Systematic Review
Periodontics

Use of platelet-rich fibrin for bone repair: a systematic review and meta-analysis of preclinical studies

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Abstract: This systematic review evaluated the potential utility of platelet-rich fibrin (PRF) in bone repair in animals. The question is: can the use of PRF in bone defects in healthy rats induce bone repair compared to clot? This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Prisma). The protocol was registered with Prospero (CRD [42020162319]). The literature search involved nine databases, including grey literature. All studies evaluated the bone defects created in rats filled with PRF and clots (control). Biomaterial evaluation was also performed in this study. The risk of bias was assessed using the Systematic Review Center for Laboratory Animal Experimentation (Syrcle) tool for animal studies. A meta-analysis of quantitative data was performed to estimate the effect of PRF on bone repair in rats. Heterogeneity among the studies was assessed using the I^2 statistic. The literature search retrieved 685 studies, 10 of which fulfilled the eligibility criteria, and 4 were included in the quantitative assessment. Analysis of the risk of bias revealed that most studies had a high risk of bias in performance and detection. Meta-analysis yielded divergent results and the absence of a statistically significant effect: PRF with control (standardized mean difference 2.54, 95% confidence interval -0.80–5.89; p = 0.14). In general, study heterogeneity was high (I2 ≥ 75.0%). The quality of the studies that influenced the conclusion of the review was based on the PICO, the sources and form of the search, the study selection criteria, the form of evaluation of publication bias, the evaluation of the quality of the studies, and data extraction by two researchers. PRF did not provide significant benefits for bone repair, resulting in unpredictable effects.

Keywords: Platelet-Rich Fibrin; Bone Regeneration; Meta-Analysis; Systematic Review; Wound Healing.

Introduction

Platelet-rich fibrin (PRF) is a natural scaffold composed of fibrin, platelets, and some fragments,[1,2] leukocytes, cytokines, and growth factors.[3-5] PRF is obtained by collecting and centrifuging patient blood without adding exogenous components.[6,7] Its characteristics are affected by the speed of blood collection and centrifugation protocol.[8,9]

The three-dimensional fibrin gel-like scaffold provided by PRF represents the final stage of the coagulation cascade, in which proteins arranged in different directions[10,11] work as "molecular rails" facilitating cell migration. During the centrifugation process, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor I and II (IGFs), and cytokines such as interleukin (IL)-4, IL-10, and transforming growth factor-beta (TGF-β),[9,10] which are retained in the fibrin mesh, are slowly released at the tissue site where PRF is placed.[12,13]

PRF accelerates epithelial and connective tissue healing, including in bone tissue.[14] Especially in dentistry, PRF may be useful in treating bone defects, such as in periodontal disease, tooth loss,[15] trauma,[16,17] or in patients with systemic conditions that impair bone healing, such as diabetes, radiotherapy, and/or osteomyelitis.[18] Several studies have evaluated the effect of PRF alone[19,20] or combined with biomaterials[21,18,22] on bone defects using small animals as preclinical models.

The use of rats as animal models enables several surgical approaches to the creation of bone defects, including the calvaria[18,21,23] and long bones,[26] which are the most commonly used. However, there are no standardized PRF protocols for rats or definitive conclusions about their role in bone repair, which can help other researchers design their studies. Thus, this study aimed to perform a systematic review of the literature to assess and provide the best available data supporting the potential utility of PRF for bone repair in rats.

Methodology

Protocol and registration

The protocol is reported in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (Prisma-P),[27] and registered in the International Prospective Register of Systematic Reviews database under the number CRD42020162319 (http://www.crd.york.ac.uk/Prospero). This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Prisma),[28] and was conducted according to the Joanna Briggs Institute (JBI) Manual.[29]

This systematic review was based on the patient, intervention, comparison, outcome, and study design (*i.e.*, "PICOS") strategy, which aimed to answer the following question: "can the use of PRF in bone defects in healthy rats induce bone repair compared to clot?" Population: healthy rats; intervention, use of PRF in bone defects; comparator, clot; outcome, effect of PRF on bone repair; study design, preclinical studies.

Only studies that reported data regarding the use of PRF in bone defects, without restrictions on the year of publication, language, or publication status (published, accepted/ahead of print articles) were eligible for inclusion. Studies not related to the topic, literature reviews, case reports/case series, human studies, pilot studies, letters to the editor, editorials, indices, congress summaries, and *in vivo* studies with female rats, *in vitro* studies, absence of a clot group, absence of a PRF group, and animals other than rats were excluded.

Information sources and literature search

The bibliographic search was completed in November 2019 and was updated in March 2021. Medical subject headings (MeSH), Health Sciences Descriptors (DeCS), and Emtree (Embase Subject Headings) were used to select search descriptors. Several combinations of descriptors were used with the Boolean operators "AND" / "OR," respecting the syntax standards of each database. Electronic surveys were performed using the Embase, Medline (via PubMed), *Literatura Latino Americana e do Caribe em Ciências da Saúde* (Lilacs), SciELO, LIVIVO, Scopus, and Web of Science databases. The Open Thesis and Open Grey citation databases were used to partially capture the "gray literature." Additional details of the search strategy and databases are presented in Table 1. The references of provisionally eligible articles were carefully reviewed to check for those that were not retrieved using the main search strategy.

Study selection

Study selection was performed in four stages. In the first stage, the registers were identified after a bibliographic search of the databases. The retrieved

Table 1. Strategies for database search.

Database	Search strategy (MARCH, 2021)
PubMed (http://www.ncbi.nlm.nih.gov/pubmed)	(("Rats" OR "Rat" OR "Rattus" OR "Rattus norvegicus" OR "Rats, Norway" OR "Rats, Laboratory" OR "Laboratory Rat" OR "Laboratory Rats" OR "Rat, Laboratory" OR "Rats, Wistar" OR "Wistar Rats" OR "Rats, Sprague Dawley" OR "Sprague-Dawley Rats" OR "Rats, Sprague Dawley" OR "Sprague Dawley Rats" OR "Rats, Holtzman" OR "Holtzman Rats") AND ("Platelet-Rich Fribrin" OR "Fibrin, Platelet-Rich" OR "Platelet Rich Fibrin"))
LIVIVO	("Rats" OR "Rat" OR "Rattus" OR "Rattus norvegicus" OR "Rats, Norway" OR "Rats, Laboratory" OR "Laboratory Rat" OR "Laboratory Rats" OR "Rat, Laboratory") AND ("Platelet-Rich Fribrin" OR "Fibrin, Platelet-Rich" OR "Platelet Rich Fibrin")
Scopus (http://www.scopus.com/)	((("Rats" OR "Rat" OR "Rattus" OR "Rattus norvegicus" OR "Rats, Norway" OR "Rats, Laboratory" OR "Laboratory Rat" OR "Laboratory Rats" OR "Rat, Laboratory") AND ("Platelet-Rich Fribrin" OR "Fibrin, Platelet-Rich" OR "Platelet Rich Fibrin")))
LILACS (http://lilacs.bvsalud.org/)	("Rats" OR "Rat" OR "Rattus") AND ("Platelet-Rich Fribrin" OR "Fibrin, Platelet-Rich" OR "Platelet Rich Fibrin") ("Rats" OR "Rat" OR "Rattus" OR "Rattus norvegicus" OR "Rats, Norway" OR "Rats, Laboratory" OR "Laboratory Rat" OR "Laboratory Rats" OR "Rat, Laboratory") AND ("Platelet-Rich Fribrin" OR "Fibrin, Platelet-Rich" OR "Platelet Rich Fibrin")
SciELO (http://www.scielo.org/)	(("Rats" OR "Rat" OR "Rattus" OR "Rattus norvegicus" OR "Rats, Norway" OR "Rats, Laboratory" OR "Laboratory Rat" OR "Laboratory Rats" OR "Rat, Laboratory") AND ("Platelet-Rich Fribrin" OR "Fibrin, Platelet-Rich" OR "Platelet Rich Fibrin")) ("Rats" OR "Rat" OR "Rattus" OR "Rats, Laboratory" OR "Laboratory Rat" OR "Laboratory Rats" OR "Rat, Laboratory") AND ("Platelet-Rich Fribrin" OR "Platelet Rich Fibrin")

results were exported to EndNote web software (Thomson Reuters, Toronto, ON, Canada), in which the duplicates were removed. The remaining results were exported to Word 2019 (Microsoft Corporation, Redmond, WA, USA) and the duplicate articles were manually removed. Two reviewers (NTAR and JLCP) independently performed each stage, and disagreements between examiners were discussed with a third reviewer (LRP) to reach a consensus.

In the second stage, methodological analysis was performed and selected based on the titles. In the third stage, the abstracts of the selected studies were read, and the exclusion criteria were applied. The titles that matched the objectives of the study but did not have available abstracts were fully analyzed in the third stage. Thus, in the fourth stage, the full texts of provisionally eligible studies were retrieved and evaluated to verify whether they fulfilled the eligibility criteria.

These strategies were used to minimize the selection and publication bias. As many databases as possible, including grey literature, were used to avoid human selection bias. There were no language restrictions to minimize selection bias. The publication bias resulted in the largest number of databases. All languages, year, and other restriction strategies were used to minimize the selection bias for eligible articles.

Data collection

To ensure consistency among the reviewers, a calibration exercise was performed before data extraction, in which data from three eligible studies were extracted together. After calibration, two reviewers (NTAR and JLCP) independently and blindly extracted data from eligible articles. In cases where there was a divergence in data extraction, a third reviewer (LRP) adjudicated the conflict(s). Kappa was performed and the result was greater than 0.81. Data extracted for this study included the author, year of publication, methodologies, characteristics of the animals that comprised the sample (breed, number of animals used in the study, weight, and age), amount and method of blood collection, bone repair time, control group, and main outcomes (Table 2). Funding and conflicts of interest were also assessed in eligible studies. The ethics criteria reported in the studies, as well as the checklist used, were collected. Data

Table 2. Main outcomes of the eligible studies

Author, year	Population qualification				Intervention characteristics				
	Specie	Amount	Average weight	Age of rats	Blood volume	Methods of collect	Methods centrifugation	Bone repair evaluation time	Methodology analysis
Oliveira et al., 2015[21]	Wistar	48	450– 550g	+	3.5 ml to produce autogenous PRF and 10 ml for the homogeneous PRF from donor rats.	Intracardiac puncture	3000 rpm for 10 min.	30, 60 days	Histomorphometric. Bone area (%)
Abdullah, 2016[23]	Sprague – Dawley	45	350– 450g	20–22 weeks	4mL of each rat.	Orbital sinus	3000 rpm for 12 minutes.	7, 14, 21, 28, 42 days	MCT. Bone Volume (mm^3)
Sindel et al., 2017[38]	Wistar	40	+	+	3.5 ml	Ventral tail artery	3000 rpm for 10 min.	21 days	Histomorphometric. Longest trabecule (%)
Dülgeroglu & Metineren, 2017[8]	Wistar	16	300–350g	Mature	Blood taken from 4 rats.	+	3000 rpm for 10 minutes.	28 days	Histological
Akyildiz et al., 2018[26]	Sprague – Dawley	23	300–380g	12 months	4mL from the donor animal.	Cardiac puncture	3000 rpm for 10 minutes.	14, 42 days	Histomorphometric. Bone area (%)
Raafat et al., 2018[19]	Wistar	48	150–200g	Adult	Around 5 ml	Human venous blood	3000 rpm for 10 min.	30, 60 days	Histomorphometric. Bone area (%)
Lago et al., 2020[18]	Wistar	40	350–450g	9–11 weeks	3.5 ml to produce autogenous PRF	Intracardiac puncture	2700 rpm for 12 min.	28, 56 days	Histomorphometric. Bone area (%)
Grecu et al., 2019[20]				Newer than 6 months	10 ml from a donor rat			45 days	Descriptive histology
Queiroz, 2019[40]	Wistar	35	220–420g	+	5ml	Intracardiac puncture	1300 rpm for 8 minutes.		
	Wistar	128	450–500g			Intracardiac puncture	400 G for 12 minutes	2, 7, 14 e 28 days	MCT and Histomorphometric
Pola, 2013[39]	Wistar	90	350-450 g	5–6 months	3,5 ml	Intracardiac puncture	400 G for 12 minutes	7, 15, 30 days	Histomorphometric. Bone area (%)

Note: + Not mentioned by the author.

were extracted from the text, tables, and images. In case of missing data, the authors of the studies were contacted to clarify the text.

Risk of bias

The risk of bias analysis of the pre-clinical studies was blindly and independently assessed by two reviewers (NTAR and JLCP), and a third reviewer (LRP) was invited to discuss the risk of bias when no consensus was achieved, as suggested by the Prisma checklist.[28] This was performed using the Syrcle RoB tool (Systematic Review Center for Laboratory Animal Experimentation) for intervention studies involving animals.[30]

The SYRCLE tool contains some questions about the methodological design in each domain called "review author jugdment". The Syrcle tool was created to be used by domains, and not by individual studies.[30] The reviewers answered these questions with data from papers in all domains. Calibration exercises and interobserver agreement testing between the authors were performed individually.

Sponsorship status evaluation

Information regarding the source(s) of funding in the selected studies was also assessed (Table 3). These data were extracted because industry sponsorship may be associated with the risks for publication,

Table 3. Sponsorship status of the studies.

Authors (year)	Sponsorship status		
	Sponsored	Non-sponsored	Unclear
Pola, 2013			x
Oliveira et al., 2015		x	
Abdullah, 2016		x	
Sindel et al., 2017		x	
Dülgeroglu; Metineren, 2017			x
Akyildiz et al., 2018		x	
Raafat et al., 2018			x
Grecu et al., 2019		x	
Queiroz, 2019		x	
Lago et al., 2020		x	

reporting, and selection biases.[31] Sponsorship status was classified as follows:[32] Unclear, when it was not possible to confirm the sponsorship status even after attempt(s) to contact the authors by email; non-sponsored, the authors declared that the study did not receive any type of financial support from companies related to PRF generator devices; and sponsored, when the authors reported any financial contribution (financial support, provision of equipment or supplies, discounts) from companies related to PRF generator devices. The main text and acknowledgements were checked to collect this information. In cases of missing information or unclear data, the authors were contacted twice by e-mail.

Summary measures and data synthesis

The data collection process was performed through an analysis of the selected studies and presented in a descriptive/narrative manner. Bone repair associated with the use of PRF was analyzed qualitatively and quantitatively for bone area by histomorphology (data presented as a percentage) or bone volume according to computed microtomography (microCT) analysis (data presented in mm^3). Comparisons were made between the groups that received PRF in bone defects and the control group (clot) in experimental time at 14 to 60 days, with n = 4 and n = 6, respectively. These data were collected from the selected studies for quantitative analysis.

Meta-analyses of continuous outcomes were performed to estimate the effects of PRF on bone repair in rats. Differences in outcomes were reported using forest plots, considering the random-effects model to determine the standardized mean difference (SMD), corresponding 95% confidence interval (CI), and p-value.[33-35] Heterogeneity among studies was assessed using the I^2 statistic, and was classified as low ($I^2 \leq 25\%$), moderate ($I^2 = 50\%$), or high ($I^2 \geq 75\%$).[36] Publication bias could not be evaluated because no more than 10 studies were grouped in a funnel plot. The assessment of publication bias may be inaccurate if the number of included studies is small. In our study, there were only four eligible quantitative articles; therefore, it would be inaccurate to perform the funnel plot.[37] Review Manager version 5.4 (RevMan, Cochrane Collaboration) was used to perform all statistical analyses.

Results

Study selection

During the first phase of study selection, 685 articles were distributed among nine electronic databases, including the gray literature. After removing repeated/duplicate results, 432 articles remained for the analysis of titles and abstracts. After detailed analysis, 102 studies were eligible for full-text analysis. The references of the 102 potentially eligible studies were carefully

evaluated, and no additional articles were selected, resulting in 102 studies for full-text reading. After reading the full text, 92 studies did not fulfil the inclusion criteria and were eliminated, and 10 articles underwent qualitative analysis. Therefore, only four articles were selected for the quantitative synthesis. The studies were eliminated with the respective reasons for exclusion, and illustrated the search, identification, and inclusion processes (Figure 1).

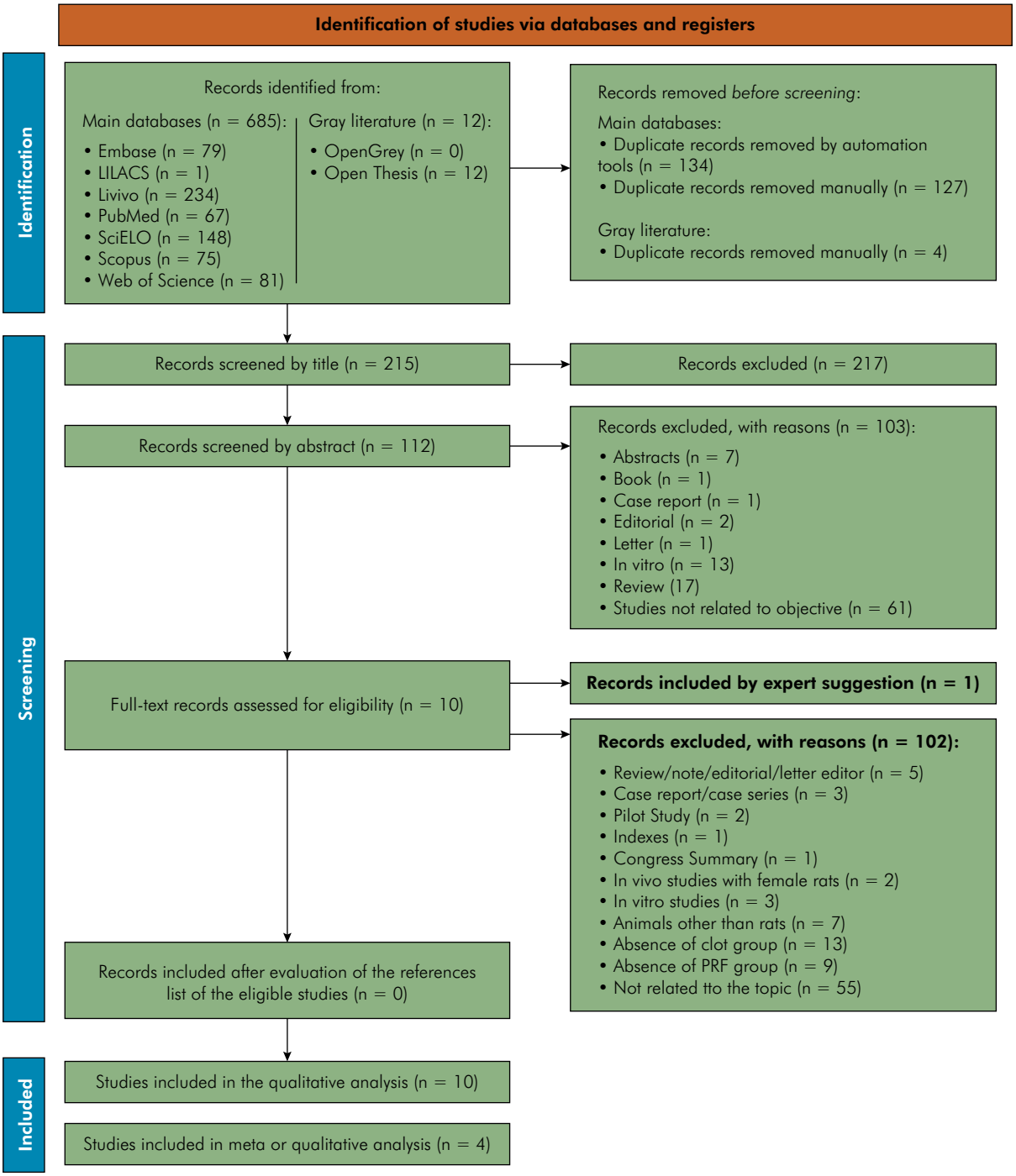

Figure 1. Flowchart adapted from the PRISMA statement showing the literature search and selection processes.

Characteristics of eligible studies

The characteristics of the eligible studies are summarized in Table 2. Three studies were conducted in Turkey,[8,26,38] four in Brazil,[18,21,39,40] one in Saudi Arabia,[23] one in Romania,[20] and one in Egypt.[19] These studies were published between 2013 and 2020. Of the 10 selected studies, seven collected PRF using intracardiac puncture[8,18,20,21,26,39,40] and the three others used venous blood,[19] orbital sinus,[23] and ventral tail artery.[38] The type of PRF (intervention) was fibrin clot form compared to the isolated clot (control and comparative). Studies have also used biomaterials such as hyaluronic acid (HA),[26,38] particulate autogenous bone, Bio-Oss,[18,21] simvastatin,[19] demineralized bone matrix,[38,18] beta-tricalcium phosphate bone graft material,[23] and anti-inflammatory non-steroidal drugs.[40]

The type of PRF, quantity, method of collection, and period of bone repair evaluation were considered as aspects of interest. The time and blood quantification methods used were not standardized because there are several available methods to obtain PRF, and no study has reported a checklist applicable to animal research. All applicable international, national, and/or institutional guidelines, and ethical criteria for the care and use of animals were followed in this study.

Risk of bias within studies

The risk of bias analysis of the included studies is shown (Figure 2). The reviewers accessed 10 checkpoints of Syrcle's risk of bias tool: three for selection bias, two for performance bias, two for detection bias, one for attrition bias, one for reporting bias, and one for others bias. It was considered "YES," when it was inside the established criteria and indicated low risk of bias, "NO" when it was outside it and represented high risk of bias, and it was "UNCLEAR" when it did not have enough information to answer the questions or was not described in the text, designated as unclear risk of bias.

Within the domains checked, performance bias was the most representative, with 43% of the studies presenting an unclear risk of bias and 36% presenting a risk of bias. The domain "detection" also presented an important value of detection bias: 21%. The other domains did not have a high risk of bias. The most important value was selection bias, with a 24% risk of bias and 14% unclear bias. For attrition and reporting 86% had no risk of bias, and to the domain "other" 100% had no risk of bias.

The quality of the included studies influenced the conclusion of the review; the lower the bias, the higher the methodological quality. In general, the quality of the included studies was good according to the risk of bias in the verified domains, since most of the bias rates were not low, as can be seen in the results. However, it is possible to infer that due to the differences between the studies, a high risk of bias was also obtained in some domains.

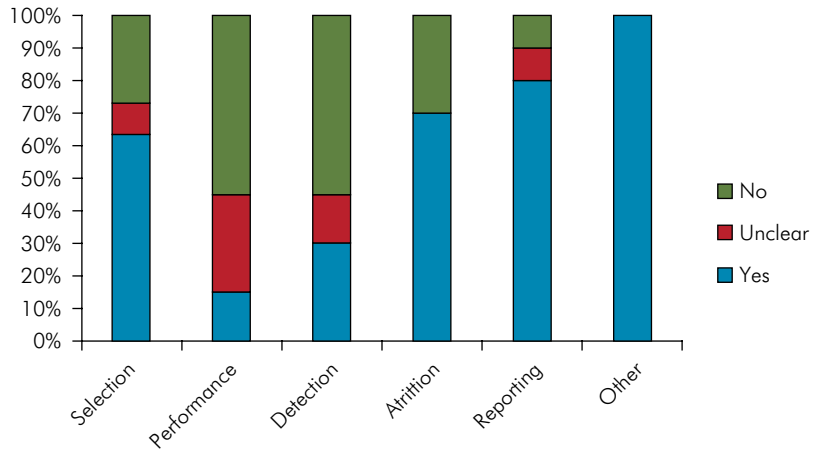

Figure 2. Summary of the types of bias risk across all the included studies assessed by the SYRCLE tool.

Industry sponsorship status

The sponsorship status is summarized in Table 3. None of the studies reported any financial support from companies related to PRF generator devices. The authors declare that they have no conflicts of interest. None of the authors of this study provided information via email. Unclear information was reported in three of the selected studies.[8,19,39] In these cases, it was not possible to confirm the sponsorship status even after attempting to contact the authors via email. Three studies[8,19,39] did not report whether they had a conflict of interest. We tried to contact them via email for two attempts, but we did not get a response, therefore, it is not clear whether they have a conflict of interest. The contact was made twice through email with the authors of the eligible studies requesting new studies and unidentified materials, however, no response was obtained from any author.

Outcomes of each study and meta-analyses

A summary of the parameters and specific results collected from the studies included in this qualitative analysis are presented in Table 4. Six studies[18,19,21,26,38,39] evaluated the bone area using histomorphometry (%) in 288 samples from 289 rats. Bone cells were evaluated using histology in 218 samples from 179 rats (reported no units of measurement).[8,20,40] Other studies evaluated bone volume using microCT (mm^3) using 218 samples from 183 rats.[23,40] Considering the individual results of the included studies, six[8,20,23,26,39,40] reported that PRF alone improved bone repair; all studies[8,21,23,38,18,19,26] only evaluated PRF and reported bone repair similar to the clot and biomaterial; three[19,21,38] found no difference between isolated PRF and control groups; and four[18,19,21,23] reported that PRF with biomaterial was efficient in improving bone repair.

The results of the meta-analysis of studies evaluating the effect of PRF on bone repair are shown (Figure 3). Comparing PRF with the control, there were divergent results among studies and no statistically significant effect (SMD 2.54 [95% CI -0.80–5.89]; $p = 0.14$). In general, heterogeneity among the studies was high ($I^2 \geq 75.0\%$).

"Heterogeneity may be high due to heterogeneity among the studies, which suggests that more studies should be conducted. There is no difference in preclinical studies between PRF and clot, so you have to consider clinical studies in humans. First, it needs specialized people, it is expensive, and it is already in so much use. It is necessary to have a protocol defined in rats for use in humans, more studies in rats are needed, and better protocols in pre-clinics are required until finding a protocol with a difference to be used in humans, and with effective results."

Table 4. Main characteristics of the eligible studies.

Author	Main outcomes
Pola, 2013	PRP resulted in accelerated bone formation when compared to control and PRF.
Oliveira et al., 2015	The use of only PRF did not enhance bone repair. The association of PRF and Bio Oss© enhanced bone repairs.
Abdullah, 2016	At an initial time, the use of only PRF did not enhance bone repair.
Sindel et al., 2017	The use of PRF did not enhanced the bone repair process at the early time point.
Dülgeroglu; Metineren, 2017	The results indicate that PRF enhances the bone repair in long bones.
Akyildiz et al., 2018	The use of PRF showed to be efficacy on bone repair process at the early time point.
Raafat et al., 2018	To the one-month analysis, PRF was not as efficacy as SIM or SIM/PRF.
Lago et al., 2020	At first time of analysis, the results of PRF and Bio-Oss were similar.
Grecu et al., 2019	New bone formations have been shown to be prevalent in the PRF augmented defect
Queiroz, 2019	The PRF was favorable from the initial to the later periods, assisting in the inflammatory response and bone neoformation.

Note: + Not mentioned by the author.

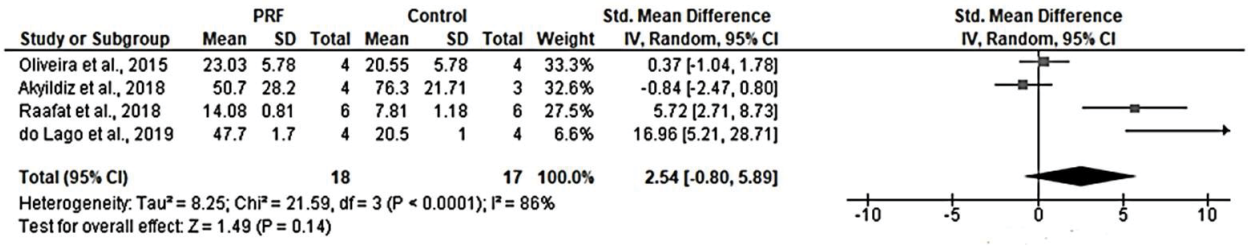

Figure 3. Forest plot of estimates reported by eligible studies that assessed the effect of PRF on bone repair histomorphometry. The standardized mean differences of the evaluated parameters and their respective 95% confidence intervals are represented by squares for the individual studies. The diamonds at the end represent the general average differences estimated from the included studies.

Discussion

This study investigated the effectiveness of PRF for bone regeneration compared to clots in preclinical animal models. PRF has been used alone[20] or as a graft complement (autogenous or non-autogenous) to reduce the length of the healing period.[18,19,21,23,26,38] PRF is easily obtained and cost effective[26,41,38,41-43] which makes PRF quite interesting. However, comparisons of the included studies revealed that the use of PRF for bone defects in healthy rats was not superior to that in the control group.

The mechanism of PRF on bone repair is due to its autologous strong fibrin clot rich in leukocytes, fibrin, platelets, and circulating stem cells; healing cytokines; the pro-inflammatory cytokines interleukin-1ß, interleukin-6, and tumor necrosis factor-α; the anti-inflammatory cytokines interleukin-4 and interleukin-10; vascular endothelial growth factor; insulin-like growth factor-I and II; epidermal growth factor; transforming growth factor-β1; and platelet-derived growth factor.[7] Furthermore, the surgical site expedites integration, maturation, and remodeling, while increasing the bone graft density if the matched PRF permits significant postoperative protection.[53,54]

Only three studies found superior results to PRF when compared to clot, and both evaluated the repair process in long bones.[8,19,26] It is widely known that the healing process in long bones requires both osteoblastic and chondroblast cells, with predominating endochondral ossification.[44,45] This process involves the presence of hard callus, which probably underwent a delay in its maturation in the PRF group, causing more fibrosis and less new bone formation at 6 weeks, despite initially favorable results.[26] Similarly, we also found superior results for the PRF group only in the first analysis period but equaling the control group in the subsequent period.[19] Other studies evaluated bone repair in the calvaria, which is subject to the process of intramembranous ossification, and to less movement than the tibia or femur, probably resulting in a modified repair pattern when subjected to bone defects. Thus, with these data, it is possible to observe differences and similarities in the results of the studies included in the present review.

PRF is collected and centrifuged in patient blood without adding exogenous components.[6,7] It is important to note that the methods for obtaining PRF varied among the studies included in this review (Table 4). There is no standardized method for determining the rotation time, speed, or radius of the centrifuge used. The sum of these factors defines the *g* force applied to the tubes inside the centrifuge.[46,47] The fibrin architecture is related to the *g* force, and the centrifugation time applied to the tube.[47,48] The *g* force was not reported in any study included in this review, thus precluding an adequate discussion regarding the effect of fibrin structure on bone healing. All eligible studies had a similar collection and obtained PRF in the membrane form.

The size and localization of the defect can also affect the results of the histomorphometric analysis. It is necessary to create critical defects that do not heal spontaneously within a certain period;[1,49,50] however

the defect sizes and localization varied in the included studies, as well as the periods of analysis. Therefore, an adequate analysis of PRF alone is difficult. In contrast, when combined with biomaterials, three studies reported promising results for PRF.[18,21,23]

The graft materials used to improve bone regeneration varied considerably among the studies included in this review.[18,19,21,23,26,38] Inorganic particulate biomaterials, such as β-TCP and Bio-Oss, provide a scaffold for neovascularization and cell penetration, which depends on the porosity of the material.[18,23] These materials possess osteoconductive properties that may be improved by sustained and gradual release of growth factors by PRF.[18]

In particular, β-TCP dissolves after grafting, providing high concentrations of calcium and phosphate, which may be related to the greater bone regeneration observed by micro-CT analysis in the PRF/β-TCP groups compared to PRF alone in the first two weeks.[9,11,23] However, differences in the volume and density of the newly formed bone between the PRF and PRF/β-TCP groups were not significant at 3, 4, and 6 weeks postoperatively,[23] indicating that the presence of β-TCP was no longer significant. In contrast to β-TCP, Bio-Oss did not exhibit complete reabsorption of its particles, presenting histological sections with residual particles at 4 and 8 weeks of analysis.[10,18] This did not prevent the association between Bio-Oss/PRF and PRF alone in either period.[21]

HA occurs naturally in the initial bone callus and possesses osteoconductive properties, which justifies its comparison with PRF in two studies.[38,26] However, the results of such research are conflicting. While one attributed better results to PRF when compared with HA,[38] the other demonstrated the total superiority of the latter.[26] It is not possible to make direct comparisons because they differ in relation to the region chosen for the creation of the bone defect, and in relation to the period(s) of analysis. Simvastatin, a drug commonly used to reduce cholesterol levels, has also recently been evaluated as an osteoinductive agent combined with different carriers[51,19] and compared with PRF alone or in combination. The latter significantly increased the maturation of collagen two months after surgery, indicating a stimulatory effect on osteoblasts.

The impact of the study design was to verify the effectiveness of PRF compared to clot in rats, and to establish a standard in rats to develop several studies using PRF. Based on the results of this review, the use of PRF did not provide significant benefits for bone repair, yielding unpredictable effects. After defining a pattern for the use of PRF in rats, which are the animals used in our research group, we intend to conduct several studies involving the use of PRF. The clinical implications will be after we use PRF in studies that will have a positive effect on rats to test it in humans.

Surprisingly, the data obtained from studies using rats as the animal model also lacked an adequate description of the preparation of PRF and standardization of the characteristics and defects. Another limitation was possible sponsorship bias among the selected studies. Some authors did not report whether they received any type of funding or provision of devices and/or products from companies related to PRF, although six studies reported no conflicts of interest. Sponsorship from corporate entities, mainly in studies evaluating specific devices or products, may lead to bias. The publication of selected results has been associated with industry sponsorship because of the risks of publication, reporting, and selection bias.[31,52] Therefore, the results of this study should be interpreted with caution, and future studies should be conducted to evaluate the effect of sponsorship bias among studies evaluating the effectiveness of PRF.

Conclusion

Despite the limitations of the current literature, PRF does not provide significant benefits for bone repair and yields unpredictable effects. Further animal studies with greater standardization of factors are necessary to make strong recommendations for its use in humans in other studies investigating PRF. Nevertheless, this systematic meta-analysis is valuable in informing, guiding, and supporting future studies. Although several studies have shown that PRF presents superior results to the control group in human studies for the control of postoperative

discomfort and periodontal defects, no study has evaluated its potential for bone repair as measured by histomorphometry. This is of fundamental importance considering the bone quality of the newly formed tissue. In addition, preclinical studies allow greater control of the intervention with less bias than in human studies.

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