 2015 Assessed PRP-gel and PRF	L-PRP used to produce L-PRP gel	Leukocyte: not stated Platelet: not stated	ACD-A used to process L-PRP	• L-PRP: clinical-grade centrifuge • L-PRP clot/gel formed by mixing: - blood with ACD-A, centrifuged in GPSIII (Biomet Inc separation tube at 1,900 ×*g* for 15 min at room temperature) - Blood with ACD-A in Clotalyst® disposable tube containing TPD™ Thrombin reagent; centrifuged at 1,900 ×*g* for ?? min • L-PRF: - Laboratory-grade centrifuge - Blood in glass-coated plastic tube - 400 ×*g* for 12 min, room temperature	PRP gel: yes	TPD™ Thrombin reagent
Perut *et al.*, 2013	Both P-PRP and L-PRP used to produce PG	Leukocyte: 5.7×10^3/μL (L-PRP) 0×10^3/μL (P-PRP) Platelet: 912×10^3 platelets/μL (L-PRP) 194×10^3 platelets/μL (P-PRP)	Sodium citrate	• Laboratory-grade centrifuge • P-PRP: - 460 ×*g* for 8 min - Supernatant collected = P-PRP • L-PRP: - 150 mL venous sample in 21 mL sodium citrate - 730 ×*g* for 15 min - 2nd centrifugation: 3,800 g × 10 min of resultant plasma and buffy coat - Supernatant collected = L-PRP • Both P-PRP and L-PRP then undergo activation to produce PG	Yes	10 % $CaCL_2$
PRF						
Moradian *et al.*, 2017	Not applicable	Leukocyte: not stated Platelet: not stated	Not applicable	• Laboratory-grade centrifuge • 400 ×*g* for 10 min • Compression of fibrin clot	Not stated	Not stated
Lucarelli *et al.*, 2010	Not applicable	Leukocyte: not stated Platelet count (on residual serum): $2{,}600 \pm 547.72/mm^3$	Not applicable	• Clinical grade centrifuge (FIBRINET®, Cascade Medical Enterprises, Wayne, USA) • 1,100 ×*g* for 6 min to obtain PRP • PRP transferred to $CaCl_2$ containing glass bottle (0.25 mL $CacL_2$ 1 mol/L and gently swirled) • 2nd centrifugation: 4,500 ×*g* for min, 25 °C to form PRF matrix	Yes	$CaCl_2$ used to activate PRP
Dohan Ehrenfest *et al.*, 2010	Not applicable	Leukocyte: not stated Platelet: not stated	Not applicable	• Laboratory-grade centrifuge for 12 min to allow for 3-layer separation (PRF clot in middle layer) • PRF clot harvested with pliers • Soft compression of PRF clot with sterile gauze into a membrane • PRF membranes remained in culture plate for first week	No	Not applicable

Table 5a. Preparation and processing of BM-MSCs. ‡: same study cohort and methodology.

Author (year)	Centrifugation system used	Centrifugation condition(s)	Subcultured cells used in laboratory assays	Passage number	Tissue culture medium used for expansion	Method of cellular detachment (enzymatic or mechanical)
PRP-CM						
Yin *et al.*, 2016	Not stated	Not stated	Yes	Passage 5	α-MEM + 10 % FBS + 1 % antibiotics (penicillin G and streptomycin) + 10 % FBS	Enzymatic by trypsinisation (trypsin-EDTA, Invitrogen)
PL						
Karadjian *et al.*, 2020	Laboratory density-centrifugation	Not stated	Yes	Proliferation: passage 0 Osteogenesis: passage 1	25 mmol/L HG-DMEM + penicillin/streptomycin 100 mg/L + L-glutamine 200 mmol/L + MEM non-essential amino acids solution + 2-mercaptoethanol 50 mmol/L + 4 µg/L FGF2	Not stated
Moisley *et al.*, 2019	Laboratory centrifugation into pellet, manual counting of nucleated mononuclear cells prior to seeding	Not stated	Yes	Differentiation assays: passage 4	MSC expansion media (Miltenyi Biotec)	Enzymatic by trypsinisation
Skific *et al.*, 2018	Laboratory density-centrifugation	Density gradient centrifugation: Ficoll-Paque™ Plus (GE Healthcare)	Yes	Proliferation: throughout passages 1 to 4 Differentiation: passage 2	α-MEM + 2 mmol/L L-glutamine + 1 % penicillin-streptomycin-neomycin solution	Enzymatic by trypsinisation
Infante *et al.*, 2017	Not stated	Not stated	Yes	Passage 4-5	DMEM GlutaMAX™ + 1 % antibiotics + 10 % FBS	Enzymatic by trypsinisation
Muraglia *et al.*, 2015	Laboratory centrifugation into pellet, manual counting of nucleated mononuclear cells prior to seeding	Bone marrow washed twice with PBS; nucleated cells counted (with 0.1 % methyl violet in 0.1 mol/L citric acid as nuclear stain) before culturing in culture medium	Yes	Passage 1	Coon's-modified Ham's F-12 medium + 100 IU/mL penicillin + 100 mg/mL streptomycin + 2 mmol/L L- glutamine	Enzymatic by trypsinisation
Muraglia *et al.*, 2014	Laboratory centrifugation into pellet, manual counting of nucleated mononuclear cells prior to seeding	Bone marrow washed twice with PBS; nucleated cells counted (using 0.1 % methyl violet in 0.1 mol/L citric acid as nuclear stain) before culturing in culture medium	No fresh mononuclear cells rich in BM-MSCs used	Not applicable	Coon's-modified Ham's F-12 medium + 10 %FBS + 100 IU/mL penicillin + 100 µg/mL streptomycin + 2 mmol/L L-glutamine (changed to serum-free medium equivalent at medium change)	Not applicable
Jonsdottir-Buch *et al.*, 2013	Not stated	Not stated	Yes	Differentiation and gene expression: passage 3 Proliferation: total of 6 passages	Standard Mesenchymal Stem Cell Basal medium (Lonza)	Enzymatic by trypsinisation
Bernardi *et al.*, 2013	Laboratory density-centrifugation	Ficoll-Premium	Yes	Proliferation: throughout all 7 passages Differentiation: passage 7	DMEM Advanced Therapy Medical Product (DMEM ATMP-Ready)	Enzymatic by TrypLE™ Select

Table 5b. Preparation and processing of BM-MSCs. ‡: same study cohort and methodology.

Author (year)	Centrifugation system used	Centrifugation condition(s)	Subcultured cells used in laboratory assays	Passage number	Tissue culture medium used for expansion	Method of cellular detachment (enzymatic or mechanical)
PL						
Murphy *et al.*, 2012	Not stated	Not stated	Yes	Passage 3-6 (cryopreserved)	α-MEM + 10 % FBS with 1 % antibiotics for 24 h; followed by serum-free media ± PRP	Not stated
Lange *et al.*, 2012	Laboratory density-centrifugation	Percoll-gradient density (density 1.068 g/mL) gradient centrifugation	Yes	Passage 2 to 3	LG-DMEM + 1 % penicillin/streptomycin + 10 % pre-selected FBS	Enzymatic by trypsinisation
Gottipamula *et al.*, 2012	Not clearly stated	Not clearly stated	Proliferation: yes Other assays: not clearly specified	Proliferation: passages 1 to 5 Other assays: not clearly specified	LG- DMEM or DMEM-KO + 2 mmol/L Glutamax and penicillin/streptomycin	Enzymatic by trypsinisation
Ben Azouna *et al.*, 2012 ‡	Laboratory density-centrifugation	Ficoll Hypaque solution	Proliferation: no Other assays: yes	Proliferation: not applicable Other assays: passage 2	αMEM + 100 U/mL penicillin + 0.1 mg/mL streptomycin + 2 mmol/L L-glutamine + 0.025 mg/mL amphotericin B	Enzymatic by trypsinisation [0.05 % (vol/vol) trypsin/1 mmol/L EDTA solution]
Jenhani *et al.*, 2011 ‡	Laboratory density-centrifugation	Ficoll Hypaque solution at 800 ×*g* for 20 min at room temperature	Proliferation: no Other assays: yes	Proliferation: not applicable Other assays: passage 2	αMEM + 100 U/mL penicillin + 0.1 mg/mL streptomycin + 2 µm L-glutamine + 0.025 mg/mL fungizone	Enzymatic by trypsinisation (trypsin/EDTA solution)
Goedecke *et al.*, 2011	Laboratory density-centrifugation	Biocoll solution density gradient centrifugation: 900 ×*g* for 30 min at room temperature	Yes	Passage 1	LG-DMEM + 10 %FBS or PRP for 48 h	Enzymatic by TrypZean (recombinant enzyme derived from corn)
Verrier *et al.*, 2010	Laboratory density-centrifugation	Ficoll (Histopaque-1077), centrifuged at 800 ×*g* for 20 min, room temperature	Yes	Passage 2-4 used	IMDM containing 10 % FBS + nonessential amino-acids + penicillin/streptomycin (100 U/mL); followed by addition of fresh medium containing 5 ng/mL basic-FGF at day 5	Enzymatic by trypsinisation (0.05 % trypsin/EDTA)
Prins *et al.*, 2009	Laboratory density-centrifugation	Ficoll 1.077 g/cm^3	Yes	Proliferation: until passage 3 Differentiation: not stated	αMEM + 100 U/mL penicillin + 100 µg/mL streptomycin	Enzymatic by trypsinisation
Vogel *et al.*, 2006	Laboratory density-centrifugation	Density gradient centrifugation: Ficoll-Paque™ Plus (Amersham Biosciences)	Yes	Passage 4	60 % LG-DMEM, 40 % MCDB-201 + ITS + linoleic acid bovine serum albumin + 10^{-9} mol/L dexamethasone + 10^{-4} mol/L ascorbic acid 2-phosphate + 100 U penicillin + 1,000 U streptomycin + 2 % FBS	Not stated
PR						
Nguyen *et al.*, 2019	Not applicable; commercially purchased BM-MSCs used	Not applicable; commercially purchased BM-MSCs used	Yes	Passage 4	DMEM: nutrient mixture F-12 + 10 % FBS + 100 µg/mL streptomycin + 100 IU/mL penicillin	Enzymatic by trypsinisation (0.25 %trypsin/EDTA)
Liou *et al.*, 2018	Yes, but type not specified	Not stated	Yes	Not stated	α-MEM + 1 % v/v antibiotic-antimycotic	Not stated

Table 5c. Preparation and processing of BM-MSCs. ‡: same study cohort and methodology.

Author (year)	Centrifugation system used	Centrifugation condition(s)	Subcultured cells used in laboratory assays	Passage number	Tissue culture medium used for expansion	Method of cellular detachment (enzymatic or mechanical)
PR						
Kosmacheva *et al.*, 2014	Laboratory density-centrifugation	Ficoll-verograffin (1.077 g/cm^3), centrifuged at 450 ×*g* for 25 min	Yes	Osteogenesis: passage 2	α-MEM + 10 % FBS + penicillin (100 U/mL) + streptomycin (100 µg/mL) + ultraglutamine (2 mmol/L)	Enzymatic by trypsinisation (0.25 % trypsin/EDTA)
Amable *et al.*, 2014	Laboratory density-centrifugation and magnetic-activated cell sorting	Ficoll-Paque PLUS density gradient centrifugation: 700 ×*g* for 15 min	Yes	Not clearly specified	α-MEM + 10 % FBS + 10 µg/mL ciprofloxacin	Not stated
Agis *et al.*, 2009	Laboratory density-centrifugation	Not stated	Yes	Not more than 10 passages	α-MEM + 10 % FBS + penicillin (100 U/mL) + streptomycin (100 µg/mL)	Enzymatic by trypsinisation
Gruber *et al.*, 2004	Laboratory density-centrifugation	Cells were resuspended in 5 mL of α-MEM, centrifuged at 135 ×*g* for 10 min	Yes	Between passages 2 and 5	α-MEM, 2 mmol/L L-glutamine + 100 U/mL penicillin + 100 µg/mL streptomycin sulphate + 20 % FBS ± 10^{-8} mmol/L dexamethasone + 10^{-4} mmol/L L-ascorbic acid phosphate magnesium salt n-hydrate when indicated	Not stated
Lucarelli *et al.*, 2003	Laboratory density-centrifugation	Not clearly stated	Yes	Proliferation assay: not stated Differentiation assay: passage 6	α-MEM + 20 % FBS + 100 units/mL penicillin + 100 mg/mL streptomycin + 2 mmol/L glutamine	Enzymatic by trypsinisation
PRC						
Samuel *et al.*, 2016	Laboratory density-centrifugation	Ficoll-Paque Premium of density 1.073 g/mL, centrifuged at 360 ×*g* for 25 min	Yes	Passage 2	L-DMEM supplemented with 10 % FBS + 1 % penicillin/streptomycin (100 U/mL) + 1 % Glutamax-1	TrypLE™ Express
Parsons *et al.*, 2008	Not applicable; commercially purchased BM-MSCs used	Not applicable; commercially purchased BM-MSCs used	Yes	Passage 2	Not clearly specified 'recommended media'	Not stated
PG-CM						
Schar *et al.*, 2015	Not applicable; BM-MSCs purchased from cell bank	Not applicable; BM-MSC purchased from cell bank	Yes	Not stated	DMEM/F-12 + 10 % FBS + 100 U/mL penicillin + 100 mg/mL streptomycin	Enzymatic by trypsinisation
Perut *et al.*, 2013	Laboratory density-centrifugation	Histopaque® (Sigma) Density gradient centrifugation: 600 ×*g* for 30 min	No; fresh mononuclear cells rich in BM-MSCs used	Not applicable	α-MEM + 100 U/mL penicillin + 0.1 mg/mL streptomycin + 100 mmol/L ascorbic acid-2 phosphate + 10 % FBS	Not applicable
PRF						
Moradian *et al.*, 2017	Laboratory density-centrifugation	Ficoll (lymphoprep), centrifuged at 338 ×*g* for 15 min	Yes	Passage 3	LG-DMEM (1,000 mg/L) + 10 % FBS	Enzymatic by trypsinisation (0.25 % trypsin)
Lucarelli *et al.*, 2010	Laboratory density-centrifugation	Not clearly stated	Yes	Passage 3	α-MEM + 20 % FBS	Enzymatic by trypsinisation
Dohan Ehrenfest *et al.*, 2010	Not clearly stated	Not clearly stated	Yes	Passage 3	HG-DMEM + 1 % antibiotics (penicillin-streptomycin) + glutamine (200 mmol/L) + 10 % FBS	Enzymatic by trypsinisation

Table 6a. Incubation period of PPs with BM-MSCs.

Author (year)	Sample size (*n*)	PP and BM-MSCs from same donor	Pooling of platelet product	Incubation period with platelet product
PRP-CM				
Yin *et al.*, 2016	PRP (*n* = 10) BM-MSCs (*n* = 14)	No	Not stated	Cell viability/apoptosis: 7 d Migration: 24 h Differentiation: 14 d Gene/cytokine/growth factor expression: 14 d
PL				
Karadjian *et al.*, 2020	PL (*n* = not stated) BM-MSCs (*n* = 10)	No	Yes	Proliferation: 7 d Osteogenesis: 21 d
Moisley *et al.*, 2019	PL (*n* = 11) BM-MSCs (*n* = 11)	No	Yes	Proliferation: 5 d Differentiation: 21 d Migration: 24 h
Skific *et al.*, 2018	PL (*n* = not stated) BM-MSCs (*n* = 13)	No	Yes	Proliferation: CFU-F 14 d; cell counting for PD: not stated Osteogenesis: 14 d Adipogenesis: 21 d
Infante *et al.*, 2017	PL (*n* = 20) BM-MSCs (*n* = 3)	No	Yes	Proliferation: up to 96 h Migration: 24 h
Muraglia *et al.*, 2015	PL and BM-MSCs (*n* = not stated)	No	Yes	Proliferation (MTT and clonogenic assays): 10-14 d
Muraglia *et al.*, 2014	PL and BM-MSCs (*n* = not stated)	No	Yes	14 d in PRP-CM
Jonsdottir-Buch *et al.*, 2013	PL (*n* = not stated) BM-MSCs (*n* = 3)	No	Yes	Proliferation: not stated Osteogenesis: 7 and 14 d Chondrogenesis: 28 d Adipogenesis: 14 d Immunomodulation: 48 h
Bernardi *et al.*, 2013	PL (*n* = 28) BM-MSCs (*n* = 4)	No	Yes	Proliferation: throughout 7 passages Differentiation: 28 d
Murphy *et al.*, 2012	PL and BM-MSCs (*n* = not stated)	No	Not stated	Proliferation: 4 and 7 d Migration: 18 h
Lange *et al.*, 2012	PL (*n* = not stated) BM-MSCs (*n* = 5)	No	Yes	Adipogenesis: 14 d Gene expression: 14 d Protein expression: 14 d
Gottipamula *et al.*, 2012	PL (*n* = 30) BM-MSCs (*n* = 4)	No	Yes	Proliferation: 14 d Osteogenesis/adipogenesis/chondrogenesis: 21 d Immunomodulation: 72 h
Ben Azouna *et al.*, 2012	PL (*n* ≥ 10) BM-MSCs (*n* = 13)	No	Yes	Proliferation: 10 d Osteogenesis: 14 d Adipogenesis: 14 d Chondrogenesis: 21 d Gene expression: day 0, 14 or 21, dependant on type of differentiation Protein expression: day 14 Cytokine expression: day 3 or 4
Jenhani *et al.*, 2011	PL (*n* ≥ 10) BM-MSCs (*n* = 13)	No	Yes	Proliferation: 10 d Cytokine expression: day 3 or 4

Table 6b. Incubation period of PPs with BM-MSCs.

Author (year)	Sample size (*n*)	PP and BM-MSCs from same donor	Pooling of platelet product	Incubation period with platelet product
PL				
Goedecke *et al.*, 2011	PL ($n = 2$) BM-MSCs ($n = 5$)	No	Yes	Differentiation: 14 d Proliferation: 14 d Migration: 20 h Chemotaxis: until culture confluence
Verrier *et al.*, 2010	PL and BM-MSCs ($n = 16$)	No	No	Osteogenesis: 14 d (von Kossa staining), 18 d (ALP activity), 21 d (45Ca2+ incorporation) BMP2 expression: 23 d
Prins *et al.*, 2009	PL ($n = 5$) BM-MSCs ($n = 9$)	No	Yes	Proliferation: 10 d Osteogenesis: 10 d Adipogenesis: 21 d Chondrogenesis: 24 d
Vogel *et al.*, 2006	PL ($n = 5$) M-MSCs ($n = 6$)	No	Yes	Not stated
PR				
Nguyen *et al.*, 2019	PR and BM-MSCs (n = not stated)	No	Not stated	Proliferation: 9 d Migration: 0, 24 and 48 h
Liou *et al.*, 2018	PR ($n = 3$) BM-MSCs ($n = 3$)	No	Yes	Proliferation: 14 d Chondrogenesis: 21 d Gene expression: 21 d
Bernardi *et al.*, 2017 Assessed PR and PL	PR ($n = 15$) PL ($n = 15$) BM-MSCs ($n = 3$)	No	Yes	Proliferation: 14 d Osteogenesis: 21 d Adipogenesis: 21 d Chondrogenesis: 28 d Immunomodulation: not stated
Kosmacheva *et al.*, 2014	PR and BM-MSCs (n = not stated)	No	Yes	Osteogenesis: 21 d Gene expression: 21 d
Amable *et al.*, 2014	PR ($n = 18$) BM-MSCs ($n = 4$)	No	Yes	Proliferation: 6 d Differentiation: 14 -21 d
Agis *et al.*, 2009	PR and BM-MSCs ($n = 6$)	No	Not stated	Fibrin dissolution assay: 25.5 h Casein zymography: 24 h qPCR-uPA, PAI-1 and UPAr levels: not stated Western blot analysis: 24 h
Gruber *et al.*, 2004	PR ($n = 4$) BM-MSCs ($n = 4$)	No	Not stated	Proliferation: 24 h Migration: 3 h Osteogenesis: 5 d Gene expression: 5 d
Lucarelli *et al.*, 2003	PR ($n = 10$) BM-MSCs ($n = 10$)	No	Not stated	Proliferation: 6 d Differentiation: 3, 6 and 9 d

Table 6c. Incubation period of PPs with BM-MSCs.

Author (year)	Sample size (*n*)	PP and BM-MSCs from same donor	Pooling of platelet product	Incubation period with platelet product
PRC				
Samuel *et al.*, 2016	PRC (*n* = 6) BM-MSCs (*n* = not stated)	No	Yes	Proliferation: day 0, 8, 16 and 24 Osteogenesis: day 8, 16, and 24 Gene expression: day 0, 8, 16 and 24
Parsons *et al.*, 2008	PRC (*n* = 11) BM-MSCs (*n* = 4)	No	Yes	Proliferation: 0 h, d 1, 2 and 3 Gene expression: 0, 6, 12, 24, and 48 h *In vitro* mineralisation: 23 d
PG-CM				
Schar *et al.*, 2015	PRP/PRF/blood clot (*n* = 11) BM-MSCs (*n* = not stated)	No	Not stated	Migration: 4 h Supernatants of all 3 platelet products (L-PRP gel, L-PRF, blood clot) harvested at different time points (8 h, 1, 3, 7, 14, and 28 d) were used for incubation with BM-MSCs in migration chambers for 4 h
Perut *et al.*, 2013	PRP (*n* = 10) BM-MSCs (*n* = 3)	No	Not stated	Proliferation: 6 d Differentiation: 14 d
PRF				
Moradian *et al.*, 2017	Not stated	No	Not stated	Proliferation: 12 d
Lucarelli *et al.*, 2010	PRF-CM (*n* = 3) BM-MSCs (*n* = 3)	No	Yes	Proliferation: 24, 48 and 72 h
Dohan Ehrenfest *et al.*, 2010	PRF-CM and BM-MSCs (*n* = 1)	Yes	Not applicable (single donor)	Proliferation: 28 d Osteogenesis: 28 d Note: PRF membranes were removed at from culture plates at day 7; authors used PRF-CM from day 7 onwards

MSCs incubated in PRP-CM (n = 1), PL (n = 6) and PRC (n = 2).

PP was found to upregulate the expression of BM-MSC gene markers for pluripotency and proliferation (*POU5F1*, *SOX2*) (Amable *et al.*, 2014); immunomodulation/inflammation (*COX-2*, *iNOS*) (Yin *et al.*, 2016) and matrix metalloproteinases (*MMP13*) (Verrier *et al.*, 2010).

The effect of PP on expression of osteogenic gene markers was variable, dependent upon the gene marker measured and choice of PP used. Osteogenic genes such as *BMP2* (Amable *et al.*, 2014; Samuel *et al.*, 2016; Verrier *et al.*, 2010), *osteocalcin* (Gottipamula *et al.*, 2012; Yin *et al.*, 2016), *osteopontin* (Jonsdottir-Buch *et al.*, 2013; Kosmacheva *et al.*, 2014; Samuel *et al.*, 2016), *bone sialoprotein 2* (Samuel *et al.*, 2016; Verrier *et al.*, 2010), *osterix* (Gottipamula *et al.*, 2012) and *COL1* (Samuel *et al.*, 2016) were upregulated in most studies. On the other hand, PP exposure resulted in equivocal expression of *ALP* (Jonsdottir-Buch *et al.*, 2013; Parsons *et al.*, 2008; Samuel *et al.*, 2016; Verrier *et al.*, 2010) and *RUNX2* (Amable *et al.*, 2014; Gruber *et al.*, 2004; Jonsdottir-Buch *et al.*, 2013; Parsons *et al.*, 2008; Samuel *et al.*, 2016; Yin *et al.*, 2016) as well as downregulation of *SPARC* (Amable *et al.*, 2014) and *osteonectin* (Samuel *et al.*, 2016).

Effects of PP on expression of adipogenic gene markers of BM-MSCs were variable: *ADIPOQ* (Amable *et al.*, 2014), *CEPBA* (Amable *et al.*, 2014) and *PPARG* (Amable *et al.*, 2014) were upregulated; whilst *L-PGDS* (Lange *et al.*, 2012), *LPL* (Ben Azouna *et al.*, 2012), *FABP4* (Lange *et al.*, 2012), *PLIN* (Lange *et al.*, 2012), *GLUT4* (Lange *et al.*, 2012) and *APOE* (Lange *et al.*, 2012) were downregulated when compared to controls. Similarly, expression of chondrogenic gene markers were variable: *COMP* (Infante *et al.*, 2017) and *COL1A1* (Verrier *et al.*, 2010) were upregulated; *COL2A1* downregulated (Infante *et al.*, 2017); *ACAN* (Amable *et al.*, 2014; Infante *et al.*, 2017; Liou *et al.*, 2018) and *SOX9* (Amable *et al.*, 2014; Infante *et al.*, 2017) equivocal. No effect on *COL2* was observed (Liou *et al.*, 2018).

Discussion

PPs and BM-MSCs are increasingly used in the current treatment of impaired bone healing. However, the clinical effectiveness of their combined use is difficult to conclude, due to differing clinical results and the huge heterogeneity in terms of harvesting, preparation techniques, contents and type of PPs used in these studies. Pre-clinical studies using animal models have their limitations, since animal cells do not always resemble human results. Systematically assessing the results of *in vitro* studies in humans should provide the best collective evidence which can guide clinical practice. This review represents the first systematic review in the literature that specifically assessed the effects of PPs on the biophysiological functions of BM-MSCs in human.

This systematic review has identified 33 *in vitro* studies in humans that compared the combined use of PP and BM-MSCs to BM-MSCs alone. It was evident that there is a lack of consistent terminology amongst these studies. For instance, several studies used the term PRP, which upon closer look at their methodology, were in fact using frozen-thawed PRP (Goedecke *et al.*, 2011; Infante *et al.*, 2017; Muraglia *et al.*, 2014; Murphy *et al.*, 2012; Vogel *et al.*, 2006). The freeze-thawing process of the PRP in these studies would have resulted in lysis of platelets. Thus, PL would have been a more appropriate term to accurately reflect the acellularity and contents of the PP used in these studies. Therefore, the present study attempted to clearly categorise these studies into PRP-CM, PL, PR, PRC, PG-CM and PRF to reflect (i) the specific type of PP used, (ii) its consistency (*i.e.* liquid, solid) and (iii) its contents (*e.g.* cellularity, lysed platelets, leukocyte). Hopefully, this would aid comparison between the important findings of these *in vitro* studies with future laboratory work and aid in its transferability into clinical work.

The majority of these studies had a small sample size ($n \leq 6$) (Agis *et al.*, 2009; Amable *et al.*, 2014; Bernardi *et al.*, 2017; Bernardi *et al.*, 2013; Dohan Ehrenfest *et al.*, 2010; Goedecke *et al.*, 2011; Gottipamula *et al.*, 2012; Gruber *et al.*, 2004; Infante *et al.*, 2017; Liou *et al.*, 2018; Lucarelli *et al.*, 2010; Parsons *et al.*, 2008; Perut *et al.*, 2013; Prins *et al.*, 2009; Vogel *et al.*, 2006). Key aspects such as the health and demographics of patients/volunteers, harvest volume of BM-MSCs and PPs as well as the leukocyte and platelet concentration in PP were not reported by most studies, which would have otherwise substantiated the correct interpretation of the results reported. Harvested samples were processed using different centrifugation systems, mostly being laboratory grade (Agis *et al.*, 2009; Amable *et al.*, 2014; Ben Azouna *et al.*, 2012; Bernardi *et al.*, 2017; Dohan Ehrenfest *et al.*, 2010; Goedecke *et al.*, 2011; Gottipamula *et al.*, 2012; Gruber *et al.*, 2004; Infante *et al.*, 2017; Jenhani *et al.*, 2011; Jonsdottir-Buch *et al.*, 2013; Kosmacheva *et al.*, 2014; Lange *et al.*, 2012; Liou *et al.*, 2018; Muraglia *et al.*, 2014; Muraglia *et al.*, 2015; Murphy *et al.*, 2012; Perut *et al.*, 2013; Prins *et al.*, 2009; Samuel *et al.*, 2016; Schar *et al.*, 2015; Skific *et al.*, 2018; Verrier *et al.*, 2010; Vogel *et al.*, 2006). Comparisons between PL produced using a laboratory and clinical grade centrifugation system on proliferation and migration of BM-MSCs have been attempted (Moisley *et al.*, 2019), but not yet on the other categories of PPs. Furthermore, the practice of pooling harvested PPs was common practice within the majority of *in vitro* studies analysed (Amable *et al.*, 2014; Ben Azouna *et al.*, 2012; Bernardi *et al.*, 2017; Bernardi *et al.*, 2013; Goedecke *et al.*, 2011; Gottipamula *et al.*, 2012; Infante *et al.*, 2017; Jenhani *et al.*, 2011; Jonsdottir-Buch *et al.*, 2013; Karadjian *et al.*, 2020; Kosmacheva *et al.*, 2014; Lange *et al.*, 2012; Liou *et al.*, 2018; Lucarelli *et al.*, 2010; Moisley *et al.*, 2019; Muraglia *et al.*, 2014; Muraglia *et al.*, 2015; Parsons *et al.*, 2008; Prins *et al.*, 2009; Samuel

et al., 2016; Skific *et al.*, 2018; Vogel *et al.*, 2006). This is not reflective of clinical practice, where patients are commonly treated with autologous PP and BM-MSCs (*i.e.* donor-matched) processed using a clinical-grade automated centrifugation system (Lee *et al.*, 2014; Martin *et al.*, 2013).

Being a biological reservoir containing a panel of potent biological factors, it is unsurprising that most *in vitro* studies have been focused upon harnessing these platelet secretomes (*e.g.* cytokines, growth factors, chemokines) either through physical lysis (*e.g.* freeze-thawing) or through exogenous chemical activation (*e.g.* $CaCl_2$, thrombin). Noteworthy, these methods in essence lead to the immediate secretion of the potent platelet secretomes upon delivery (Marx, 2001; 2004). Therefore, it is unsurprising that most *in vitro* studies utilised PL (Agis *et al.*, 2009; Ben Azouna *et al.*, 2012; Bernardi *et al.*, 2017; Bernardi *et al.*, 2013; Goedecke *et al.*, 2011; Gottipamula *et al.*, 2012; Gruber *et al.*, 2004; Infante *et al.*, 2017; Jenhani *et al.*, 2011; Jonsdottir-Buch *et al.*, 2013; Kosmacheva *et al.*, 2014; Lange *et al.*, 2012; Moisley *et al.*, 2019; Muraglia *et al.*, 2014; Muraglia *et al.*, 2015; Murphy *et al.*, 2012; Prins *et al.*, 2009; Skific *et al.*, 2018; Vogel *et al.*, 2006), PR (Agis *et al.*, 2009; Amable *et al.*, 2014; Bernardi *et al.*, 2017; Gruber *et al.*, 2004; Kosmacheva *et al.*, 2014; Liou *et al.*, 2018; Lucarelli *et al.*, 2003; Nguyen *et al.*, 2019) and PG-CM (Perut *et al.*, 2013; Schar *et al.*, 2015), all of which were induced to release platelet secretomes through its manufacturing process. Notably, a significantly larger number of studies used PL, traditionally regarded as a stable, xeno-free product, with less homogeneity when compared to FBS and vastly used in the mass *ex vivo* expansion of BM-MSCs for clinical applications (Schallmoser *et al.*, 2020). However, a recent study by Scherer *et al.* (2012) comparing activated and non-activated platelets demonstrated better tissue healing and angiogenesis in the non-activated platelet group. The authors suggested that natural activation occurring intrinsically 'on demand' may be more effective than a bolus of exogenously activated platelets (Scherer *et al.*, 2012). As yet, no *in vitro* studies have compared the effects of activated and non-activated PPs on the biophysiological functions of human BM-MSCs, which could guide clinical practice on whether these PPs should be activated *ex vivo* prior to delivery.

Another important aspect to consider is whether leukocyte content in PP would influence the biophysiological behaviour of BM-MSCs. P-PRP-CM was found to result in stronger osteogenic gene expression in BM-MSCs (Yin *et al.*, 2016). However, the effects of leukocyte content on proliferation, differentiation and migration remain unclear, due to the conflicting findings from the small number of studies available (Moisley *et al.*, 2019; Perut *et al.*, 2013; Yin *et al.*, 2016). Furthermore, the effect of leukocyte content on other functions of BM-MSCs, such as cytokine expression and immunomodulation, were not assessed.

Expansion of harvested BM-MSCs and the subsequent use of subcultured (or passaged) BM-MSCs were common in most studies (Agis *et al.*, 2009; Amable *et al.*, 2014; Bernardi *et al.*, 2017; Bernardi *et al.*, 2013; Dohan Ehrenfest *et al.*, 2010; Goedecke *et al.*, 2011; Gottipamula *et al.*, 2012; Gruber *et al.*, 2004; Infante *et al.*, 2017; Jonsdottir-Buch *et al.*, 2013; Kosmacheva *et al.*, 2014; Lange *et al.*, 2012; Liou *et al.*, 2018; Lucarelli *et al.*, 2003; Lucarelli *et al.*, 2010; Moisley *et al.*, 2019; Moradian *et al.*, 2017; Muraglia *et al.*, 2015; Murphy *et al.*, 2012; Nguyen *et al.*, 2019; Parsons *et al.*, 2008; Prins *et al.*, 2009; Samuel *et al.*, 2016; Schar *et al.*, 2015; Skific *et al.*, 2018; Verrier *et al.*, 2010; Vogel *et al.*, 2006; Yin *et al.*, 2016), since larger quantities of BM-MSCs were desired for laboratory assays. However, studies have demonstrated the 'internalisation' of bovine proteins in BM-MSCs expanded in culture medium containing FBS, a commonly used animal serum (Spees *et al.*, 2004). The stemness of passaged BM-MSCs and, therefore, how closely it reflects fresh BM-MSCs used in clinical practice is questionable. Furthermore, *in vitro* passage of BM-MSCs could also result in the loss of homing (Rombouts and Ploemacher, 2003), migration (Bustos *et al.*, 2014; Choumerianou *et al.*, 2010), proliferation (Ganguly *et al.*, 2017) and differentiation (Kim *et al.*, 2012) capabilities of BM-MSCs. The use of fresh or minimally cultivated BM-MSCs would have been more representative of clinical practice.

Incubation time with PP reflects the duration which BM-MSCs were exposed to the inductive molecules contained in PP, such as growth factors and cytokines (Di Matteo *et al.*, 2015; Roffi *et al.*, 2017). BM-MSCs in most of the studies were exposed to a continuous dose of PP through prolonged periods of co-incubation. Differently, in clinical practice, autologous PPs and BM-MSCs are commonly harvested on the day of surgery and incubated for a short duration prior to implantation (Lee *et al.*, 2014; Martin *et al.*, 2013). *In vitro* studies reflecting the short incubation periods seen in clinical settings would have been more informative, as it will elucidate whether short PP exposure is effective at optimising the potent biophysiological functions of BM-MSCs necessary for advantageous bone repair.

Despite the inherent weaknesses limiting transferability into clinical practice, these *in vitro* studies in humans have nonetheless demonstrated promising findings. Most *in vitro* studies focused on the impact of PP on proliferation and differentiation of BM-MSCs (Amable *et al.*, 2014; Ben Azouna *et al.*, 2012; Bernardi *et al.*, 2017; Bernardi *et al.*, 2013; Dohan Ehrenfest *et al.*, 2010; Goedecke *et al.*, 2011; Gottipamula *et al.*, 2012; Gruber *et al.*, 2004; Infante *et al.*, 2017; Jenhani *et al.*, 2011; Jonsdottir-Buch *et al.*, 2013; Karadjian *et al.*, 2020; Kosmacheva *et al.*, 2014; Lange *et al.*, 2012; Liou *et al.*, 2018; Lucarelli *et al.*, 2003; Lucarelli *et al.*, 2010; Moisley *et al.*, 2019; Moradian *et al.*, 2017; Muraglia *et al.*, 2014; Muraglia *et al.*, 2015; Murphy *et al.*, 2012; Nguyen *et al.*, 2019; Parsons *et al.*,

2008; Perut *et al.*, 2013; Prins *et al.*, 2009; Samuel *et al.*, 2016; Skific *et al.*, 2018; Verrier *et al.*, 2010; Vogel *et al.*, 2006; Yin *et al.*, 2016). The huge focus thrown upon these two biophysiological functions were perhaps unsurprising, given that the successful proliferation of BM-MSCs and its subsequent differentiation into the desired mesenchymal lineage are key determinants for successful bone repair. Overall, PP has been shown to increase cellular proliferation (Amable *et al.*, 2014; Ben Azouna *et al.*, 2012; Bernardi *et al.*, 2013; Dohan Ehrenfest *et al.*, 2010; Goedecke *et al.*, 2011; Gottipamula *et al.*, 2012; Gruber *et al.*, 2004; Infante *et al.*, 2017; Jenhani *et al.*, 2011; Jonsdottir-Buch *et al.*, 2013; Karadjian *et al.*, 2020; Lucarelli *et al.*, 2003; Moradian *et al.*, 2017; Muraglia *et al.*, 2014; Muraglia *et al.*, 2015; Murphy *et al.*, 2012; Nguyen *et al.*, 2019; Perut *et al.*, 2013; Prins *et al.*, 2009; Samuel *et al.*, 2016; Skific *et al.*, 2018; Yin *et al.*, 2016) and osteogenic differentiation of BM-MSCs in the majority of these studies (Amable *et al.*, 2014; Dohan Ehrenfest *et al.*, 2010; Gottipamula *et al.*, 2012; Karadjian *et al.*, 2020; Kosmacheva *et al.*, 2014; Lucarelli *et al.*, 2003; Parsons *et al.*, 2008; Perut *et al.*, 2013; Samuel *et al.*, 2016; Skific *et al.*, 2018; Verrier *et al.*, 2010; Yin *et al.*, 2016). The use of passaged BM-MSCs, known to affect differentiation potential (Kim *et al.*, 2012), could explain these mixed findings in the remaining studies [no difference ($n = 3$) (Ben Azouna *et al.*, 2012; Goedecke *et al.*, 2011; Prins *et al.*, 2009); reduced ($n = 1$) (Vogel *et al.*, 2006); equivalent ($n = 2$) (Gruber *et al.*, 2004; Jonsdottir-Buch *et al.*, 2013)], therefore, warranting further research using fresh or minimally cultured BM-MSCs. On the other hand, chondrogenic and adipogenic differentiation were not improved by PPs in most studies (Amable *et al.*, 2014; Ben Azouna *et al.*, 2012; Goedecke *et al.*, 2011; Gottipamula *et al.*, 2012; Lange *et al.*, 2012; Liou *et al.*, 2018; Prins *et al.*, 2009; Vogel *et al.*, 2006). Increasing PP concentrations up to 10 % have also been demonstrated to improve proliferation (Amable *et al.*, 2014; Bernardi *et al.*, 2017; Bernardi *et al.*, 2013; Goedecke *et al.*, 2011; Gottipamula *et al.*, 2012; Jenhani *et al.*, 2011; Karadjian *et al.*, 2020; Lucarelli *et al.*, 2003; Muraglia *et al.*, 2014; Muraglia *et al.*, 2015; Murphy *et al.*, 2012; Nguyen *et al.*, 2019; Perut *et al.*, 2013; Prins *et al.*, 2009; Skific *et al.*, 2018; Yin *et al.*, 2016). However, no studies have yet investigated the correlation between PP concentration and the differentiation potential of BM-MSCs.

The ability to migrate is a potent biophysiological function possessed by BM-MSCs, allowing it to be signalled and recruited to areas requiring more osteogenic cells (Su *et al.*, 2018). Hence, being able to influence its migratory capacity is crucial, thereby improving the chances of successful bone repair. PP represents such a promising chemotactic stimulus since it possesses multiple osteoinductive molecules (Di Matteo *et al.*, 2015; Roffi *et al.*, 2017; Schallmoser *et al.*, 2020). This was further confirmed by the improved migratory capacity in BM-MSCs exposed to PP (Goedecke *et al.*, 2011; Gruber *et al.*, 2004; Infante *et al.*, 2017; Moisley *et al.*, 2019; Murphy *et al.*, 2012; Nguyen *et al.*, 2019; Schar *et al.*, 2015; Yin *et al.*, 2016). The effects of PP concentration were compared up to 10 % only, whereby increasing PP concentrations enhances the migratory capacity of BM-MSCs (Goedecke *et al.*, 2011; Gruber *et al.*, 2004; Murphy *et al.*, 2012; Nguyen *et al.*, 2019).

Comminuted, segmental and open fractures represent injuries with a higher risk of impaired bone healing. This is because the zone of injury in these high-risk fractures are subjected to immense amounts of inflammatory, oxidative and apoptotic stressors (Wang *et al.*, 2017). Despite the paucity of research on immunomodulation and response towards apoptotic stress, findings from Yin *et al.* (2016) were encouraging. Yin *et al.* (2016) demonstrated PRP-CM to (i) have a protective effect and preserved the viability of BM-MSCs in the face of apoptotic stress and (ii) improve immunomodulatory functions in BM-MSCs. Despite the use of subcultured BM-MSCs and PPs that were not donor-matched, it has nonetheless uncovered important biophysiological functions of BM-MSCs primed by PPs relevant to improving bone repair in high-risk fractures. Therefore, further research using fresh donor-matched BM-MSCs and PP is warranted given it bears closer resemblance to clinical practice.

An important question to ask when applying these biologics during surgery is whether the priming effect of PPs on BM-MSCs observed in *in vitro* studies would be lost following surgery, since it is subjected to the homeostatic and dilutional effects of circulation once inside the human body. Albeit not assessed by many studies, results from Lucarelli *et al.* (2003) were encouraging, where BM-MSCs were demonstrated to retain their improved osteogenic and chondrogenic capability despite PP withdrawal. This suggested that the priming effect of PPs is perhaps not transient and limited to the incubation period only, as could be expected.

In the attempt to uncover the underlying cellular and molecular mechanisms explaining the effects of PP on the bio-physiology of BM-MSCs, several studies have investigated the release of cytokines, growth factors and proteins. Following PP exposure, BM-MSCs were found to upregulate their release of (i) pro-inflammatory cytokines (IL-2R, IL-6, IL-7, IL-8, IL-12, IL-15, PGE2, NO) (Amable *et al.*, 2014; Ben Azouna *et al.*, 2012; Jenhani *et al.*, 2011; Yin *et al.*, 2016); (ii) chemokines (eotaxin, IP-10, MIP-1β, MCP-1, RANTES, SDF-1α) (Amable *et al.*, 2014; Ben Azouna *et al.*, 2012; Goedecke *et al.*, 2011; Jenhani *et al.*, 2011); (iii) pro-angiogenic factors (thrombospondin, PLGF, aFGF, VEGF-D) (Amable *et al.*, 2014) and (iv) osteogenic proteins (osteocalcin, RUNX2 and BMP2) (Samuel *et al.*, 2016; Verrier *et al.*, 2010; Yin *et al.*, 2016). All of these are important for successful bone repair, since higher levels of pro-inflammatory cytokines advantageous for mediating the early inflammatory phase of fracture healing, whilst inducing an angiogenic and chemotactic state in BM-MSCs, lead to improved vascularity and recruitment of non-resident BM-MSCs, cytokines and growth factors to

Table 7a. Seeding density of BM-MSCs and choice of culture medium used for assays. *used as basal medium before addition of PP/other.

Author (year)	Seeding density of BM-MSCs	Tissue culture medium (base)*
PRP-CM		
Yin *et al.*, 2016	• Proliferation assay: 4,000 cells/well (96-well plate) • Cell viability and apoptosis assay: 1 × 10^5cells/well (6-well plate) • Migration assay: 2.1 × 10^4 cells • Differentiation and gene expression: not stated	α-MEM containing 10 % FBS and 1 % antibiotics (penicillin G and streptomycin)
PL		
Karadjian *et al.*, 2020	• Osteogenesis: 35,000 cells/well (24-well plate)	Proliferation: 25 mmol/L HG-DMEM + 100 mg/L penicillin/streptomycin, 200 mmol/L L-glutamine, MEM non-essential amino acids solution, 50 mmol/L 2-bercaptoethanol, 4 μg/L FGF2 Osteogenesis: 25 mmol/L HG-DMEM + L-glutamine, 100mg/mL penicillin/streptomycin, 0.1 μmol/L dexamethasone, 2.5 mg/L ascorbic acid-2-phosphate, 10 mmol/L β-glycerophosphate
Moisley *et al.*, 2019	• Proliferation assay: 500 cells/well (96-well plate) • Differentiation: not stated • Migration: not stated	Proliferation: MSC expansion medium for 24 h, followed by DMEM Osteogenesis: OsteoDiff Media (Miltenyi Biotec) Adipogenesis: AdipoDiff Media (Miltenyi Biotec) Chondrogenesis: ChondroDiff Media (Miltenyi Biotec)
Skific *et al.*, 2018	• Proliferation: 1 × 10^5 to 6 × 10^5 cells/cm^2 (225 cm^2 tissue culture flasks) • Osteogenesis and adipogenesis: 3,000 cells/cm^2 (6-well plate)	Proliferation: α-MEM + 2 mmol/L L-glutamine, 1% penicillin-streptomycin-neomycin solution Adipogenesis: basal medium + 1 μmol/L dexamethasone, 0.5 mmol/L 3-isobutyl-1-mehylxanthine, 50 μmol/L indomethacin, 5 μg/mL insulin Osteogenesis: basal medium + 0.1 μmol/L dexamethasone, 10 mmol/L glycerol 2-phosphate, 0.2 mmol/L L-ascorbic acid
Infante *et al.*, 2017	• Proliferation: 4,000 cells/cm^2 (96-well plate) • Migration: 12,000 cells/chamber [m-Slide Chemotaxis 3D chamber (Ibidi GmbH, Martinsried, Germany)] • 2D cultures (gene expression): 2 × 10^5 cells/cm^2 (12-well plate)	Proliferation/cells from 2D constructs used for gene expression: DMEM GlutaMAX™ Migration: DMEM GlutaMAX™ + 2 % FBS
Muraglia *et al.*, 2015	• Proliferation (clonogenic assay): 1 × 10^5/dish (60 mmol/L Petri dish) • Proliferation (MTT assay): 5,000 cells/well (24-well plate)	Coon's-modified Ham's F-12 medium + 100 IU/mL penicillin, 100 mg/mL streptomycin, 2 mmol/L L-glutamine
Muraglia *et al.*, 2014 *lyophilised PL	• Proliferation assay: 5,000 cells/well (24-well plate)	Serum-free Coon's-modified Ham's F-12 medium + 100 IU/mL penicillin, 100 μg/mL, streptomycin, 2 mmol/L L-glutamine
Jonsdottir-Buch *et al.*, 2013	• Osteogenic differentiation: 3,000 cells/cm^2 • Chondrogenic differentiation: 250,000 cells/pellet • Adipogenic differentiation: 10,000 cells/cm^2 • Immunomodulation: 25,000 MSC/cm^2 (24-well plates)	Proliferation: DMEM/F12 media + 1 % penicillin/streptomycin, 4 IU/mL heparin Osteogenesis: MSC Osteogenic Differentiation Media (Lonza) Adipogenesis: StemProH Adipogenesis Differentiation medium (Gibco) Chondrogenesis: MSC Chondrocyte Differentiation Medium (Lonza) Immunomodulation: RPMI 1640 medium
Bernardi *et al.*, 2013	• Proliferation: not stated • Osteogenesis and adipogenesis: 1,500 cells/cm^2 (24-well plate) • Chondrogenesis: 2.5 × 10^5 cells (15 mL culture tube)	Proliferation: DMEM Advanced Therapy Medical Product (DMEM ATMP-Ready) Osteogenesis: StemPro osteogenesis differentiation kit Adipogenesis: StemPro adipogenic differentiation kit Chondrogenesis: Chondrogenic differentiation medium
Murphy *et al.*, 2012	• Proliferation assay: • 2,000 BM-MSC/well (24-well plate) • Migration assay: • 50,000 BM-MSC/transwell	Proliferation assay: α-MEM + 1 % antibiotics Migration assay: 250 μL serum-free medium (DMEM)

Table 7b. Seeding density of BM-MSCs and choice of culture medium used for assays. *used as basal medium before addition of PP/other.

Author (year)	Seeding density of BM-MSCs	Tissue culture medium (base)*
PL		
Lange *et al.*, 2012	Not stated	Growth medium: LG-DMEM + 1 % penicillin/streptomycin Adipogenesis: Growth medium + 1 μmol/L dexamethasone, 0.5 mmol/L isobutyl-methylxanthine, 100 μmol/L indomethacin, 10 μmol/L insulin for 2-3 d, followed by 1 d of growth medium + insulin as maintenance medium
Gottipamula *et al.*, 2012	• Proliferation: 100 BM-MSCs on 100 mm/L^2 cell culture dish • Differentiation: not stated • Immunosuppression: 2 × 10^5 cells/well (96-well plate)	Proliferation: LG-DMEM or DMEM-KO + 2 mmol/L, penicillin/streptomycin Osteogenesis: 10 % FBS + 2 mmol/L Glutamax, 10^{-8} mol/L dexamethasone, 30 μg ascorbic acid/mL, 10 mmol/L b-glycerophosphate Adipogenesis: 10 % FBS + 2 mmol/L Glutamax, 1 μmol/L dexamethasone, 0.5 mmol/L isobutylmethylxanthine, 1 μg insulin/mL, 100 μmol/L indomethacin Chondrogenesis: Stempro (Invitrogen) chondrogenesis differentiation medium Immunosuppression: RPMI (Rose-well Park Memorial Institute) medium ± phytohaemaggultinin
Ben Azouna *et al.*, 2012	• Proliferation: 1 × 10^5 cells (T25 flasks) • Osteogenesis and adipogenesis: 1 × 10^3 cells/cm^2 • Chondrogenesis: 3 × 10^5 cells	Proliferation: αMEM + 100 U/mL penicillin, 0.1 mg/mL streptomycin, 2 mmol/L L-glutamine, 0.025 mg/mL amphotericin B Osteogenesis: HG-DMEM (with 4.5 g/L glucose) + 2 % FBS, 0.1 μmol/L dexamethasone + 2 mmol/L b-glycerolphosphate, 100 μmol/L ascorbate-2-phosphate Adipogenesis: LG-DMEM (with 1 g/L glucose) + 20 % FBS, 1 μmol/L dexamethasone, 0.5 mmol/L isobutylmethylxanthine, 60 μmol/L indomethacin Chondrogenesis: HG-DMEM + 0.1 μmol/L dexamethasone, 1 mmol/L sodium pyruvate + 170 μmol/L ascorbic acid-2-phosphate, 350 μmol/L proline, 1× ITS, 10 ng/mL TGF-b1
Jenhani *et al.*, 2011	• Proliferation: 1 × 10^5 cells (T25 flasks) • Passaged for cytokine: 1 × 10^{3}cells/cm^2	Proliferation and expansion media - αMEM + 100 U/mL penicillin + 0.1 mg/mL streptomycin, 2 μm L-glutamine, 0.025 mg/mL fungizone
Goedecke *et al.*, 2011	• Differentiation assay: 5,000 cells/cm^2 (6-well plate) • Proliferation assay: 100 cells/well (6-well plate) • Migration assay: 5 × 10^3 cells/well (24-well plate) • ELISA: 5,000 cells/cm^2 (12-well plate)	Differentiation: 4 d culture in DMEM followed by 'adipogenic medium or osteogenic medium' (not clearly specified) × 14 d Proliferation: 14 d in medium (not clearly specified) Migration: DMEM for 20 h Chemotaxis: DMEM until culture confluence
Verrier *et al.*, 2010	• 30,000 cells/cm^2 were seeded in 24-well or 12-well plates	IMDM + 10 % FBS, nonessential amino acids, 0.1 mmol/L ascorbic acid-2-phosphate, 10 mmol/L β-glycerophosphate
Prins *et al.*, 2009	• Proliferation: various density: 0.5, 1, and 2 × 10^6 cells/25 cm^2 • Osteogenesis: 4 × 10^4 cells/well (24-well plate) • Adipogenesis: 5 × 10^4 cells/well (24-well plate) • Chondrogenesis: 2.5 × 10^5 cells were pelleted in 15 mL polypropylene tubes	Proliferation: αMEM supplemented with 100 U/mL penicillin + 100 μg/mL streptomycin Osteogenesis: NH Osteodiff human medium Adipogenesis: DMEM + 10 % FBS, 1 % penicillin/streptomycin, 2 mmol/L L-glutamine, 1 μmol/L dexamethasone, 0.5 mmol/L isobutyl methyl xanthine, 0.2 mmol/L Indomethacin, 10 ng/mL insulin Chondrogenesis: NH Chondrodiff medium
Vogel *et al.*, 2006	• Chondrogenesis assay: micromasses of 1 × 10^6 cells • Osteogenesis assay: 3.5 × 10^4 cells/well (24-well plate) • Adipogenesis assay: 3.5 × 10^4 cells/well (24-well plate)	Osteogenesis: 500 μL osteogenic induction medium containing HG-DMEM + 10 % FBS, 100 IE/mL penicillin, 100 μg/mL streptomycin, 0.1 μM dexamethasone, 10 mmol/L β-glycerol-phosphate, 0.17 mmol/L ascorbic acid-2-phosphate Chondrogenesis: HG-DMEM + 5 μg/mL insulin + 5 μg/mL transferrin, 5 μg/mL selenous acid, 0.1 μmol/L dexamethasone, 0.17 mmol/L ascorbic acid-2-phosphate, 1 mmol/L sodium pyruvate, 0.35 mmol/L proline, 1.25 mg/mL BSA, 10 ng/mL TGF-β3 Adipogenesis: HG-DMEM + 10 % FBS, 0.01 mg/mL insulin, 1 μmol/L dexamethasone, 0.2 mmol/L indomethacin, 0.5 mmol/L 3-isobutyl-1-methyl xanthine, 100 units/mL penicillin, 100 μg/mL streptomycin

Table 7c. Seeding density of BM-MSCs and choice of culture medium used for assays. *used as basal medium before addition of PP/other.

Author (year)	Seeding density of BM-MSCs	Tissue culture medium (base)*
	PR	
Nguyen *et al.*, 2019	• Cell counting assay (proliferation assay): 1 × 10^4cells/well (96-well plate) • CFU-F assay (proliferation assay): 500 cells/well (6-well plate) • Migration assay: 2 × 10^4 cells/well (6-well plate)	DMEM/F-12 + 10 % FBS, 100 µg/mL streptomycin, 100 IU/ mL penicillin
Liou *et al.*, 2018	• Proliferation: 100 cells/10 cm Petri dish • Chondrogenesis: 2 × 10^5 cells	Proliferation: DMEM + 1 % v/v antibiotic-antimycotic Chondrogenesis: DMEM + 1 % v/v antibiotic-antimycotic, 1× ITS, 0.1 µmol/L dexamethasone, 40 µg/mL proline, 50 µg/mL ascorbic acid, 10 ng/mL recombinant human TGF-β3
Bernardi *et al.*, 2017 *Assessed PR and PL	• Proliferation: 200 cells in 10 mmol/L diameter culture dishes • Osteogenesis: 4,000 cells/cm^2 (24-well plate) • Adipogenesis: 4,000 cells/cm^2 (24-well plate) • Chondrogenesis: 10 × 10^4 cells in 100 µL (24-well plate) • Immunomodulation: not stated	Proliferation: LG-DMEM + penicillin/streptomycin, heparin 30 U/mL Osteogenesis: StemPro® osteogenic differentiation kit Adipogenesis: StemPro® adipogenic differentiation kit Chondrogenesis: StemPro® chondrogenic differentiation kit Immunomodulation: not stated
Kosmacheva *et al.*, 2014	• Osteogenesis: 40,000 cells/mL (8,000 cells/cm^2) in T25 flasks or 40 mmol/L Petri dishes	αMEM + 10 % FBS, 10 mmol/L β-glycerophosphate, 50 µg ascorbic acid, 0.1 mmol/L dexamethasone
Amable *et al.*, 2014	• Proliferation: 6 × 10^3 cells/ mL (24-well plate) • Adipogenic differentiation: 26,000 cells/mL • Osteogenic differentiation: 10,000 cells/mL • Chondrogenic differentiation: pellets containing 10,000 cells	Adipogenesis: LG-DMEM + 1 µmol/L dexamethasone, 0.5 mmol/L 3-isobutyl-1-methylxanthine + 10 mg/mL ciprofloxacin, 10 µmol/L Humulin-N, 0.2 mmol/L indomethacin, penicillin/streptomycin Osteogenesis: LG-DMEM + 10 nmol/L dexamethasone, 10 mmol/L beta-glycerophosphate, 50 µmol/L L-ascorbic acid 2-phosphate, penicillin/streptomycin Chondrogenesis: LG-DMEM + 50 µg/mL L-ascorbic acid 2-phosphate, 10 ng/mL TGF-β3 (Sigma-Aldrich), 0.169 UI/mL human insulin, 6.25 µg/mL human transferrin
Agis *et al.*, 2009	• 5 × 10^5 cells/cm^2	αMEM + 10 % FBS, 100 U/mL penicillin G, 100 µg/mL streptomycin
Gruber *et al.*, 2004	• Proliferation: 5 × 10^4 cells/cm^2 (96-well plate) • Migration: 5 × 10^5cells/mL (48-well Boyden chamber) • Osteogenesis: 5 × 10^4cells/cm^2 (96-well plate) • Gene expression: not stated	Proliferation: αMEM and antibiotics Migration: αMEM and antibiotics Osteogenesis: αMEM + antibiotics, 50 µmol/L ascorbic acid-2 phosphate Gene expression: αMEM and antibiotics
Lucarelli *et al.*, 2003	• Proliferation: 2 × 10^4 cells/well (96-well plate) • Differentiation assay: 2 × 10^4 cells/ cm^2	αMEM containing: 20 % FBS, 100 units/mL penicillin, 100 mg/mL streptomycin, 2 mmol/L glutamine
	PRC	
Samuel *et al.*, 2016	• Proliferation: 1.5 × 10^3 cells/well (24-well plate) • Osteogenesis: 800 cells/cm^2 (24-well plates) • Gene expression: not stated	L-DMEM + 1 % penicillin/streptomycin (100 U/mL), 1 % Glutamax-1
Parsons *et al.*, 2008	• 5000 cells/cm^2 (24-well plate)	Not clearly specified
	PG-CM	
Schar *et al.*, 2015	• 20,000 calcein-AM stained BM-MSCs in lower chamber of Boyden chamber	DMEM/F-12 + 10 % FBS, 100 U/mL penicillin, 100 mg/mL streptomycin
Perut *et al.*, 2013	• Proliferation and differentiation assay: 1 × 10^4 cells/well (24-well plate)	αMEM supplemented with 100 U/mL penicillin, 0.1 mg/mL streptomycin, 100 µmol/L ascorbic acid-2 phosphate
	PRF	
Moradian *et al.*, 2017	• 2 × 10^5 cells/ ml in each group	Osteogenesis and adipogenesis: LG-DMEM + 1 M/L hydrocortisone, 0.05 g/L ascorbic acid, 0.05 g/L indomethacin, 10^{-6} mol/L/L dexamethasone
Lucarelli *et al.*, 2010	• 5 × 10^3 cells/well (96-well plate)	Serum free α-MEM
Dohan Ehrenfest *et al.*, 2010	• Proliferation: 1,000 cells/well (96-well) • Differentiation: 20,000 cells/plate (60 mm Nunc culture plates)	Proliferation: standard medium – HG-DMEM + 1 % antibiotics (penicillin-streptomycin), glutamine (200 mmol/L), 10 % FBS Differentiation: standard medium + Vitamin C (50 µg/mL), B-glycerophosphate (10 mmol/L), dexamethasone (0.1mmol/L)

Table 8a. Effects of PPs on proliferation of BM-MSCs.

Author (year)	Assay used	Groups compared	Findings
		PRP-CM	
Yin *et al.*, 2016	Cell counting on days 1, 3, 5 and 7 using Cell Counting Kit-8 (CCK-8; Dojindo)	• 10 % FBS (control) • 10 % L-PRP-CM • 10 % P-PRP-CM	• PRP promotes proliferation BM-MSCs significantly ($p < 0.05$) • Highest proliferation observed in presence P-PRP *vs.* L-PRP ($p < 0.05$)
		PL	
Karadjian *et al.*, 2020	Cell counting (Neubauer haemocytometer) to count PD rate of 7 d	• 10 % FBS (control) • 10 % PL	• 10 % PL demonstrated superior PD > control ($p < 0.01$)
Moisley *et al.*, 2019	XTT assay (Roche)	• 10 % FBS (control) • 10 % cPL • 10 % PL	• 10 % PL > 10 % FBS > 10 % cPL ($p < 0.001$) • Leukocyte content found not to influence proliferation
Skific *et al.*, 2018	Cell counting to assess PD, cumulative PD and PD time CFU-F assay	• 10 % FBS (control) • 10 % PL • 5 % PL	• Cumulative PD: 10 % PL > 5 % PL > 10 % FBS ($p < 0.05$) • Rate of decline in number of colonies with increased passages is less in PL *vs.* 10 % FBS • Colonies in 5 % and 10 % PL are larger and contain more cells *vs.* 10 % FBS
Infante *et al.*, 2017	XTT colorimetric assay (Cell Proliferation Assay Kit, Roche)	• ITS (control) • PL • PL + 10 ng/mL TGF-β1 + 10^{-7} mol/L dexamethasone + 50 μg/mL ascorbic acid	• Significant increase in proliferation with PL (*vs.* controls, $p < 0.001$) • TGF- β1 has no effect on proliferation and does not modify proliferative potential of PL
Muraglia *et al.*, 2015	MTT assay Cell counting to assess PD	• 10 % FBS (− Ve control) • 10 % FBS + 1 ng/mL FGF-2 (+ Ve control) • PL (0.5 %; 1 %; 2.5 %) • PL/PPP (0.5 %/4.5 %; 1 %/4 %; 2.5 %/2.5 %)	• PL/PPP > controls in terms of (a) total and ALP positive colonies (all concentrations) and (b) middle/large-size colonies • Both proliferation and PD of BM-MSCs improve with increasing PL concentration • 2.5 % PL alone > 10 % FBS in proliferation (0.5 % and 1 % PL has similar proliferative capacity *vs.* 10 % FBS) • PL/PPP combination superior to FBS at all concentrations ($p < 0.05$)
Muraglia *et al.*, 2014	MTT assay CFU-F assay	• 10 % FBS (control) • 5 % PL (from PRP-CM)	• CFU-F assay: colony numbers in 5 % PL (stored at − 80 °C) > 5 % PL (4 °C) > 10 % FBS ($p < 0.05$) • MTT assay: proliferation in PL-CM several times higher than control (p not stated)
Jonsdottir-Buch *et al.*, 2013	PD assay	• 10 % FBS (control) • HPLF • HPLO	• HPLF/HPLO proliferated faster than FBS • Average generation time between PD shorter in HPLF/HPLO > FBS, $p < 0.05$ • No difference between HPLF and HPLO in terms of PD and generation time • Morphology of MSCs in HPL-supplemented medium shows denser cell bodies and more spindle-shaped cells than FBS group
Bernardi *et al.*, 2013	PD assay	• 10 % FBS (control) • 10 % FT PL • SN PL (various concentrations: 10 %, 7.5 %, 5 %, 2.5 %)	• PD time (hours): 10 % SN PL (PD: 65) > 10 % FT PL (PD: 67.6) > 10 % FBS (PD: 137.5) at passage 7 • Cumulative PD of FTPL: perform better than control • Cumulative PD and PD time of SNPL: SN PL of concentrations ≥ 5 % performs better than control
Murphy *et al.*, 2012	Cell counting using Quant-iT PicoGreen quantification kit (Invitrogen)	• 10 % FBS (control) • 20 % FBS (control) • 1 % PL • 10 % PL	• 1 % PL: similar performance compared to 20 % FBS • 10 % PL: more cell proliferation than 1 % PL, 10 % FBS and 20 % FBS (p not stated) • PPP: found to be effective alternative to FBS • (10 % aPPP = 10 % FBS in performance)

Table 8b. Effects of PPs on proliferation of BM-MSCs.

Author (year)	Assay used	Groups compared	Findings
PL			
Gottipamula *et al.*, 2012	CFU assay PD assay	• 10 % FBS (control) • 10 % PL • In 2 different media: LG-DMEM and KO-DMEM	• CFU-F: LG-PL significantly higher > KO-PL, LG-FBS, OR KO-FBS ($p < 0.05$) • PD time: LG-FBS (66.4 h) > LG-PL (36.5 h) • PD: PL > FBS ($p < 0.05$)
Ben Azouna *et al.*, 2012	CFU assay PD assay	• 10 % FBS + 1 ng/mL FGF2 • 10 % FBS + 5 % PL • 10 % PL • 5 % PL	• Colony counts: no difference between groups • Colony morphology: larger, denser colonies with very small spindle-shaped cells in PL-supplemented expansion culture media *vs.* control medium • PD time: cultures containing FBS longer than PL-supplemented medium ($p < 0.0007$)
Jenhani *et al.*, 2011	CFU assay PD assay	• 10 % FBS + 1 ng/mL FGF2 • 10 % FBS + 5 % PL • 10 % PL • 5 % PL • 10 % FBS + 10 % PL • 10 % FBS	• CFU-F assay: no clear difference in colony counts (FBS *vs.* PL) • PD time: PL > FBS alone /FBS + FGF2 ($p < 0.0007$)
Goedecke *et al.*, 2011	CFU assay	• 10 % FBS (control) • 5 % PL • 10 % PL	• Both 5 % and 10 % PL had significantly higher median yield of MSCs as compared to 10 % FBS (10 %PL > 5 % PL > 10 % FBS, $p = 0.03$) • PD: faster BM-MSC proliferation in PL > FBS (median PD of PL: 2-fold of 10 % FBS; 10 % PL > 5 % PL > 10 % FBS) • Median number of colonies not statistically significant between all groups
Prins *et al.*, 2009	CFU assay PD assay	• 10 % FBS (control) • 5 % PL	• Colony numbers: PL > FBS (p not stated) • BM-MSCs cultured in PL-CM: increased yield at each passage • PD by passage 3: PL > FBS ($p = 0.008$) • Time to reach passage 3: PL significantly shorter than FBS ($p = 0.001$)
PR			
Nguyen *et al.*, 2019	Cell-number counting with haemocytometer CFU-F assay	• 10 % FBS in DMEM/F12 medium (+ Ve control) • DMEM/F12 medium alone (− Ve control) • 1 % PR • 2 % PR • 5 % PR	• 1 % PR: similar proliferation compared to + Ve control group • 2 % PR and 5 % PR: prolonged proliferation period of BM-MSCs ($p \leq 0.001$) • Cell counting assay at day 9: cell number larger in 5 % PR > 2 % PR > + Ve control > 1 % PRP group > − Ve control (5 % PR *vs.* all groups: $p < 0.05$; 2 % PR *vs.* 1 % PR: $p < 0.01$; 1 % PR *vs.* − Ve control: $p = 0.007$)
Liou *et al.*, 2018	CFU assay	• TGF-b3 (control) • 10 % PR	• Increased duration of treatment with PR does not affect proliferation of BM-MSCs
Bernardi *et al.*, 2017	• CFU assay • PD assay	• 10 % FBS (control) • 7.5 % PL • 7.5 % PR	• PD assay: PR > FBS > PL ($p < 0.05$) • CFU-F assay: FBS > PR > PL ($p < 0.05$)
Amable *et al.*, 2014	Cell counting (Neubauer haemocytometer) to extrapolate proliferation curve	• 10 % FBS (control) • PR at various concentrations (1 %; 2.5 %; 5 %; 10 %; 20 %; 30 %; 40 % and 50 %)	• 10 % PR sustains highest proliferation rate and shortest doubling time (p not stated) • PR concentrations higher than 10 % inhibits cell growth

Table 8c. Effects of PPs on proliferation of BM-MSCs. .

Author (year)	Assay used	Groups compared	Findings
		PR	
Gruber *et al.*, 2004	Liquid scintillation counting of cells pulse labelled with [3H] thymidine	• Serum-free medium (control) • PR with different platelet concentrations: 2×10^8/mL (PR1) 4×10^7/mL (PR2) 8×10^6/mL (PR3) 1.6×10^6/mL (PR4)	• Highest concentration (PR 1), 10.0 ± 1.8-fold over controls ($p < 0.01$) • Dose-dependent increase (platelets/mL of PR) in proliferation/mitogenic activity
Lucarelli *et al.*, 2003	MTT-assay	3 comparisons: • BM-MSCs + 1 % PR *vs.* BM-MSCs + 10 % PR • BM-MSCs + 20 % FBS *vs.* BM-MSCs + 20 % FBS + 10 % PR • BM-MSCs + medium + 10 % PR *vs.* BM-MSCs + 10 % PR alone	• PR improves cellular proliferation of BM-MSCs; increase in proliferation is dose dependent (10 % > 1 % > control) (p not stated) • Induction of proliferation by PR is maintained throughout the entire period of testing (up to 9 d) • Serum is not necessarily required for PR-mediated induction of BM-MSC proliferation • PD of BM-MSCs in 10 % PR twice that in control (4 PD *vs.* 2 PD in control at day 6) • BM-MSCs return to regular proliferation (2 PD) within 6 d of withdrawing PR
		PRC	
Samuel *et al.*, 2016	AlamarBlue® assay at days 0, 8, 16, 24	• 10 % FBS (control) • 15 % PRC	• PRC significantly increases cell proliferation as compared to control at days 8 and 16 ($p < 0.05$)
Parsons *et al.*, 2008	Pico Green reagent and Fluroskan fluorometer	• 2.5 % serum (control) • 2.5 % PRC	• Earlier onset of proliferation (24 h): PRC > serum ($p < 0.001$) • 48 h: no difference • 72 h: serum > PRC ($p < 0.001$)
		PG-CM	
Perut *et al.*, 2013	Cell counting using Coulter LH 750 Hematology analyzer AlamarBlue test: to assess cell viability and proliferation	• PPP gel (control) • L-PRP gel • fL-PRP gel • P-PRP gel	• L-PRP > fL-PRP > P-PRP > PPP gels efficiency in terms of BM-MSC cellular proliferation (L-PRP *vs.* P-PRP gels, $p = 0.014$; fL-PRP *vs.* P-PRP gels, $p = 0.027$) • bFGF release: faster in both forms of L-PRP gel (fresh/frozen), with higher concentrations than P-PRP gel [L-PRP (both forms) *vs.* P-PRP and PPP gels, $p < 0.05$] • BM-MSC proliferation is significantly correlated with amount of bFGF released
		PRF	
Moradian *et al.*, 2017	MTT assay	• 10 % FBS (control) • PRF	• PRF increases proliferation of BM-MSCs significantly throughout the 12-d incubation period (*vs.* control, $p < 0.05$)
Lucarelli *et al.*, 2010	Methylene blue assay and NucleoCounter	• Serum free α-MEM (− Ve control) • 20 % FBS + α-MEM (+ Ve control) • 5 % PRF-CM • 10 % PRF-CM • 20 % PRF-CM at 24, 48 and 72 h	• PRF-CM (all concentrations) *vs.* serum-free α-MEM: statistically significant different proliferation at all time points • 5 % PRF-CM < 20 % FBS at all time points ($p < 0.01$) • 10 % PRF-CM = 20 % FBS up to 48 h, significantly decreased after 72 h ($p < 0.001$) • 20 % PRF-CM > 20 % FBS significantly up to 48 h ($p < 0.001$); no significant difference after 72 h
Dohan Ehrenfest *et al.*, 2010	MTT assay	• Standard medium (− Ve control) • PRF + standard medium (PRF) • 2 PRF membranes + standard medium (2PRF) • Differentiation medium (+ Ve control) • PRF + differentiation medium (DPRF) • 2 PRF membranes + differentiation medium (D2PRF) PRF membranes were removed from culture plates at day 7	• 1 or 2 PRF membranes > control cultures (+ Ve/− Ve) at all time points ($p < 0.01$) • Standard medium: dose-dependent effect in number of PRF membrane used: 2 PRF > 1 PRF for first 14 d ($p < 0.01$), disappearing beyond day 14 • Differentiation medium: dose-dependent effect in number of PRF membrane used: 2 PRF > 1 PRF throughout whole experiment ($p < 0.01$) • Proliferation from day 14 onwards: 2 PRF more proliferation *vs.* all other conditions ($p < 0.01$)

Table 9. Effects of PPs on migration of BM-MSCs.

Author (year)	Assay used	Groups compared	Findings
PRP-CM			
Yin *et al.*, 2016	WimScratch assay	• L-PRP-CM • P-PRP-CM • 10 % FBS (control)	• Both L-PRP-CM and P-PRP-CM significantly promoted migration of BM-MSCs (compared to FBS; $p < 0.05$) • P-PRP-CM promoted more BM-MSC migration, compared to L-PRP-CM ($p < 0.05$)
PL			
Moisley *et al.*, 2019	Transwell migration assay (Inucyte)	• cPL • PL • 10 % FBS (control)	• Both PL and cPL induce significantly more migration than FBS (PL *vs.* FBS $p = 0.0006$; cPL *vs.* FBS $p = 0.0008$) • Removal of leukocytes seen in fPL reduces migratory response slightly, with BM-MSCs still significantly more migratory towards fPL *vs.* 10 %FBS
Skific *et al.*, 2018	μSlide chemotaxis 3D chamber (Ibidi)	• ITS/PL • PL • PL/PL (+ Ve control) • DMEM/DMEM (− Ve control)	• Only PRP is chemotactic for BM-MSCs ($p = 1.02 \times 10^{-10}$) • ITS, used as basal medium supplement to reduce amount of FBS needed to culture cells: not chemotactic for BM-MSCs
Murphy *et al.*, 2012	Transwell migration assay	• 1 % PL • 1 % ucPL • 10 % PL • 10 % ucPL • 10 % aPPP • 20 % FBS (control)	• 10 % ucPL > 10 % aPL ($p < 0.03$) • 1 % ucPL > 1 % PL ($p < 0.01$) • 10 % > 1 % PL > 20 % FBS in terms of migration capacity (p not stated) • 10 % PL *vs.* 1 % PL: comparable
Goedecke *et al.*, 2011	Transwell migration assay	• 10 % FBS (control) • 5 % PL • 10 % PL	• Migration capacity of BM-MSCs was higher in 10 % FBS • 10 % FBS *vs.* 5 %PL ($p = 0.06$); 10 %FBS *vs.* 10 %PL ($p = 0.04$) • 10 % PL > 5 % PL (p not stated)
PR			
Nguyen *et al.*, 2019	Scratch wound-healing assay	• 1 % PR • 2 % PR • 5 % PR • 10 % FBS in DMEM/F12 medium (+ Ve control) • DMEM/F12 medium alone (− Ve control)	• 1 % PR: no difference in migration compared to + Ve control group • 2 % and 5 % PR: BM-MSC migration better than 1 % PR and control groups ($p < 0.001$) • 2 % PR *vs.* 5 % PR: no difference
Gruber *et al.*, 2004	Boyden Chamber assay	• Serum-free medium (control) • PR with different platelet concentrations: 1. 2×10^8/mL (PR1) 2. 4×10^7/mL (PR2) 3. 8×10^6/mL (PR3) 4. 1.6×10^6/mL (PR4)	• Highest concentration (PR 1), 6.7 ± 0.8-fold over controls ($p < 0.01$) • Dose-dependent (platelets/mL) increase of cell migration *vs.* controls
PG-CM			
Schar *et al.*, 2015	Boyden Chamber assay	Supernatants collected from: • 10 % FBS (control) • L-PRP • L-PRF • Blood clot At different time points were compared (8 h, days 1, 3, 7, 14 and 28)	• BM-MSC migration: occurred mainly at day 1 (L-PRP gel and blood clot) and at day 3 (L-PRF) • Migration *vs.* FBS (control): L-PRF > L-PRP at days 3, 7 and 14 ($p < 0.05$) • IL-1 β secreted by platelet products: + Ve correlation with migration • PDGF-AB and IGF-1 secreted by platelet products: − Ve correlation with migration • VEGF secreted by platelet products: no influence on migration

Table 10a. Effects of PPs on differentiation of BM-MSCs.

Author (year)	Type of differentiation assessed	Staining used/variables measured	Groups compared	Findings
PRP-CM				
Yin *et al.*, 2016	Osteogenesis	Alizarin red staining	• 10 % FBS (control) • L-PRP-CM • P-PRP-CM	• More calcium nodules in groups treated with PRP-CM ($p < 0.05$) • P-PRP-CM > L-PRP-CM ($p < 0.05$)
PL				
Karadjian *et al.*, 2020	Osteogenesis	Alizarin red staining and ALP staining	• 1 % FBS (control) • 10 % FBS (control) • 1 % PL • 10 % PL	• ALP activity in hPL ≥ FBS (control) of same concentration at any time point. 1 % PL: statistically significant on days 1 and 7. 10 % PL: statistically significant on days 7 and 21 • Alizarin red staining: BM-MSCs incubated with PL differentiate faster than controls (PL reached maximum calcium expression at day 14 *vs.* day 21 in FBS). 1 % PL > 1 % FBS at all time points. 10 % PL > 10 % FBS at days 1, 7 and 14. End calcium content similar between two groups
Skific *et al.*, 2018	Osteogenesis Adipogenesis	Osteogenesis: Nuclear Fast Red Solution and von Kossa staining Adipogenesis: oil red O	• 10 % FBS (control) • 5 % PL • 10 % PL	• Numbers of differentiated adipocytes and osteoblasts in PL > FBS (p not stated)
Jonsdottir-Buch *et al.*, 2013	Osteogenesis Adipogenesis Chondrogenesis	Osteogenesis: alizarin red staining and ALP activity Adipogenesis: oil red O Chondrogenesis: toluidine blue	• FBS (control) • HPLF • HPLO	• ALP activity: day 7: FBS > HPLO > HPLF; day 14: HPLO > HPLF > FBS • Alizarin red staining: slightly more mineralisation in HPLF and HPLO > FBS • No comparisons made on adipogenesis and chondrogenesis. Authors reported on ability of BM-MSCs to differentiate similar to control
Bernardi *et al.*, 2013	Osteogenesis Adipogenesis Chondrogenesis	Osteogenesis: von Kossa staining Adipogenesis: oil red O Chondrogenesis: Carazzi haematoxylin	• 10 % FBS (control) • 10 % FT PL • 10 % SN PL	• No comparison made in terms of differentiation between SNPL, FTPL and FBS. Authors reported that "BM-MSC were able to differentiate in all three lineages"
Lange *et al.*, 2012	Adipogenesis	Sudan red staining	• FBS (control) • PL	• Significant reduction in lipid accumulation in PL group (*vs.* FBS, $p = 0.00007$)
Gottipamula *et al.*, 2012	Osteogenesis Adipogenesis Chondrogenesis	Osteogenesis: von Kossa staining Adipogenesis: oil red O staining Chondrogenesis: safranine O	• LG-PL • LG-FBS (control) • KO-PL • KO-FBS (control)	• Osteogenesis: PL > FBS in differentiation (p not stated) • Adipogenesis: similar between PL and FBS. (p not stated) • Chondrogenesis: PL > FBS in differentiation (p not stated)
Ben Azouna *et al.*, 2012	Osteogenesis Adipogenesis Chondrogenesis	Osteogenesis: von Kossa and Alizarin red staining Adipogenesis: Nile red staining Chondrogenesis: alcian blue and toluidine blue staining	• 10 % FBS + 1 ng/mL FGF2 • 10 % FBS + 5 % PL • 10 % PL • 5 % PL	• No difference between groups
Goedecke *et al.*, 2011	Osteogenesis Adipogenesis	Microscopic analysis following culturing in osteogenic and adipogenic medium	• 10 % FBS (control) • 5 % PL • 10 % PL	• No difference observed in the osteogenic and adipogenic differentiation potential
Verrier *et al.*, 2010	Osteogenesis	ALP activity assay 45Ca2+ incorporation assay von Kossa staining	• IMDM/10 % FBS (control) • 10 nmol/L dexamethasone (Dexa) • 10 % PL	• ALP activity: Dexa > PL ($p < 0.01$) > control • 45Ca2+ incorporation assay: PL > Dexa > control. PR was 10- and 400-fold more than Dexa (p not stated) and control groups ($p < 0.005$) respectively • von Kossa staining: PL > Dexa > unsupplemented control (p not stated)

Table 10b. Effects of PPs on differentiation of BM-MSCs.

Author (year)	Type of differentiation assessed	Staining used/variables measured	Groups compared	Findings
			PL	
Prins *et al.*, 2009	Osteogenesis Adipogenesis Chondrogenesis	Osteogenesis: ALP staining with BCIP/NBT substrate Adipogenesis: oil red O Chondrogenesis: DAPI	• 10 % FBS (control) • 5 % PL	• No difference in differentiation potential (osteo/adipo/chondrogenesis)
Vogel *et al.*, 2006	Osteogenesis Adipogenesis Chondrogenesis	Osteogenesis: alizarin red S Adipogenesis: oil red O Chondrogenesis: toluidine blue	• 2 % FBS (control) • 3 % PL	• Osteogenic differentiation: FBS > PL on calcification at day 14 ($p \leq 0.05$) • Chondrogenesis: no difference between groups ($p > 0.05$) • Adipogenesis: no difference between groups ($p > 0.05$)
			PR	
Liou *et al.*, 2018	Chondrogenesis	Safranin O/fast green Alcian blue/Nuclear Fast Red Collagen II immunohistochemistry	• TGF-b3 (control) • PR Compared effect of: duration (with 10 % PR: days 1, 3, 7, 21) and concentration (1 %, 5 %, 10 %, 20 %) of PR	• Duration of treatment with 10 % PR: increased duration = inhibition of chondrogenesis (decreased deposition of cartilage specific extracellular matrix production). FBS > PR throughout • PR concentration: only 20 % showed slight reduction in staining intensity. No difference between 1 %, 5 % and 10 % and control
Bernardi *et al.*, 2017	Osteogenesis Adipogenesis Chondrogenesis	Osteogenesis: von Kossa staining Adipogenesis: oil red O solution Chondrogenesis: alcian blue 1 % acetic acid	• 10 % FBS (control) • 7.5 % PL • 7.5 % PR-SRGF	• BM-MSCs able to maintain differentiation potential • No comparisons made
Kosmacheva *et al.*, 2014	Osteogenesis	Alizarin red staining ALP staining	• Nutrient medium (− Ve control) • Osteogenic medium (+ Ve control) • Nutrient medium + 2.5 % PR • Osteogenic medium + 2.5 % PR	• Alizarin red staining: PR leads to pronounced accumulation of calcium ossificates *vs.* + Ve controls. Degree of calcium ossification is directly dependent on PR concentration added in differentiation medium • − Ve control and nutrient medium + PR groups: no calcium ossificates • ALP staining: PR increases ALP synthesis slightly when added into osteogenic medium (*vs.* + Ve control)
Amable *et al.*, 2014	Osteogenesis Adipogenesis Chondrogenesis	Osteogenesis: alizarin red S Adipogenesis: oil red O Chondrogenesis: toluidine blue	• 10 % FBS (control) • 1 % PR	• Adipogenesis: reduced by PR • Chondrogenesis: increased by PR • Osteogenesis: increased by PR (p not stated)
Gruber *et al.*, 2004	Osteogenesis	ALP activity assay	• Serum free medium (control) • PR with different platelet concentrations: (1) 2×10^8/mL (PR1) (2) 4×10^7/mL (PR2) (3) 8×10^6/mL (PR3)	• ALP activity: decrease with increased concentrations of PR. Only PR 3 > FBS. P1 and PR2 < FBS
Lucarelli *et al.*, 2003	Osteogenesis Chondrogenesis	Osteogenesis: ALP staining Osteogenesis: alizarin red staining Chondrogenesis: von Kossa staining	• 20 % FBS • 10 % PR Note: compared the effect of PR withdrawal on osteogenesis and chondrogenesis of each group	• BM-MSCs cultured in 10 % PR retaine osteogenic and chondrogenic ability despite PR withdrawal

Table 10c. Effects of PPs on differentiation of BM-MSCs.

Author (year)	Type of differentiation assessed	Staining used/variables measured	Groups compared	Findings
PRC				
Samuel *et al.*, 2016	Osteogenesis	Alizarin red staining: days 8, 16 and 24 ALP activity: days 8, 16 and 24 Immunohistochemistry (using Hoechst stain) for RUNX2, Osteocalcin, osteopontin: days 8, 16 and 24.	• 10 % FBS (− Ve control) • Osteogenic medium, StemPro® (+ Ve control) • 15 % PRC	• Alizarin red staining: 15 % PRC > 10 % FBS ($p < 0.05$) at all time points; 15 % PRC comparable to osteogenic medium • ALP activity: 15 % PRC > osteogenic medium > 10 % FBS; significant increase on days 8 and 16 (15 % PRC > osteogenic medium, $p < 0.05$) • Immunohistochemistry: RUNX2 − 15 % PRC > osteogenic medium; osteocalcin and osteopontin − 15 % PRC = osteogenic medium. Absent staining for all markers in 10 % FBS
Parsons *et al.*, 2008	Osteogenesis	Alizarin red staining ALP activity	• 2.5 % FBS (control) • 2.5 % PRC	• Alizarin red staining (23 d): PRC > control during first 5 d (p not stated) • ALP activity (after day 3): PRC > control (2.5-fold, $p < 0.001$)
PG-CM				
Perut *et al.*, 2013	Osteogenesis	Alizarin red staining	• PPP (control) • L-PRP gel • fL-PRP gel • P-PRP gel	• L-PRP gel and fL-PRP gel more effective at inducing osteoblast differentiation and mineralisation of BM-MSCs (*vs.* P-PRP and PPP gels) (p not stated) • bFGF release is significantly positively correlated to mineralisation ability of BM-MSCs (p = not stated) • BM-MSC mineralisation was not influenced by platelet numbers in L-PRP and fL-PRP gels
PRF				
Dohan Ehrenfest *et al.*, 2010	Osteogenesis	ALP activity von Kossa staining SEM	• Standard medium (− Ve control) • PRF + standard medium (PRF) • 2 PRF membranes + standard medium (2PRF) • Differentiation medium (+ Ve control) • PRF + differentiation medium (DPRF) • 2 PRF membranes + differentiation medium (D2PRF)	• ALP activities: 1 or 2 PRF > other conditions at all time points ($p < 0.01$) • von Kossa staining: 1 or 2 PRF > other conditions at all time points ($p < 0.01$) • Dose-dependent effect of PRF on BM-MSC differentiation (2PRF > 1 PRF, $p < 0.01$; standard medium up to day 21; differentiation medium at all time points) • SEM: − Ve control: no differentiation nodule. In presence of PRF: numerous module evident (differentiation > standard medium)

Table 11a. Effects of PP on growth factor/cytokine/protein expression of BM-MSCs.

Author year	Platelet product	Cytokines/growth factors/proteins/MMPs assessed	Groups compared	Assay used	Findings
Inflammation					
Yin *et al.*, 2016	PRP-CM	PGE2 NO	• 10 % FBS (control) • L-PRP-CM • P-PRP-CM	ELISA: to measure PGE2 Griess reaction using NO sssay kit: to measure NO	• L-PRP-CM upregulates production of PGE2 and NO ($p < 0.05$; *vs.* FBS or P-PRP-CM) • No significant difference between P-PRP-CM and FBS
Ben Azouna *et al.*, 2012; Jenhani *et al.*, 2011	PL	GM-CSF IL-6 IL-8	• 10 % FBS + 1 ng/mL FGF2 (control) • 10 % FBS + 5 % PL • 10 % PL • 5 % PL	Multiplex bead-based immunoassay (Bioplex cytokine assay system)	• IL-6 and IL-8: PL (5 % and 10 %) > control/FBS + PL, $p < 0.05$ • GM-CSF: undetectable
Amable *et al.*, 2014	PR	Pro-inflammatory cytokines: GM-CSF, IL-1β, IL-2, IL-2R, IL-6, IL-7, IL-8, TNF- α, IFN-g, IL-12p40/p70, IL-15 and IL-17 Anti-inflammatory cytokines: IL-1RA, IL-4, IL-5, IL-10, IL-13 and IFN-α	• 10 % FBS (control) • 10 % PR	Commercial luminex kit	• PR increases pro-inflammatory profile of BM-MSCs (in particular cytokines IL-2R, IL-6, IL-7, IL-12 and IL-15) (p not stated) • Undetectable proinflammatory cytokines: GM-CSF, IL-1β, TNF-α, IFN-g, IL-2 and IL-17 • Most anti-inflammatory cytokines were undetectable. No clear conclusion on the effect of PR on release of anti-inflammatory cytokines
Osteogenesis					
Yin *et al.*, 2016	PRP-CM	Osteocalcin (OC) RUNX2	• 10 % FBS (control) • L-PRP-CM • P-PRP-CM	ELISA: to measure OC Western blot: RUNX2	• Both L-PRP-CM and P-PRP-CM upregulate expression of OC and RUNX2 significantly when compared against FBS (control) ($p < 0.05$) • Upregulation stronger in P-PRP-CM > L-PRP-CM ($p < 0.001$)
Ben Azouna *et al.*, 2012	PL	CaSR, PTHR	• 10 % FBS + 1ng/mL FGF2 (control) • 10 % FBS + 5 % PL • 10 % PL • 5 % PL	Western blot	• 10 % FBS + 5 % PL = control group in expression • Expression decreased in 10 % PL and 5 % PL
Verrier *et al.*, 2010	PL	BMP-2	• Culture medium (un-supplemented, as control) • 10 nmol/L dexamethasone (control) • 10 % PL	ELISA	• Only PL shows a clear increase in BMP-2 protein levels over time • BMP-2 protein levels significantly upregulated by 10-fold at day 23 compared to day 1 ($p < 0.05$) • Undetectable BMP-2 levels in control groups
Samuel *et al.*, 2016	PRC	Osteocalcin (OC)	• 10 % FBS (− Ve control) • Osteogenic medium, StemPro® (+ Ve control) • 15 % PRC	ELISA	• 15 % PRC > osteogenic medium > 10 % FBS ($p < 0.05$)
Adipogenesis					
Lange *et al.*, 2012	PL	L-PGDS	• FBS (control) • PL	Western blot	• Higher levels of L-PGDS in FBS *vs.* PL group
Ben Azouna *et al.*, 2012	PL	Leptin	• 10 % FBS + 1 ng/mL FGF2 (control) • 10 % FBS + 5 % PL • 10 % PL • 5 % PL	Western blot	• 10 % FBS + 5 % PL = control group in expression • Expression decreased in 10 % PL and 5 % PL

Table 11b. Effects of PP on growth factor/cytokine/protein expression of BM-MSCs.

Author year	Platelet product	Cytokines/growth factors/proteins/MMPs assessed	Groups compared	Assay used	Findings
Vascular smooth muscle					
Ben Azouna *et al.*, 2012	PL	ASMA and SM22a	• 10 % FBS + 1 ng/mL FGF2 (control) • 10 % FBS + 5 % PL • 10 % PL • 5 % PL	Western blot	• No difference between groups
Chemotaxis					
Ben Azouna *et al.*, 2012; Jenhani *et al.*, 2011	PL	RANTES	• 10 % FBS + 1 ng/mL FGF2 (control) • 10 % FBS + 5 % PL • 10 % PL • 5 % PL	Multiplex bead-based immunoassay (Bioplex cytokine assay system)	• Present at high concentrations with all PL-supplemented medium; low concentrations in control medium
Goedecke *et al.*, 2011	PL	SDF-1α	• 10 % FBS (control) • 5 % PL • 10 % PL	ELISA Supernatants collected at 24, 48 and 72 h	• SDF-1α levels are significantly higher in FBS-cultured MSCs, compared to PL-cultured MSCs (5 % and 10 % PRP) ($p = 0.04$)
Amable *et al.*, 2014	PR	eotaxin, IP-10, MCP-1, MIG, MIP-1α, MIP-1β and RANTES	• 10 % FBS (control) • 10 % PR	Commercial luminex kit	• PR increases chemokines IP-10, MIP-1β and MCP-1, mild increase with eotaxin (p not stated) • MIG, RANTES and MIP-1α undetectable in all conditions
Angiogenesis					
Ben Azouna *et al.*, 2012; Jenhani *et al.*, 2011	PL	VEGF	• 10 % FBS + 1 ng/mL FGF2 (control) • 10 % FBS + 5 % PL • 10 % PL • 5 % PL	Multiplex bead-based immunoassay (Bioplex cytokine assay system)	• VEGF: control > PL ($p < 0.01$)
Amable *et al.*, 2014	PR	Pro-angiogenic factors: VEGF, VEGF-D, endostatin, aFGF, thrombospondin-2, angiopoietin-1, angiogenin and PLGF	• 10 % FBS (control) • 10 % PR	Commercial luminex kit	• PR activates pro-angiogenic factors in BM-MSCs (thrombospondin, PLGF, aFGF, VEGF-D, VEGF) (p not stated) • Angiogenin, endostatin and angiopoietin-1 undetectable in both groups
MMPs					
Amable *et al.*, 2014	PR	MMP-1, -3, -7, -8 and -13	• 10 % FBS (control) • 10 % PR	Commercial luminex kit	• PR increases secretion of MMP1, MMP3, MMP7, MMP8 and MMP13 (p not stated)
Other growth factors					
Amable *et al.*, 2014	PR	EGF, HGF, bFGF, G-CSF, TGF-β1, TGF-β2, TGF-β3, PDGF-AA, PDGF-AB, PDGF-BB and IGF-1	• 10 % FBS (control) • 10 % PR	Commercial luminex kit ELISA: PDGF-AB	• PR increases HGF, G-CSF, TGF-β1 and TGF-β2 secretion (p not stated) • PR downregulates PDGF-BB secretion (p not stated) • EGF, bFGF, TGF-β3, PDGF-AA, PDGF-AB and IGF-1 not secreted in both groups
Ben Azouna *et al.*, 2012; Jenhani *et al.*, 2011	PL	FGF2, G-CSF	• 10 % FBS + 1 ng/mL FGF2 (control) • 10 % FBS + 5 % PL • 10 % PL • 5 % PL	Multiplex bead-based immunoassay (Bioplex cytokine assay system)	• Undetectable
Extracellular matrix proteins and molecules					
Amable *et al.*, 2014	PR	Heparan sulphate, aggrecan, decorin, elastin, laminin, perlecan, fribronectin, collagens I, II, III and IV	• 10 % FBS (control) • 10 % PR	ELISA	• PR activates heparan sulphate, elastin, laminin, collagen I and III (p not stated) • Aggrecan, perlecan, decorin, fibronectin and collagens II and IV not detectable in all groups

Table 12. Effects of PPs on other BM-MSC functions.

Author (year)	Platelet product	BM-MSC function assessed	Assay used	Groups compared	Findings
Yin *et al.*, 2016	PRP-CM	Response of BM-MSCs to apoptotic stress Immunomodulation	Flow cytometry using annexin V-FITC/PI staining (apoptotic markers) Western blotting	• Apoptotic stress: serum-starved BM-MSCs treated for 7 d in following medium, before camptothecin-induced apoptosis: 1. L-PRP-CM 2. P-PRP-CM 3. 10 % FBS (control) • Nearly confluent BM-MSCs serum-starved for 24 h and treated with: 1. 10 % FBS 2. L-PRP-CM 3. P-PRP-CM	• Apoptotic stress: both L-PRP-CM- and P-PRP-CM-inhibited camptothecin-induced apoptosis to enhance viability of BM-MSCs ($p < 0.05$) • Apoptotic stress: L-PRP-CM > P-PRP-CM in inhibiting apoptosis ($p < 0.05$) • Immunomodulation: L-PRP-CM induces activation of NF-κB signalling pathway, evidenced by the translocation and accumulation of NF-κB p65 in the nucleus of BM-MSCs (p not stated)
Bernardi *et al.*, 2017	PR and PL	Immunomodulation, inhibition of $CD45^+7AAD$ peripheral blood mononuclear cell subset by BM-MSCs	Flow cytometry enumeration (CD44 PC7m, 7-AAD) on inhibition of $CD45^+$ 7AAD cell by BM-MSCs	• 10 % FBS (control) • 7.5 % PR • 7.5 % PL at PBMC : BM-MSC ratios 20 : 1, 10 : 1 and 5 : 1	• Immunomodulation of BM-MSCs expanded in PL at 20 : 1 and PR (all ratios) significantly lower han FBS ($p < 0.05$)
Jonsdottir-Buch *et al.*, 2013	PL	Immunomodulation	Mixed lymphocyte reaction assays	• BM-MSCs (FBS) + MNCs • BM-MSCs (PL) + MNCs • MNCs alone	• Co-culture with BM-MSCs reduces proliferation of MNCs *vs.* MNCs alone ($p \leq 0.03$) • PL and control do not affect ability of BM-MSCs to reduce MNC proliferation
Bernardi *et al.*, 2013	PL	Immunomodulation, effect of PL expanded BM-MSCs on inhibition of T-cell proliferation	5-bromo-2-deoxyuridine proliferation assay	• No controls used • BM-MSCs expanded in 10 % SN PL and seeded at different ratios with T-cells • (0, 1 : 1,000, 1 : 100, 1 : 10 1 : 5)	• Suppression of lymphocyte proliferation is proportional to the ratio of BM-MSCs : T-cells seeded (significant at 1 : 5)
Gottipamula *et al.*, 2012	PL	Immunomodulation	MTT assay	• LG-PL *vs.* LG-FBS • KO-PL *vs.* KO-FBS	• BM-MSCs cultured in PL show dose-dependent (ratio of BM-MSCs : PBMCs) inhibition on mitogen-activated T-cell proliferation ($p < 0.05$) • Cells grown in FBS > PL in terms of immunosuppression
Goedecke *et al.*, 2011	PL	Chemotactic effect on HSCs	Transwell migration assay	Migration of $CD34^+$ HSCs in response to: • SDF-1 (+ Ve control) • BM-MSCs + 10 % FBS • BM-MSCs + 5 % PL • BM-MSCs + 10 % PL	• 10 % FBS induces a stronger chemoattraction towards $CD34^+$ HSCs, when compared to 5 % PL ($p = 0.002$) and 10% PL ($p = 0.003$)
Agis *et al.*, 2009	PR	Stimulation of fibrinolytic capacity Capacity of BM-MSCs to activate plasminogen – through afibrin zymography/casein zymography/qPCR assays	Fibrin film dissolution assay Fibrin zymography Casein zymography qPCR – uPA and PAI-1 levels released by BM-MSCs	• BM-MSCs pre-incubated with PR • BM-MSCs incubated in serum-free medium	• BM-MSCs pre-incubated with PR cause large lysis zone in fibrin film dissolution assay and increased caseinolysis • Fibrin and casein zymographies: increased plasminogen activation in BM-MSCs exposed to PR • uPA and PAI-1 levels of BM-MSCs increased by PR ($p < 0.01$)

Table 13a. Gene expression of BM-MSCs following PP exposure.

Author (year)	Platelet product	Genes assessed	Groups compared	Findings
Osteogenesis				
Yin *et al.*, 2016	PRP-CM	*Osteocalcin*, *RUNX2*	• 10 % FBS (control) • L-PRP-CM • P-PRP-CM	• Upregulation of both genes by P-PRP-CM > L-PRP-CM > FBS ($p < 0.001$)
Infante *et al.*, 2017	PL	*RUNX2*	• ITS (control) • PL • PL + 10 ng/mL TGF-b1 + 10^{-7} mol/L dexamethasone + 50 µg/mL ascorbic acid, day 14 and 21	• *RUNX2* not modified by PL
Jonsdottir-Buch *et al.*, 2013	PL	*ALP*, *RUNX2*, *SPP1*	• FBS (control) • HPLF • HPLO	• *RUNX2*: increased expression HPLO > HPLF > FBS at both day 7 and 14 ($p \leq 0.05$) • *ALP* and *SPP1*: no differences between groups at all time points
Gottipamula *et al.*, 2012	PL	*Osteocalcin*, *Osterix*	• FBS (control) • PL	• Both *osteocalcin* and *Osterix* were higher in PL • *Osteocalcin* higher than *Osterix* in PL
Ben Azouna *et al.*, 2012	PL	*ALP*, *RUNX2*	• 10 % FBS + 1 ng/mL FGF2 • 10 % FBS + 5 % PL • 10 % PL • 5 % PL taken before (day 0) and after induction of differentiation (day 14)	• Day 0 (before differentiation): expression of both *ALP* and *RUNX2* decreased upon addition of PL • Day 14: not stated
Verrier *et al.*, 2010	PL	*BMP2*, *bone sialoprotein 2*, *osteopontin*, *Osterix*, *RUNX2*	• Culture medium (un-supplemented, as control) • 10 nmol/L dexamethasone (Dexa) • 10 % PL	• PL upregulates expression of *BMP2* ($p < 0.05$), *bone sialoprotein II* ($p < 0.05$) and *MMP13* ($p < 0.01$); displays trend toward downregulation of *ALP*, *osteopontin*, *RUNX2* as well as a tendency toward downregulation of *Osterix* • Dexa group: same trend as PL observed
Kosmacheva *et al.*, 2014	PR	*Osteopontin*	• No PR (in NM, as − Ve control) • 1 % PR (NM) • 2.5 % PR (NM) • 5 % PR (NM) • No PR (in OM, as + Ve control) • 1 % PR (OM) • 2.5 % (OM) • 5 % PR (OM)	• 2.5 to 5 % PR in NM increases levels of osteopontin expression, with a significant increase noted in 2.5 % PR ($p < 0.001$) • 2.5 to 5 % PR in OM increases levels of osteopontin expression significantly [compared to controls, $p < 0.05$ (2.5 % PR); $p < 0.001$ (5 % PR)]
Amable *et al.*, 2014	PR	*BMP2*, *RUNX2*, *SPARC*	• 10 % FBS (control) • 10 % PR	• PR upregulates *BMP2* expression • PR downregulates *SPARC* and *RUNX2*, *p* not stated

Table 13b. Gene expression of BM-MSCs following PP exposure.

Author (year)	Platelet product	Genes assessed	Groups compared	Findings
Osteogenesis				
Gruber *et al.*, 2004	PR	*RUNX-2*, *osteocalcin*	• Serum free medium (control) • PR at 2 × 10^8/mL (platelets/mL)	• Expression of both genes: control > PR, *p* not stated
Samuel *et al.*, 2016	PRC	*ALP*, *BMP2*, *COLI*, *osteonectin*, *osteopontin*, *RUNX2*	• Osteogenic medium, StemPro® (control) • 15 % PRC	• Day 8 (PRC *vs.* osteogenic medium): higher upregulation in PRC for *RUNX2* (3-fold), *COL1* (2-fold) at day 8 ($p < 0.05$) • Day 16 (PRC *vs.* osteogenic medium): higher expression of ALP (1-fold) and osteopontin (5-fold) in PRC group ($p < 0.05$) • Day 24 (PRC *vs.* osteogenic medium): higher expression of *BMP2* (6-fold) and *osteopontin* (4-fold) in PRC group ($p < 0.05$) • Osteonectin: persistently upregulated in osteogenic medium when compared to PRC ($p < 0.05$)
Parsons *et al.*, 2008	PRC	*ALP*, *BMP2*, *RUNX2*	• 2.5 % FBS (control) • 2.5 % PRC	• ALP at 12 h: PRC > control (PRC 8.2-fold higher than serum) • *ALP* at 24 h: no difference between groups • *BMP2* up to 24 h: PRC > control at 6, 12, 24 h ($p < 0.05$); level of *BMP2* peaked at 12 h • *BMP2* at 0 and 48 h: no difference • *RUNX2* at 12 h: PRC > control ($p < 0.05$)
Chondrogenesis				
Infante *et al.*, 2017	PL	*ACAN*, *COL2A1*, *COMP*, *SOX9*	• ITS (control) • PL • PL + 10 ng/mL TGF-b1 + 10^{-7} mol/L dexamethasone + 50 µg/mL ascorbic acid), days 14 and 21	• *COL2A1* and *SOX9*: reduced by PL [*COL2A1*: 4-fold at day 21 ($p < 0.019$); *SOX9*: 2-fold at days 14 ($p = 0.024$) and 21 ($p = 0.05$)] • *ACAN* and *COMP*: increased by PL [*ACAN*: 3-fold at day 14 ($p = 0.050$); *COMP*: 3-fold at day 14 and 6-fold day 21 ($p < 0.001$)]
Verrier *et al.*, 2010	PL	*COL1A1*	• Culture medium (un-supplemented, as control) • 10 nmol/L dexamethasone (Dexa) • 10 % PL	• PL upregulates expression of *COL1A1* ($p < 0.05$)
Liou *et al.*, 2018	PR	*ACAN*, *COL2*	• TGF-b3 (control) • PR Compared effect of duration (with 10 % PR: days 1, 3, 7, 21) and PR concentration (1 %, 5 %, 10 %, 20 %)	• PR concentrations do not alter expression of *ACAN*, *COL2* and *MMP13* • Duration of treatment with 10 % PR: increased duration of PR exposure = inhibition of chondrogenesis
Amable *et al.*, 2014	PR	*ACAN*, *COL2A1*, *SOX9*	• 10 % FBS (control) • 10 % PR-CM	• aPRP upregulates *SOX9* • aPRP downregulates *ACAN*, *p* not stated • *COL2A1* undetectable in all conditions
Adipogenesis				
Lange *et al.*, 2012	PL	Adipocyte master regulator: *PPARG* and *CEPBA* Adipocyte markers: *FABP4*, *PLIN*, *GLUT4* and *APOE* Lipocyte transcription factors: *SREBP1*, *LXRα* and *L-PGDS*	• FBS (control) • PL	• *PPARg3*, *CEBPA*, *FABP4*, *PLIN*, *GLUT4* and *APOE*: FBS > PL (5-100-fold higher expression) • *LXRα*: significantly induced in PL • *SREBP1*: unaltered by PL • *L-PGDS*: FBS > PL ($p = 0.028$); levels low in *L-PDGS* throughout whole differentiation period

Table 13c. Gene expression of BM-MSCs following PP exposure.

Author (year)	Platelet product	Genes assessed	Groups compared	Findings
Adipogenesis				
Ben Azouna *et al.*, 2012	PL	*PPARG, LPL*	• 10 % FBS + 1 ng/mL FGF2 • 10 % FBS + 5 % PL • 10 % PL • 5 % PL taken before and after induction of differentiation	• Day 0 (before differentiation): expression of both PPARg decreased upon addition of PL • Day 14 (after osteogenic induction): decreased expression of PPARg in 10 % FBS + 5 % PL; effect on other culture conditions not stated
Amable *et al.*, 2014	PR	*ADIPOQ, CEPBA, PPARG*	• 10 % FBS (control) • 10 % PR	• All adipogenic markers upregulated by PR, *p* not stated
Pluripotency and proliferation				
Amable *et al.*, 2014	PR	*POU5F1, SOX2, TERT*	• 10 % FBS (control) • 10 % PR-CM	• PR slightly upregulates *SOX2* and *POU5F1*, *p* not stated • *TERT* not detectable
Immunomodulation/inflammation				
Yin *et al.*, 2016	PRP-CM	*COX-2, iNOS*	• 10 % FBS (control) • L-PRP-CM • P-PRP-CM	• Both *COX-2* and *iNOS* significantly upregulated by L-PRP-CM when compared with FBS or P-PRP-CM ($p < 0.001$)
MMP				
Verrier *et al.*, 2010	PL	*MMP13*	• Culture medium (un-supplemented, as control • 10 nmol/L dexamethasone (Dexa) • 10 % PL	• PL upregulated expression of *MMP13* ($p < 0.01$)
Hypertrophy				
Infante *et al.*, 2017	PL	*COL1A1, VEGF, COL10A1*	• ITS (control) • PL • PL + 10 ng/mL TGF-b1 + 10^{-7} mol/L dexamethasone + 50 µg/mL ascorbic acid), day 14 and 21	• *COL1A1, VEGF* and *COL10A1*: not modified by PL
Liou *et al.*, 2018	PR	*COL10, MMP13*	• TGF-b3 (control) • 10 % PR	• Changes in concentrations of PR do not affect expression of *COL10* and *MMP13*

the zone of injury – further guaranteeing successful bone repair. Moreover, the release of growth factors (HGF, G-CSF, TGF-β1, TGF-β2), MMPs (MMP1, MMP3, MMP7, MMP8, MMP13) and extracellular matrix molecules (heparan sulphate, elastin, laminin, collagen I and III), with multiple regulatory roles that enhance bone regeneration, was likewise larger in BM-MSCs exposed to PP (Amable *et al.*, 2014). Taken together, these results agreed with findings from functional assays.

BM-MSC gene expression has also been assessed by several studies. Proliferative gene markers in BM-MSCs were found to be consistently upregulated following PP exposure, confirming the findings from differentiation assays (Amable *et al.*, 2014). The effects of PP on the expression of BM-MSC gene markers for osteogenesis, chondrogenesis, adipogenesis and immunomodulation were rather variable and dependant on the gene assessed. Alterations to the physiological behaviour and characteristics of subcultured BM-MSCs following multiple passages using highly variable media (Ganguly *et al.*, 2019) could potentially explain the inconsistency in gene expression observed.

The present systematic review highlights the usefulness of PPs, many of which could be harvested and delivered autologously. Until research would advance in a manner whereby the array of most potent growth factors, cytokines and proteins could be commercially produced and compliant with the Good Manufacturing Practice, PPs remain a useful adjunct to bone healing. To ensure that the strengths of these PPs are put to the best use and not risk them being disregarded in the history of medicine as another 'mythic wonder drug' (Wang and Avila, 2007), there are several aspects that the clinical, scientific community and the industry are responsible for, including: (1) standardised use of terminology; (2) standardised preparation protocols; (3) standardised protocols for clinical application; (4) consistent description of source, volume, method of harvesting/processing and contents of these PPs used. Only then, further progress and breakthrough would be made in the fields of bone regeneration and tissue engineering.

Conclusion

I*n vitro* studies in human have demonstrated the multi-faceted 'priming effect' of PPs on the biophysiological functions of BM-MSCs. PPs have been shown to improve proliferation, migration, osteogenic differentiation, immunomodulation, reaction to apoptotic stress as well as pro-angiogenic and pro-inflammatory capacities of BM-MSCs. The lack of standardised terminology and protocols surrounding the use of PPs was highlighted, along with other factors that unfortunately restrict the transferability of these findings into clinical practice. Furthermore, the impact of short-term exposure of BM-MSCs to PPs, as seen commonly in clinical practice, has not been investigated. Therefore, further collaborative multi-centre *in vitro* research in humans modelled to reflect clinical practice is required to better understand the effects of PP exposure on the biophysiological function(s) of BM-MSCs in human.

Conflicts of interest and source of funding

None of the authors has any conflict of interest relevant to the study and no funding was received for the completion of this project.

References

Agis H, Kandler B, Fischer MB, Watzek G, Gruber R (2009) Activated platelets increase fibrinolysis of mesenchymal progenitor cells. J Orthop Res **27**: 972-980.

Amable PR, Teixeira MV, Carias RB, Granjeiro JM, Borojevic R (2014) Mesenchymal stromal cell proliferation, gene expression and protein production in human platelet-rich plasma-supplemented media. PLoS One **9**: e104662. DOI: 10.1371/journal.pone.0104662.

Andia I, Maffulli N (2019) New biotechnologies for musculoskeletal injuries. Surgeon **17**: 244-255.

Ben Azouna N, Jenhani F, Regaya Z, Berraeis L, Ben Othman T, Ducrocq E, Domenech J (2012) Phenotypical and functional characteristics of mesenchymal stem cells from bone marrow: comparison of culture using different media supplemented with human platelet lysate or fetal bovine serum. Stem Cell Res Ther **3**: 6. DOI: 10.1186/scrt97.

Bernardi M, Agostini F, Chieregato K, Amati E, Durante C, Rassu M, Ruggeri M, Sella S, Lombardi E, Mazzucato M, Astori G (2017) The production method affects the efficacy of platelet derivatives to expand mesenchymal stromal cells *in vitro*. J Transl Med **15**: 90. DOI: 10.1186/s12967-017-1185-9.

Bernardi M, Albiero E, Alghisi A, Chieregato K, Lievore C, Madeo D, Rodeghiero F, Astori G (2013) Production of human platelet lysate by use of ultrasound for *ex vivo* expansion of human bone marrow-derived mesenchymal stromal cells. Cytotherapy **15**: 920-929.

Bielecki T, Gazdzik TS, Szczepanski T (2008) Benefit of percutaneous injection of autologous platelet-leukocyte-rich gel in patients with delayed union and nonunion. Eur Surg Res **40**: 289-296.

Bishop JA, Palanca AA, Bellino MJ, Lowenberg DW (2012) Assessment of compromised fracture healing. J Am Acad Orthop Surg **20**: 273-282.

Bustos ML, Huleihel L, Kapetanaki MG, Lino-Cardenas CL, Mroz L, Ellis BM, McVerry BJ, Richards TJ, Kaminski N, Cerdenes N, Mora AL, Rojas M (2014) Aging mesenchymal stem cells fail to protect because of impaired migration and antiinflammatory response. Am J Respir Crit Care Med **189**: 787-798.

Calori GM, Giannoudis PV (2011) Enhancement of fracture healing with the diamond concept: the role of the biological chamber. Injury **42**: 1191-1193.

Calori GM, Mazza E, Colombo M, Ripamonti C, Tagliabue L (2011) Treatment of long bone non-unions with polytherapy: indications and clinical results. Injury **42**: 587-590.

Calori GM, Tagliabue L, Gala L, d'Imporzano M, Peretti G, Albisetti W (2008) Application of rhBMP-7 and platelet-rich plasma in the treatment of long bone non-unions: a prospective randomised clinical study on 120 patients. Injury **39**: 1391-1402.

Chiang CC, Su CY, Huang CK, Chen WM, Chen TH, Tzeng YH (2007) Early experience and results of bone graft enriched with autologous platelet gel for recalcitrant nonunions of lower extremity. J Trauma **63**: 655-661.

Choumerianou DM, Martimianaki G, Stiakaki E, Kalmanti L, Kalmanti M, Dimitriou H (2010) Comparative study of stemness characteristics of mesenchymal cells from bone marrow of children and adults. Cytotherapy **12**: 881-887.

DeLong JM, Russell RP, Mazzocca AD (2012) Platelet-rich plasma: the PAW classification system. Arthroscopy **28**: 998-1009.

Di Matteo B, Filardo G, Kon E, Marcacci M (2015) Platelet-rich plasma: evidence for the treatment of patellar and Achilles tendinopathy–a systematic review. Musculoskelet Surg **99**: 1-9.

Dohan Ehrenfest DM, Doglioli P, de Peppo GM, Del Corso M, Charrier JB (2010) Choukroun's platelet-rich fibrin (PRF) stimulates *in vitro* proliferation and differentiation of human oral bone mesenchymal stem cell in a dose-dependent way. Arch Oral Biol **55**: 185-194.

Dohan Ehrenfest DM, Rasmusson L, Albrektsson T (2009) Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol **27**: 158-167.

Einhorn TA, Gerstenfeld LC (2015) Fracture healing: mechanisms and interventions. Nat Rev Rheumatol **11**: 45-54.

Ekegren CL, Edwards ER, de Steiger R, Gabbe BJ (2018) Incidence, costs and predictors of non-union, delayed union and mal-union following long bone fracture. Int J Environ Res Public Health **15**: 2845. DOI: 10.3390/ijerph15122845.

Etulain J (2018) Platelets in wound healing and regenerative medicine. Platelets **29**: 556-568.

Galasso O, Mariconda M, Romano G, Capuano N, Romano L, Ianno B, Milano C (2008) Expandable intramedullary nailing and platelet rich plasma to treat long bone non-unions. J Orthop Traumatol **9**: 129-134.

Ganguly P, El-Jawhari JJ, Burska AN, Ponchel F, Giannoudis PV, Jones EA (2019) The analysis of *in vivo* aging in human bone marrow mesenchymal stromal cells using colony-forming unit-fibroblast assay and the CD45(low)CD271(+) phenotype. Stem Cells Int **2019**: 5197983. DOI: 10.1155/2019/5197983.

Ganguly P, El-Jawhari JJ, Giannoudis PV, Burska AN, Ponchel F, Jones EA (2017) Age-related changes in bone marrow mesenchymal stromal cells: a potential impact on osteoporosis and osteoarthritis development. Cell Transplant **26**: 1520-1529.

Gianakos AL, Sun L, Patel JN, Adams DM, Liporace FA (2017) Clinical application of concentrated bone marrow aspirate in orthopaedics: a systematic review. World J Orthop **8**: 491-506.

Giannoudis PV, Einhorn TA, Marsh D (2007) Fracture healing: the diamond concept. Injury **38 Suppl 4**: S3-6.

Giannoudis PV, Einhorn TA, Schmidmaier G, Marsh D (2008) The diamond concept–open questions. Injury **39 Suppl 2**: S5-8.

Goedecke A, Wobus M, Krech M, Münch N, Richter K, Hölig K, Bornhauser M (2011) Differential effect of platelet-rich plasma and fetal calf serum on bone marrow-derived human mesenchymal stromal cells expanded *in vitro*. J Tissue Eng Regen Med **5**: 648-654.

Golos J, Walinski T, Piekarczyk P, Kwiatkowski K (2014) Results of the use of platelet rich plasma in the treatment of delayed union of long bones. Ortop Traumatol Rehabil **16**: 397-406.

Gottipamula S, Sharma A, Krishnamurthy S, Majumdar AS, Seetharam RN (2012) Human platelet lysate is an alternative to fetal bovine serum for large-scale expansion of bone marrow-derived mesenchymal stromal cells. Biotechnol Lett **34**: 1367-1374.

Gruber R, Karreth F, Kandler B, Fuerst G, Rot A, Fischer MB, Watzek G (2004) Platelet-released supernatants increase migration and proliferation, and decrease osteogenic differentiation of bone marrow-derived mesenchymal progenitor cells under *in vitro* conditions. Platelets **15**: 29-35.

Hak DJ, Fitzpatrick D, Bishop JA, Marsh JL, Tilp S, Schnettler R, Simpson H, Alt V (2014) Delayed union and nonunions: epidemiology, clinical issues, and financial aspects. Injury **45 Suppl 2**: S3-7.

Hutton B, Salanti G, Caldwell DM, Chaimani A, Schmid CH, Cameron C, Ioannidis JP, Straus S, Thorlund K, Jansen JP (2015) The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. Ann Intern Med **162**: 777-784.

Infante A, Rubio-Azpeitia E, Sanchez P, Alberdi R, Rodriguez CI, Andia I (2017) Platelet rich plasma and culture configuration affect the matrix forming phenotype of bone marrow stromal cells. Tissue Eng Regen Med **14**: 567-577.

Jenhani F, Durand V, Ben Azouna N, Thallet S, Ben Othmen T, Bejaoui M, Domenech J (2011) Human cytokine expression profile in various conditioned media for *in vitro* expansion bone marrow and umbilical cord blood immunophenotyped mesenchymal stem cells. Transplant Proc **43**: 639-643.

Jonsdottir-Buch SM, Lieder R, Sigurjonsson OE (2013) Platelet lysates produced from expired

platelet concentrates support growth and osteogenic differentiation of mesenchymal stem cells. PLoS One **8**: e68984. DOI: 10.1371/journal.pone.0068984.

Karadjian M, Senger AS, Essers C, Wilkesmann S, Heller R, Fellenberg J, Simon R, Westhauser F (2020) Human platelet lysate can replace fetal calf serum as a protein source to promote expansion and osteogenic differentiation of human bone-marrow-derived mesenchymal stromal cells. Cells **9**: 918. DOI: 10.3390/cells9040918.

Kasten P, Vogel J, Beyen I, Weiss S, Niemeyer P, Leo A, Lüginbuhl R (2008) Effect of platelet-rich plasma on the *in vitro* proliferation and osteogenic differentiation of human mesenchymal stem cells on distinct calcium phosphate scaffolds: the specific surface area makes a difference. J Biomater Appl **23**: 169-188.

Kim M, Kim C, Choi YS, Kim M, Park C, Suh Y (2012) Age-related alterations in mesenchymal stem cells related to shift in differentiation from osteogenic to adipogenic potential: implication to age-associated bone diseases and defects. Mech Ageing Dev **133**: 215-225.

Kosmacheva SM, Danilkovich NN, Shchepen AV, Ignatenko SI, Potapnev MP (2014) Effect of platelet releasate on osteogenic differentiation of human mesenchymal bone marrow stem cells. Bull Exp Biol Med **156**: 560-565.

Lana J, Purita J, Paulus C, Huber SC, Rodrigues BL, Rodrigues AA, Santana MH, Madureira JL, Jr., Malheiros Luzo AC, Belangero WD, Annichino-Bizzacchi JM (2017) Contributions for classification of platelet rich plasma - proposal of a new classification: MARSPILL. Regen Med **12**: 565-574.

Lange C, Brunswig-Spickenheier B, Eissing L, Scheja L (2012) Platelet lysate suppresses the expression of lipocalin-type prostaglandin D2 synthase that positively controls adipogenic differentiation of human mesenchymal stromal cells. Exp Cell Res **318**: 2284-2296.

Lee DH, Ryu KJ, Kim JW, Kang KC, Choi YR (2014) Bone marrow aspirate concentrate and platelet-rich plasma enhanced bone healing in distraction osteogenesis of the tibia. Clin Orthop Relat Res **472**: 3789-3797.

Liou JJ, Rothrauff BB, Alexander PG, Tuan RS (2018) Effect of platelet-rich plasma on chondrogenic differentiation of adipose- and bone marrow-derived mesenchymal stem cells. Tissue Eng Part A **24**: 1432-1443.

Lucarelli E, Beccheroni A, Donati D, Sangiorgi L, Cenacchi A, Del Vento AM, Meotti C, Bertoja AZ, Giardino R, Fornasari PM, Mercuri M, Picci P (2003) Platelet-derived growth factors enhance proliferation of human stromal stem cells. Biomaterials **24**: 3095-3100.

Lucarelli E, Beretta R, Dozza B, Tazzari PL, O'Connel SM, Ricci F, Pierini M, Squarzoni S, Pagliaro PP, Oprita EI, Donati D (2010) A recently developed bifacial platelet-rich fibrin matrix. Eur Cell Mater **20**: 13-23.

Magalon J, Chateau AL, Bertrand B, Louis ML, Silvestre A, Giraudo L, Veran J, Sabatier F (2016) DEPA classification: a proposal for standardising PRP use and a retrospective application of available devices. BMJ Open Sport Exerc Med **2**: e000060. DOI: 10.1136/bmjsem-2015-000060.

Malhotra R, Kumar V, Garg B, Singh R, Jain V, Coshic P, Chatterjee K (2015) Role of autologous platelet-rich plasma in treatment of long-bone nonunions: a prospective study. Musculoskelet Surg **99**: 243-248.

Mariconda M, Cozzolino F, Cozzolino A, D'Agostino E, Bove A, Milano C (2008) Platelet gel supplementation in long bone nonunions treated by external fixation. J Orthop Trauma **22**: 342-345.

Martin JR, Houdek MT, Sierra RJ (2013) Use of concentrated bone marrow aspirate and platelet rich plasma during minimally invasive decompression of the femoral head in the treatment of osteonecrosis. Croat Med J **54**: 219-224.

Marx RE (2001) Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent **10**: 225-228.

Marx RE (2004) Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg **62**: 489-496.

Mautner K, Malanga GA, Smith J, Shiple B, Ibrahim V, Sampson S, Bowen JE (2015) A call for a standard classification system for future biologic research: the rationale for new PRP nomenclature. PM R **7**: S53-S59.

Mills LA, Simpson AH (2013) The relative incidence of fracture non-union in the Scottish population (5.17 million): a 5-year epidemiological study. BMJ Open **3**: e002276. DOI: 10.1136/bmjopen-2012-002276.

Mishra A, Harmon K, Woodall J, Vieira A (2012) Sports medicine applications of platelet rich plasma. Curr Pharm Biotechnol **13**: 1185-1195.

Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med **151**: 264-269.

Moisley KM, El-Jawhari JJ, Owston H, Tronci G, Russell SJ, Jones EA, Giannoudis PV (2019) Optimising proliferation and migration of mesenchymal stem cells using platelet products: a rational approach to bone regeneration. J Orthop Res **37**: 1329-1338.

Moradian H, Rafiee A, Ayatollahi M (2017) Design and fabrication of a novel transplant combined with human bone marrow mesenchymal stem cells and platelet-rich fibrin: new horizons for periodontal tissue regeneration after dental trauma. Iran J Pharm Res **16**: 1370-1378.

Muraglia A, Ottonello C, Spanò R, Dozin B, Strada P, Grandizio M, Cancedda R, Mastrogiacomo M (2014) Biological activity of a standardized freeze-dried platelet derivative to be used as cell culture medium supplement. Platelets **25**: 211-220.

Muraglia A, Todeschi MR, Papait A, Poggi A, Spanò R, Strada P, Cancedda R, Mastrogiacomo M (2015) Combined platelet and plasma derivatives enhance proliferation of stem/progenitor cells maintaining their differentiation potential. Cytotherapy **17**: 1793-1806.

Murphy MB, Blashki D, Buchanan RM, Yazdi IK, Ferrari M, Simmons PJ, Tasciotti E (2012) Adult and umbilical cord blood-derived platelet-rich plasma for mesenchymal stem cell proliferation, chemotaxis, and cryo-preservation. Biomaterials **33**: 5308-5316.

Nguyen ATM, Tran HLB, Pham TAV (2019) *In vitro* evaluation of proliferation and migration behaviour of human bone marrow-derived mesenchymal stem cells in presence of platelet-rich plasma. Int J Dent **2019**: 9639820. DOI: 10.1155/2019/9639820.

Parsons P, Butcher A, Hesselden K, Ellis K, Maughan J, Milner R, Scott M, Alley C, Watson JT, Horner A (2008) Platelet-rich concentrate supports human mesenchymal stem cell proliferation, bone morphogenetic protein-2 messenger RNA expression, alkaline phosphatase activity, and bone formation *in vitro*: a mode of action to enhance bone repair. J Orthop Trauma **22**: 595-604.

Perut F, Filardo G, Mariani E, Cenacchi A, Pratelli L, Devescovi V, Kon E, Marcacci M, Facchini A, Baldini N, Granchi D (2013) Preparation method and growth factor content of platelet concentrate influence the osteogenic differentiation of bone marrow stromal cells. Cytotherapy **15**: 830-839.

Prins HJ, Rozemuller H, Vonk-Griffioen S, Verweij VG, Dhert WJ, Slaper-Cortenbach IC, Martens AC (2009) Bone-forming capacity of mesenchymal stromal cells when cultured in the presence of human platelet lysate as substitute for fetal bovine serum. Tissue Eng Part A **15**: 3741-3751.

Roffi A, Di Matteo B, Krishnakumar GS, Kon E, Filardo G (2017) Platelet-rich plasma for the treatment of bone defects: from pre-clinical rational to evidence in the clinical practice. A systematic review. Int Orthop **41**: 221-237.

Rombouts WJ, Ploemacher RE (2003) Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture. Leukemia **17**: 160-170.

Samuel S, Ahmad RE, Ramasamy TS, Karunanithi P, Naveen SV, Murali MR, Abbas AA, Kamarul T (2016) Platelet-rich concentrate in serum free medium enhances osteogenic differentiation of bone marrow-derived human mesenchymal stromal cells. PeerJ **2016 4**: e2347. DOI: 10.7717/peerj.2347.

Sanchez M, Anitua E, Cugat R, Azofra J, Guadilla J, Seijas R, Andia I (2009) Nonunions treated with autologous preparation rich in growth factors. J Orthop Trauma **23**: 52-59.

Say F, Turkeli E, Bulbul M (2014) Is platelet-rich plasma injection an effective choice in cases of non-union? Acta Chir Orthop Traumatol Cech **81**: 340-345.

Schallmoser K, Henschler R, Gabriel C, Koh MBC, Burnouf T (2020) Production and quality requirements of human platelet lysate: a position statement from the working party on cellular therapies of the International Society of Blood Transfusion. Trends Biotechnol **38**: 13-23.

Schar MO, Diaz-Romero J, Kohl S, Zumstein MA, Nesic D (2015) Platelet-rich concentrates differentially release growth factors and induce cell migration *in vitro*. Clin Orthop Relat Res **473**: 1635-1643.

Scherer SS, Tobalem M, Vigato E, Heit Y, Modarressi A, Hinz B, Pittet B, Pietramaggiori G (2012) Nonactivated versus thrombin-activated platelets on wound healing and fibroblast-to-myofibroblast differentiation *in vivo* and *in vitro*. Plast Reconstr Surg **129**: 46e-54e.

Skific M, Golemovic M, Crkvenac-Gornik K, Vrhovac R, Golubic Cepulic B (2018) Comparative analysis of biological and functional properties of bone marrow mesenchymal stromal cells expanded in media with different platelet lysate content. Cells Tissues Organs **205**: 226-239.

Soares CS, Babo PS, Reis RL, Carvalho PP, Gomes ME (2020) Platelet-derived products in veterinary medicine: a new trend or an effective therapy? Trends Biotechnol. DOI: 10.1016/j.tibtech.2020.07.011.

Spees JL, Gregory CA, Singh H, Tucker HA, Peister A, Lynch PJ, Hsu SC, Smith J, Prockop DJ (2004) Internalized antigens must be removed to prepare hypoimmunogenic mesenchymal stem cells for cell and gene therapy. Mol Ther **9**: 747-756.

Su P, Tian Y, Yang C, Ma X, Wang X, Pei J, Qian A (2018) Mesenchymal stem cell migration during bone formation and bone diseases therapy. Int J Mol Sci **19**: 2343. DOI: 10.3390/ijms19082343.

Verboket R, Leiblein M, Seebach C, Nau C, Janko M, Bellen M, Bonig H, Henrich D, Marzi I (2018) Autologous cell-based therapy for treatment of large bone defects: from bench to bedside. Eur J Trauma Emerg Surg **44**: 649-665.

Verrier S, Meury TR, Kupcsik L, Heini P, Stoll T, Alini M (2010) Platelet-released supernatant induces osteoblastic differentiation of human mesenchymal stem cells: potential role of BMP-2. Eur Cell Mater **20**: 403-414.

Vogel JP, Szalay K, Geiger F, Kramer M, Richter W, Kasten P (2006) Platelet-rich plasma improves expansion of human mesenchymal stem cells and retains differentiation capacity and *in vivo* bone formation in calcium phosphate ceramics. Platelets **17**: 462-469.

Wang HL, Avila G (2007) Platelet rich plasma: myth or reality? Eur J Dent **1**: 192-194.

Wang T, Zhang X, Bikle DD (2017) Osteogenic differentiation of periosteal cells during fracture healing. J Cell Physiol **232**: 913-921.

Yin W, Qi X, Zhang Y, Sheng J, Xu Z, Tao S, Xie X, Li X, Zhang C (2016) Advantages of pure platelet-rich plasma compared with leukocyte- and platelet-rich plasma in promoting repair of bone defects. J Transl Med **14**: 73. DOI: 10.1186/s12967-016-0825-9.

Discussion with Reviewer

Reviewer: In addition to the variations due to many preparation protocols, it would be interesting to have the authors' opinion on the inter-individual variations. Prepared in similar conditions, PPs from

different patients will have different properties (in term of efficacy). Do you think that the preparation of PPs has more influence on its outcome than the patient of origin?

Authors: Factors affecting BM-MSC functions are multifactorial. Both PP preparation method and donor play equally important roles in determining the outcomes when using PPs clinically to optimise BM-MSC function. However, whilst patient's biology is often not-modifiable, the choice of PPs and its components used (*e.g.* only growth factors in PL/PR; combination of both cells and growth factors in PRP) as well as its preparation and incubation period with BM-MSCs are all flexible and remains at the clinician's disposal when treating patients with autologous PPs.

Editor's note: The Scientific Editor responsible for this paper was Martin Stoddart.