Concentrations of Blood Components in Commercial Platelet-Rich Plasma Separation Systems

A Review of the Literature

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Background: Platelet-rich plasma (PRP) has proven to be a very safe therapeutic option in the treatment of tendon, muscle, bone, and cartilage injuries. Currently, several commercial separation systems are available for the preparation of PRP. The concentrations of blood components in PRP among these separation systems vary substantially.

Purpose: To systematically review and evaluate the differences between the concentrations of blood components in PRP produced by various PRP separation systems.

Study Design: Systematic review.

Methods: MEDLINE/PubMed, the Cochrane Central Register of Controlled Trials (CENTRAL), and EMBASE were searched for studies that compared the concentrations of blood components and growth factors in PRP between various separation systems and studies that reported on the concentrations of blood components and growth factors of single separation systems. The primary outcomes were platelet count, leukocyte count, and concentration of growth factors (eg, platelet-derived growth factor–AB [PDGF-AB], transforming growth factor–β1 [TGF-β1], and vascular endothelial growth factor [VEGF]). Furthermore, the preparation protocols and prices of the systems were compared.

Results: There were 1079 studies found, of which 19 studies were selected for inclusion in this review. The concentrations of platelets and leukocytes in PRP differed largely between, and to a lesser extent within, the studied PRP separation systems. Additionally, large differences both between and within the studied PRP separation systems were found for all the growth factors. Furthermore, preparation protocols and prices varied widely between systems.

Conclusion: There is a large heterogeneity between PRP separation systems regarding concentrations of platelets, leukocytes, and growth factors in PRP. The choice for the most appropriate type of PRP should be based on the specific clinical field of application. As the ideal concentrations of blood components and growth factors for the specific fields of application are yet to be determined for most of the fields, future research should focus on which type of PRP is most suitable for the specific field.

Keywords: platelet-rich plasma; systematic review; concentration; platelets; leukocytes; growth factors

Platelet-rich plasma (PRP) is a small volume of autologous blood plasma that has been enriched with blood-derived platelets.[21] PRP is considered to have beneficial effects on many healing processes as a result of the growth factors contained in the platelet alpha-granules.[43] The use of PRP for clinical applications in periodontal and oral surgery, maxillofacial surgery, plastic surgery, and the treatment of chronic skin and soft tissue ulcers has been extensively investigated.[22,33,47,53] PRP has proven to be a very safe therapeutic option; complications are rarely reported, as PRP is derived from autologous blood.[42] In orthopaedic surgery and sports medicine, the use of PRP has been of increasing interest over the last decade. PRP has shown to have a beneficial effect on the healing of tendon, muscle, bone, and cartilage injuries.[15,58] Clinical studies on the

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efficacy of PRP in the treatment of symptomatic knee osteoarthritis[31,39,52] and chronic tendinopathy such as patellar tendinopathy[14,17] and lateral epicondylitis[19,23,40,41] have shown beneficial effects of PRP injections.

Currently, several commercial separation systems are available for the preparation of PRP.[15] The concentrations of blood components in PRP (platelets, leukocytes, and growth factors) among these separation systems vary substantially.[15] Studies comparing the differences in blood components in PRP from these separation systems report varying outcomes in terms of the concentrations of blood components and growth factors.[7,36,50] To gain more insight into the differences between the concentrations of blood components and growth factors in PRP produced by the different separation systems, we conducted a systematic review of the literature on studies investigating the blood components and growth factors in PRP.

METHODS

Inclusion Criteria

The literature search performed for this review was limited to studies that compared the concentrations of blood components and growth factors in PRP between different PRP separation systems and studies that reported on the concentrations of blood components and growth factors of single PRP separation systems. We only included studies investigating human blood taken from healthy adult (age >18 years) volunteers. The literature search was limited to articles in the English, German, French, and Dutch languages. Only studies reporting on PRP separation systems that are currently commercially available were included.

Outcome Measures

This review primarily focused on the platelet count, leukocyte count, platelet enrichment factor ([platelet concentration in PRP]/[platelet concentration in whole blood]), and growth factors (platelet-derived growth factor–AB [PDGF-AB], platelet-derived growth factor–BB [PDGF-BB], transforming growth factor–β1 [TGF-β1], vascular endothelial growth factor [VEGF], epidermal growth factor [EGF], fibroblast growth factor–2 [FGF-2], hepatocyte growth factor [HGF], and insulin-like growth factor [IGF]). Furthermore, the preparation protocols (amount of whole blood needed, number of centrifugations, time of centrifugation) and prices of the different PRP separation systems were compared.

Search Strategy

We searched MEDLINE/PubMed, the Cochrane Central Register of Controlled Trials (CENTRAL), and EMBASE up until March 2017 to identify relevant studies concerning the concentrations of blood components in PRP. There were no constraints based on the publication status. In MEDLINE, the following search strategy was used and modified for other databases:

1. Humans
2. Platelet-rich plasma
3. 1 AND 2
4. Blood platelets or platelet count
5. Leukocytes or leukocyte count
6. Platelet-derived growth factor
7. 3 AND 4 AND 5
8. 3 AND 6
9. 7 OR 8

The search was performed by one of the authors (B.W.O.). References of retrieved publications were also used to add studies potentially meeting the inclusion criteria that were missed by the electronic search. Abstracts from scientific meetings and review articles were excluded.

Review Process

To identify relevant articles for this review, the title and abstract of the articles found by the abovementioned search strategy were reviewed. After selection, the full articles were reviewed for definitive selection. All identified studies were independently reviewed by 2 reviewers (B.W.O. and J.C.P.) for inclusion using the abovementioned criteria. In case of disagreement, a third reviewer (A.J.H.V.) was consulted to resolve the disagreement.

Data Collection

The following data were extracted from the included trials: study design (comparative study or study describing one separation device), study characteristics (eg, number of blood samples), concentration analysis methods, type of outcome, results of the study, and main conclusion(s) of the study. This information was extracted by one author (B.W.O.). If necessary, authors were contacted for additional information about their specific article.

The companies producing the PRP separation systems were contacted to gain information about the specific preparation protocols. In case a company did not respond to the request, the literature was searched for the preparation protocol.

Statistical Analysis

First, 95% CIs were calculated for each of the blood components studied in the included studies using the mean concentration, SD, and number of samples. The following formula was used: $x \pm \gamma \times \frac{\sigma}{\sqrt{n}}$, where x is the mean concentration, γ the critical value of the t distribution based on the sample size of the study, σ the SD, and n the number of samples studied. Forest plots were created using the mean and 95% CI. Differences in concentrations within and between the different PRP separation systems were explored informally by the eyeball test. Additional statistical analyses of differences within and between the different separation systems were not conducted. As a substantial part of the data in the included studies was presented in graphs, which led to missing quantitative data, descriptive results of the studies that compared ≥2 PRP preparation systems were summarized

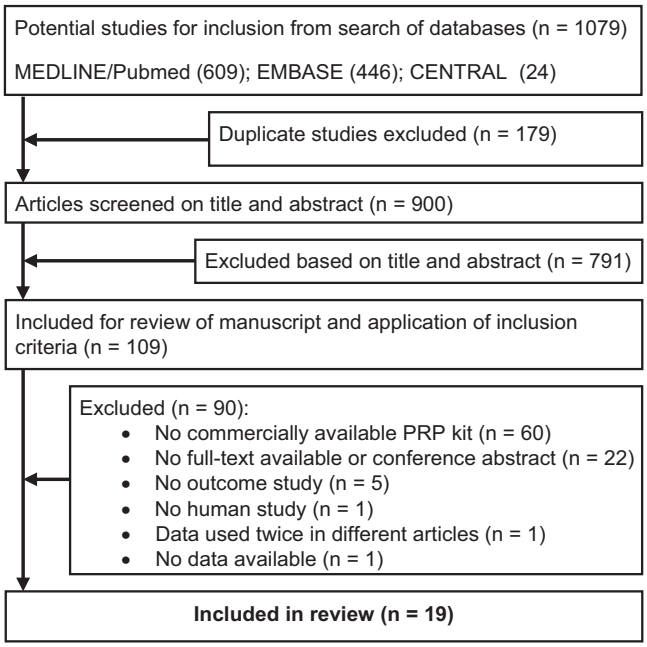

Figure 1. Flow diagram of the search process.

in a table. Analyses were conducted in SPSS (version 15.0; SPSS) and Excel (Microsoft).

RESULTS

Search Results

The search was performed on September 17, 2016, with a final search update to check for recently published relevant articles on April 11, 2017. The search of MEDLINE/PubMed, the Cochrane Central Register of Controlled Trials (CENTRAL), and EMBASE databases provided 1079 citations, of which 179 were duplicates. After reviewing the titles and abstracts of the 900 remaining studies, 791 studies were excluded for not meeting the inclusion criteria. The articles of the remaining 109 studies were reviewed, after which 90 studies were excluded: 19 studies were selected for inclusion in this review (Figure 1). No additional studies were found by checking the references of the selected articles.

Characteristics of Included Studies

The characteristics of the included studies are summarized in Table 1. Fourteen studies compared the concentrations of blood components in PRP between different PRP separation systems. In 8 studies, commercially available separation systems were compared. Five studies reported the concentrations of blood components of single separation systems. The number of samples analyzed varied between 3 and 102. Ten different commercially available separation systems were studied. The GPS III system (Zimmer Biomet) was studied the most, with 10 articles in total, followed by the ACP system (Arthrex), which was studied in 5 articles. The Endoret (BTI Biotechnology Institute), Magellan (Arteriocyte), and SmartPrep (Harvest Technologies) systems were all studied in 3 articles; the Cascade (Musculoskeletal Transplant Foundation) and RegenPRP (RegenLab) systems were studied in 2 articles; and the Prosys (Prodizen), KYOCERA (Kyocera Medical), and GLO (Glofinn Oy) systems were only studied in 1 article.

Outcome Measures

The platelet concentration was the most studied outcome measure, studied in 13 of 17 articles. Other outcome measures were the leukocyte concentration (12/17), red blood cell concentration (5/17), and platelet enrichment factor (7/17). With regard to growth factors, TGF-β1 was studied the most (9/17), followed by PDGF-AB and VEGF (both 8/17). Other reported growth factors were IGF (4/17), PDGF-BB (3/17), EGF (3/17), HGF (2/17), and FGF-2 (1/17). As TGF-β1, PDGF-AB, and VEGF were by far the most studied growth factors, further statistical analyses were only performed for these 3 growth factors.

PRP Separation Systems

The preparation protocols for the different PRP separation systems are summarized in Table 2. The majority of the systems use a dual spin method (6/10). Both the centrifugal force (range, 350-2008*g*) and the total centrifugation time (range, 5-21 minutes) differed largely between the systems. Also, a wide variation in price per kit (range, US$50-US$500) was found between the systems.

Laboratory Results

Platelets, Leukocytes, and Platelet Enrichment Factors. The concentrations of platelets and leukocytes found in the included studies are presented in Figure 2. The concentration of platelets in PRP differed largely between, and to a lesser extent within, the studied PRP separation systems. The highest concentration of platelets was produced by the Cascade system; the lowest concentration of platelets was produced by the ACP system. Regarding the concentration of leukocytes in PRP, large differences were found between, but not within, the separation systems. The highest concentration of leukocytes was found in PRP produced by the GPS III system; PRP produced by the ACP system contained the lowest number of leukocytes. Although only reported in 4 studies, large differences between PRP separation systems were found for the platelet enrichment factor. The highest platelet enrichment factors were found for the GPS III and SmartPrep systems (3.93[32] and 3.79,[30] respectively) and the lowest for the ACP, RegenPRP, and Cascade systems (1.31,[32] 1.59,[32] and 1.62,[7] respectively).

Growth Factors. The concentrations of the growth factors PDGF-AB, TGF-B1, and VEGF found in the included studies are presented in Figure 3. Large differences both between and within the studied PRP separation systems were found for all the growth factors. Additionally, no differences in the concentrations of PDGF-AB and TGF-B1

TABLE 1
Characteristics of the Included Studies[a]

	No. of Samples	No. of PRP Kits Studied	PRP Kits Studied	Outcome Measures
Anitua et al[3] (2013)	3	1	Endoret	PEF, WBCC, PDGF-AB, VEGF, HGF, IGF
Castillo et al[7] (2011)	5	3	GPS III, Cascade, Magellan	PC, WBCC, RBCC, PEF, PDGF-AB, PDGF-BB, TGF-B1, VEGF, PCE, FC
Dragoo et al[13] (2012)	40	1	GPS III	PDGF-BB, TGF-B1, VEGF, IGF
Evanson et al[16] (2014)	102	1	ACP	PC, WBCC, RBCC, PDGF-AB, PDGF-BB, TGF-B1, VEGF, EGF, FGF-2, HGF, IGF
Everts et al[18] (2008)	20	1	Magellan	PC, WBCC, PEF
Hamilton et al[24] (2015)	10	1	GPS III	PC, WBCC, PDGF-AB, HGF, IGF, VEGF
Howard et al[25] (2014)	4	2	Cascade, SmartPrep	PC, PEF, PDGF-AB, TGF-B1
Kaux et al[27] (2011)	6	1	GPS III	PC, WBCC, RBCC
Kaux et al[26] (2011)	5	1	GPS III	WBCC, RBCC, PEF
Kushida et al[29] (2014)	5	3	GLO, KYOCERA, Magellan	PC, PDGF-AB, TGF-B1, VEGF
Leitner et al[30] (2006)	3	1	SmartPrep	PC, WBCC, RBCC
Magalon et al[32] (2014)	10	3	ACP, GPS III, RegenPRP	PC, WBCC, PEF, PDGF-AB, TGF-B1, VEGF, EGF, PCE
Mazzocca et al[36] (2012)	8	2	ACP, GPS III	PC, WBCC, RBCC, PDGF-AB, TGF-B1, VEGF, EGF, FGF-2, HGF, IGF
Mazzucco et al[37] (2009)	Not provided	1	RegenPRP	PC, PEF, PDGF-BB, TGF-B1, VEGF, EGF, IGF
Oh et al[46] (2015)	14	3	ACP, GPS III, Prosys	PC, WBCC
Schar et al[51] (2015)	11	1	GPS III	TGF-B1, VEGF
Sundman et al[54] (2011)	11	2	ACP, GPS III	PC, WBCC, PEF
Weibrich et al[56] (2005)	51	1	Endoret	PC, WBCC, PDGF-AB, TGF-B1, PCE
Weibrich et al[57] (2012)	54	2	Endoret, SmartPrep	PC, WBCC, PDGF-AB, TGF-B1, IGF

[a]EGF, epidermal growth factor; FC, fibrinogen concentration; FGF-2, fibroblast growth factor–2; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; PC, platelet concentration; PCE, platelet capture efficiency; PDGF-AB, platelet-derived growth factor–AB; PDGF-BB, platelet-derived growth factor–BB; PEF, platelet enrichment factor; PRP, platelet-rich plasma; RBCC, red blood cell concentration; TGF-B1, transforming growth factor–B1; VEGF, vascular endothelial growth factor; WBCC, white blood cell concentration.

TABLE 2
Preparation Protocols and Costs for the Different PRP Separation Systems[a]

			Centrifugal Force, *g*		Centrifugation Time, min			
	Type of System	Whole Blood Volume, mL	First Spin	Second Spin	First Spin	Second Spin	Final Volume of PRP, mL	Cost/Kit, $
ACP	Plasma	11	350	—	5	—	2.0-5.0	150
GPS III	Buffy coat	54	1100	—	15	—	6.0	350
Cascade	Plasma	9	1100	1450	6	15	2	NP
Endoret	NP	9	580	—	8	—	2.0	NP
GLO	Buffy coat	9	1200	600	5	2	0.6	50-75
SmartPrep	Buffy coat	60	1250	1050	14	7.0-10.0	NP	NP
KYOCERA	NP	20	600	2000	7	5	2	60
Magellan	Buffy coat	60	610	1240	4	6	3	500
Prosys	NP	30	1660	2008	3	3	3	NP
RegenPRP	NP	8	1500	—	5	—	4	NP

[a]NP, not provided by manufacturer (unknown); PRP, platelet-rich plasma.

were found between the higher (GPS III, SmartPrep, and Magellan) and lower platelet-yielding devices (ACP, Cascade, Endoret, and RegenPRP) as for the higher (GPS III, SmartPrep, Magellan, and RegenPRP) and lower leukocyte-yielding devices (ACP and Cascade). However, the concentration of VEGF tended to be higher in PRP produced by systems that yield higher concentrations of platelets and leukocytes (GPS III and Magellan).

Comparative Studies

As not all selected studies provided exact data, descriptive results of the studies comparing ≥2 PRP separation systems were used.[7,25,29,32,36,46,54,57] The ACP and GPS III were the only systems that have been compared in more than 1 study: the concentrations of platelets, leukocytes, and growth factors were significantly higher in favor of

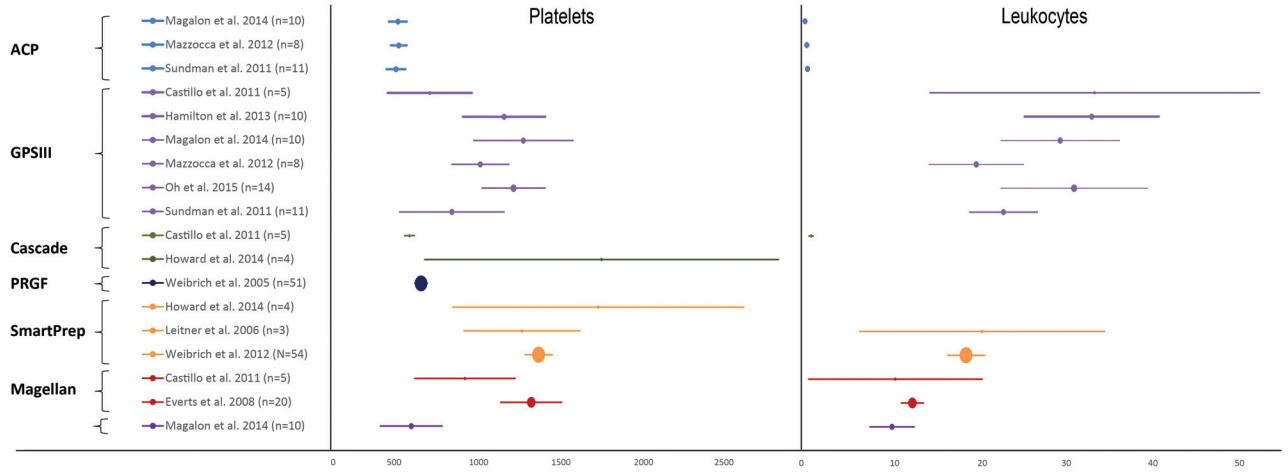

Figure 2. Concentrations of platelets ($\times 10^3$ μL) and leukocytes ($\times 10^3$ μL) found in the included studies.

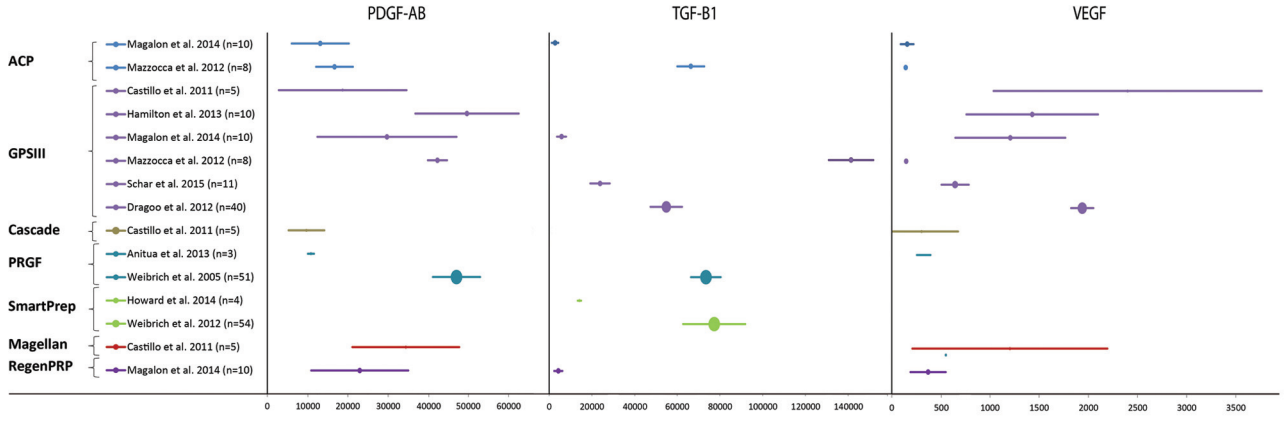

Figure 3. Concentrations of PDGF-AB (pg/mL), TGF-B1 (pg/mL), and VEGF (pg/mL) found in the included studies.

the GPS III.[32,36,46,54] Overall, the ACP showed lower platelet and leukocyte concentrations in studies comparing the ACP with systems other than the GPS III; the concentrations of growth factors, however, were largely comparable.[32,46] The GPS III, on the other hand, showed a significantly higher concentration of leukocytes compared with other systems.[7,32,46] Furthermore, the GPS III produced a higher concentration of platelets than the RegenPRP and Prosys,[32,46] but no significant differences in the platelet concentration were found between the GPS III and the Cascade and Magellan.[7] The concentrations of growth factors did not significantly differ in most of the studies.

DISCUSSION

The objective of this review was to assess the differences between the concentrations of blood components and growth factors in PRP between the various PRP separation systems. The findings in this review demonstrate that there is a large heterogeneity among various systems regarding the concentrations of platelets and leukocytes. Regarding the concentrations of growth factors, there is a large heterogeneity both between and within the different systems. Furthermore, the concentration of VEGF tended to be higher in PRP produced by systems that produce higher concentrations of platelets and leukocytes.

Concentration of Platelets

There was a large difference in the concentration of platelets between the systems studied in this review. Roughly, the systems studied in this review can be divided into high- and low-yielding devices. This division into high- and low-yielding devices has been described before by Dhurat and Sukesh.[11] Dhurat and Sukesh[11] described that PRP devices can usually be divided into lower (2.5-3 times the baseline concentration) and higher (5-9 times the baseline concentration) systems. The low-yielding devices in this review produce PRP with a platelet concentration around 500×10^3 μL, whereas the high-yielding devices generally produce a platelet concentration over 750×10^3 μL. Among the high-yielding devices were the

GPS III, SmartPrep, and Magellan systems; the lower concentration systems were the ACP, Cascade, Endoret, and RegenPRP. These findings correlate well with the findings in this review.

The concentration of platelets in PRP is of importance, as the mechanism of action of PRP is mainly based on the growth factors and cytokines found in the alpha-granules in the platelets. However, there is no consensus about the optimal concentration of platelets in PRP: some authors have reported platelet concentrations greater than 200×10^3 uL[37] to be therapeutic, whereas others have reported concentrations of 1000×10^3 uL.[34] In the present study, the platelet concentrations of all of the PRP separation systems exceeded a platelet concentration of $>200 \times 10^3$ uL, which implies that all the devices met the definition for therapeutic and effective PRP as defined by Mazzucco et al.[37]

Concentration of Leukocytes

Comparable with the concentration of platelets in PRP, the concentration of leukocytes differed largely between the systems studied in this review. Additionally, no large differences within the systems were found. PRP separation systems can be divided into systems producing a high and a low concentration of leukocytes. The concentration of leukocytes in PRP is a direct result of the preparation method that is used. Buffy coat–based systems, for example, produce PRP with a high concentration of leukocytes, as the buffy coat is rich in leukocytes. Plasma-based systems, in contrast, are designed to separate only the platelet and plasma portions of whole blood and therefore contain a low concentration of leukocytes.[11,15,50] The majority of separation systems in the current literature yield leukocyte-rich PRP. As also shown in this review, the ACP, Cascade, and Endoret systems are known to produce leukocyte-poor PRP. Currently, the inclusion of leukocytes in PRP is subject to debate, as both beneficial and adverse effects of leukocyte inclusion have been suggested.[50] Potential beneficial effects of leukocyte inclusion include their role in tissue remodeling and their increased antibacterial and immunological resistance.[12,44] Furthermore, the presence of leukocytes in PRP is associated with an increased concentration of growth factors, especially VEGF.[9,10,28,64] On the other hand, the inclusion of leukocytes might have catabolic and inflammatory effects on the targeted tissue as a result of the release of proinflammatory cytokines by leukocytes, which is associated with decreased proliferation and increased apoptosis.[2,4,5,8,38,49,59-62] As the aim of this review was to evaluate the differences between the concentrations of blood components in PRP produced by the various PRP separation systems, no definitive answer can be provided on whether leukocyte-rich or leukocyte-poor PRP is best based on the results of this review. There is, however, increasing evidence that the type of PRP (leukocyte-rich or leukocyte-poor) should be matched to the specific clinical field of application. In the treatment of knee osteoarthritis, for example, the use of leukocyte-poor PRP seems to be more beneficial than leukocyte-rich PRP.[48] In the treatment of chronic tendinopathy, in contrast, the use of leukocyte-rich PRP is superior to leukocyte-poor PRP.[20] To gain more insight in the specific indications for the different types of PRP, future research should focus on which type of PRP is most suitable for the specific fields of application.

Concentrations of Growth Factors

A wide variation was found regarding the concentrations of growth factors both between different systems as well as within systems. These differences can partly be explained by the use of the specific enzyme-linked immunosorbent assay kits. The assays of growth factors contained in the platelets may be influenced by the incomplete removal of platelets and red blood cells and therefore give variable results.[36] Data within the studies are comparable, but a comparison between studies is less reliable, which limits the relevance of these findings. In this review, it seemed, however, that the concentration of VEGF tended to be higher in PRP produced by systems with higher concentrations of platelets and leukocytes. Higher amounts of growth factors have indeed been correlated with higher amounts of platelets and leukocytes.[55,63] Although evidence about the role of the specific growth factors is scarce, in vitro studies have suggested that PDGF and TGF-B are the 2 most important growth factors in PRP.[1,6,35,45] In contrast to the platelet and leukocyte concentrations, there is no evidence about ideal concentrations of growth factors in PRP for tissue regeneration. Therefore, future studies are necessary to reveal the exact mechanisms of growth factors in PRP and their role in tissue regeneration.

Preparation Protocols

Besides a large heterogeneity in the concentrations of platelets, leukocytes, and growth factors between systems, the preparation protocols for the different systems also differed largely. Wide ranges were found for both the centrifugal force (350-2008*g*) and the total centrifugation time (5-21 minutes). There are many ways of preparing PRP; the most common methods are the plasma-based and buffy coat–based methods.[29] Although not known for all systems in this review, most systems use the buffy coat–based method. As mentioned earlier, buffy coat–based systems produce PRP with a high concentration of leukocytes, as the buffy coat is rich in leukocytes.[11,15,50] Although the ideal concentrations of blood components and growth factors for the specific fields of application have yet to be determined, the field of application should play an important role in the choice for the most appropriate PRP separation system. Other factors such as the volume of whole blood needed, the final volume of PRP, and the usability and reliability of the separation system could also be taken into consideration. Finally, the price of the systems can be taken into consideration, as a wide variation in price per kit ($95-$500) was found.

Strengths and Limitations

This is the first systematic review that offers a comprehensive overview of the concentrations of blood components in PRP produced by all the commercially available PRP

separation systems and that analyzes the differences between the systems in terms of the concentrations of blood components and growth factors. Initially, this study was designed as a meta-analysis. Unfortunately, despite all the authors who were contacted, we had to deal with a lot of missing data, and no raw data were available for the majority of the studies. This limited the statistic options available for analyzing the differences between systems, and therefore, a meta-analysis could not be conducted. To overcome the missing data, descriptive results of the studies that compared $\geq$2 PRP preparation systems were summarized. Furthermore, the number of samples studied in the included studies was rather small; only 5 of the 19 studies used $\geq$20 samples, and 10 of the 19 studies used $\leq$10 samples, which also limits a comparison between systems.

However, as this review of the literature showed, future research on the components of PRP should not focus on the concentrations of the components but rather on the optimal concentrations of platelets, leukocytes, and growth factors for the different fields of application. The use of leukocyte-rich PRP in chronic tendinopathy has been extensively investigated and been proven to be superior to leukocyte-poor PRP.[20] For other applications, osteoarthritis, for example, the evidence is limited, and well-designed clinical studies are necessary to gain more insight to which formulation of PRP is most suitable.

In conclusion, this review demonstrates that there is a large heterogeneity among different systems with regard to the concentrations of platelets, leukocytes, and growth factors in PRP. Also, the preparation protocols for the different systems differ largely. The choice for the most appropriate type of PRP should be based on the specific clinical field of application. As the ideal concentrations of blood components and growth factors for the specific fields of application are yet to be determined for most of the fields, future research should focus on which type of PRP is most suitable for the specific field.

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