



Systematic Review / Meta-analysis

Platelet-rich fibrin as a tissue engineering material in accelerate bone healing in rat bone defects: A systematic review and meta-analysis

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ARTICLE INFO

Keywords:

Bone defects
Bone regeneration
Platelet-rich fibrin
Rats

ABSTRACT

Introduction: Various techniques for tissue engineering have been introduced to help regenerate damaged or lost bone tissue. This study aimed to see the potential implication of platelet-rich fibrin (PRF) to accelerate the bone healing process in rat bone defects.

Methods: A systematic literature search was conducted from several electronic databases on subjects looking at the use of PRF in rat bone defects and their results in bone regeneration. Specific results compared PRF vs. other methods, PRF vs. control, and PRV vs. combination PRF and other methods. Science Direct, PubMed, and Cochrane Library were the main information sources. The Cochrane Collaboration method is employed to assess the risk of bias.

Results: A total of 483 rats were used in the twelve studies, and this meta-analysis showed that the PRF vs. other methods pooled odds ratio (OR) obtained was 0.92 (95% CI 0.42–2.04; $p = 0.29$; $I^2 = 18\%$), PRF versus control OR obtained 9.45 (95% CI 4.68–19.08; $P = 0.01$; $I^2 = 0\%$), the combination of PRF compared to PRF alone OR obtained 0.12 (95% CI 0.03–0.41; $p = 0.01$; $I^2 = 0\%$).

Discussion: Platelet-rich fibrin accelerates the bone healing process in rat bone defects compared to physiologically. Platelet-rich fibrin combined with other methods can stimulate rat bone defects than utilization of platelet-rich fibrin only. The small number of articles assessed may cause limitations in sensitivity tests. This study was registered in the research registry (reviewregistry1341)

1. Introduction

Bone defects are caused by trauma, neoplasms, congenital disabilities, open fracture, infection, and failed arthroplasty. The incidence of bone diseases differs from other tissues, and bones can self-regenerate for the occurring defects. Most fractures and bone injuries can recover without forming scars [1]. Biologic factors such as the transforming growth factor-beta (TFG- β) superfamily, TNF- α , IL-1 β , IL-6, IL-17F, and bone morphogenetic proteins (BMP) are commuted in the inflammatory phase. Furthermore, the hydrostatic stress and strain as part of mechanical load also act as an essential factor for the healing of bone fracture [2,3].

Biological and mechanical factors in the bone modulate mesenchymal stem cell (MSCs) activity, the pivotal contributor to bone formation [4], endothelial cells, and chondrocytes osteoblast activity.

Nonetheless, Cellular and mechanical activity interaction are still undefined [4]. However, in pathological bone defects and fractures, the bone will fail to heal itself [1,5–7]. To catalyze bone healing and prevent its fail of it, bone grafts are introduced to improve the recovery of a bone defect. Bone graft is material implanted in the bone to help enhance bone healing through osteogenesis, osteoconduction, and osteoinduction, either with a single material or a combination material [8]. The biological approach and mechanical combination to shorten the healing process of bone defects have been studied recently. Bone grafts, platelet-rich fibrin (PRF), platelet-rich plasma (PRP), and platelet-derived growth factors have shown promising results in the healing process. PRF can be utilized as a single biomaterial or with grafting material to accelerate bone regeneration [9]. Platelet Rich Fibrin simultaneously promotes angiogenesis, immunity, and epithelialization as the healing process and soft tissue maturation [10]. Based on

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<https://doi.org/10.1016/j.amsu.2022.104869>

Received 18 August 2022; Received in revised form 8 October 2022; Accepted 6 November 2022

Available online 19 November 2022

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histological and clinical evaluations, PRF showed significantly better results in tissue healing [11]. However, the effect of PRF on the recovery of bone defects is still unclear. Therefore, this study aims to analyze the success rate of PRF in bone healing in rat bone defects.

2. Materials and methods

This research referred to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines [12], as shown in Fig. 1. This study has also been evaluated regarding AMSTAR assessment [13].

2.1. Eligibility criteria

The subsequence types included studies on rats with bone defects with available information on the application of PRF in their healing process and validated definitions of bone regeneration processes. In the systematic review and meta-analysis, only published studies were included. We exclude abstract publications only, preprints, letters without primary data, review articles, case reports, comments, non-English language papers, and particularly studies without information on the critical exposure of bone healing.

2.2. Search strategy and study options

We conducted a systematic literature search on Science Direct, PubMed, and Cochrane Library with keywords (“bone defect” OR “fracture” OR “bone defect” AND (“bone graft” OR “bone graft”) AND “platelet-rich fibrin”) published from January 1999 to July 2021. A complete search strategy can be accessed in Table 1. Duplicates were eliminated, and two authors independently assessed the title and abstract of the remaining articles with inclusion and exclusion criteria. Both authors are medical doctors experienced in systematic review and meta-analysis studies.

2.3. Data extraction

Two independent authors abstracted the data with a standardized form. It obtained information for assessing articles about the first authors, year of publication, study design, rat type (including age and weight), sample size, bone defect type, evaluation time, and the primary outcome. Data selection was dependent on the potential variables influencing PRF use and outcomes. PRF application was compared with other methods, control, and a combination of PRF. The main result was bone regeneration in each group.

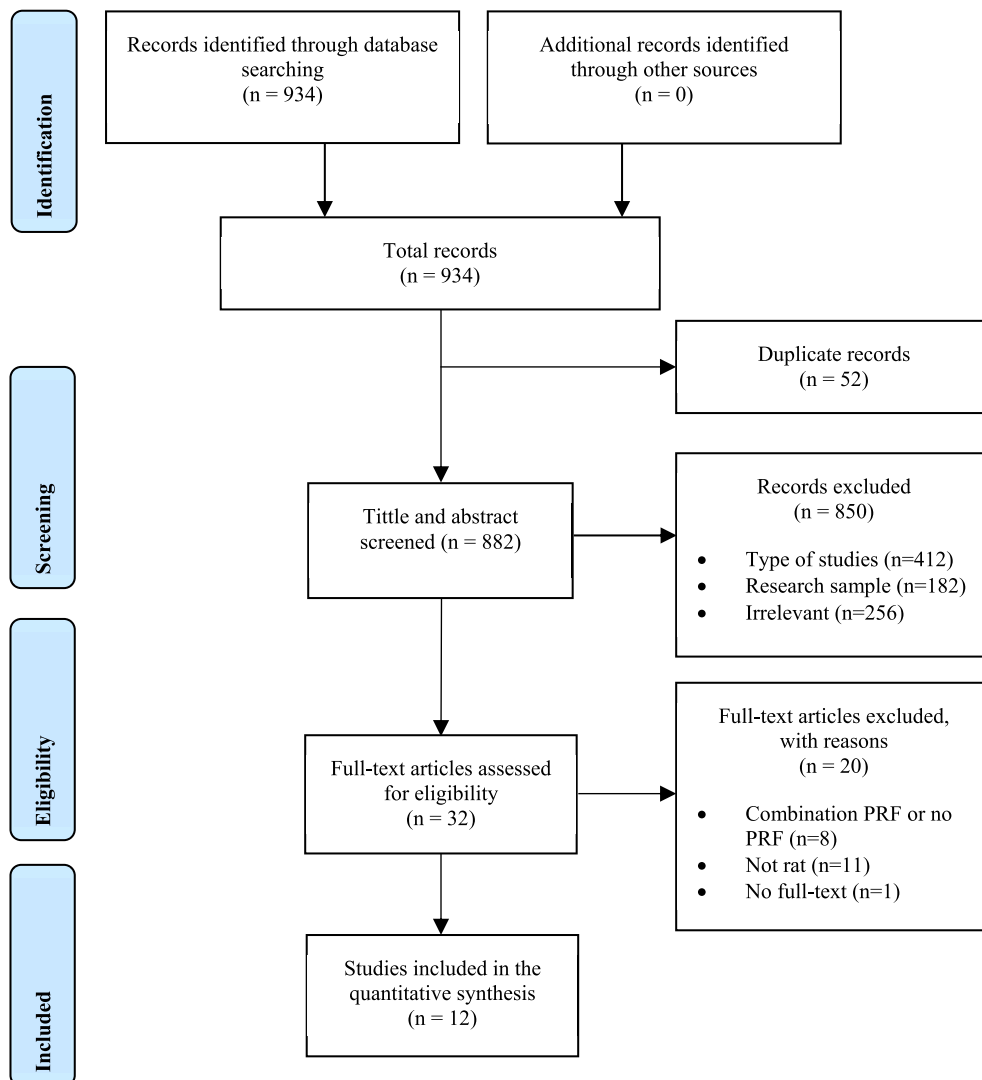


Fig. 1. Prisma flowchart.

Table 1
Study characteristic.

Author	Publication years	Type of Rat	Number of Samples	Weight of Rats (gram)	Age of Rats	Type of Bone Defects	Divition	PRF	Evaluation time	Type of Analysis Evaluation	Result
Abdullah	2015	<i>Sprague–Dawley</i>	45	350–450	20–22 weeks	Calvaria. 3 mm (2 side)	C= Without intervention P1= PRF P2= PRF + β -TCP	3000 rpm, 12 min	1 = 1 weeks 2 = 2 weeks 3 = 3 weeks 4 = 4 weeks 5 = 5 weeks 6 = 6 weeks	MCT analysis	C: 9/ 15 P1: 15/15 P2: 15/15
Alsherif et al.	2020	<i>Wistar Albino</i>	30	300–360	3–4 months	mandible. 2 mm	C = without intervention P1= Ozone P2= PRF	2000 RPM, 10 min	1 = 2 weeks 2 = 4 weeks	Histomorphometric, histology, microscop electron	C: 6/ 10 P1: 8/10 P2: 10/10
Awadeen et al.	2020	<i>Sprague–Dawley</i>	63	–	–	Mandible. 5 mm	C = without intervention P1= PRF P2= PRF + BMSC	3000 rpm, 10 min	1 = 1 weeks 2 = 2 weeks 3 = 4 weeks	Histomorphometric, histology, IHC	C: 1/ 8 P1: 15/21 P2: 21/21
Chen et al.	2021	<i>Sprague–Dawley</i>	32	–	–	Calvaria. 6 mm (2 side)	C = without intervention P1 = 3D-printed poly- ϵ -caprolactone (PCL) P2= PRF P3= PCL + PRF	3000 rpm, 10 min	1 = 4 weeks 2 = 8 m weeks	Dental radiography, micro CT, hystology	C: 7/8 P1: 8/ 8 P2: 6/8 P3: 8/ 8
Do Lago et al.	2020	<i>Rattus norvegicus, Albinus, Wistar</i>	40	350–450	9–11 weeks	Calvaria. 5 mm	P1 = blood clot (CO) P2 = DBBM (BIO) P3 = L-PRF P4 = DBBM associated with L-PRF (BIO-LPRF)	2700 rpm, 12 min	1 = 4 weeks 2 = 8 weeks	Histomorphometric and IHC	P1: 3/ 8 P2: 7/8 P3: 6/ 8 P4: 8/8
Oliveira et al.	2015	<i>Rattus norvegicus, Albinus, Wistar</i>	48	450–550	–	Calvaria. 5 mm	C = homogenous clot P1 = autogenous PRF P2 = bovine bone P3 = bovine + PRF	3000 rpm, 10 min	1 = 30 days 2 = 60 days	Histomorphometric	C: 3/8 P1: 6/ 8 P2: 7/8 P3: 8/ 8
Padilha et al.	2018	<i>Rattus norvegicus, Albinus, Wistar</i>	18	300–400	15 weeks	Calvaria. 2 mm (2 side)	P1 = blood clot (CTRL) P2 = L-PRF	3000 rpm, 12 min	1 = 5 days 2 = 15 days 3 = 30 days	Histomorphometric, histology, IHC	P1: 3/ 9 P2: 9/9
Raafat et al.	2018	<i>Albino rat</i>	48	150–200	–	Tibia. 3 mm	C = without intervention P1= PRF P2= Statins like simvastatin (SIM) P3= SIM/PRF	3000 rpm, 10 min	1 = 1 months 2 = 2 months	Histology, IHC, Radiologic	C: 3/ 12 P1: 7/12 P2: 10/12 P3: 12/12
Rady et al.	2018	<i>Wistar rat</i>	36	175–200	12–14 weeks	Tibia. 3 mm	P1= BM-MSc P2= PRF	3000 rpm, 10 min	1 = 3 days 2 = 10 days 3 = 3 weeks	Scanning electron microscopy/energy-dispersive X-ray (SEM/EDX) analysis	P1: 18/18 P2: 16/18
Sindel et al.	2017	<i>Wistar Albino</i>	40	–	–	Calvaria. 8 mm	C = without intervention P1= PRF P2= HA gel P3 = Demineralized Bone Matrix (DBM) Allograft	3000 rpm, 10 min	1 = 21 days	Histomorphometric	C: 6.10 P1: 10/10 P2: 8/ 10
Sumida et al.	2019	<i>Wistar rat</i>	23	400–450	8–10 weeks	Mandible. 2 mm (2 side)	C = without intervention P1= PRF	890 g, 13 min	1 = 4 days 2 = 6 days 3 = 12 days	Histology, IHC	C: 3/8 P1: 8/ 8
Tasyi et al.	2018	<i>Sprague–Dawley</i>	60	240 \pm 20	–	Tibia. 8 mm	C = without intervention P1 = collagen membran P2= PRF	3000 rpm, 10 min	1 = 7 days 2 = 28 days	Histomorphometric	C: 9/ 10 P1: 11/14 P2: 14/14

2.4. Risk of bias assessment publications

The risk of bias for all included studies was analyzed using the components proposed by the Cochrane Collaboration, namely random sequencing, concealment of allocation, outcome blinding raters, incomplete outcome data, and selective outcome reporting.

2.5. Data synthesis and meta-analysis

Mantel-Haenszel statistical method was employed with fixed effects. Odds ratio (OR) and Confidence interval (CI) were used to estimate the effect size of the outcome. Q test and I2 test were performed to check the cross-study heterogeneity. Values > 50% and P values ≤ 0.1 were considered significant. We performed sensitivity/meta-regression analyses and no-one studies when needed to account for significant heterogeneity.

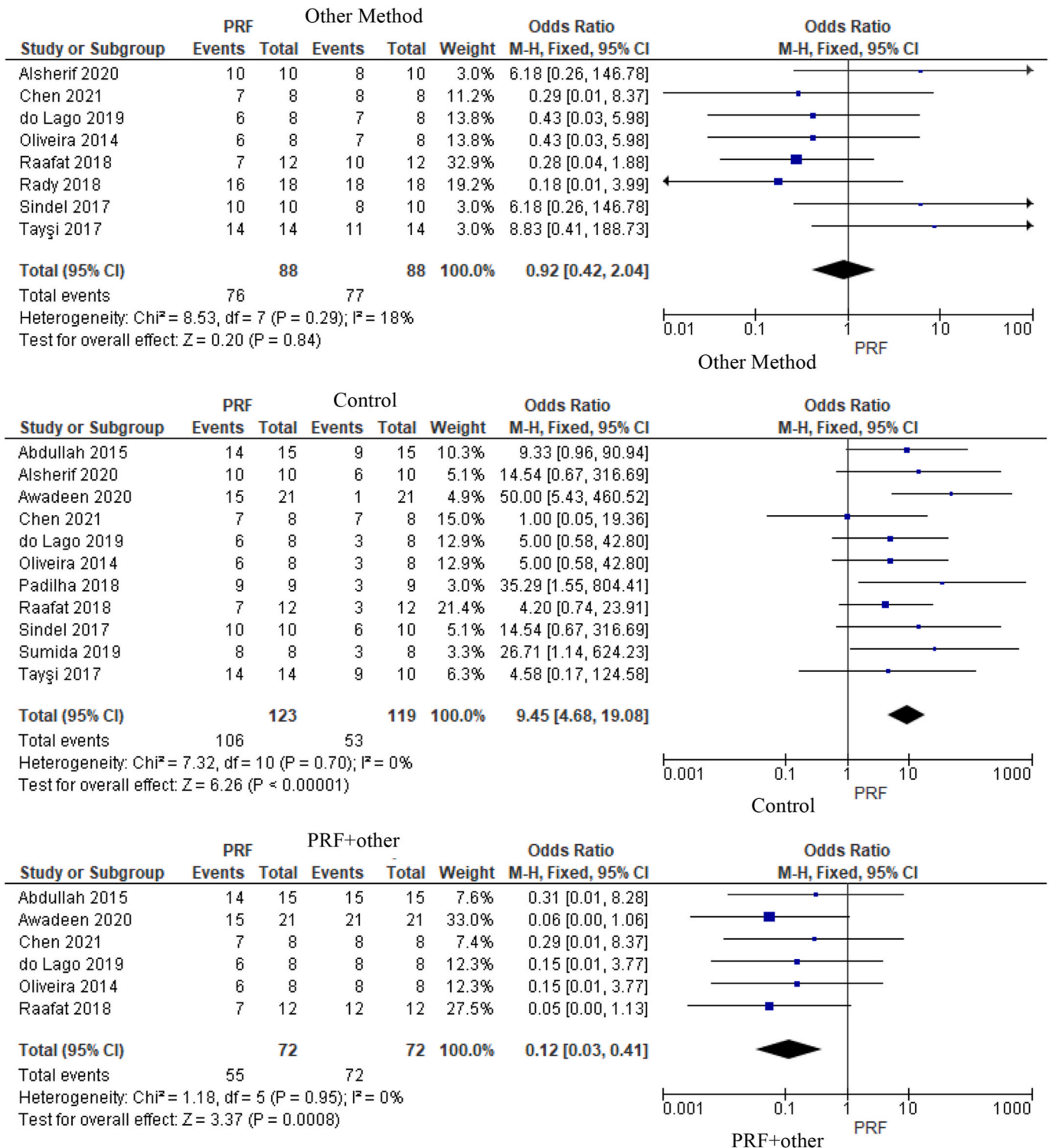


Fig. 2. Forest Plot showing the odds ratio (OR) of PRF vs other methods (A), PRF vs control (B), PRF vs combination of PRF with other methods (C).

3. Results

3.1. Study selection flow chart

Fig. 1 elucidates the study selection process, which started with the initial identification resulting in a total of 934 articles. Succeeding it, 943 papers were filtered based on the duplication of articles, and there were 882 that were not duplicated, which then the process followed directly with the abstract review. The abstract assessment resulted in 32 suitable reports based on the availability of independent and dependent variables. The filtered papers were re-evaluated regarding the study design and resulted in 12 articles to be recruited on the qualitative analysis of systematic review, which comprised 483 mice.

3.2. Study quality of risk bias

None of the twelve studies identified provided information on the seven risks of bias assessment. However, most of the study is categorized into low-risk bias in most aspects of the evaluation (Fig. 4). One study illustrated a high bias in random sequence and another in allocation concealment (selection bias). High bias on blinding of outcome was found in one study, and two studies evaluated into high risk of bias in the incomplete outcome data (attrition bias). In the compilation (Fig. 3) high risk of bias is found in four aspects of assessment (random sequence generation, allocation concealment, blinding of outcome assessment, and incomplete outcome data).

3.3. Evidence synthesis

The meta-analysis assessed three different comparisons of PRF with other bone regeneration methods. PRF, compared to other methods, resulted in a pooled odds ratio of 0.92 ((95% CI 0.42–2.04; $p = 0.29$), PRF compared to control led to a pooled odds ratio of 9.45 (95% CI 4.68–19.08; $p = 0.01$), a combination of PRF compared PRF alone resulted in a pooled ratio 0.12 ((95% CI 0.03–0.41; $p = 0.01$) (Fig. 2). There was a limited sensitivity test because of the small studies involved.

4. Discussion

Based on Fig. 2A, there is no significant relationship between the effectiveness of using PRF compared to other methods in the process of bone regeneration in bone defects, with a pooled OR of 0.92. Different ways that were compared with PRF in this study varied, including ozone, poly-3-caprolactone (PCL), deproteinized bovine bone mineral (DBBM), and statins like simvastatin (SIM), bone marrow mesenchymal stem cells

(BM-MSCs), hyaluronic acid gel (HA) and collagen membrane. Along with technology development, several methods are employed for bone defect restoration [14], which can ensue for various reasons. Many graft materials and biomaterials, like allografts [15] and autografts [16], are utilized in treating bone defects [17]. Although bone graft plays the gold standard because of its outstanding osteoinductive and osteogenic, its application is limited because of additional surgical operation factors [18], donor morbidity [19,20], and a large number of defects are significantly decreased [21].

PRF is a second-generation derivative platelet applied to escalate bone healing and regeneration [22]. PRF is also regarded as the reservoir of multi-growth factors in bone regeneration augmentation procedures in the initiation phase [11,23]. In this study, PRF was not much different from other methods used in the bone regeneration process. PRF can still compete with other methods in bone regeneration processes in bone defects [24].

Based on the analysis results in Fig. 2B, there is a very significant relationship between the effectiveness of using PRF compared to controls in the process of bone regeneration in bone defects resulting from pooled OR as much as 9.45. It can be concluded that the use of PRF is 9.45 times more effective in bone regeneration compared to the control. The use of PRF consistently accelerated the process of bone degeneration compared to the control group. The control group did not receive any intervention to improve the intervention. This explanation is supported by a previous study that assessed platelet-derived growth factors' effect on the potential of alveolar bone formation. It was reported that PRFs resulted in an incremental 3.8 fold in cellular proliferation rate accompanied by cellular migration and chemotaxis increase of 9.6 fold to the control group. The advance in cellular proliferation and migration is coherent with an overall improvement in extracellular matrix component synthesis with increased expression of ALP and procollagen type I. It jointly escalated matrix formation and mineralization rates [25].

Bone healing relies on the coordination of multi-various cells and biological events series. Bone healing is a complex process and is the primary concern in the medical field [26] Our result is similar to the previous study, which showed that PRF commuted the autologous and various growth factors that significantly activate the more robust and durable effect of osteoblast proliferation and differentiation in the mouse.

We observed a significant relationship between the effectiveness of using a combination of PRF with other methods compared to PRF alone in the process of bone regeneration in bone defects with a pooled OR of 0.12 (Fig. 2). It can be concluded that the combined use of PRF with other methods is 0.12 times more effective than the use of PRF alone in

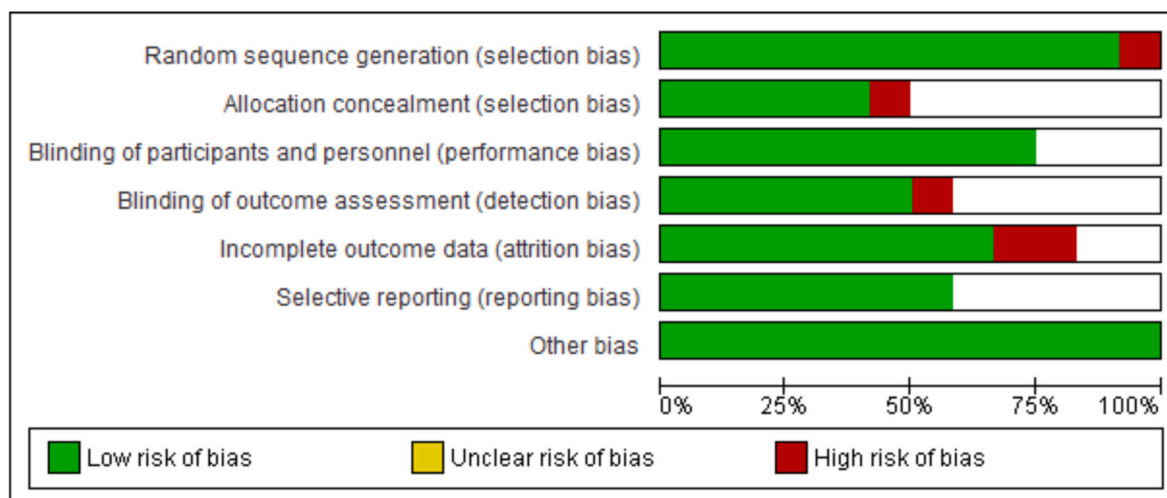


Fig. 3. Risk of bias graphic, reviewing the authors' assessment of each item's risk of bias presented as a percentage across all included studies.

	Tayşi 2017	Sumrida 2019	Sindel 2017	Rady 2018	Raafat 2018	Padilha 2018	Oliveira 2014	do Lago 2019	Chen 2021	Awadeen 2020	Alsherif 2020	Abdullah 2015
Random sequence generation (selection bias)	+	+	+	+	+	-	+	+	+	+	+	+
Allocation concealment (selection bias)			+		+	+	+	+			-	
Blinding of participants and personnel (performance bias)	+	+	+	+				+	+	+	+	+
Blinding of outcome assessment (detection bias)				-	+	+	+		+	+	+	
Incomplete outcome data (attrition bias)	+		+	+		+	+	+	-	-	+	+
Selective reporting (reporting bias)		+		+	+				+	+	+	+
Other bias	+	+	+	+	+	+	+	+	+	+	+	+

Fig. 4. Summary of risk of bias, reviewing the authors' assessment of each item's risk of bias for each of the included studies.

bone regeneration in bone defects. Scientists have studied the synergistic effect of PRF combined with different materials of bone grafts. Nonetheless, those reports have performed deeply mixed results, probably because of the bioactive properties of graft materials.

This study has several limitations; among others, our analysis was not in direct contact with the researcher, resulting in several articles that could not be analyzed. The small number of articles obtained also resulted in limitations in conducting sensitivity tests.

5. Conclusion

Our study found that PRF use could not accelerate the bone healing process compared to other methods. The combination of PRF with other methods can better enhance bone healing than the use of PRF only. However, PRF-only intervention results better than the physiological process (without any intervention). The result provides a better understanding of the most effective treatment for bone defects. Further experimental studies should emphasize more detail of the intervention and outcome assessment.

Other information

This study is registered in the research registry with the number of [reviewregistry1341](https://www.crd.york.ac.uk/PROSPERO/record/reviewregistry1341).

Funding

There is no funding supporting this study; the authors cover all expenses.

Ethical approval

This study is not involving human or patients (not applicable).

Please state any sources of funding for your research

None to disclose.

Author contribution

Mujaddid Idulhaq: Methodology construction, literature review, data collection, data analysis.

Ambar Mudigdo: Data collection, statistical analysis.

Pamudji Utomo: Literature review, and methodology construction.
 Brian Wasita: Data collection and literature review, statistical analysis.
 Fanny Indra Warman: Figures and tables, and data analysis.

Please state any conflicts of interest

None to disclose.

Registration of research studies

1. Name of the registry:
2. Unique Identifying number or registration ID:
3. Hyperlink to your specific registration (must be publicly accessible and will be checked):

Guarantor

Mujaddid Idulhaq, MD.

Consent

Not applicable.

Declaration of competing interest

We declare no conflict of interest in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.amsu.2022.104869>.

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