International Orthopaedics (SICOT) (2017) 41:221–237
DOI 10.1007/s00264-016-3342-9

REVIEW ARTICLE

Platelet-rich plasma for the treatment of bone defects: from pre-clinical rational to evidence in the clinical practice. A systematic review

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Received: 31 October 2016 / Accepted: 7 November 2016 / Published online: 26 November 2016

Abstract
Purpose The treatment of large bone defects represents a significant challenge for orthopaedic surgeons. In recent years, biologic agents have also been used to further improve bone healing. Among these, platelet-rich plasma (PRP) is the most exploited strategy. The aim of the present study was to systematically review the available literature to identify: 1) pre-clinical in-vivo results supporting the rational of PRP use for bone healing; 2) evidence from the clinical practice on the actual clinical benefit of PRP for the treatment of fractures and complications such as delayed unions and non-unions.
Methods A systematic review of the literature was performed on the application of PRP in bone healing, using the following inclusion criteria: pre-clinical and clinical reports of any level of evidence, written in English language, published in the last 20 years (1996–2016), on the use of PRP to stimulate long-bone defect treatment, with focus on fracture and delayed/non-unions healing.
Results The search in the Pubmed database identified 64 articles eligible for inclusion: 45 were preclinical in-vivo studies and 19 were clinical studies. Despite the fact that the overall pre-clinical results seem to support the benefit of PRP in 91.1 % of the studies, a more in depth analysis underlined a lower success rate, with a positive outcome of 84.4 % in terms of histological analysis, and even lower values considering radiological and biomechanical results (75.0 % and 72.7 % positive outcome respectively). This was also mirrored in the clinical literature, where the real benefit of PRP use to treat fractures and non-unions is still under debate.
Conclusion Overall, the available literature presents major limitations in terms of low quality and extreme heterogeneity, which hamper the possibility to optimize PRP treatment and translate it into a real clinical benefit despite positive preclinical findings on its biological potential to favour bone healing.

Keywords Bone defect · Bone healing · Fracture · Growth factors · Non-union · PRP

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Introduction

The treatment of large bone defects represents a significant clinical challenge for orthopaedic surgeons [1, 2]. The well-orchestrated regenerative ability of bone to heal is hampered, in the case of complex defects, by the lack of a template for regeneration and, eventually, it requires surgical intervention [3]. Autografts and allografts are considered to be the major bone substitutes, however they each have their own limitations regarding availability, donor site morbidity and chronic pain, leading to not always optimal results [4]. In order to overcome these issues, several bone substitute materials have been developed and applied in the clinical practice [5, 6]. To further improve the success rates, co-adjuvant agents have also been proposed, which may enhance implant osseointegration potential and restore bone tissue function [7]. Among these, growth factors (GFs) are expressed during different phases of tissue healing and may represent a key element in promoting tissue regeneration [8]. In fact, GFs delivered through orthopaedic devices have

been reported to enhance osteoblastic activity and favour implant integration [9, 10].

Platelet-rich plasma (PRP) is emerging as a powerful tool for tissue healing, thanks to the many GFs contained in platelet alpha-granules. PRP is defined as a blood derivative, where the platelets concentration is above the baseline levels, thus providing a large number of bioactive molecules in physiologic proportions [11]. Activated platelets can release more than 300 molecules that are responsible for the coordination of numerous cell-cell and cell-extracellular matrix (ECM) interactions [12]. The evidence for PRP osteogenic potential has been suggested by several in vitro studies, i.e. PRP addition in culture medium promoted the proliferation and differentiation of human mesenchymal stem cells (MSCs) [13, 14], and the effect of PRP on osteogenic differentiation was also seen on human adipose derived stem cells (ADSCs) [15]. Furthermore, PRP can improve cell chemokinesis and chemotaxis through cytoskeleton reorganization and accelerate cell migration, thus influencing osteoblast like cells mobility [16]. Finally, anti-microbial effects have been suggested [17, 18], which are highly desirable in relation to a surgical bone application. However, besides the beneficial role in terms of proliferation and differentiation, as well as cell migration and protection towards microbial contamination, in-vitro studies have also shown controversial results on PRP potential to favour bone healing [19–21].

Thus, the aim of this study was to systematically review the available literature to identify both preclinical in-vivo results supporting the rational of PRP use for bone healing, and the evidence from the orthopaedics practice on the actual clinical benefit of PRP for the treatment of bone disorders.

Materials and methods

A systematic review of the literature was performed on the use of PRP in both pre-clinical in-vivo setting and clinical setting for the treatment of fractures and delayed unions/non-unions. The search was conducted on the PubMed database on August 1st, 2016 using the following string: (PRP OR platelet-rich plasma OR plasma rich in growth factors OR platelet derived growth factor OR platelet derived OR platelet gel OR platelet concentrate OR PRF OR platelet rich fibrin OR platelet rich membrane OR ACP OR autologous conditioned plasma OR PRGF OR platelet lysate) AND (fracture OR trauma OR traumatic OR non union OR mal union OR post-traumatic OR pseudoarthrosis OR delayed union OR bone defect).

The screening process and analysis were conducted separately by two independent observers (BDM and GS). First, the articles were screened by title and abstract, using the following inclusion criteria: pre-clinical and clinical reports of any level of evidence, written in English language, published in the last 20 years (1996–2016), on the use of PRP to stimulate long-bone defect treatment, with focus on fracture and delayed/non-unions healing. Exclusion criteria were articles written in other languages, reviews, case reports or case series with less than ten patients included, or studies analysing other applications of PRP in bone pathology. In the second step, the full texts of the selected articles were screened, with further exclusions according to the previously described criteria. Reference lists from the selected papers were also screened. A flowchart of the systematic review process is provided in Fig. 1. Relevant data were then extracted and collected, with the consensus of the two observers, in a database including pertinent information (type of study, number of cases, follow-up, PRP preparation, cytology and application modality, type of surgical treatment or animal model used, type of bone or other materials applied, type of evaluation and results) to be analysed for the purposes of the present manuscript.

Results

In total 3160 articles were screened and 64 articles were found to be eligible for inclusion in the present review. Among these, 45 were pre-clinical in-vivo studies [1, 2, 4, 22–63] and 19 were clinical studies [64–83] (Fig. 1). The trend of publication of pre-clinical and clinical trials over years has been reported in Fig. 2. Results will be discussed separately for pre-clinical and clinical studies.

Pre-clinical studies

Animal models

Of the 45 identified studies (Table 1), 20 (44.4 %) used a rabbit model, while other animals were used in a smaller number of studies, i.e. rat in eight studies (17.8 %), sheep in seven studies (15.6 %), dog in four studies (8.9 %), pig in four studies (8.9 %) and goat in two studies (4.4 %). With regard to defect type, bone sites were tibia in 16 studies (35.6 %) (four segmental, 14 cylindrical defects), femur in 14 studies (31.1 %) (two segmental, eight cylindrical defects, four fractures), radius in ten studies (22.2 %) (nine segmental, one fracture), fibula in 1 study (2.2 %) (one fracture), ulna in three studies (6.7 %) (three segmental defects) and metatarsus in one study (2.2 %) (one segmental defects).

With regard to the delivery mode, 3/45 studies delivered PRP through percutaneous injection while the remaining studies used the surgical approach to apply PRP either alone or in combination with other materials. Only one paper described the combination of percutaneous PRP injection and surgical PRP delivery with biomaterial. Besides seven articles (16 %) documenting the effect of PRP alone, most of the studies (38 papers, 84 %) analysed the effect of PRP with other materials: 20 studies (53 %) reported the use of three dimensional scaffolds, nine (23.5 %) with auto ($n = 5$) / allo ($n = 3$) / xenografts ($n = 1$) and nine (23.5 %) with powders/granules/pastes.

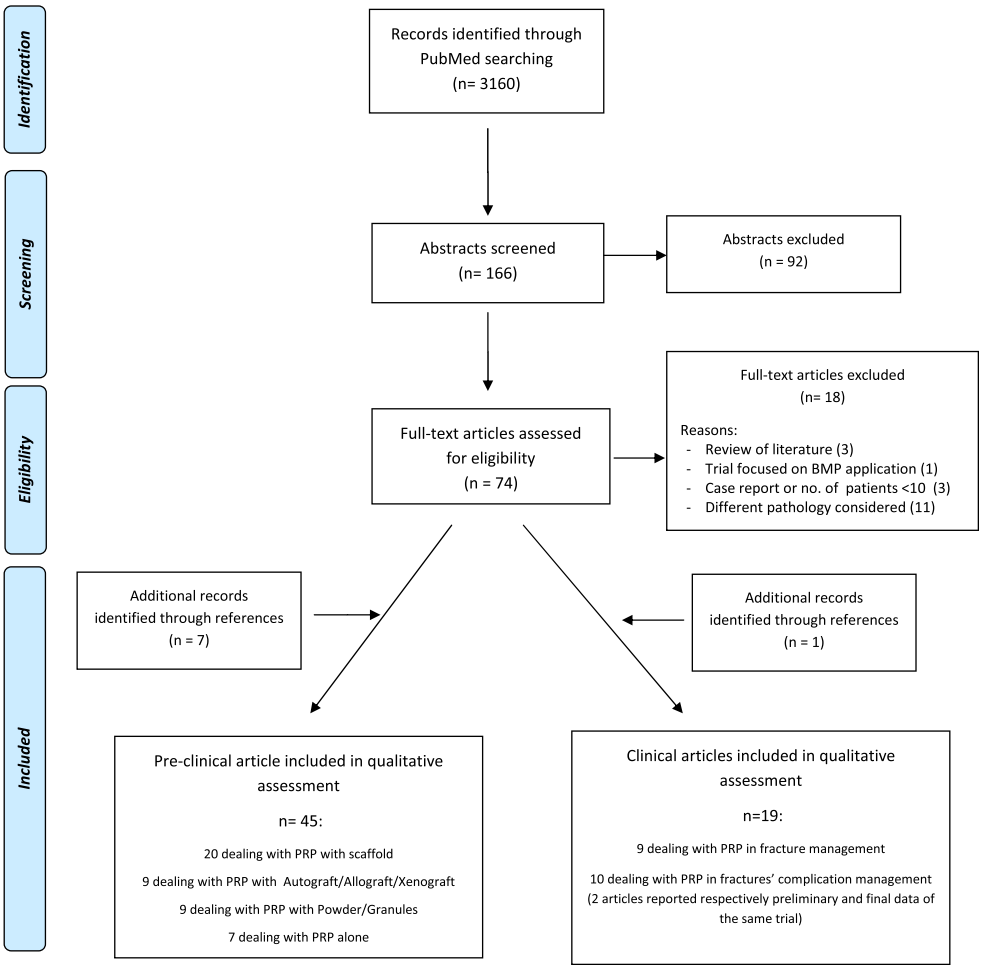

Fig. 1 PRISMA flowchart of the papers' selection process

Finally, with regard to other biological augmentations, 11 papers (24.4 %) reported the use of MSCs as a co-adjuvant osteogenic factor in their studies. BMSCs was the favourite cell source, used in eight papers, followed by bone marrow concentrate (BMC) in two articles, and BMSCs vs. ADSCs were reported in one article.

PRP preparation

Platelet count was reported in studies 32/45 (71 %) with heterogeneous platelet concentration. However, all showed significantly higher levels of platelets than in whole peripheral blood, ranging from 1.1 x to 10.1 x. Sixteen out of 45 papers

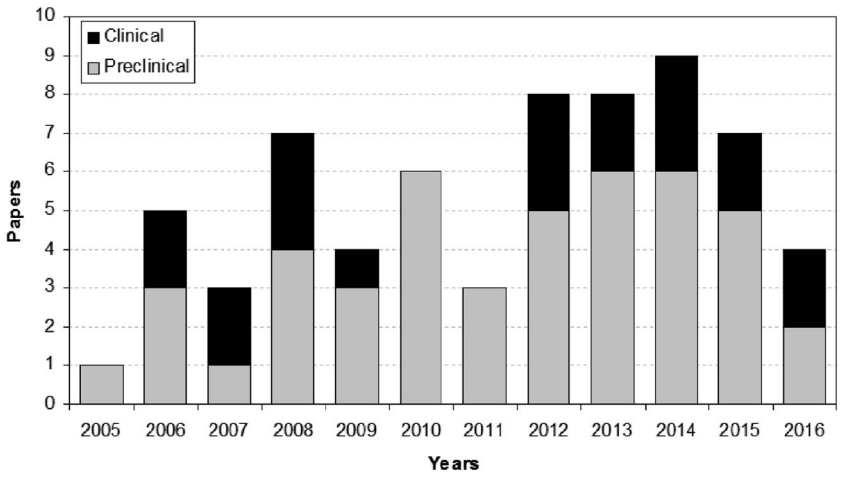

Fig. 2 Pre-clinical and clinical studies published over time

Table 1 Complete details of 45 pre-clinical papers identified in this systematic review

	Publication	Treatment groups	PRP characteristics	Animal model	Protocol	Effects
Scaffold	1. Hokugo et al. 2005 [22]	1) Gelatin 2) Gelatin + PRP 3) Fibrin-glue + PRP 4) PRP 5) Control (untreated)	Platelet concentration: NA Activation: NA Leukocytes: NA Allogenic Fresh/frozen: NA GFs: TGF-β1 and PDGF-BB	Rabbit ulna segmental defect (10 mm)	Hist: + X-Ray: + DEXA: + Positive results for PRP in combination with gelatin	+
	2. Sugimori et al. 2006 [23]	1) AF 2) PRP 3) AF + PRP 4) Control (untreated)	Platelet concentration: 2.5 x Activation: NA Leukocytes: NA Allogenic Fresh/frozen: NA GFs: NA	Rat tibial cylindrical defect (3.5 mm x 4.5 mm)	Hist: + Positive results for PRP in combination with AF	+
	3. Sarkar et al. 2006 [4]	1) Collagen + PRP 2) Collagen	Platelet concentration: 3.5 x Activation: NA Leukocytes: yes Autologous Fresh GFs: NA	Sheep tibial segmental defect (25 mm)	Hist: = X-Ray: = μCT: = Mech: =	=
	4. Rai et al. 2007 [24]	1) PCL/TCP 2) PCL/TCP + PRP	Platelet concentration: NA Activation: calcium chloride\thrombin Leukocytes: NA Allogenic Fresh GFs: NA	Rat femoral segmental defect (8 mm)	Hist: = X-Ray: + μCT: + Mech: +	+
	5. Kasten et al. 2008 [25]	1) CDHA + BMSCs 2) CDHA + PRP 3) CDHA + PRP + BMSCs 4) CDHA 5) ABG 6) Control (untreated)	Platelet concentration: 5.3 x Activation: calcium chloride\thrombin Leukocytes: NA Allogenic Frozen GFs: NA	Rabbit radius segmental defect (15 mm)	Hist: + X-Ray: + μCT: + Mech: +	+
	6. Lysiak-Drwal et al. 2008 [26]	1) BOC + BG 2) BOC + BG + PRP 3) Control (untreated)	Platelet concentration: NA Activation: NA Leukocytes: yes Allogenic/autologous: NA Fresh/frozen: NA GFs: NA	Rabbit femoral cylindrical defect (5 mm)	Hist: +	+
	7. Nair et al. 2009 [1]	1) HASi 2) HASi + BMSCs 3) HASi + BMSCs + PRP	Platelet concentration: 1.5 x Activation: NA Leukocytes: NA Autologous Fresh GFs: NA	Goat femoral segmental defect (20 mm)	Hist: + X-Ray: +	+
	8. Niemeyer et al. 2010 [27]	1) HA/collagen + BMSCs 2) HA/collagen + ASCs 3) HA/collagen + ASCs + PRP 4) Control (untreated)	Platelet concentration: 4.5 x Activation: calcium chloride/thrombin Leukocytes: No Xenogenic Frozen GFs: PDGF-AB and TGF-β1	Sheep tibial segmental defect (30 mm)	Hist: + X-Ray: +	+
	9. Bi et al. 2010 [28]	1) TCP/chitosan 2) TCP/chitosan + PRP 3) Control (untreated)	Platelet concentration: 5.3 x Activation: calcium chloride\thrombin Leukocytes: NA Allogenic/autologous: NA Fresh/frozen: NA	Goat tibia cylindrical defect (12 mm)	Hist: + X-Ray: +	+

Table 1 (continued)

Publication	Treatment groups	PRP characteristics	Animal model	Protocol	Effects
		GFs: TGF-β1, PDGF-BB, bFGF and VEGF			
10. Kon et al. 2010 [29]	1) COL/HA 2) COL/HA + PRP 3) Control (untreated)	Platelet concentration: 3.2 x Activation: calcium chloride Leukocytes: yes Autologous Fresh GFs: TGF-β1, PDGF-AB and IL1	Sheep femoral condyle cylindrical defect (7 mm x 9 mm)	Hist: − X-Ray: −	-
11. Kanthan et al. 2011 [30]	1) PRP 2) Coral 3) Coral + PRP 4) Control (untreated)	Platelet concentration: 2.6 - 4.6 x Activation: calcium chloride/thrombin Leukocytes: NA Autologous Fresh GFs: NA	Rabbit tibial segmental defect (20 mm)	Hist: + X-Ray: +	+
12. Zhang et al. 2011 [31]	1) Bioactive glass 2) Bioactive glass + PRP 3) Control (untreated)	Platelet concentration: NA Activation: calcium chloride/thrombin Leukocytes: NA Allogenic/autologous: NA Fresh/frozen: NA GFs: NA	Rabbit radius segmental defect (15 mm)	Hist: + X-Ray: + μCT: +	+
13. Parizi et al. 2012 [32]	1) Coral 2) Coral + PRP +1 injection 4 days post op 3) Control (untreated)	Platelet concentration: 10.1 x Activation: NA Leukocytes: no Xenogenic Frozen GFs: NA	Rabbit radius segmental defect (10 mm)	Hist: + X-Ray: + Mech: +	+
14. Oryan et al. 2012 [33]	1) HA 2) HA + hPRP 3) Control (untreated)	Platelet concentration: 10.1 x Activation: NA Leukocytes: No Xenogenic Frozen GFs: NA	Rabbit radius segmental defect (10 mm)	Hist: + X-Ray: + Mech: =	+
15. Kasten et al. 2012 [34]	1) CDHA 2) CDHA + PRP 3) CDHA + PRP + BMSCs 4) CDHA + BMSCs 5) CDHA + BMSCs + VEGF (transfected)	Platelet concentration: 5.3 x Activation: calcium chloride/ thrombin Leukocytes: NA Allogenic Frozen GFs: NA	Rabbit radius segmental defect (15 mm)	Hist: + μCT: +	+
16. Kim et al. 2014 [35]	1) Gelatin + PRP 2) Gelatin + SEW2871 micelles 3) Gelatin + SEW2871 micelles + PRP 4) Control (untreated)	Platelet concentration: 8–10 x Activation: calcium chloride Leukocytes: NA Allogenic Fresh/frozen: NA GFs: TGF-β1 and SDF-1	Rat ulnar segmental defect (6 mm)	Hist: + X-Ray: + μCT: +	+
17. Filardo et al. 2014 [36]	1) BioSiC(HaCol) 2) BioSiC (HaCol) + PRP 3) BioSiC (HaCol) + BMSCs	Platelet concentration: 2.8 x Activation: calcium chloride Leukocytes: yes Autologous Fresh GFs: NA	Sheep metatarsus segmental defect (20 mm)	Hist: + X-Ray: =	+
18. Zhong et al. 2014 [37]	1) β-TCP + BMSCs 2) β-TCP + BMSCs + PRP 3) Autogenic ilium (control)	Platelet concentration: NA Activation: calcium chloride/thrombin Leukocytes: NA Autologous	Dog tibia cylindrical defect (10 mm)	Hist: + X-Ray: + Mech: +	+

Table 1 (continued)

	Publication	Treatment groups	PRP characteristics	Animal model	Protocol	Effects
			Frozen GFs: NA			
	19. He et al. 2015 [2]	1) PLGA/CPC 2) PLGA/CPC + PRP	Platelet concentration: 3.3 x Activation: calcium chloride/ thrombin Leukocytes: yes Allogenic Fresh/frozen: NA GFs: NA	Rabbit femoral cylindrical defect (6 mm x 10 mm) Rabbit radius segmental defect (15 mm)	In femoral defect: Hist: + X-Ray: + μCT: + In radial defect: Hist: + X-Ray: + μCT: +	+
	20. Chen et al. 2016 [38]	1) CS + PRP 2) CS 3) PRP 4) BMP-2 (positive control)	Platelet concentration: 8–10 x Activation: calcium chloride/thrombin Leukocytes: NA Autologous Fresh GFs: NA	Rabbit radius segmental defect (12 mm)	Hist: + X-Ray: + : + Superior results for CS + PRP with respect to CS and PRP alone, but similar results with BMP-2	+
Autograft/ allograft/ xenograft	21. Dallari et al. 2006 [39]	1) BMSCs + PRP 2) FDBA + BMSCs 3) FDBA + PRP 4) FDBA + PRP + BMSCs	Platelet concentration: 2.8 x Activation: calcium chloride/thrombin Leukocytes: yes Autologous Fresh GFs: NA	Rabbit femoral cylindrical defect (diameter: 6 mm; depth: 10 mm)	Hist: − Superior results for FDBA + PRP + BMSCs	+
	22. Kroese-Deutman et al. 2008 [40]	1) Ti 2) Ti-Bone chips 3) Ti-Bone chips + PRP	Platelet concentration: 1000–1500 x 10^9 / L Activation: bovine thrombin Leukocytes: NA Autologous Fresh GFs: NA	Rabbit radial segmental defect (15 mm)	Hist: + X-Ray: =	+
	23. Molina-Minano et al. 2009 [41]	1) Autograft 2) PRP 3) Autograft + PRP 4) Control (untreated)	Platelet concentration: NA Activation: calcium chloride Leukocytes: NA Allogenic Fresh GFs: NA	Rabbit tibial cylindrical defect (4 mm)	Hist: = X-Ray: + at 1 m = at 2 m	+
	24. Hakimi et al. 2010 [42]	1) Autograft 2) Autograft + PRP	Platelet concentration: 4.9 x Activation: calcium chloride/thrombin Leukocytes: yes Autologous Fresh GFs: TGF-β1 and PDGF-BB	Pig tibial cylindrical defect (11 x 25 mm)	Hist: + X-Ray: =	+
	25. Nather et al. 2012 [43]	1) Autograft 2) Allograft 3) Allograft + PRP	Platelet concentration: NA Activation: thrombin Leukocytes: NA Autologous Fresh GFs: NA	Rabbit tibial segmental defect (15 mm)	Hist: + at 12 w = at 24 w X-Ray: = for all the aspects related to mineralization and bone volume + for resorption index	+
	26. Kurikchy et al. 2013 [44]	1) Xenograft 2) Xenograft + PRP 3) Control (untreated)	Platelet concentration: 2–3 x Activation: calcium chloride Leukocytes: NA Autologous Fresh GFs: NA	Rabbit femoral cylindrical defect (3 mm)	Hist: +	+

Table 1 (continued)

	Publication	Treatment groups	PRP characteristics	Animal model	Protocol	Effects
	27. Zhang et al. 2013 [45]	1) DPB + PRP + BMSCs 2) DPB + BMSCs 3) DPB + PRP 4) DPB	Platelet concentration: 4 x Activation: calcium chloride\thrombin Leukocytes: NA Allogenic Fresh/frozen: NA GFs: TGF-β1, PDGF, EGF and VEGF	Rabbit radial segmental defect (15 mm)	Hist: + X-Ray: + DEXA: + for new bone tissue quality	+
	28. Schneppendahl et al. 2015 [46]	1) Autograft + PRP 2) Autograft	Platelet concentration: 5.4 x Activation: calcium chloride/thrombin Leukocytes: NA Autologous Fresh GFs: TGF-β1; PDGF-BB; VEGF	Rabbit radial segmental defect (15x4x3 mm)	Hist: + X-Ray: +	+
	29. Park et al. 2016 [47]	1) Synthetic bone 2) CGF 3) PRF 4) Control (untreated)	Platelet concentration: NA Activation: NA Leukocytes: NA Autologous Fresh GFs: TGF-β and VEGF	Dog femur cylindrical defect (8 mm)	Hist: +	+
Powder/granules	30. Rabillard et al. 2009 [48]	1) CaP 2) CaP + PRP	Platelet concentration: 3-6x Activation: calcium Borogluconate + batroxobin Leukocytes: NA Autologous Fresh GFs: NA	Dog ulnar segmental defect (20 mm)	Hist: = X-Ray: = SEM: =	=
	31. Jungbluth et al. 2010 [49]	1) CaP 2) CaP + PRP	Platelet concentration: 4.4 x Activation: calcium chloride/ thrombin Leukocytes: NA Autogenous Fresh GFs: TGF-β1 and PDGF-BB	Pig tibial defect cylindrical defect (11x25 mm)	Hist: + X-Ray: +	+
	32. Batista et al. 2011 [50]	1) β - TCP + PRP 2) β - TCP + BMCs	Platelet concentration: 6.2 x Activation: calcium gluonate Leukocytes: NA Allogenic Fresh GFs: NA	Rabbit tibial cylindrical defect (3.3 mm)	Hist: + X-Ray: = μCT: +	+
	33. Bolukbasi et al. 2013 [51]	1) BCP 2) BCP + PRF 3) PRF 4) Control (untreated)	Platelet concentration: NA Activation: no Leukocytes: NA Autologous Fresh GFs: NA	Sheep tibial cylindrical defect (5 mm)	Hist: +	+
	34. Hakimi et al. 2014 [52]	1) CPG 2) CPG + BMCs 3) CPG + BMCs + PRP 4) Autografts	Platelet concentration: 4.7 x Activation: calcium chloride/thrombin Leukocytes: yes Autologous Fresh GFs: TGF-β1 and PDGF-BB	Pig tibial cylindrical defect (25 x 11 mm)	Hist: + X-Ray: + μCT: + X-Ray superior for CPG + BMSCs + PRP compared to CPG alone and CPG + BMSCs, but similar results compared to autograft	+
	35. Yilmaz et al. 2014 [53]	1) PRF 2) β-TCP 3) β-TCP + PRF	Platelet concentration: NA Activation: no Leukocytes: NA	Pig tibia cylindrical defect (5 mm)	Hist: + Superior results for β-TCP + PRF	+

Table 1 (continued)

	Publication	Treatment groups	PRP characteristics	Animal model	Protocol	Effects
		4) Control (untreated)	Autologous Fresh GFs: NA			
	36. Malhotra et al. 2014 [54]	1) BCP + 33 % PRP 2) BCP + 66 % PRP 3) BCP + 100 % PRP 4) Autografts 5) BCP 6) Control (untreated)	Platelet concentration: 2.9 x Activation: calcium chloride/thrombin Leukocytes: 0.6 x Fibrinogen: 0.6 x Autologous Fresh GFs: TGF-β1	Sheep femoral cylindrical defect (11x20 mm)	Hist: + X-Ray: + μCT: + Highest dose of PRP: greater micro-CT bone volume compared with BCP alone All PRP doses: better histomorphometric parameters vs BCP alone	+
	37. Qi et al. 2015 [55]	1) BMSCs + PRP gel 2) CaP 3) CaP + PRP gel 4) CaP + BMSCs 5) CaP + BMSCs + PRP gel 6) Control (untreated)	Platelet concentration: 6 x Activation: calcium chloride/ thrombin Leukocytes:NA Allogenic Fresh/frozen: NA GFs: NA	Rat femur cylindrical defect (2.5 x 5 mm)	Hist: + X-Ray: + SEM:+ Superior results for CaP + BMSCs + PRP gel	+
	38. Velev et al. 2015 [56]	1) TCP cement 2) TCP cement + PRP 3) TCP cement + GH	Platelet concentration: NA Activation: No Leukocytes: No Autologous Fresh GFs: NA	Rabbit tibia cylindrical defect (6 mm)	Hist: + for qualitative analysis = for quantitative analysis	+
PRP alone	39. Simman et al. 2008 [57]	1) PRP 2) Control (untreated)	Platelet concentration: 2.7 x Activation: calcium chloride/thrombin Leukocytes: NA Allogenic Fresh/frozen: NA GFs: TGF-β1, P-selectin and BMP-2	Rat femoral fracture	Hist: + X-Ray: + Mech: =	+
	40. Gumieiro et al. 2010 [58]	1) PRP 2) Control (untreated)	Platelet concentration: 4x Activation: calcium chloride Leukocytes: NA Allogenic Fresh GFs: NA	Rat tibial cylindrical defect (3 mm)	Hist: + The effect of PRP is present more in the first 14 days	+
	41. Souza et al. 2012 [59]	1) PRP 2) Control (untreated)	Platelet concentration: 338 % Activation: calcium chloride Leukocytes: NA Autologous Fresh GFs: NA	Dog radial fracture with 2 mm gap	Hist: + X-Ray: + DEXA: + Mech: +	+
	42. Chen et al. 2013 [60]	1) High conc PRP 2) Medium conc PRP 3) Low conc PRP 4) PPP 5) Control (untreated)	Platelet concentration (high/medium/low) Activation: calcium chloride/thrombin Leukocytes: NA Allogenic Fresh/frozen: NA GFs: TGF-β1 and PDGF-BB	Rat femoral fracture	Hist: + X-Ray: + Mech: + Superior results for medium conc. of PRP	+
	43. Neves et al. 2013 [61]	1) PRP 2) HBO 3) PRP+ HBO 4) Control (untreated)	Platelet concentration: 2x Activation: No Leukocytes: No Autologous Fresh GFs: NA	Rabbit fibula segmental fracture	Hist: +	+

Table 1 (continued)

Publication	Treatment groups	PRP characteristics	Animal model	Protocol	Effects
44. Hernandez – Fernandez et al. 2013 [62]	1) PRP 2) Control (untreated)	Platelet concentration: 4.6 x Activation: calcium chloride Leukocytes: No Autologous Fresh/frozen: NA GFs: NA	Sheep femur osteotomy model	Hist: = μCT: =	=
45. Guzel et al. 2015 [63]	1) PRP 2) Control (untreated)	Platelet concentration: NA Activation: calcium chloride Leukocytes: No Allogenic Fresh/frozen: NA GFs: NA	Rat femoral fracture	Hist: + Mech: +	+

PRP Platelet- rich Plasma, *AF* Apatite foam, *PCL/TCP* Polycaprolactone-tricalcium phosphate, *CDHA* Calcium deficient hydroxyapatite, *BOC + BG* Bio-Oss Collagen + BioGide Perio membrane, *HASi* Triphasic ceramic-coated hydroxypatite, *HA* Hydroxapatite, *TCP* Tri-calcium phospahte, *COL/HA* Collagen + Hydroxapatite, *hPRP* Human platelet rich plasma, *BioSic* Biomorphic silicon carbide, *β-TCP* Beta-tricalcium phospahte, *PLGA/CPC* Poly (lactic-*co*-glycolic acid) + calcium phosphate cement, *CS* Calcium sulphate, *FDBA* Fried dried bone allograft, *Ti* Titanium, *DPB* Deproteinized bone matrix, *CGF* Concentrated growth factor, *PRF* Platelet rich fibrin, *CaP* Calcium phospahte, *BCP* Biphasic calcium phosphate, *CPG* Calcium phosphate granules, *BMSCs* Bone marrow derived mesenchymal stem cells, *ASCs* Adipose derived stem cells, *BMCs* Bone marrow concentrate, *HBO* Hyperbaric oxygen, *TGF-β1* Transforming growth factor beta 1, *PDGF-BB* Platelet-derived growth factor, *bFGF* Basic fibroblast growth factor, *VEGF* Vascular endothelial growth factor, *IL-1* Interleukin-1, *SDF-1* Stromal cell-derived factor 1, *EGF* Epidermal growth factor, *BMP-2* Bone morphogenetic protein 2, *Hist* Histology analysis, *μCT* Micro-computed tomography, *DEXA* Dual-energy x-ray absorptiometry, *Mech* Mechanical analysis, *NA* Not available

reported information about leukocytes content in PRP, nine of which described the use of leukocyte-rich PRP and seven of leukocyte-poor PRP. Only one paper out of 45 reported fibrinogen content. Fourteen studies analysed PRP for GFs, which included TGF-β1 (in 14), PDGF (in ten), VEGF (in four), bFGF (in one), SDF-1 (in one), p-selectine (in one), BMP-2 (in one), IL-1 (in one) and EGF (in one).

Thirty-two out of 45 papers (71 %) reported information about the use of fresh or frozen PRP: 26 were fresh and six frozen; while 42 (93.3 %) papers reported the type of PRP in terms of autologous (in 24), allogeneic (in 15), and xenogeneic (in three) origin. Finally, PRP activation modality was not reported in 12 studies; among studies where this was specified (33 papers, 73.3 %), 20 (60.6 %) reported a combination of $CaCl_2$ and thrombin, nine (27.3 %) used $CaCl_2$ alone, two (6.1 %) thrombin alone, one (3 %) calcium gluconate, one (3 %) calcium borogluconate and one (3 %) batroxobin.

Pre-clinical findings

Overall positive results were shown in 41 studies (91 %), while three studies (7 %) showed the same results as control and one study (2 %) showed negative effects of PRP use. In particular, among the 20 studies reporting the use of PRP with scaffolds (hydroxyapatite alone or in combination with collagen was the most frequent material used), 18 showed positive results; among the nine papers using bone grafts, all reported good results. Among the nine studies on PRP with powder/granules, eight showed a good outcome. Finally, among the seven studies where PRP was used alone, six reported good results while one study using PRP injections failed to show significant effects. Finally, the use of MSCs always provided a benefit, with PRP further increasing the outcome in all 11 studies.

Further analyses have been performed according to the results of each specific evaluation performed. The histological outcome has been reported in 45 papers, with 38 (84.4 %) showing significant improvement in bone healing. The radiographic outcome was reported in 32/45 papers; among these studies 24 (75.0 %) showed significant bone consolidation. The micro-computed tomography (CT) outcome was reported in 11 papers and significant bone area formation was reported in nine (81.8 %) studies. DEXA analysis was reported only in three studies, with good results in favour of PRP in all papers. Finally, biomechanical tests in regard to torsion, torque and bone strength, were performed in ten papers and seven (70 %) of these studies documented significantly better biomechanical properties.

Clinical studies

A total of 19 clinical trials were found to be eligible for inclusion in the present review: nine dealt with fracture

management [64–72] whereas ten focused on fracture complications [73–83] (i.e. delayed unions or non-unions; Table 2).

Treatment of fractures

Eight out of nine studies were RCTs and one was a retrospective comparative trial [64]. Three studies aimed at understanding the role of PRP in stimulating healing after iatrogenic fractures: two trials in opening-wedge high tibial osteotomy (HTO) [65, 66] and one in tibial distraction osteogenesis for limb lengthening [67]. The other six trials focused on disparate traumatic injuries: two papers on hip fractures [68, 69], one paper on calcaneus fractures [70], one (the retrospective comparative trial) on complex bimalleolar fractures [64], one on tibial pilon fractures [71] and one on intra-articular distal radial fractures [72]. With regard to the application strategies, PRP was locally applied during surgery in eight studies, whereas in one case a delayed PRP injection was applied (3–6 weeks after primary surgery) [70]. PRP was leukocyte-rich in five papers and leukocyte-depleted in two studies, while in two papers authors did not provide specific details about the PRP formulation adopted. In six trials PRP was associated to other "augmentation strategies", such as MSCs and/or bone graft (Table 2).

Overall, eight out of nine papers reported radiologic outcomes: six of them revealed better results with PRP, one found no difference, whereas one paper documented worse radiologic results with the biologic augmentation. Clinical outcomes were reported in only five papers, i.e. functional subjective scores and/or objective measurements and/or complications after PRP treatment: in three cases PRP did not provide any beneficial contribution, whereas in the remaining two studies it contributed to a superior clinical outcome. Finally, only one trial documented histomorphometric results, showing that the addition of PRP to bone chips, even more if combined with BMC augmentation, was able to promote a superior healing of the high tibial osteotomy gap site (Table 2).

Treatment of delayed unions/non unions

Out of the ten studies (11 papers in total) on the application of PRP to manage delayed unions or non-unions [73–83], four used a minimally invasive approach through percutaneous PRP injections, six studies used PRP as topical enhancer during revision surgery, whereas one study reported results of both treatment approaches (Table 2). Only one trial (which was the object of two different publications, one reporting the preliminary results and the other reporting the complete data) was a RCT [73, 74], whereas all the others were case series. In four studies PRP was used together with other augmentation strategies (bone graft or synthetic bone). PRP was leukocyte-rich in two studies and leukocyte-depleted in three studies, while in five trials authors did not provide specific details about the PRP formulation adopted (Table 2).

All but one [75] study considered patients affected by delayed union/non-union in different anatomical districts pooled together (mainly humerus, femur and tibia, which are the most common sites for this kind of complication), thus reducing the homogeneity of the cohorts of patients. With regard to the outcome, two case series (where PRP was injected at the site of non-union or delayed union) failed to documented a beneficial effect of the biological stimulation [76, 77]. The other reports suggested instead a positive role of PRP, although the only RCT documented a clear advantage of bone morphogeneic protein 7 (BMP-7) over PRP in stimulating bone healing (Table 2) [73, 74].

Discussion

This systematic research of the literature documented a growing interest on PRP use for bone disorders, with an increasing number of papers published over time which show a complex scenery and more controversial results than previously thought.

In fact, while basic science suggests several favourable potential effects of PRP for bone healing, and platelet concentrates have also been successfully used in other medical fields to enhance bone and soft tissue regeneration [5, 84–86], the evidence on its real benefit is questionable. The first phase of the literature analysis focused on preclinical in-vivo evidence, which should allow us to understand potential and indications for PRP use. This systematic review documented 45 papers reporting heterogeneous models both in terms of animal model and defect type chosen. This is an important factor to consider in terms of results transferability to the clinical field. In fact, although several models are appropriate for the evaluation of bone regeneration, not all of them closely reproduce human tissue characteristics. Chosen models should present physiological and pathophysiological analogies to favour results transferability: for this reason larger models more closely resemble the human condition [87]. For example, pig models present bone features similar to humans in terms of mineral density, concentration and healing capacity, with a 1.2 to 1.5 mm per day growth rate, similar to bone regeneration capacity in humans [88]. Among the studies found in this systematic review, only four chose the pig model, while the majority adopted smaller models with rabbits or even rats. Moreover, these animal models analysed surgically-created acute lesions which were treated immediately, oppositely to what happens in the clinical practice. Despite the inherent limits of these models in terms of transferability to humans, these studies still showed overall promising findings, with a positive outcome documented in 91.1 % of the papers. However, a more in depth analysis shed some doubts on the

Table 2 Synopsis of clinical trials dealing with PRP application in fractures and non-union/delayed union management

Fractures

Publication	Study design	N of patients	Pathology	Therapeutic protocol	Platelet count and leukocytes	Activation	F-up	Main findings
Namazi H et al., Orthop Tramatol Surg Res.2016 [72]	Randomized controlled trial	30 patients: *n* = 15 percutaneous fixation + PRP *n* = 15 percutaneous fixation	Distal radius fracture	Intra-articular injection of PRP immediately after percutaneous pinning fixation	Platelet count: NA Leukocytes: no	no	6 months	PRP has significant effect on reduction of pain and functional recovery
Rodriguez-Collazo ER et al., Strategies Trauma Limb Reconstr. 2015 [64]	Case series	20 patient with relevant comorbidities *n* = 10 BMC + PRP + DBM+ EF n =10 DBM+ EF (historical controls)	Bimalleolar fractures	PRP and BMC mixed with DBM and injected locally at the fracture site	Platelet count: NA Leukocytes: yes	no	18 months	External fixation + DBM, BMA and PRP promoted fracture healing of the distal tibia and fibula in patients with significant co-morbidities
Samy AM Int orthop. 2015 [68]	Randomized controlled trial	60 patients: n =30 screw fixation n =30 screw fixation + PRP	Femoral neck fracture	PRP locally applied during surgery	Platelet count: NA Leukocytes: no	n.a.	48 months	PRP was a beneficial adjuvant to the classical internal fixation technique: both radiologic and clinical outcome were better in PRP group
Lee Dh et al., Clin Orthop Relat Res.2014 [67]	Randomized controlled trial	20 patients: *n* = 10 external fixation n =10 external fixation + BMC + PRP	Tibal distraction osteogenesis (limb lenghtening)	PRP + BMC locally injected at the osteotomy gap site	Platelet count: NA Leukocytes: yes	no	24 months	BMC + PRP significantly improved bone healing in distraction osteogenesis of the tibia, allowing earlier return to weightbearing
Griffin XL et al., BMJ Open. 2013 [69]	Randomized controlled trial	200 patients *n* = 99 screw fixation *n* = 101 screw fixation + PRP	Femoral neck fracture	PRP locally applied during surgery	Platelet count: NA Leukocytes: yes	no	12 months	There was no significant clinical difference following to PRP therapy. Only a shorter hospital stay was registered for PRP group.
Liebergall M et al., Mol. Ther.2013 [71]	Randomized controlled trial	24 patients *n* =12 ORIF *n* = 12 ORIF + DBM+ MSCs + PRP	Disttal tibial fractures	PRP and MSCs mixed with DBM and injected at the fracture site 3–6 weeks after primary surgery	Platelet count: 1.10 X 10^6 per mm^3 Leukocytes: NA	n.a.	12 months	The combination of PRP + MSCs + DBM is a safe therapeutic option and contributed to reduce the time of bone fusion
Wei LC et al., J Orthop Res.2012 [70]	Randomized controlled trial	254 patients n =101 ORIF + autograft n =85 ORIF + allograft + PRP n =90 ORIF + allograft	Calcaneal fractures	PRP mixed with bone allograft and applied locally during ORIF	Platelet count: 780000 platelets/ uL Leukocytes: NA	Thrombin + CaCl	72 months	PRP-augmented allografts showed better radiological results compared to allograft alone
Perbooms JC et al., Int Orthop. 2012 [66]	Randomized controlled trial	41 patients n =20 bone chips + PRP n =21 bone chips	High tibial osteotomy	PRP mixed with the bone chips and put in the osteotomy gap site	Platelet count: NA Leukocytes: yes	Thrombin	12 weeks	PRP provided detrimental effects with significant lower bone density around the osteotomy wedge at one and 12 weeks after surgery
Dallari D et al., J bone Joint surg Am. 2007 [65]	Randomized controlled trial	33 patients n =11 bone chips + PRP *n* = 12 bone chips + PRP+ BMC n =10 bone chips	High tibial osteotomy	PRP mixed with the bone chips and put in the osteotomy gap site	Platelet count: 1×10^6 per mm^3 Leukocytes: yes	Thrombin	12 months	PRP increased the osteogenic potential of the bone chips and provided better outcome in terms of radiologic healing of the osteotomy site. Further beneficial effects with the addition of BMC
Non-union/delayed union								
Malhotra R et al., Musculoskelet Surg. 2015 [78]	Case series	94 patients	Long bone non union	PRP injection at the site of non-union	Platelet count: minimum 2 x 10^6 per mm^3 Leukocytes: no	n.a.	4 months	PRP was a safe and effective treatment for managing non-unions
Say F et al.,	Case series	20 patients			Platelet count: NA	CaCl	12 months	

Table 2 (continued)

Fractures								
Acta Chir Orthop Traumatol Cech. 2014 [77]			Long bone non union/delayed union	3 PRP injections at the site of non-union at one week interval	Leukocytes: no			PRP had no additional healing potential
Golos J et al., Ortop Traumatol Rehabil. 2014 [79]	Case series	132 patients	Long bone non union	PRP injection at the site of non-union	Platelet count: NA Leukocytes: n.a.	n.a.	4 months	PRP was effective in treatment of delayed union of long bones
Tarallo L et al., Eur J Orthop Surg Traumatol. 2012 [75]	Case series	10 patients	Isolated non-union of the ulna	PRP + bone autograft locally applied during revision surgery	Platelet count: NA Leukocytes: yes	Thrombin + calcium gluconate	minimum 3 months – maximum 36 months	High rate of clinical and radiologic healing with the biologic augmentation
Galasso O et al., J Orthop Traumatol. 2008 [80]	Case series	22 patients	Long bone non union	PRP applied locally during revision surgery	Platelet count : NA Leukocytes: NA	Batroxobin and CaCl	13 months	Intramedullary nailing and PRP produced comparable results with less complications
Sanchez M et al., J Orthop Trauma. 2009 [81]	Case series	15 patients	Non hypertrophic non union/delayed unions	PRP mebrane locally applied during revision surgery (sometimes with bone graft) PRP injection at the site of non-union (without surgery)	Platelet count: NA Leukocytes: no	CaCl	8 months	PRP was clinically safe and enhanced the healing of non-hypertrophic non unions
Mariconda M et al., J Orthop Trauma. 2008 [76]	Case series	20 patients	Long bone non union	PRP locally applied during revision surgery (external fixation)	Platelet count: NA Leukocytes: NA	Thrombin + calcium gluconate	9 months	The study failed to show any clinical usefulness of PRP
Bielecki T et al., Eur Surg Res. 2008 [82]	Case series	32 patients	Long bone non union	PRP injection at the site of non-union	Platelet count: 241 ± 64 X 10^6 per mm^3 Leukocytes : yes	Thrombin + CaCl	6 months	PRP injection was a valid strategy to obtain union
Calori et al., Injury. 2008 [73] + Calori et al., Injury. 2006 [74]	Randomized controlled trial	120 patients *n* = 60 BMP-7 *n* = 60 PRP	Long bone non union	PRP or BMP-7 locally applied during revision surgery (sometimes with bone graft)	Platelet count: 1.582 x 10^6 per mm^3 Leukocytes: NA	CaCl	mean 12.3 months	BMP-7 is significantly more effective than PRP in promoting bone healing, both at radiolofic and clinical evaluation
Chiang CC et al., J Trauma. 2007 [83]	Case series	12 patients	Long bone non union	PRP + bone graft applied locally during revision surgery	Platelet count: n.a. Leukocytes: NA	Thrombin + CaCl	mean 32.4 months	There was a beneficial potential of PRP in treating non unions

PRP Platelet-rich Plasma, *ORIF* open reduction and internal fixation, *EF* External Fixation, *DBM* Demineralized Bone Matrix *BMC* Bone Marrow Concentrate, *BMP-7* Bone Morphogeneic Protein-7, *MSC* Mesenchymal Stem cells, *NA* Not available

real potential of PRP. In fact, looking at the specific evaluations performed, the percentage of success decreased. While histological analysis showed an improvement in 84.4 % of the experiments, imaging analysis showed a lower success, with a 75.0 % success rate documented through radiological analysis. Moreover, the higher quality of the regenerated tissue should lead to a stronger tissue, but biomechanical analysis could prove superior results related to PRP use only in 72.7 % of the studies. This is an important factor, because it raises some caution on the interpretation of the positive results of several studies based on different and more successful outcomes. An improved regeneration should allow for higher biomechanical properties to be considered significant, otherwise the real usefulness of this biological augmentation remains questionable. Besides the lack of biomechanical evaluations, performed only in a minority of preclinical studies, other aspects hinder the understanding of PRP potential. These lie in the limitations of the current preclinical literature, in terms of extreme heterogeneity of the published studies as well as often poor study quality.

Most of the studies fail to report key aspects that may influence the final outcome and hinder a correct interpretation of the results: platelet concentrations, leukocyte components, activation modality etc. are often overlooked. Even though several products with a wide range of cell concentration are included in the PRP family [89], the number and type of cells applied is not a secondary aspect. A platelet concentration of approximately (1,000,000/μl) has been linked to positive biological effects in bone regeneration by Weibrich et al. [90]. Chen et al. [60] showed how a medium concentration of PRP ($2.65 \pm 0.2 \times 10^9$/ml) induced osteogenic differentiation of BMSCs and improved fracture healing, while a high concentration of PRP ($8.21 \pm 0.4 \times 10^9$/ml) inhibited osteogenic differentiation of BMSCs and delayed callus remodelling in a rat femoral osteotomy. Other than the importance of platelet number, Perut et al. [21] underlined the contribution of other cells to the overall effect: leukocyte-rich PRP induced significantly higher proliferation of BMSCs compared to leukocyte-depleted PRP. Moreover, other studies emphasized the importance of further aspects related to PRP, such as storage or activation modality that, together with the autologous or allogeneic nature, the characteristics of the donor and the preparation method etc., may influence the molecules released and the biological results of platelet concentrates [88, 91, 92]. PRP effects may also depend on the treatment condition, i.e. the application modality, in particular in terms of injective or surgical delivery (a more solid state with more fibrin may imply a different release of the GFs from the fibrin net [93]), and even more with combined augmentation procedures. Preclinical studies are highly heterogeneous in terms of materials combined with PRP, which has been suggested to influence cell-signalling molecules that promote osteogenesis [20] and, in the end, the success rate [5]. Moreover, PRP has also been combined with MSCs: while overall results of the combined treatment seemed positive, the independent contribution of PRP remains questionable.

All these factors contribute to the complexity of the preclinical findings and leave many open questions on the optimization and transferability of PRP potential for humans use. Overall clinical findings confirm the preclinical scenery. The lack of clear indications on the best way to apply platelet concentrates is reflected by an extreme heterogeneity in terms of PRP preparations, as well as in targets and delivery methods. Moreover, the quality of the studies is limited: as for the preclinical field, many key parameters are omitted, the study design is poor and even for randomized trial low patients numbers and the presence of concomitant confounding factors hinder the possibility to have clear results. This is a key aspect since a tendency to report better results with lower quality studies compared to more scientific robust ones has already been reported [5]. Finally, it has to also be emphasized for clinical studies that not all positive results may be clinically significant. In fact, the rational of a biological augmentation is to favour a faster recovery. Earlier weight bearing and mobilization may lead to fewer adhesions, higher postoperative range of motion and earlier return to physical activity [88]. To this aim, positive histological or imaging findings may be insufficient if not coupled by a significant increase in biomechanical quality of bone. The analysis of the clinical studies in terms of documented clinical benefit shows an even lower outcome than what is suggested by imaging findings, which further questions the real benefit provided by PRP use to favour bone regeneration in the clinical practice.

Thus, while positive findings have been suggested by the pre-clinical literature, a more in depth evaluation shed some doubts on the real role of PRP, which are confirmed by the limited benefits documented in the human experience. Currently, there is no evidence to support the routine use of PRP to enhance bone healing. Therefore, until trials with high methodological quality will allow the optimization of its biological potential and clearly prove results and indications, PRP use should be restricted to controlled studies investigating its real benefit for the treatment of bone pathologies.

Conclusions

This systematic research of the literature documented a growing interest on PRP use for bone disorders. While the overall pre-clinical results seem to support the benefit of PRP in 91.1 % of the studies, a more in depth analysis underlines a lower success rate, with a positive outcome of 84.4 % in terms of histological analysis, and even lower when considering radiological and biomechanical analysis, 75.0 % and 72.7 %, respectively. This is reflected by controversial findings also documented in the clinical literature, where the real

benefit of PRP use to treat fractures and non-unions is questionable. Overall, the available literature presents major limitations in terms of low quality and extreme heterogeneity, which hamper the possibility to optimize PRP treatment and translate positive preclinical findings on its biological potential to favour bone healing into a real clinical benefit.

Acknowledgment This work was partially supported by European Union Seventh Framework Programme FP7- PEOPLE-2013-ITN under Grant Agreement no. 607051-Bioinspire: Training program on new bioinspired bone regeneration.

Compliance with ethical standards

Conflict of interest Elizaveta Kon
Zimmer-Biomet (USA): Paid presenter or speaker
Cartiheal (Israel): Paid consultant; Stock or stock options
Fidia (Italy): Paid presenter or speaker
Finceramica (Italy): Paid presenter or speaker
International Cartilage Repair Society: Board or committee member
Journal of Experimental Orthopedics: Editorial or governing board
Giuseppe Filardo
Zimmer-Biomet (USA): Institutional Support
Cartiheal (Israel): Consultant and Institutional Support
Fidia (Italy): Consultant and Institutional Support
Finceramica (Italy): Consultant and Institutional Support
Green Bone (Italy): Consultant and Institutional Support
DSM Biomedical (USA): Institutional Support
IGEA Clinical Biophisic: Institutional Support
PIRAMAL/ Smith-Nephew: Institutional Support
All the other authors declare that there are no competing interests regarding the publication of this paper.

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