Platelet-rich fibrin as palatal bandage in gingival graft harvest: a systematic review and meta-analysis

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Abstract

Aim: To systematically review the effects of platelet-rich fibrin applications on the palatal wounds of patients undergoing gingival graft harvest.

Materials and Methods: An electronic search was performed (May 2020) in six databases: Cochrane Library, EMBASE, LILACS, PubMed, Scopus, and Web of Science. Pain and delayed bleeding were the outcomes assessed. The randomized clinical trials were submitted to risk analysis of bias (RoB 2.0 Cochrane Collaboration), and the Grading of Recommendations Assessment, Development and Evaluation (GRADE) assessed the quality of scientific evidence.

Results: From the ten studies included, the majority showed unclear or high risk of bias. Patients who received PRF had lower pain scores within 7-13 days (SMD = -3.01; 95% CI = -5.22 - -0.80; I2 = 95%). The PRF group showed no statistically significant difference in delayed bleeding outcome (RR = 0.38; 95% CI = 0.05 - 3.18; I2= 80%). For both outcomes, GRADE analysis showed a low quality of scientific evidence.

Conclusion: Despite the limited evidence currently available, analysis found that PRF can reduce postoperative pain, enabling a postoperative period more comfortable for the patient, but does not decrease the risk of delayed bleeding.

Keywords: Biomaterial; Regeneration; Periodontal surgery; Growth Factors; Systematic Review/Meta-analysis.

1. Introduction

Gingival recessions are characterized by marginal gingiva displacement apically to the cementoenamel junction. They can bring unfavorable conditions to the patient such as an area more conducive to root caries, non-carious cervical lesions, greater dentinal sensitivity, and even aesthetic dissatisfaction (Cairo *et al*., 2014; Deo *et al*., 2019). Among the techniques used for root coverage, the free gingival graft and the subepithelial connective tissue graft stand out, as they have the advantage of having an increase in the area of adherent gingiva, simultaneously with the root coverage (Yadav *et al*., 2016; Windisch and Molnar, 2019). In this sense, its performance becomes dependent on a tissue graft which comes from the palate in most cases (Zucchelli *et al*., 2018; Deo *et al*., 2019). However, the inclusion of

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a second surgical area can bring about greater morbidity in the postoperative aspects of patients undergoing this procedure (Alkan and Parlar, 2011; Windisch and Molnar, 2019).

Therefore, alternatives are proposed to improve these parameters, especially to the experience of postoperative pain and healing of the graft donor area (Dragan *et al*., 2017). Several studies analyzed the options to achieve this goal: the application of cyanoacrylate adhesive, absorbable gelatin sponge, collagen membrane, and latex membrane on the palatal wound (Thoma *et al*., 2016; Stavropoulou *et al*., 2019; Ehab *et al*., 2020).

More recently, the benefits of using the platelet-rich fibrin (PRF) for tissue repair and biostimulation have been observed (Naik *et al*., 2013). This material consists of a highly biocompatible matrix, acting as a reservoir of tissue growth factors, which proliferates and differentiates osteoblasts, endothelial cells, and various sources of fibroblasts, and thus contributes to the process of healing (Miron *et al*., 2017a). Its preparation only requires blood collection and centrifugation, with no need for biochemical blood management (Ozcan *et al*., 2017). Depending on the centrifugation protocol used, it can be presented in liquid form (i-PRF) or solid form (A-PRF; L-PRF; T-PRF), which can also interfere with its biological properties (El Bagdadi *et al*., 2019). i-PRF is characterized by reduced centrifugation time and speed, resulting in a fluid biomaterial rich in platelets, leukocytes and growth factors (Miron *et al*., 2017b; Choukroun and Ghanaati, 2018; Varela *et al*., 2019). For solid preparations, a longer resorption time and better mechanical properties are observed, respectively, for T-PRF, A-PRF and L-PRF membranes, providing a better use of growth factors over time. (Ravi and Santhanakrishnan, 2020). Considering that the membrane resorption time is a determining factor in terms of its effectiveness, it is reported in the literature that the increase in the number of layers on the wound optimizes this aspect, providing a homogeneous and more concentrated distribution of its biological components (Shivashankar *et al.*, 2013; Culhaoglu *et al.*, 2017).

To the best of our knowledge, there are still no systematic reviews about the application of this biomaterial on palatal wounds resulting from the harvesting of gingival grafts, although the literature has already demonstrated its benefits in the most diverse areas of dentistry, such as periodontics and oral surgery (Tarallo *et al*., 2020). This suggests it as an alternative to improve the post-operative parameters of root coverage surgery, to avoid the occurrence of postoperative complications, and to increase post-surgical comfort, thus aiming to increase patients' adherence to treatment.

Therefore, the objective of this systematic review was to evaluate the healing properties of platelet-rich fibrin, through an analysis of postoperative pain levels and delayed bleeding events experienced by patients undergoing harvesting of palatal gingival graft.

2. Materials and Methods

2.1 Protocol and registration

The protocol of this systematic review was developed following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA Checklist) (Moher *et al*., 2009), and registered in the International Prospective Register of Systematic Reviews (PROSPERO), under the identification number CRD42020185863.

2.2 Eligibility criteria

2.2.1 Inclusion criteria

Studies that met the following criteria using the acronym "PICOS" were considered eligible for this systematic review:

1. Population (P): Patients undergoing root coverage surgery with a gingival graft harvested from the palate;
2. Intervention (I): PRF application on palatal wound;
3. Control (C): No procedure (only hemostasis with gauze), synthesis (suture or adhesive), or non-autologous biomaterials;
4. Outcomes (O): Lower pain scores and fewer delayed bleeding events;
5. Study types (S): Randomized, non-randomized or pseudo-randomized clinical trials.

2.2.2 Exclusion criteria

Articles were excluded based on the following criteria: (1) pre-clinical studies; (2) studies that did not provide information about the PRF preparation protocol; (3) literature reviews, cards, books, conference abstracts, case reports, case series, opinion article, technical articles, retrospective studies.

2.3 Information sources

The electronic search was carried out on May 1, 2020, using keywords in different combinations, based on the terms of the Medical Subject Heading (MeSH) (Appendix 1). Cochrane Library, EMBASE, Latin American and Caribbean Health Sciences (LILACS), PubMed (including Medline), Scopus, and Web of Science databases were consulted and configured to generate an email alert to notify users of new studies that emerged after this initial search. Also, on the same date, a search was made in the gray literature, through searches in Google Scholar, Open Grey, and Proquest (theses and dissertations). The reference's list of relevant studies was consulted, and experts were

contacted to improve the search strategy. References have been managed and duplicates have been removed using the EndNote®X7 reference manager (Thomson Reuters, Philadelphia, PA).

2.4 Study selection

The selection of articles to be included after the search was carried out by four independent reviewers. In the first phase, the reviewers read the titles and abstracts to which they applied the eligibility criteria. In phase two, the same reviewers read the full text in order to apply the established inclusion and exclusion criteria. At the end of each phase, in case of disagreement between the reviewers, a fifth reviewer, with experience in the subject, also analyzed the study.

2.5 Data collection process and data list

The same four independent reviewers collected data from the selected studies. The articles' data were crossed, and the reviewers discussed disagreements in search of a consensus. For all studies included after the complete reading, the following information was collected: author, publication year, country, sample characteristics, root coverage technique, follow-up period, data of outcomes (pain and delayed bleeding), and main conclusion. In case of the absence of data in the studies, the authors were contacted.

2.6 Risk of bias in individuals study

The same four independent reviewers analyzed the risk of bias for each outcome of the included studies using the RoB 2.0 tool (Cochrane Collaboration) (Sterne *et al*., 2019). If there was any divergence between the four reviewers, they consulted a fifth experienced reviewer. For each outcome analyzed, a judgment of low risk, some concerns, or high risk of bias was made using five domains: randomization process, deviations from the intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result.

2.7 Summary measures and synthesis of results

Meta-analyses were performed with models of random effects for continuous (pain scores) and binary (delayed bleeding) outcomes for the quantitative evaluation of results. To ensure better visualization of the estimated effect of the PRF group when associated with another material, the control groups included in the analysis were paired with the corresponding material associated with the PRF.

For the pain outcome, due to the difference in the scales to measure this outcome (range 1-5, 1-10 and 1-100), the standardized difference between the means was calculated, thus dividing the difference in the means between the two arms of analysis by the standard deviation common to them, generating the value corresponding to the difference in standard deviations between the two interventions (experimental and comparator). The meta-analysis was performed for the following postoperative time intervals: 0-6 days; 7-13 days; and 28-30 days. Due to the clinical and statistical heterogeneity present in the analysis, subgroup analyses were performed, dividing the analysis based on the category of material used in the control and experimental group, thus visualizing the individual effect of each of these categories. In addition, if substantial heterogeneity was detected in the analysis (above 50%) (Higgins and Thompson, 2002), the sensitivity analysis was performed using the leave-one-out method, thus detecting how each individual study affected the overall estimate and statistical heterogeneity.

The association between the presence or absence of delayed bleeding in the postoperative period between the experimental group (PRF) and the comparator group was assessed by calculating the relative risk, according to the prospective design of all studies included in the analysis. The calculation of statistical heterogeneity was performed using the Higgins inconsistency index (I2) (Higgins and Thompson, 2002). This analysis was also divided into subgroups, and for the calculation of statistical heterogeneity, the Higgins inconsistency index was used (I2) (Higgins and Thompson, 2002), together with the sensitivity analysis in cases of detection of substantial heterogeneity.

The significance level adopted was 5%. Forest plots and leave-one-out analyses were generated using Review Manager 5.3 software (RevMan 5.3, Copenhagen, Denmark) and the Rstudio version statistical software 1.2.1335 (Rstudio Inc, Boston, USA).

2.8 Risk of bias across studies

The certainty of the evidence from the studies was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) (Guyatt *et al*., 2008). The final judgment, for each outcome, was defined as high, moderate, low or very low, being based on five study criteria: design, methodological limitations, inconsistency, indirect evidence and imprecision.

3. Results

3.1 Study selection

A total of 396 articles were identified in the searches. After removing duplicates, 244 studies were included for the reading of titles and abstracts, of which 17 were selected for full reading. The configuration for alerts, by the databases, generated the inclusion of one more article.

The search in the gray literature resulted in 861 articles, but as well as the consultation of reference lists and specialists, it did not result in the inclusion of additional studies. Finally, in view of the 18 studies selected for full reading, ten were included for the qualitative synthesis, and seven, for the quantitative synthesis (Appendix 2). Figure 1 shows the flowchart of this process.

3.2 Study characteristics

The included articles were written in English and published between the years 2016 to 2020. All of them had at least one intervention group characterized by the PRF application at the graft donor site. However, there was a variation concerning to the type of PRF prepared. L-PRF was the most commonly described preparation, used by Femminella *et al.* (2016); Ozcan *et al.* (2017); Bahammam (2018); İşler *et al.* (2019); Patarapongsanti *et al.* (2019); Sharma *et al.* (2019); and Alpan and Cin (2020). Kiziltoprak and Uslu (2020) used i-PRF; Sousa *et al.* (2020), A-PRF and Ustaoğlu *et al.* (2016), T-PRF. PRF was fixed with sutures in all of them, except by Ozcan *et al.* (2017), who used adhesive to fix this biomaterial. Regarding the number of layers of biomaterial applied to the palatal wound, only Femminella *et al.* (2016) and Sousa *et al.* (2020) reported this detail and state that the patients in the test group (PRF) received four layers of L-PRF and two from A-PRF, respectively.

It is also possible to observe a variation between the studies concerning to the comparator groups. The studies of Ustaoğlu *et al*. (2016), Ozcan *et al*. (2017), İşler *et al*. (2019), and Kızıltoprak and Uslu (2020) presented a negative control group, that is, patients that did not receive any type of intervention in the palatal wound. Considering the occlusive procedures, Ozcan *et al*. (2017) applied cyanoacrylate adhesive to a group in their study, whereas Bahammam (2018) and Alpan and Cin (2020) used only the suture in one of the studied groups. Finally, some other studies carried out the application of some other biomaterial in the comparator groups, such as gelatin sponge (Femminella *et al*., 2016), collagen membrane (Sharma *et al*., 2019), cellulose (Patarapongsanti *et al*., 2019) and collagen sponge (Sousa *et al*., 2020).

The follow-up periods comprised of 14 (İşler *et al*., 2019) to 180 days (Ustaoğlu *et al*., 2016). In addition, the number of participants ranged from 18 (Patarapongsanti *et al*., 2019) to 141 patients (Ozcan *et al*., 2017). In four studies, the patients' palatal wounds received any type of protection, such as plate protection (Ustaoğlu *et al*., 2016; Sousa *et al*., 2020) and dental cement (Bahammam, 2018; Kızıltoprak and Uslu, 2020). The inclusion criteria for these studies, in general, were adult patients (18 to 65 years old) without contraindications to undergo periodontal surgery, without a history of periodontal operations at the surgical site, and with good oral hygiene conditions. The exclusion criteria included the presence of systemic diseases, a smoking habit, or consumption of anticoagulant drugs or those that may interfere with periodontal health. The detailed characteristics of the included studies are described in Table 1.

3.3 Risk of bias within studies

For the pain outcome, three studies were classified as some concerns, while the others were classified as a high risk of bias (Femminella *et al*., 2016; Sharma *et al*., 2019;Sousa *et al*., 2020) (Figure 2). The high risk was especially attributed to the domain of outcome measurement, since the studies did not bring information related to the blinding of the patients. For the delayed bleeding outcome, one study was classified as high risk (Ustaoğlu *et al*., 2016), five, as some concerns (Ozcan *et al*., 2017; İşler *et al*., 2019; Kızıltoprak and Uslu, 2020; Alpan and Cin, 2020; Sousa *et al*., 2020), and two, as low risk of bias (Femminella *et al*., 2016; Sharma *et al*., 2019) (Figure 3).

3.4 Results of individual studies

3.4.1 Pain

The Visual Analogue Scale (VAS) was used by all studies to assess pain levels. From the four studies that assessed this outcome between the PRF group and the negative control group (Ustaoğlu *et al*., 2016; Ozcan *et al*., 2017; İşler *et al*., 2019; Kızıltoprak and Uslu, 2020), two found statistically significant differences in favor of the PRF group (Ustaoğlu *et al*., 2016; Ozcan *et al*., 2017). Ozcan *et al*. (2017), who used L-PRF fixed with adhesive, found this difference during the first six days, and as Ustaoğlu *et al*. (2016), who used T-PRF, found a difference for the 7th and 14th postoperative days. The two studies that had as a comparator group patients who underwent only the suture, also found lower levels of pain, in favor of the PRF group, for the 3rd, 4th, 5th, 6th, 7th (Bahammam, 2018; Alpan and Cin, 2020) and 14th postoperative days (Alpan and Cin, 2020).

When compared to those who received some material (Femminella *et al*., 2016; Ozcan *et al*., 2017; İşler *et al*., 2019; Sharma *et al*., 2019; Patarapongsanti *et al*., 2019; Sousa *et al*., 2020; Kızıltoprak and Uslu, 2020), three studies reported differences in favor of the PRF group ($p<0.05$) (Femminella *et al*., 2016; Ozcan *et al*., 2017; Sousa *et al*., 2020). The results of Sousa *et al*., (2020), who used two layers of A-PRF, showed statistical significance only for the second day after surgery. Ozcan *et al*. (2017) found differences between groups during the first five days of evaluation and Femminella *et al*. (2016), who used four layers of L-PRF, for the four weeks after surgery.

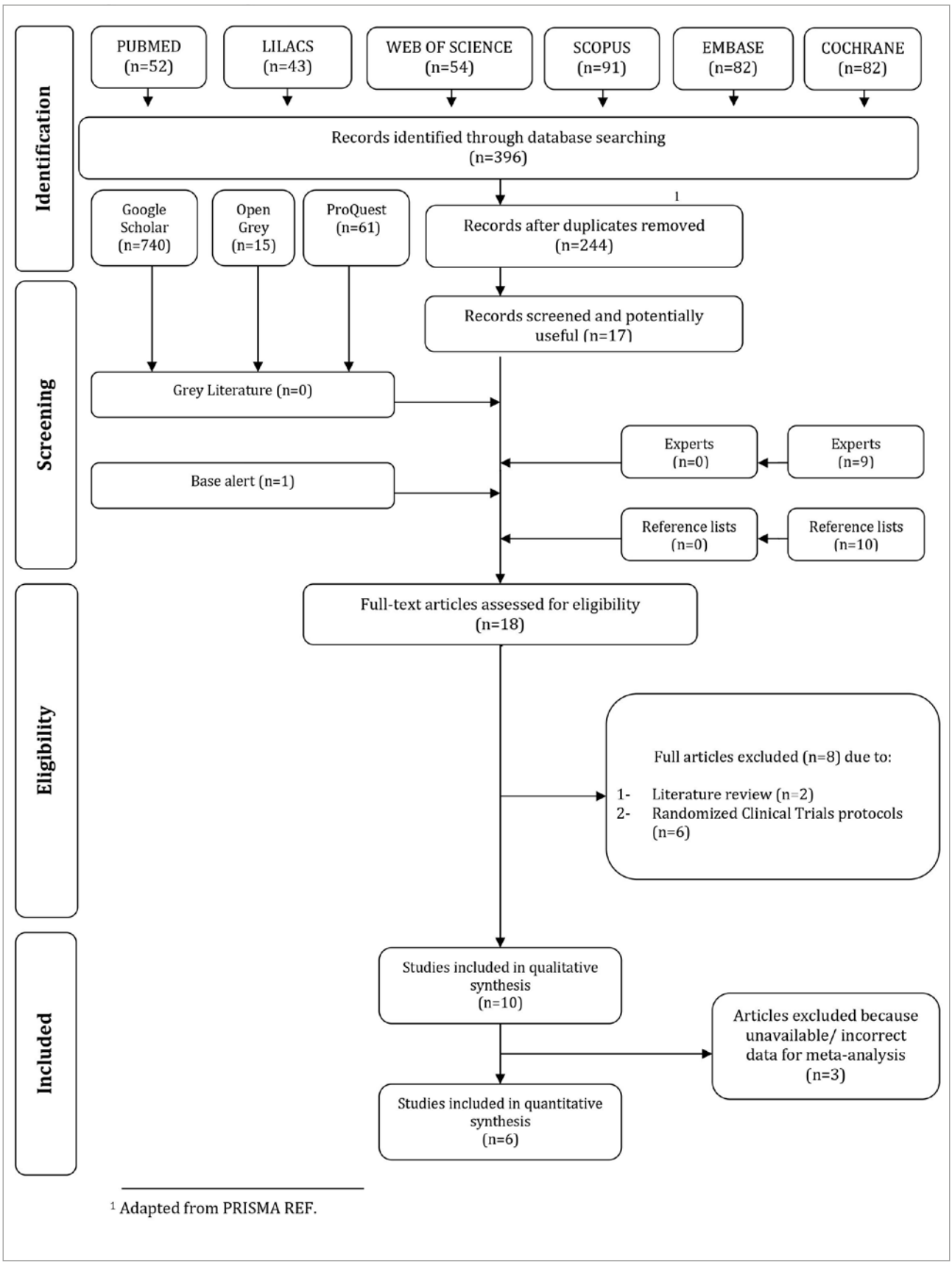

Figure 1. Flow diagram of literature search and selection criteria.

Table 1. Summary of descriptive characteristics of included studies (n=10).

Author, Year; Country	Groups (n)	Age range ± Standard Deviation (years)	Key technique utilized	Graft thickness (mm)	Follow-up time	PRF's protocol	Pain- ¹mean or ²median	Delayed bleeding (number of patients)	Main conclusion
Alpan and Cin, 2020 Turkey	G1 (L-PRF + suture) n = 20 G2 (Suture) n = 20	G1 30,6 ± 6,45 G2 30,89 ± 6,92	Subepithelial connective tissue graft	2	T0) Baseline T1) 1 day T2) 3 days T3) 7 days T4) 10 days T5) 14 days T6) 30 days	20 mL of venous blood (10mL/tube) centrifuged at 2800 rpm for 12 minutes	G1 x G2 VAS¹ 0-100 T0: 52,5 x 71,58* T1: 27,3 x 44,78 T2: 19,85 x 38,42 T3: 6,5 x 28,95 T4: 3 x 14,21*	Patient's report G1: 2 G2: 4	PRF application decreases pain and postoperative complications
Bahammam, 2018; Saudi Arabia	G1 (L-PRF + suture) n = 12 G2 (Suture) n = 12	G1 27,8 ± 4.3 G2 28.5 ± 3.7	Free gingival graft	1.5	T0) Baseline T1) 1 days T2) 2 days T3) 3 days T4) 4 days T5) 7 days T6) 14 days T7) 21 days T8) 30 days T9) 60 days	10 mL of venous blood centrifuged at 3000 rpm for 10 minutes	G1 x G2 a VAS¹ 0-10 T1: 1,4 x 2,94 T2: 0,8 x 3,3 T3: 0 x 1,3* T4: 0 x 0,16* T5: 0 x 0,04*	Has not been reported	PRF application accelerates healing and reduces postoperative pain
Femminella et al., 2016; Italy	G1 (L-PRF + suture) n = 20 G2 (Gelatin sponge + suture) n = 20	All subjects 32,4 ± 5	Subepithelial connective tissue graft	2	T0) Baseline T1) 7 days T2) 14 days T3) 21 days T4) 30 days	40 mL of venous blood (10mL/tube) centrifuged at 3000 rpm for 10 minutes. The fibrin clots were squeezing with a specific mechanical press	G1 x G2 VAS¹ 0-10 T1: 2,4 x 4,6* T2: 1,75 x 3,75* T3: 1,1 x 2,6* T4: 0,15 x 1,35*	Patient's report None of patients reported delayed bleeding	PRF application accelerates healing and reduces the patient's morbidity
Isler et al., 2019; Turkey	G1 (L-PRF + suture) n = 10 G2 (Collagen sponge + suture) n = 10 G3 (Secondary healing) n = 10	All subjects 39,5 ± 12,7	Free gingival graft	1.5	T0) Baseline T1) 1 day T2) 2 days T3) 3 days T4) 4 days T5) 5 days T6) 6 days T7) 7 days T8) 14 days	10 mL of venous blood centrifuged at 3000 rpm for 10 minutes b	G1 x G2 x G3 VAS¹ 0-10 T1: 0,7 x 1,3 x 2,2 T2: 0,3 x 2,1 x 2,1 T3: 0 x 0,7 x 0,3 T4: 0,1 x 2,4 x 2,4 T5: 0,3 x 2,8 x 3,9 T6: 0,1 x 1,6 x 2,4 T7: 0,4 x 1,6 x 1,9	Patient's report None of patients reported delayed bleeding	PRF application can improve post-operative discomfort of patients undergoing to free gingival graft
Kızıltoprak and Uslu, 2020; Turkey	G1 (i-PRF + suture) n = 12 G2 (AFG) n = 12 G3 (Secondary healing) n = 12	G1 28.92 ± 9.66 G2 33.25 ± 10.97 G3 32.08 ± 9.46	Free gingival graft	1.5-2mm	T0) Baseline T1) 3 days T2) 7 days T3) 14 days T4) 30 days T6) 90 days	9 mL of venous blood centrifuged at 2300 RPM for 3 minutes. i-PRF at the top was collected and transferred to a metal bowl for 20-25 minutes for polymerization	G1 x G2 x G3 VAS¹ 0-100 T1: 27,92 x 16,08 x 33,67 T2: 32,42 x 11,67 x 33,33 T3: 6,33 x 1,08 x 16,67 T4: 0,83 x 0 x 0	Clinical analysis G1: 2 G2: 0 G3: 9*	The evaluated platelet concentrates improved postoperative morbidity, with emphasis on fibrin glue

Table 1 continued overleaf...

Table 1 continued

Ozcan et al., 2017; Turkey	G1 (L-PRF + Adhesive) n = 42 G2 (Adhesive) n = 42 G3 (Second healing) n = 41	G1 (34.55 ± 7.64) G2 (37.11 ± 4) G3 (37.61 ± 6.64)	Free gingival graft	1.3-1.5	T0) Baseline T1) 1 days T2) 2 days T3) 3 days T4) 4 days T5) 5 days T6) 6 days T7)7 days T8) 14 days T9) 21 days T10) 28 days	10 mL of venous blood centrifuged at 2700 rpm for 12 minutes	G1 x G2 x G3 VAS[1] 0-10 T1: 2,0 x 4,6* x 6,10* T2: 1,3 x 5,9* x 5,2* T3: 0,36 x 1,9* x 3,2* T4: 0,12 x 1,21* x 2,41* T5: 0,02 x 0,88* x 1,98* T6: 0 x 0,12 x 1,29* T7: 0 x 0 x 1,02* T8: 0 x 0 x 0,32* T9: 0 x 0 x 0,15 T10: 0 x 0 x 0,01	Patient's report G1: 0 G2: 34 * G3: 37 *	PRF application can accelerate healing and reduce postoperative morbidity
Patarapongsanti et al., 2019; Thailand	G1 (L-PRF membrane + suture) n = 18 G2 (Cellulose + suture) n = 18	All subjects 60 ± 8,45	Free gingival graft	0.75-1.25	T0) Baseline T1) 1 day T2) 3 days T3) 7 days T4) 14 days T5) 21 days T6) 30 days	10 mL of venous blood centrifuged at 3000 rpm for 10 minutes. The fibrin clot was placed on a mechanical press device which transformed the clot in membrane	G1 x G2 VAS [1]a 0-10 T1: 0,28 x 0,72 T2: 0 x 0,15 T3: 0 x 0	Has not been reported	Both materials can accelerate healing, but PRF may have more advantages in reducing postoperative morbidity
Sharma et al., 2019; India	G1 (L-PRF membrane + suture) n = 10 G2 (Collagen membrane + suture) n = 10	Has not been reported	Free gingival graft	Has not been reported	T0) Baseline T1) 1 day T2) 7 days T3) 8 days T4) 12 days T5) 13 days T6) 18 days T7) 19 days T8) 24 days T9) 25 days T10) 30 days T11) 31 days	10 mL of venous blood centrifuged at 3000 rpm for 10 minutes b	G1 x G2 VAS[1] 0-5 T0: 2,2 x 1,5 T1: 1,9 x 3,2 T4: 0,8 x 1,5 T6: 0,3 x 0,6 T8: 0,4 x 0,5 T10: 0,1 x 0,1	Patient's report G1: 3 G2: 3	PRF and collagen membrane improve post-operative parameters, but PRF is easier to handle and suture
Sousa et al., 2020; Portugal	G1 (A-PRF + suture) n = 14 G2 (Collagen sponge + suture) n = 11	All subjects 36,4 ± 14,9	Free gingival graft	1.5	T0) Baseline T1) 2 days T2) 7 days T3) 14 days T4) 30 days T5) 90 days	10 mL of venous blood centrifuged at 1500 RPM for 8 minutes. The PRF clot was squeezed between a sterile metal plate and a metal box	G1 x G2 VAS[2] 0-10 T1: 0 x 2* T2: 0 x 1 T3: 0 x 0 T4: 0 x 0 T5: 0 x 0	Patient's report G1: 2 G2: 1	PRF accelerated the healing process and reduced postoperative morbidity
Ustaoglu et at., 2016; Turkey	G1 (T-PRF + suture) n = 16 G2 (Secondary healing) n = 18	Has not been reported	Free gingival graft	1.5	T0) Baseline T1) 3 day T2) 7 days T3) 14 days T4) 21 days T5) 30 days T6) 90 days T7) 180 days	20 mL of venous blood centrifuged in titanium tube at 2800 rpm for 12 minutes. The clot fibrin was pressed between two pieces of gauze to obtain a membrane	Has not been reported	Patient's report G1: 14 G2: 18	PRF application can accelerate the healing process

i-PRF injectable-platelet fibrin; L-PRF leukocyte-platelet rich fibrin; AFG autologous fibrin glue; A-PRF advanced-platelet rich fibrin; T-PRF titanium-platelet rich fibrin. aEstimated by the reviewers of this systematic review; b Referenced information; *p < 0,05 in favor of platelet-rich fibrin group.

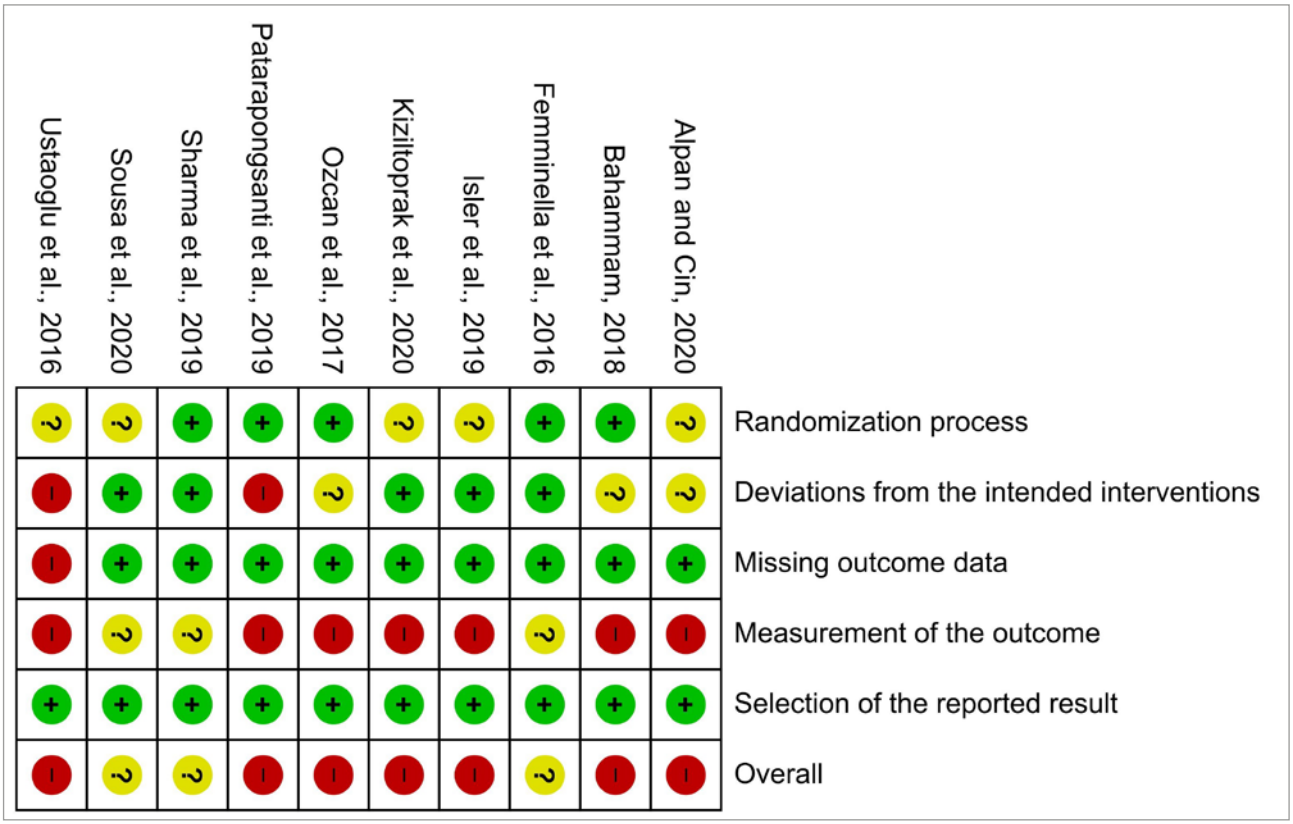

Figure 2. Cochrane's tool to assessed risk of bias in randomized controlled trials (pain outcome). Green indicates a low risk of bias, yellow indicates some concerns, and red indicates a high risk of bias.

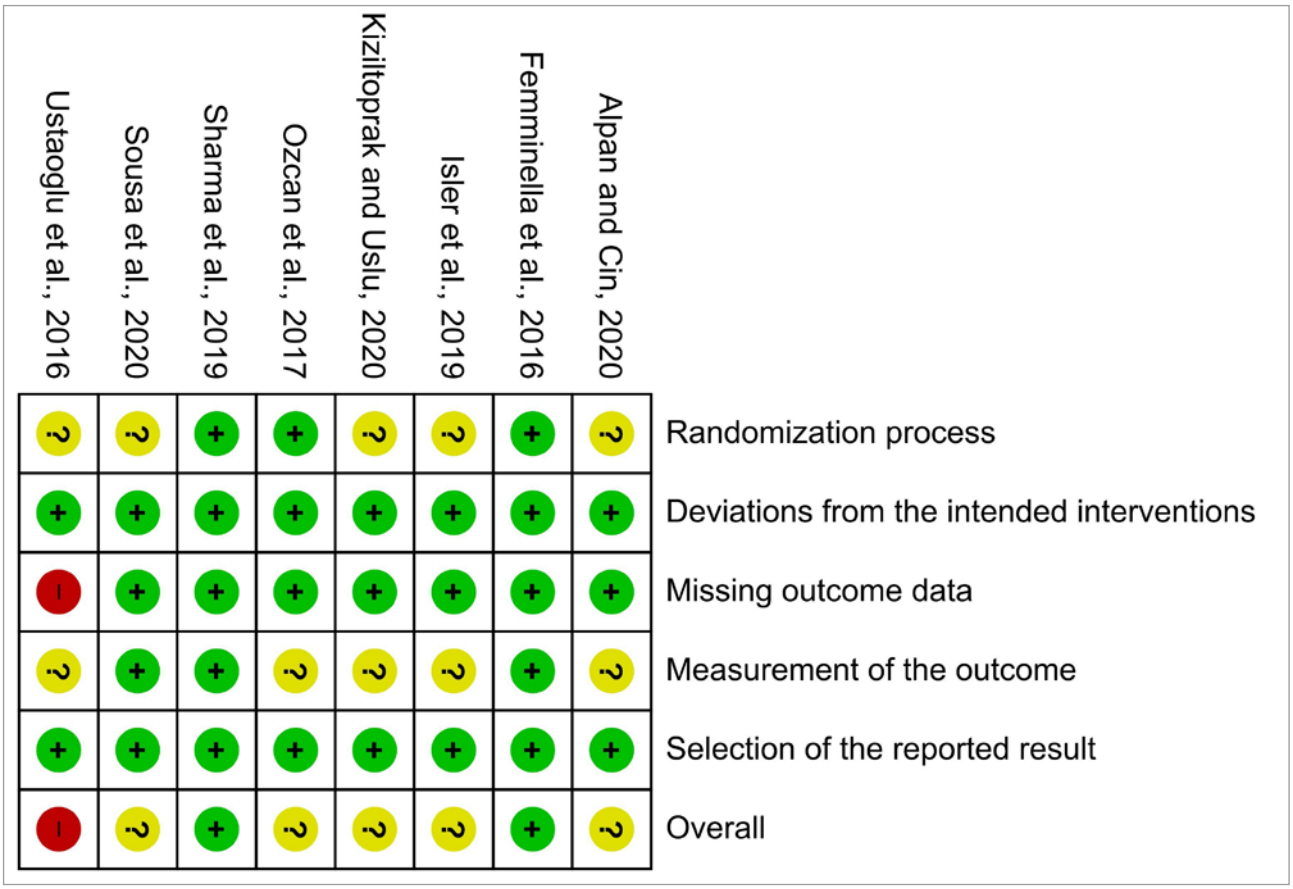

Figure 3. Cochrane's tool to assessed risk of bias in randomized controlled trials (delayed bleeding outcome). Green indicates a low risk of bias, yellow indicates some concerns, and red indicates a high risk of bias.

3.4.2 Delayed bleeding

Eight included studies brought the assessment of the occurrence of this event, among which seven considered it from the patient's report (Femminella *et al.*, 2016; Ustaoğlu *et al.*, 2016; Ozcan *et al.*, 2017; İşler *et al.*, 2019; Sharma *et al.*, 2019; Alpan and Cin, 2020; Sousa *et al.*, 2020) and one carried out the evaluation through clinical analysis (Kızıltoprak and Uslu, 2020). Four studies reported a reduction in the occurrence of these events, in favor of the PRF group (Ustaoğlu *et al.*, 2016; Ozcan *et al.*, 2017; Kızıltoprak and Uslu, 2020; Alpan and Cin, 2020), being statistically significant for Kiziltoprak and Uslu (2020), who used i-PRF, compared to the negative control group, and Ozcan *et al.* (2017), in wich L-PRF was fixed with cyanoacrylate adhesive, compared to the negative control group and the group that received only cyanoacrylate adhesive.

3.5 Synthesis of results

Six studies were included in the quantitative synthesis. When the difference in pain scores was assessed between the groups that used PRF and did not use it, a lower pain score was found in the PRF group ($p<0.05$), in the period from the seventh to the thirteenth postoperative day (SMD = -3.01; 95% CI = -5.22 - -0.80; I^2 = 95%) (Figure 4). Two periods of analysis showed high heterogeneity (7-13 days and 28-30 days). The sensitivity analysis performed for the period of 7-13 days showed that no studies had an influence on the analysis to the point of changing the statistical significance or not (Figure 5). No sensitivity analysis was performed for the 28-30 day period, because there were only two studies in that period. In both analyses, the study of Femminella *et al.* (2016), was the one that had the greatest influence on the amount of heterogeneity.

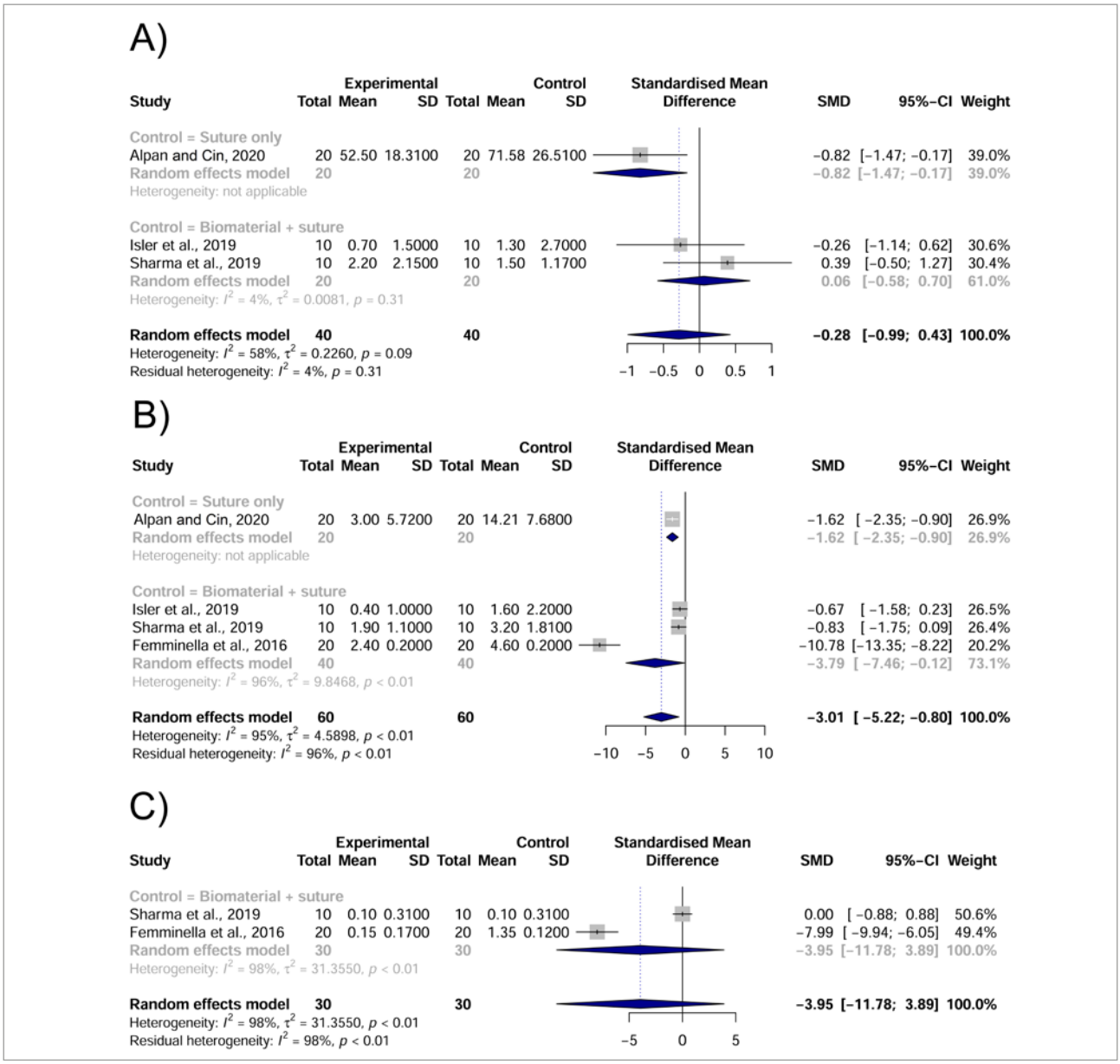

Figure 4. Forest plot detailing standardized mean difference and 95% CI for the postoperative pain score between PRF and control groups: a) 0-6 days; b) 7-13 days; c) 28-30 days.

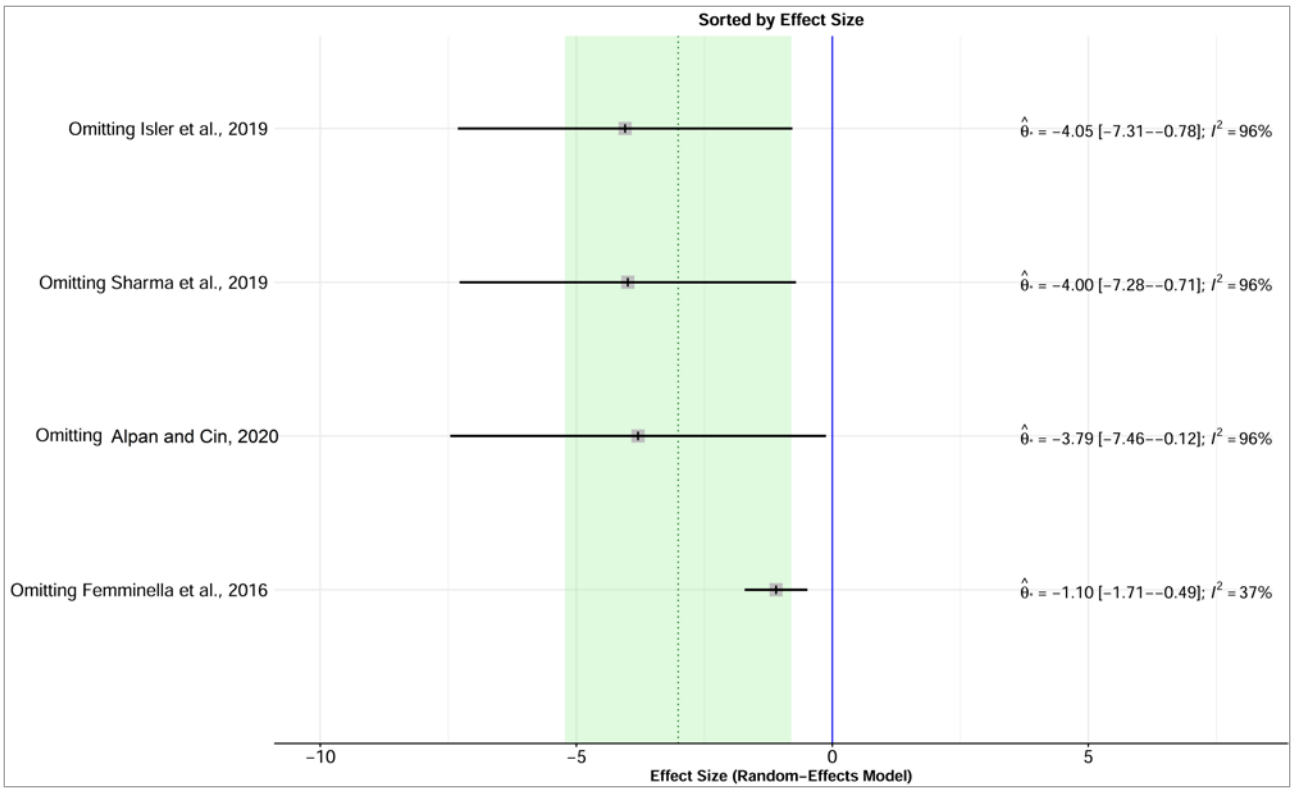

Figure 5. Leave-one-out sensitivity analysis of the postoperative pain scores: 7-13 days.

There was no statistical significance (p>0.05) for the relative risk of the presence or absence of postoperative bleeding between the experimental group and controls (RR = 0.38; 95% CI = 0.05 - 3.18; I^2 = 80%) (Fig. 6). The sensitivity analysis revealed that no study demonstrated an influence on the analysis to the point of altering the statistical non-significance. In addition, the source of the existing statistical heterogeneity was due to the results found by Ozcan *et al.* (2017). However, even with the removal of this study from the analysis, there was no statistical significance (RR = 0.86; 95% CI = 0.35 – 2.12; I^2 = 0%) (Fig. 7).

3.6 Risk of bias across studies

The evaluation of the quality of scientific evidence resulted in a very low level for pain and delayed bleeding outcomes (Table 2). This result was mainly due to the high risk of bias in the studies, as well as the clinical and statistical heterogeneity among them, directly impacting the assessment of inconsistency and imprecision.

4. Discussion

The present systematic review sought to evaluate the effects of the application of PRF on the palatal wounds of patients submitted to the collection of gingival graft. The individual analysis of the included studies showed that the PRF group had better postoperative parameters, when compared, especially, to those who did not receive any type of treatment. The meta-analytical analysis revealed that these patients had lower pain scores for one of the four periods evaluated but did not have a lower risk of delayed bleeding.

The meta-analysis revealed that PRF significantly reduced pain levels between the 7th and 13th postoperative days (SMD = -3.01; 95% CI = -5.22 - -0.80; I^2 = 95%). This result is directly related to the fact that the PRF acts as an autologous reservoir that favors the expression of TGF-β (transforming growth factor-β); PDGF (platelet-derived growth factor) and VEGF (vascular endothelial growth factor) (Miron *et al.*, 2017a; Dohle *et al.*, 2018; Kasnak *et al.*, 2019; Wang *et al.*, 2019). Mesenchymal, endothelial, epithelial and immune cells have their proliferation stimulated when exposed to PRF (Fujioka-Kobayashi *et al.*, 2017). In addition, the fibrin matrix, a three-dimensional structure, concentrates leukocytes and platelets and optimizes the release of these products gradually, ensuring that the effects associated with healing are effective and prolonged (Fan *et al.*, 2020). It is also possible to verify the consequence of the application of this biomaterial in other aspects beyond pain since patients who received PRF had epithelialization faster than the other groups (Femminella *et al.*, 2016; Sousa *et al.*, 2020), further reduction of the wound area (Sousa *et al.*, 2020), better tissue color matching (Ustaoğlu *et al.*, 2016; Bahammam, 2018), sensitivity levels closer to normal (Ozcan *et al.*, 2017) and less interference with eating habits (Femminella *et al.*, 2016; Ozcan *et al.*, 2017).

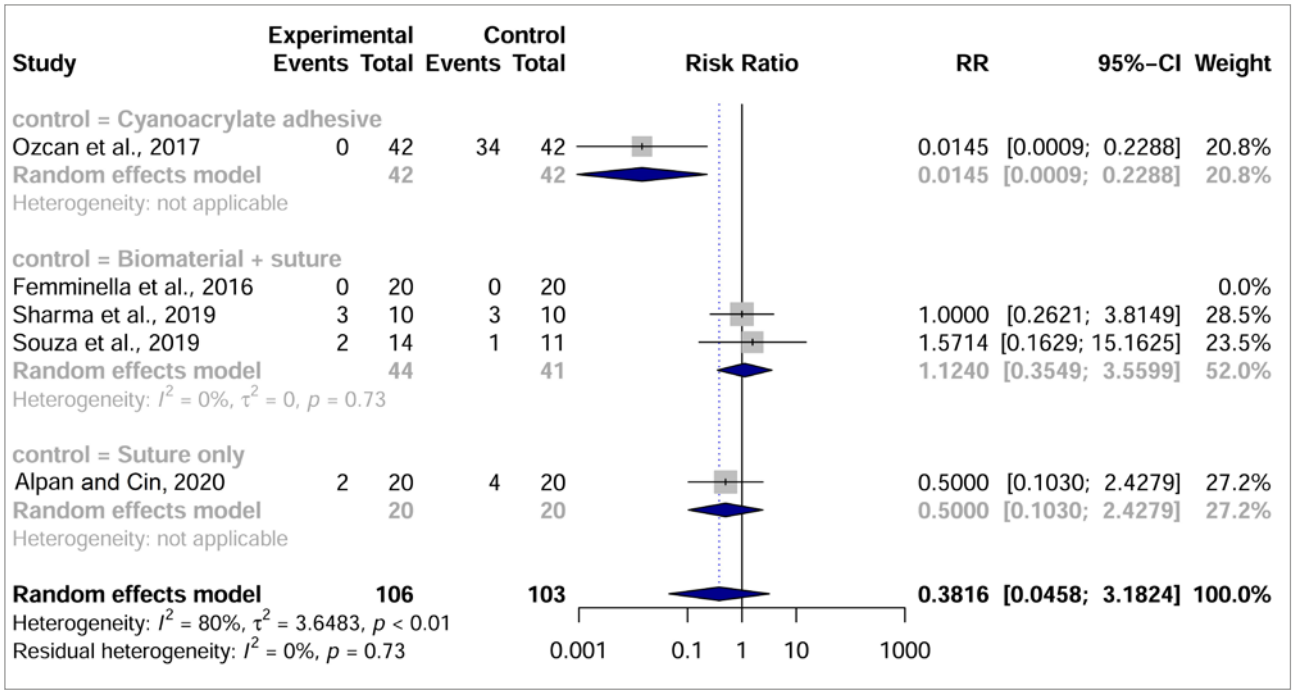

Figure 6. Forest plot detailing risk relative and 95% CI for the rate of postoperative bleeding events.

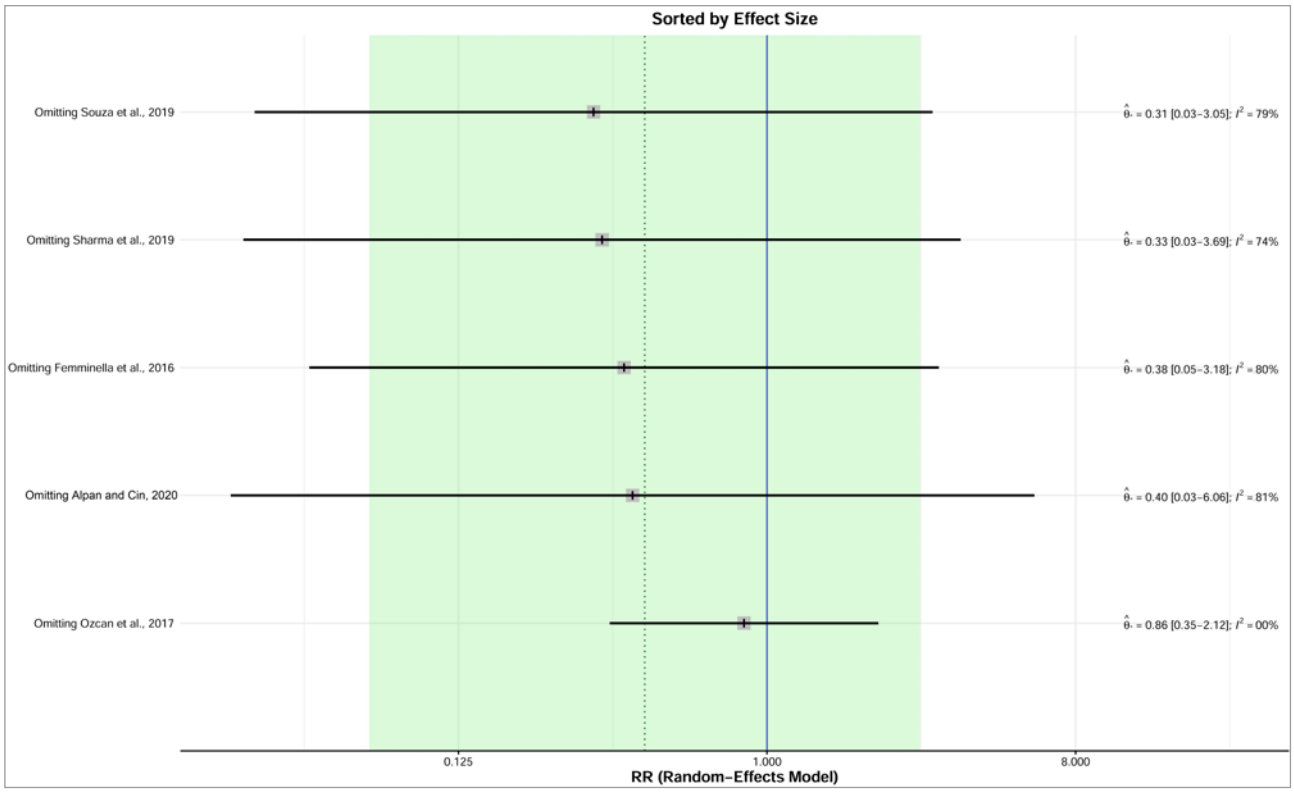

Figure 7. Leave-one-out sensitivity analysis of the rate of postoperative bleeding events.

Table 2. Risk of bias across studies.

Outcome	Anticipated absolute effects (95% CI)		Relative effect (95% CI)	Nº of participants (studies)	Certainty	Comments
	Risk with placebo	*Risk with PRF*				
Pain	-	0-6 days: SMD **0.31 lower** (0.81 lower to 0.19 higher) 7-13 days: SMD **2.29 lower** (4.03 lower to 0.56 lower) 14-20 days: SMD **0.62 lower** (1.23 lower to 0.01 lower) 28-30 days: SMD **2.38 lower** (5.85 lower to 1.09 higher)	-	0-6 days: SMD 0.31 lower (0.81 lower to 0.19 higher) 7-13 days: SMD 2.29 lower (4.03 lower to 0.56 lower) 14-20 days: SMD 0.62 lower (1.23 lower to 0.01 lower) 28-30 days: SMD 2.38 lower (5.85 lower to 1.09 higher)	⊕◯◯◯ VERY LOW[a,b,c]	a. Studies did not perform patient blinding (-2); b. Clinical and statistical heterogeneity across studies (-1); c. Studies are imprecisions (-1).
Delayed bleeding	443 per 1.000	**160 per 1.000** (35 to 692)	**RR 0.36** (0.08 to 1.56)	233 (6 RCTs)	⊕◯◯◯ VERY LOW[a,b,c]	a. Studies did not perform patient blinding (-1); b. Clinical and statistical heterogeneity across studies (-1); c. Studies are imprecisions (-1).

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** risk ratio.
GRADE Working Group grades of evidence.
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

In general, it is observed in the literature that the first days after gingival graft surgery are the main responsible for the highest levels of pain in patients (Sharma *et al*., 2019). This reason, associated with the general fact that PRF has a prolonged degradation time, may explain why there is no significant difference in pain levels for the first period analyzed in the meta-analysis. Nevertheless, we observed that in the qualitative synthesis, some studies demonstrated the effectiveness of PRF in the first postoperative days, but most were not included in the meta-analysis due to the lack of fundamental data required for this analysis. It is possible that, if these studies were included for quantitative analysis, we would have a global effect from that period and on. On the other hand, it is observed that untreated sites that were submitted to the collection of the free gingival graft may present healing from the 2nd week of surgery (Farnoush, 1978), corroborating the findings of this meta-analysis, which did not find an advantage in pain levels from the 14th postoperative day.

The qualitative synthesis of the pain outcome revealed that Ustaoğlu *et al*. (2016) and Ozcan *et al*. (2017) found lower levels of pain in the PRF group compared to negative controls. This outcome is in line, therefore, with the effects of PRF on the palatal wound. However, İşler *et al*. (2019) and Kızıltoprak and Uslu (2020) did not show significant differences, even with comparator groups with the negative control. We can associate this with the fact that, although there are no well-defined reports, the sutures, used by these two studies to fix the PRF membranes, can promote biofilm accumulation, generate inflammation, promote discomfort for the patient or even lead to the loss of the membrane, which directly impacts the patient's pain levels (Ozcan *et al*., 2017). This reason may have led to a difference in results when comparing these studies with Ozcan *et al*. (2017), who used cyanoacrylate adhesive to fix the biomaterial. Ustaoğlu *et al*. (2016), who also showed an advantage in the use of PRF, the use of the suture does not seem to have influenced this outcome and this may be due to the superiority of the T-PRF membrane in terms of the long time of reabsorption and organization of the fibrin matrix, assisting in healing and bypassing the possible negative effects of the suture. (Ustaoğlu *et al*., 2016; Ravi and Sathan *et al*., 2020). For Kızıltoprak and Uslu (2020), despite having used i-PRF, which has a higher concentration of platelets and leukocytes, in addition to using

the suture as a form of fixation, it presented a greater depth of the palatal wound than the others, a fact this corroborates the understanding of its non-significant results for the PRF group. In terms of depth, according to Zucchell *et al*. (2010), a soft tissue remnant with a minimum thickness of 2 mm plays an important role in postoperative morbidity.

This aspect is further reinforced when we verify that Bahammam (2018), Alpan and Cin (2020) also found lower levels of pain in the PRF group compared to the group that only received sutures, showing that regardless of the type of graft technique (subepithelial conjunctive or free gingival, respectively), through an isolated view (since the suture was used in both groups), the PRF shows benefits in reducing postoperative pain. As in Bahammam (2018), Alpan and Cin (2020), it is possible to have an isolated view of the biomaterial in Ozcan *et al*. (2017), whose group that received PRF bonded with adhesive showed lower levels of pain during the first five days after surgery compared to those who received only the cyanoacrylate adhesive.

Still considering the qualitative synthesis, we found that less than half of the studies that carried out the application of another biomaterial in some comparator group reported significant differences regarding the level of pain concerning the PRF group. Of the three who made this report, Sousa *et al*. (2020) showed a difference for the second day, compared to the collagen sponge. Ravi and Santhanakrishnan (2020) compared chemical, mechanical and structural properties of three different PRF preparations and found that A-PRF showed slower degradation, better mechanical properties and greater release of growth factors compared to L-PRF. This, associated with the use of two layers of A-PRF, may have contributed to a higher performance of this biomaterial concerning to the collagen sponge, unlike other studies that used L-PRF and performed comparisons with the same biomaterial, such as İşler *et al*. (2019). Femminella *et al*. (2016) stood out, among all the studies presented in this review, as it can also be seen by the sensitivity analysis, due to the persistence of the statistically significant difference for the pain outcome, in favor of the group that received L- PRF, compared to the group that received collagen sponge. This can be attributed to how the PRF is used, since the palatal wound was covered by 4 layers of this material. Thus, since leukocytes and platelet aggregates are concentrated at one end of the membrane, a greater number of layers provides a slower degradation and a more homogeneous distribution of its components, corroborating with a more effective healing (Shivashankar *et al*,. 2013; Sousa *et al*., 2020).

For the delayed bleeding outcome, there was no association between the risk of bleeding when using PRF or not, demonstrated by the overall effect of the analysis (RR = 0.38; 95% CI = 0.05 - 3.18; I^2= 80%). Since most studies performed comparisons with groups that also received the application of some biomaterial (Femminella *et al*., 2016; İşler *et al*., 2019; Sharma *et al*., 2019; Sousa *et al*., 2020), it is comprehensible that few differences have been observed regarding this parameter, when considering the joint contribution of biological and mechanical properties to the occlusion of the wound, reduction of physical damage to the region and the consequent reduction in the possibility of delayed bleeding. In the same way, Ehab *et al*. (2020) observed that patients who underwent free gingival graft that covered their palatal wounds with biomaterials did not present late hemorrhagic events in any of the evaluated group. This association of properties is important when we observe the sensitivity analysis, as it appears that Ozcan *et al*. (2017), when comparing the PRF group with the group that only received cyanoacrylate adhesive, whose properties are exclusively mechanical (acting as a protective stent, for example), found that PRF still had advantages, reducing the risk of delayed bleeding events ($p<0.05$). Escobar *et al*. (2020) in a systematic review, evaluated the effect of cyanoacrylate adhesive on palatal wounds resulting from the collection of gingival graft, and found that, for the free gingival graft technique, its advantage over gauze or suture was exacerbated by the association with a biomaterial.

On the other hand, among the studies that made comparisons regarding this outcome between PRF groups and those negative controls (Ozcan *et al*., 2017; İşler *et al*., 2019; Kızıltoprak and Uslu, 2020), differently from what is expected, since the palatal wound is subject to constant friction, only Ozcan *et al*. (2017) e Kızıltoprak and Uslu (2020) revealed that PRF demonstrated to reduce the risk of delayed bleeding. For Ozcan *et al*. (2017), we observed that there was an association between a material that provides mechanical protection to a biomaterial that has biological and occlusive characteristics, once again demonstrating that these aspects are fundamental when dealing with this outcome. For Kızıltoprak and Uslu (2020), who used i-PRF, by the increasing of approximately 33% of platelets and leukocytes to the fibrinolytic network of this biomaterial, it may favor a better hemostatic property concerning to other PRF preparations. These questions may explain why İşler *et al*. (2019), when using L-PRF fixed by the suture and comparing it with his negative control, and also Alpan and Cin (2020), who used L-PRF fixed by the suture and comparing it with the suture group, did not obtain better answers in the PRF groups regarding delayed bleeding. However, it is worth to mention that these studies did not determine the measurement of these parameters in their methodology and therefore, the conclusion to be obtained from these studies must be made cautiously.

Considering the analysis presented, it is important to note that our study has limitations. Due to the scarcity of studies with similar comparator groups, carrying out an analysis of the different types of PRF preparation protocol was not possible. Also, although the ten studies included in this systematic review are recent randomized controlled trials, the analysis of the risk of bias showed methodological weaknesses. Regarding the blinding of patients, there was no report of this fact in any of the studies, although it is fundamental when the patient is responsible for evaluating the outcome, especially in a subjective evaluation, such as pain. In addition, as the outcomes are reported by the patient in most studies, it is important to pay attention to possible memory bias, since only two of them (Bahammam, 2018; Alpan and Cin, 2020) reported providing diaries where the patient could also record pain levels. Also, no specification has been reported on the patients' bleeding measures. Still, six of them did not describe measures of standard deviation for the averages found in the VAS scale, directly compromising the estimate of the effect for this result. Another point to be considered is the fact that the evidence generated may be inconsistent, due to the fact that there is a difference between the control groups of the registered studies included in the analysis. These aspects are observed in GRADE and reflect the uncertainty of the effect estimates, as well as demonstrate the possibility of future work impacting on the confidence of these estimates (Guyatt *et al*., 2008). Despite this, the present systematic review is of fundamental importance, as its analyzes, in addition to guiding the conduct of future studies with a better methodological design, alert periodontists about the aspects that must be taken into account when choosing a material to be applied to the palatal wound.

5. Conclusions

Despite the limitation involving the current scientific evidence available, this systematic review and meta-analysis demonstrated that platelet-rich fibrin can reduce postoperative pain in patients undergoing gingival graft harvesting in the period from 7 to 13 days after surgery, enabling a more comfortable postoperative period for the patient. There is still no evidence on its effectiveness in reducing the risk of delayed bleeding events. More randomized clinical trials with greater standardization of control groups and better designs are needed for greater clinical relevance of the findings.

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Appendix 1. Database search strategy.

Database	Search (May 1th 2020)
Cochrane	("Gingival recession" OR "gingival recessions" OR "Atrophy of Gingiva" OR "Gingiva Atrophies" OR "Gingiva Atrophy" OR "Gingival Atrophies" OR "Gingival Atrophy" OR "root coverage" OR "free gingival graft" OR "sub-epithelial connective tissue graft" OR "Miller Class" OR "Gingival Augmentation" OR "gingival graft" OR "palate, hard" OR "hard palate" OR "hard palates" OR "palates, hard" OR "palatal" OR "palate") in Title Abstract Keyword AND ("Platelet-rich fibrin" OR "l-prf" OR "leukocyte and platelet rich fibrin" OR "leukocyte and platelet-rich fibrin" OR "platelet rich fibrin" OR "autologous platelet concentrate" OR "leukocyte platelet-rich fibrin" OR "pure platelet-rich fibrin" OR "LPRF" OR "advanced platelet-rich fibrin" OR "thrombocyte rich plasma" OR "APRF" OR "A-PRF") in Title Abstract Keyword AND ("Pain, Postoperative" OR "Postoperative Pain" OR "Postoperative Pains" OR Pain OR "Pain Measurement" OR "Measurement, Pain" OR "Measurements, Pain" OR "Pain Measurements" OR "Assessment, Pain" OR "Assessments, Pain" OR "Pain Assessments" OR "Pain Assessment" OR "Pain Perception" OR "Pain Perceptions" OR "Perception, Pain" OR "Perceptions, Pain" OR "Pain, Procedural" OR "Procedural Pain" OR "Wound Healing" OR "Healing, Wound" OR "Healings, Wound" OR "Wound Healings" OR "healing" OR "Hemorrhage" OR "Hemorrhages" OR "Bleeding" OR "Re-Epithelialization" OR "Epithelialization, Wound" OR "Re Epithelialization" OR "Wound Epithelialization") in Title Abstract Keyword
EMBASE	('gingival recession' OR 'gingival recessions' OR 'atrophy of gingiva' OR 'gingiva atrophies' OR 'gingiva atrophy' OR 'gingival atrophies' OR 'gingival atrophy' OR 'root coverage' OR 'free gingival graft' OR 'sub-epithelial connective tissue graft' OR 'miller class' OR 'gingival augmentation' OR 'gingival graft' OR 'palate, hard' OR 'hard palate' OR 'hard palates' OR 'palates, hard' OR 'palatal' OR 'palate') AND ('platelet-rich fibrin' OR 'l-prf' OR 'leukocyte and platelet rich fibrin' OR 'leukocyte and platelet-rich fibrin' OR 'thrombocyte rich plasm' OR 'platelet rich fibrin' OR 'autologous platelet concentrate' OR 'leukocyte platelet-rich fibrin' OR 'pure platelet-rich fibrin' OR 'lprf' OR 'advanced platelet-rich fibrin' OR 'aprf' OR 'a-prf') AND ('pain, postoperative' OR 'postoperative pain' OR 'postoperative pains' OR pain OR 'pain measurement' OR 'measurement, pain' OR 'measurements, pain' OR 'pain measurements' OR 'assessment, pain' OR 'assessments, pain' OR 'pain assessments' OR 'pain assessment' OR 'pain perception' OR 'pain perceptions' OR 'perception, pain' OR 'perceptions, pain' OR 'pain, procedural' OR 'procedural pain' OR 'wound healing' OR 'healing, wound' OR 'healings, wound' OR 'wound healings' OR 'healing' OR 'hemorrhage' OR 'hemorrhages' OR 'bleeding' OR 're-epithelialization' OR 'epithelialization, wound' OR 're epithelialization' OR 'wound epithelialization')
LILACS	(tw:("retração gengival" OR "gingival recession" OR "recesion gengival" OR "palato duro" OR "palate, hard" OR "paladar duro" OR palato OR palate OR paladar)) AND (tw:("fibrina rica em plaquetas" OR "platelet-rich fibrin" OR "fibrina rica en plaquetas" OR "fibrina rica em leucócitos e plaquetas" OR "leukocyte and platelet-rich fibrin" OR "leucocitos y fibrina rica en plaquetas" OR "l-prf")) AND (tw:("wound healing" OR "healing" OR "cicatrizacion de heridas" OR cicatrização OR cicatrizacion OR pain OR dor OR dolor))
PubMed	((("Gingival recession"[Mesh] OR "gingival recessions" OR "Atrophy of Gingiva" OR "Gingival Atrophy" OR "root coverage" OR "free gingival graft" OR "sub-epithelial connective tissue graft" OR "Gingival Augmentation" OR "gingival graft" OR "palate, hard"[Mesh] OR "hard palate" OR "hard palates" OR "palatal" OR "palate")) AND (("Platelet-rich fibrin"[Mesh] OR "l-prf" OR "leukocyte and platelet rich fibrin" OR "leukocyte and platelet-rich fibrin" OR "platelet rich fibrin" OR "autologous platelet concentrate" OR "leukocyte platelet-rich fibrin" OR "pure platelet-rich fibrin" OR "LPRF" OR "thrombocyte rich plasma" OR "advanced platelet-rich fibrin" OR "APRF" OR "A-PRF"))) AND (("Pain, Postoperative"[Mesh] OR "Postoperative Pain" OR "Postoperative Pains" OR "Pain"[Mesh] OR "Pain Measurement"[Mesh] OR "Measurement, Pain" OR "Pain Measurements" OR "Pain Assessments" OR "Pain Assessment" OR "Pain Perception"[Mesh] OR "Pain Perceptions" OR "Perception, Pain" OR "Perceptions, Pain" OR "Pain, Procedural"[Mesh] OR "Procedural Pain" OR "Wound Healing"[Mesh] OR "Healing, Wound" OR "Healing" OR "Wound Healings" OR "Hemorrhage"[Mesh] OR "Hemorrhages" OR "Bleeding" OR "Re-Epithelialization"[Mesh] OR "Epithelialization, Wound" OR "Re Epithelialization" OR "Wound Epithelialization"))

Appendix 1 continued.

Scopus	(TITLE-ABS-KEY ("Gingival recession") OR TITLE-ABS-KEY ("gingival recessions") OR TITLE-ABS-KEY ("recession, gingival") OR TITLE-ABS-KEY ("recessions, gingival") OR TITLE-ABS-KEY ("Atrophy of Gingiva") OR TITLE-ABS-KEY ("Gingiva Atrophies") OR TITLE-ABS-KEY ("Gingiva Atrophy") OR TITLE-ABS-KEY ("Gingival Atrophies") OR TITLE-ABS-KEY ("Gingival Atrophy") OR TITLE-ABS-KEY ("root covering") OR TITLE-ABS-KEY ("root coverage") OR TITLE-ABS-KEY ("free gingival graft" OR TITLE-ABS-KEY "sub-epithelial connective tissue graft" OR TITLE-ABS-KEY "Miller Class" OR TITLE-ABS-KEY "Gingival Augmentation" OR TITLE-ABS-KEY "gingival graft") OR TITLE-ABS-KEY ("palate, hard") OR TITLE-ABS-KEY ("hard palate") OR TITLE-ABS-KEY ("hard palates") OR TITLE-ABS-KEY ("palates, hard") OR TITLE-ABS-KEY (palatal) OR TITLE-ABS-KEY (palate) AND TITLE-ABS-KEY ("Platelet-rich fibrin") OR TITLE-ABS-KEY ("fibrin, platelet-rich") OR TITLE-ABS-KEY ("l-prf") OR TITLE-ABS-KEY ("leukocyte and platelet rich fibrin") OR TITLE-ABS-KEY ("leukocyte and platelet-rich fibrin") OR TITLE-ABS-KEY ("platelet rich fibrin") OR TITLE-ABS-KEY ("autologous platelet concentrate") OR TITLE-ABS-KEY ("leukocyte platelet-rich fibrin") OR TITLE-ABS-KEY ("pure platelet-rich fibrin") OR TITLE-ABS-KEY ("LPRF") OR TITLE-ABS-KEY ("thrombocyte rich plasma") OR TITLE-ABS-KEY ("advanced platelet-rich fibrin") OR TITLE-ABS-KEY ("APRF") OR TITLE-ABS-KEY ("A-PRF") AND TITLE-ABS-KEY ("Pain, Postoperative") OR TITLE-ABS-KEY ("Postoperative Pain") OR TITLE-ABS-KEY ("Postoperative Pains") OR TITLE-ABS-KEY (pain) OR TITLE-ABS-KEY ("Pain Measurement") OR TITLE-ABS-KEY ("Measurement, Pain") OR TITLE-ABS-KEY ("Measurements, Pain") OR TITLE-ABS-KEY ("Pain Measurements") OR TITLE-ABS-KEY ("Assessment, Pain") OR TITLE-ABS-KEY ("Assessments, Pain") OR TITLE-ABS-KEY ("Pain Assessments") OR TITLE-ABS-KEY ("Pain Assessment") OR TITLE-ABS-KEY ("Pain Perception") OR TITLE-ABS-KEY ("Pain Perceptions") OR TITLE-ABS-KEY ("Perception, Pain") OR TITLE-ABS-KEY ("Perceptions, Pain") OR TITLE-ABS-KEY ("Pain, Procedural") OR TITLE-ABS-KEY ("Procedural Pain") OR TITLE-ABS-KEY ("Wound Healing") OR TITLE-ABS-KEY ("Healing, Wound") OR TITLE-ABS-KEY ("Healings, Wound") OR TITLE-ABS-KEY ("Wound Healings") OR TITLE-ABS-KEY ("healing") OR TITLE-ABS-KEY (hemorrhage) OR TITLE-ABS-KEY (hemorrhages) OR TITLE-ABS-KEY (bleeding) OR TITLE-ABS-KEY ("Re-Epithelialization") OR TITLE-ABS-KEY ("Epithelialization, Wound") OR TITLE-ABS-KEY ("Re Epithelialization") OR TITLE-ABS-KEY ("Wound Epithelialization"))
Web of Science	TOPIC: (("Gingival recession" OR "gingival recessions" OR "Atrophy of Gingiva" OR "Gingiva Atrophies" OR "Gingiva Atrophy" OR "Gingival Atrophies" OR "Gingival Atrophy" OR "root coverage" OR "free gingival graft" OR "sub-epithelial connective tissue graft" OR "Miller Class" OR "Gingival Augmentation" OR "gingival graft" OR "palate, hard" OR "hard palate" OR "hard palates" OR "palates, hard" OR palatal OR palate)) AND TOPIC: (("Platelet-rich fibrin" OR "l-prf" OR "thrombocyte rich plasma" OR "leukocyte and platelet rich fibrin" OR "leukocyte and platelet-rich fibrin" OR "platelet rich fibrin" OR "autologous platelet concentrate" OR "leukocyte platelet-rich fibrin" OR "pure platelet-rich fibrin" OR LPRF OR "advanced platelet-rich fibrin" OR "APRF" OR "A-PRF")) AND TOPIC: (("Pain, Postoperative" OR "Postoperative Pain" OR "Postoperative Pains" OR Pain OR "Pain Measurement" OR "Measurement, Pain" OR "Measurements, Pain" OR "Pain Measurements" OR "Assessment, Pain" OR "Assessments, Pain" OR "Pain Assessments" OR "Pain Assessment" OR "Pain Perception" OR "Perceptions, Pain" OR "Pain, Procedural" OR "Procedural Pain" OR "Wound Healing" OR "Healing, Wound" OR "Healings, Wound" OR "Wound Healings" OR healing OR Hemorrhage OR Hemorrhages OR Bleeding OR "Re-Epithelialization" OR "Epithelialization, Wound" OR "Re Epithelialization" OR "Wound Epithelialization"))
Google Scholar	"gingival recession" OR "root coverage" OR "palate" OR "hard, palate" OR "palatal" AND PRF OR "l-prf" OR "platelet rich fibrin" AND healing OR Pain OR "wound healing" filetype:PDF
Open Grey	"Gingival Recession" OR "hard palate" AND "Platelet-rich fibrin" doctype:(U - Thesis)
ProQuest	"Gingival recession" OR "gingival recessions" OR "Atrophy of Gingiva" OR "Gingival Atrophy" OR "root coverage" OR "free gingival graft" OR "sub-epithelial connective tissue graft" OR "Gingival Augmentation" OR "gingival graft" OR "palate, hard" OR "hard palate" OR "hard palates" OR "palatal" OR "palate" AND "Platelet-rich fibrin" OR "l-prf" OR "leukocyte and platelet rich fibrin" OR "leukocyte and platelet-rich fibrin" OR "platelet rich fibrin" OR "autologous platelet concentrate" OR "thrombocyte rich plasma" OR "leukocyte platelet-rich fibrin" OR "pure platelet-rich fibrin" OR "LPRF" OR "advanced platelet-rich fibrin" OR "APRF" OR "A-PRF" AND "Pain, Postoperative" OR "Postoperative Pain" OR "Postoperative Pains" OR "Pain" OR "Pain Measurement" OR "Measurement, Pain" OR "Pain Measurements" OR "Pain Assessments" OR "Pain Assessment" OR "Pain Perception" OR "Pain Perceptions" OR "Perception, Pain" OR "Perceptions, Pain" OR "Pain, Procedural" OR "Procedural Pain" OR "Wound Healing" OR "Healing, Wound" OR "Wound Healings" OR "Hemorrhage" OR "Hemorrhages" OR "Bleeding" OR "healing"

Appendix 2. Excluded articles and reasons for exclusion (n=8).

Author, Year	Reason for exclusion
Alpan and Cin, 2018	3
Bahammam, 2016	3
İşler, 2018	3
Kumar, 2017	3
Kumar and Shubhashini, 2013	3
Martín-Del-Campo et al., 2019	3
Scaramuzza, 2018	3
Sharma et al., 2019	3

1. Pre-clinical studies (n=0); 2. Studies that did not provide information about the PRF preparation protocol (n=0); 3. Literature reviews; cards; books; conference abstracts; case reports; clinical trial protocols; case series; opinion article; technical articles; retrospective studies (n=8).

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Kumar A. Comparative evaluation of the efficacy of a commercially available collagen dressing (Collacote ®) with autologous Platelet Rich Fibrin (PRF) membrane on the wound healing of palatal donor site of free gingival graft. ICTRP Search Portal, 2017; Apps.who.int. https://apps.who.int/trialsearch/Trial2.aspx?TrialID=CTRI/2017/01/007719. Accessed 10 May 2020

Kumar RV and Shubhashini N. Platelet rich fibrin: a new paradigm in periodontal regeneration. Cell Tissue Bank 2013;14:453-463.

Martín-Del-Campo M, Rosales-Ibañez R and Rojo L. Biomaterials for Cleft Lip and Palate Regeneration. Int J Mol Sci 2019;20:2176-218

Scaramuzza E. Patient Morbidity After Palatal Free Gingival Grafts With or Without PRF Membranes Coverage. ClinicalTrials.gov 2018; https://clinicaltrials.gov/ct2/show/NCT03685396. Accessed 10 May 2020

Sharma V. Effect of two different dressings on palatal wound healing. Cochranelibrary.com 2019; https://www.cochranelibrary.com/central/doi/10.1002/central/CN-01814860/full. Accessed 10 May 2020