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The adjunctive use of platelet-rich plasma in the therapy of periodontal intraosseous defects: a systematic review

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Background and Objective: The evidence for the efficacy of the adjunctive use of platelet-rich plasma (PRP) in periodontal intraosseous defects has not been systematically evaluated. The objective of this review was to address the focused question, 'What is the efficacy, with respect to clinical, radiographical and patient-centred outcomes, of combinations of PRP with other therapeutic bioactive agents/procedures, compared with the efficacy of the same agents/procedures without the adjunctive use of PRP in the therapy of periodontal intraosseous defects in patients with chronic periodontitis and without systemic diseases that could potentially influence the outcome of periodontal therapy?' by performing a systematic review of randomized controlled clinical trials (RCTs) published in the dental literature in any language, up to and including September 2008.

Material and Methods: Data sources principally included electronic databases, manually searched journals and contact with experts. In the first phase of study selection, the titles and abstracts, and in the second phase, full papers were screened independently and in duplicate by two reviewers.

Results: In the first phase, 6124 potentially relevant titles and abstracts were examined. In the second phase, the full text of 20 publications was thoroughly evaluated. Eventually, 10 RCTs were selected.

Conclusion: Diverse outcomes (positive and negative) have been reported for the efficacy of PRP combined with various therapeutic bioactive agents/procedures, reflecting the limited and heterogeneous data available and possibly suggesting that the specific selection of agents/procedures combined with PRP could be important. Additional research on the efficacy of each specific combination of PRP is necessary.

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Platelet-rich plasma (PRP) is a preparation, serving as an autologous source of highly concentrated doses of platelets [330 (1) or 338% (2) relative to their concentration within patient blood], which in their α-granules contain and may release a variety of polypeptide growth factors, principally platelet-derived growth factor (PDGF), transforming growth factors-β1 and -β2 (TGF-β1 and -β2) and insulin-like growth factor-1 (IGF-1; 2–5).

Although the precise biological mechanisms of action of PRP have not been completely clarified as yet, PRP appears to possess the potential to enhance and accelerate both soft (e.g. epithelialization) and hard tissue (e.g.

osseous regeneration) healing processes (2,6–10), thereby often providing an improved aesthetic outcome (6), a shortened duration of therapy (7) and limiting inflammation (11).

The first systematic review (12) on all clinical applications of PRP in Dentistry concluded that evidence was found 'for beneficial effects of PRP in the treatment of periodontal defects.' However, a more recent conventional (not systematic) review (13) on the clinical effect of the use of various bioactive agents, including PRP, either alone or combined with grafts and/or guided tissue regeneration (GTR), for the treatment of intraosseous and furcation defects concluded that 'when the additional effect of PRP over a graft was evaluated, contrasting results were reported, ranging from a significant enhancement for PRP to a null effect.'

To our knowledge, until September 2008, a systematic review providing the highest level of scientific evidence on the efficacy of the adjunctive use of PRP specifically in the therapy of periodontal intraosseous defects had not been published.

Consequently, it is not clear whether the addition of PRP to various therapeutic bioactive agents/procedures could provide an increased efficacy, compared with the efficacy of the same therapeutic bioactive agents/procedures without the adjunctive use of PRP, in the therapy of periodontal intraosseous defects.

Therefore, the aim of the present study was to address the focused question, 'What is the efficacy, with respect to clinical, radiographical and patient-centred outcomes, of combinations of PRP with other therapeutic bioactive agents/procedures, compared with the efficacy of the same agents/procedures without the adjunctive use of PRP in the therapy of periodontal intraosseous defects in patients with chronic periodontitis and without systemic diseases that could potentially influence the outcome of periodontal therapy?' by performing a systematic review of randomized controlled clinical trials (RCTs) published in the dental literature in any language, up to and including September 2008.

Material and methods

Search strategy for identification of RCTs

Electronic search — For the identification of RCTs to be considered for inclusion in this systematic review, the *PubMed* database of the US National Library of Medicine and *The Cochrane Library* (CENTRAL) of The Cochrane Collaboration® were employed as electronic databases, and a literature search was carried out with a personal computer (PC) on articles published up to and including September 2008. Articles available online in electronic form prior to their publication in material form (according to the so-called 'Epub ahead of print') were considered eligible for inclusion in the present paper; last electronic search was carried out on 30 September 2008. Since the applications of PRP in oral surgery were first reported in the literature (3) in November 1997, no search was carried out for articles published prior to this date. Furthermore, no language restriction was applied.

The terms and key words used in the search were as follows: 'platelet-rich plasma' OR 'PRP' OR 'Platelet'.

During the search in the *PubMed* database, the following limits were applied, using the specially designed *Limits* tab:

- *Dates: Published in the Last: Specify date range (YYYY/MM/DD). Published Date:* 1997/11/01 to 2008/09/30.
- *Humans or Animals:* Humans (only).
- *Type of article:* Clinical Trial, Randomized Controlled Trial.

It was deemed safer to examine any publication referred to as 'Clinical Trial' than only the type of article 'Randomized Controlled Trial', because certain studies could potentially be randomized, without explicitly mentioning the presence of randomization in the title/abstract or within the published text.

Manual search — Additionally, various journals were searched manually from November 1997 up to and including September 2008, as reported below in alphabetical order:

American Journal of Dentistry, *Australian Dental Journal*, *British Dental Journal*, *Clinical Oral Investigations*, *European Journal of Oral Sciences*, *The International Journal of Periodontics and Restorative Dentistry*, *The Journal of the American Dental Association*, *Journal of Biomaterials Applications*, *Journal of the Canadian Dental Association*, *The Journal of Clinical Dentistry*, *Journal of Clinical Periodontology*, *Journal of Dental Education*, *Journal of Dental Research*, *Journal of Dentistry*, *Journal of Periodontal Research* and *Journal of Periodontology*.

Other data sources — Eventually, the bibliographies of all original research and review papers identified (through electronic and manual search) relevant to the subject were scanned. An effort was made to search for the so-called 'grey literature' (i.e. literature not formally published), including as many as possible proceedings of possibly relevant previous workshops, position papers and theses. Whenever considered necessary, contact with the corresponding author of a study would be made, in order to acquire missing, unclear or unpublished data.

Inclusion/exclusion criteria and selection of RCTs

In the first phase of study selection, the titles and abstracts (when available) of all identified publications were screened independently and in duplicate by two reviewers (S.K. and N.M.) for potential selection in the review, based on predefined (at the beginning of this study) inclusion/exclusion criteria. The broadest possible inclusion criteria were determined, aimed at making the results of this systematic review as generalizable as possible.

The inclusion criteria were accepted by all reviewers as follows:

- Publication in the dental literature in any language, up to and including September 2008.
- RCT, either of a parallel group or of a split-mouth design.
- All patients included in the RCT should exhibit exclusively chronic periodontitis (of any extent and severity).

- All patients included in the RCT should have no systemic diseases that could potentially influence the outcome of periodontal therapy.
- Presence of at least one experimental group, in which PRP was clinically applied as an adjunct to other therapeutic bioactive agents/procedures for the therapy of periodontal intraosseous defects.
- Presence of an appropriate (concurrent with the experimental group) non-PRP control group, in which the same therapeutic bioactive agents/procedures as those employed in at least one experimental group (or these materials/procedures plus a placebo material/procedure) were clinically applied for the therapy of periodontal intraosseous defects, without the adjunctive use of PRP.
- Report of change in clinical attachment level between baseline and the end of follow-up period as the primary outcome variable and at least of change in probing pocket depth between baseline and the end of follow-up period as secondary outcome variable.
- Follow-up period of at least 6 mo.

The exclusion criteria were agreed by all reviewers as follows:

- Mixed RCT design, including both parallel group and split-mouth design.
- Use of historical control group.
- History of periodontal therapy within the preceding 12 mo or less.
- Periodontal intraosseous defect(s) extending into furcation area(s) or located around teeth presenting furcation involvement(s).
- Patients receiving any medication reported to interfere with wound healing, cause gingival overgrowth or known to affect the number or function of platelets over a period of 3 mo or less prior to the baseline of the RCT.
- Patients with abnormal platelet counts.
- Patients receiving antibiotics at the baseline of the RCT and/or during the previous 3 mo or less.
- History of radiotherapy in the head and neck region of the patients.
- Teeth presenting endodontic problems at the baseline of the RCT or endodontically treated prior to baseline, but still exhibiting endodontic pathology (clearly defined) at baseline.

In the second phase of selection, the full text was obtained of all studies previously singled out in the first phase, as well as the full text of publications without abstract or publications with insufficient data in the title and abstract to allow an unambiguous evaluation. Subsequently, these studies were examined independently and in duplicate by two reviewers (S.K. and N.M.), based on the aforementioned inclusion/exclusion criteria.

In case of any potential disagreement between the reviewers, consensus would have to be achieved by discussion. If disagreement still continued to remain unresolved, it would have to be subsequently reported and analysed in the text of the present paper.

Primary outcome variable

Change in clinical attachment level between the baseline and the end of follow-up period was the primary outcome variable in this systematic review.

Secondary outcome variables

Change in probing pocket depth, change in gingival recession (depth, width etc.), changes in alveolar bone (radiographical and/or hard tissue probing at surgical re-entry) between the baseline and the end of the follow-up period and tooth loss were used as secondary outcome variables. Secondary outcome variables also included patient-centred parameters, such as aesthetics (unaltered, improved or deteriorated, according to the patient), postoperative complications (such as pain, swelling, infection, abscess etc.) and adverse events. Finally, the rate of healing, whenever assessed by the investigators, served as a secondary outcome variable.

Data extraction

As described in the literature (14), a standardized procedure of extracting data from the selected RCTs, using specially designed data extraction forms, was planned to be performed in duplicate and independently by two reviewers (S.K. and N.M.), regarding the main characteristics (study design, methods, participants, interventions, outcome measures/variables etc.) and outcomes of RCTs, as demonstrated in Tables 2–6, with particular emphasis on the primary and secondary outcome variables settled in this systematic review. Any other information deemed scientifically interesting was also recorded. Authors of studies were contacted for clarification or missing information.

Quality assessment of selected RCTs

The quality assessment of RCTs remaining after the second phase of selection was planned to be carried out autonomously and in duplicate by two reviewers (S.K. and N.M.), applying certain criteria proposed in the dental literature (15,16). The quality assessment system employed in the present systematic review was almost identical to a previous one (16), using slight modifications/improvements for quality criteria A and F, as described below.

Quality criterion A — This criterion assessed sample size calculation and adequacy, i.e. whether the authors of the RCT had estimated the minimum number of participants required to detect a statistically significant difference among compared study groups and, furthermore whether sample size actually included in the RCT was adequate.

Grading:

0: Sample size calculation was not performed/not mentioned/not clear (unless sample size could be estimated as clearly adequate and therefore grade 3 was immediately applied).
1: Sample size calculation was reported, but not confirmed and, furthermore, sample size was inadequate.
2: Sample size calculation was reported and confirmed, but sample size was inadequate.
3: Sample size calculation was reported and confirmed and, furthermore, sample size was adequate (grade 3 might also apply to specific cases, where sample size calculation was not performed/not mentioned/not clear, but sample size could be estimated as adequate).

The reason for the slight modification of quality criterion A, compared with that previously described (16), was that the presence of an adequate sample size is even more important for study quality than the calculation of adequate sample size *per se*.

Quality criterion B — This criterion assessed the method of randomization and allocation concealment.

Grading:
0: Did not exist/not described/not clear.
1: Clearly inadequate, i.e. when the method of randomization was other than a table of random numbers, computer-based random number generator, tossed coin, shuffled cards; hence, for example, odd/even birth date is a clearly inadequate method of randomization.
2: Possibly adequate, i.e. when an adequate randomization method was applied, but the therapist(s) was (were) informed about the randomization sequence prior to or at the beginning of the procedure and accordingly could potentially be biased during intervention(s).
3: Clearly adequate, i.e. when an adequate randomization method was applied and the therapist(s) was (were) kept unaware of the randomization sequence until immediately prior to the therapeutic procedure implemented.

Quality criterion C — This criterion assessed clear definition of inclusion and/or exclusion criteria.

Grading:
0: No.
1: Yes.

Quality criterion D — This criterion assessed completeness of follow-up (specified reasons for withdrawals and dropouts in each study group).

Grading:
0: No/not mentioned/not clear.
1: Yes/no withdrawals or dropouts occurred.

Quality criterion E — This criterion assessed whether experimental and control groups were comparable at study baseline for important prognostic factors.

Grading:
0: No.
1: Unclear/possibly not comparable for one or more important prognostic factors.
2: Yes.

Quality criterion F — This criterion assessed the presence of masking.

Quality criterion F was subdivided into quality criteria F1 and F2, both of which had precisely the same grading, as previously reported (16). Quality criterion F1 assessed the presence of examiner masking, while quality criterion F2 assessed the presence of operator masking.

Grading:
0: No.
1: Unclear/not complete; not for all study measurements or evaluations.
2: Yes.

A third type of masking that might exist (patient masking) was not included in quality criterion F, because the adjunctive use of PRP in intraosseous defects requires the collection of blood from the patient and therefore can hardly be masked.

Quality criterion G — This criterion assessed the appropriateness of statistical analysis.

Grading:
0: No.
1: Unclear/possibly not the best method applied.
2: Yes.

In the context of this systematic review, 'appropriate' statistical analyses were considered to include the following:

- In parallel group RCTs, for intergroup comparisons with regard to primary and secondary outcome variables (clinical attachment level, probing pocket depth etc.) and their changes: Student's (unpaired) *t*-test was an appropriate analytical statistical method if the data followed a normal distribution, whereas the Mann–Whitney *U*-test was an appropriate analytical statistical method if the data did not follow a normal distribution.
- In split-mouth RCTs, for intergroup comparisons with regard to primary and secondary outcome variables (clinical attachment level, probing pocket depth etc.) and their changes: Student's paired *t*-test was an appropriate analytical statistical method if the data followed a normal distribution, whereas the Wilcoxon signed-rank matched-pair test was an appropriate analytical statistical method if the data did not follow a normal distribution.

Quality assessment was conducted in two phases (16). In the first phase, quality assessment was based entirely on the published text of studies and was carried out separately and in duplicate by two reviewers (S.K. and N.M.) by use of the criteria mentioned above. In the second phase of quality assessment, studies were re-evaluated independently and in duplicate by the same reviewers, using the same quality assessment criteria, but considering the supplementary information provided by the corresponding author.

Agreement between the two reviewers (S.K. and N.M.) with regard to quality assessment scorings for each quality criterion was determined by the proportion (%) of inter-reviewer agreement and, likewise, by κ score, which additionally incorporates an adjustment for the degree of agreement to be expected entirely by chance (17–19). In the event of any discrepancy between the authors, an agreement had to be accomplished by discussion; otherwise, the diverse assessments would have to be explained within the present text. After forming the scorings in the second phase of quality assessment, an overall estimation of plausible risk of bias (low, moderate or high) would be made for each RCT selected, based on proposed Cochrane definitions of the degree of bias (20).

Results

Study selection (Tables 1–3)

The electronic search in both databases (*PubMed* and *Cochrane CENTRAL*) provided a total of 6124 potentially relevant titles and abstracts, and the subsequent manual search provided no additional papers. Following the first phase of evaluation, 6104 publications were rejected on the basis of the title and the abstract. In the second phase,

the full text of the remaining 20 publications was retrieved for more detailed evaluation. During this evaluation, 10 papers (21–30), corresponding to nine RCTs, were excluded, based on reasons reported in Table 1. Two publications (24,25) were part of the same RCT. Finally, 10 RCTs (31–40) were selected (Tables 2 and 3).

For the first phase of selection, the proportion of inter-reviewer agreement (18,19) was 99.92% and κ score (17–19) was 0.860 ± 0.062. For the second phase of selection, the proportion of inter-reviewer agreement was 100% and κ score was 1.000. Based on proposed interpretations of the magnitude of κ score (18,19), its value in both cases was well above 0.75 and therefore could be considered to represent an excellent level of agreement beyond chance.

Main characteristics and classification of selected RCTs (Tables 2–4)

Seven RCTs (31–37) had a parallel group design (Table 2) and three RCTs (38–40) exhibited a split-mouth design (Table 3).

With regard to their location, all 10 RCTs selected (31–40) were university-based studies. Four RCTs (33–36) were conducted by the same research group. The source of funding of the selected RCTs was as follows: one RCT (31) was supported by a grant from a scientific foundation and another grant from a university foundation; two RCTs (32,34) were funded by a university foundation; two RCTs (35,36) were funded by the author's own institution (university) and part of the grafting material was kindly provided by an industrial company; one RCT (40) was funded by an industrial company; and a potential source of funding was not stated in the published text of the remaining RCTs (33,37–39).

The following methods/instruments were used in the RCTs selected for clinical and/or radiographical measurements:

- All clinical measurements were performed by a calibrated examiner using a manual periodontal probe (31–37,40), often with a customized acrylic stent (31,32,40), or using a force-controlled periodontal probe (39).
- Calibration of the examiner (32,38) and/or the instrument used for performing clinical measurements were not mentioned in the published text of certain RCTs (38,40).
- Standardized radiographs were taken with the paralleling technique (31,37,39) or using a standardized individually manufactured holder (40).

The following types of periodontal intraosseous defects were treated in the RCTs selected, according to their number of osseous walls:

- one-wall intraosseous defects (31,32).
- one- to two-wall intraossous defects (33–36).
- two-wall intraosseous defects (31–40).
- three-wall intraosseous defects (31,32,34,35,37–39).

As demonstrated in Table 4, various parameters of PRP preparation and application differed substantially among RCTs selected, namely the type of cell separator device (centrifuge), the pattern of centrifugation steps (number of centrifugation steps, frequency and duration of centrifugation in each step), baseline and treatment concentration of platelets (platelet count), concentration of growth factors of PRP and the use of coagulation activators.

Overall, PRP was combined with bone grafts or substitutes, such as allogenous bone grafts or allografts (37), xenogenous bone grafts or xenografts (33,34,36,38), alloplastic materials (31, 32,35,40), and/or GTR by the use of non-resorbable (33,35) or resorbable barrier membranes (34,39). None of the RCTs selected provided information on the combination of PRP and autogenous bone grafts or autografts.

Table 1. Studies excluded in the second phase of selection and the reason for the exclusion of each study

Excluded study	Reason for exclusion
Camargo *et al.* 2002 (21)	Inappropriate control group (use of GTR instead of GTR + BM)
Lekovic *et al.* 2002 (22)	Inappropriate control group (use of BM + PRP instead of BM + GTR)
Camargo *et al.* 2005 (23)	Inappropriate control group (use of OFD instead of GTR + BM)
Christgau *et al.* 2006, 2006 (24,25)	Presence of an aggressive periodontitis patient
Czuryszkiewicz-Cyrana & Banach, 2006 (26)	Not a randomized controlled clinical trial
Ouyang & Qiao, 2006 (27)	Mixed (parallel group/split-mouth) design
Ilgenli *et al.* 2007 (28)	Inappropriate control group (use of PRP instead of DFDBA)
Yassibag-Berkman *et al.* 2007 (29)	Mixed (parallel group/split-mouth) design
Yamamiya *et al.* 2008 (30)	Inappropriate control group (use of HA + PRP instead of HA + HCP)

Abbreviations: BM, bovine-derived porous bone mineral; DFDBA, demineralized freeze-dried bone allograft; GTR, guided tissue regeneration; HA, hydroxyapatite; HCP, human cultured periosteum; OFD, open flap debridement; and PRP, platelet-rich plasma.

Main outcomes of selected RCTs (Tables 5 and 6 and Fig. 1)

Platelet-rich plasma combined only with allografts —

- Platelet-rich plasma combined with demineralized freeze-dried bone allograft (DFDBA; experimental group) vs. the combination of DFDBA and saline as placebo (control group; 37; Tables 2 and 5).

o *Primary outcome variable* (*change in clinical attachment level*). When compared with the use of DFDBA and a non-therapeutic substance (saline), the addition of PRP to DFDBA resulted in statistically significantly ($p < 0.001$) higher clinical attachment level gain (intergroup difference of mean change in clinical attachment level, 1.2 mm; 95% confidence intervals (CI), 0.2–2.2 mm; Fig. 1) at the end of the 12 mo follow-up period (relative to baseline values).

Table 2. Main characteristics of selected parallel group randomized controlled clinical trials

Study	Study groups	Experimental interventions	Control interventions	Follow-up (months)
Okuda *et al*. 2005 (31)	70 patients: 49 females and 21 males; age 55.5 ± 8.2 years. In each study group, 35 patients and 35 defects.	PRP + HA	Saline + HA	12
Demir *et al*. 2007 (32)	29 patients: 16 females and 13 males; age 36.03 ± 12.02 years. Experimental group, 15 patients and 15 defects; control group, 14 patients and 14 defects.	PRP + BG	BG	9
Döri *et al*. 2007 (33)	24 patients:14 females and 10 males; age 26–55 years. In each study group, 12 patients and 12 defects.	PRP + BM + e-PTFE	BM + e-PTFE	12
Döri *et al*. 2007 (34)	30 patients: 16 females and 14 males; age 28–56 years. In each study group, 15 patients and 15 defects.	PRP + BM + COL	BM + COL	12
Döri *et al*. 2008 (35)	28 patients: 16 females and 12 males; age 28–58 years. In each study group, 14 patients and 14 defects.	PRP + β-TCP + e-PTFE	β-TCP + e-PTFE	12
Döri *et al*. 2008 (36)	26 patients: 14 females and 12 males; age 32–56 years. In each study group, 13 patients and 13 defects.	PRP + BM + EMD	BM + EMD	12
Piemontese *et al*. 2008 (37)	60 patients: 29 females and 31 males; age 47–72 years. In each study group, 30 patients and 30 defects.	PRP + DFDBA	Saline + DFDBA	12

Abbreviations: β-TCP, β-tricalcium phosphate; BG, bioactive glass; BM, bovine-derived porous bone mineral; COL, collagen membrane; DFDBA, demineralized freeze-dried bone allograft; EMD, enamel matrix protein derivative; e-PTFE, expanded polytetrafluoroethylene membrane; HA, hydroxyapatite; and PRP, platelet-rich plasma.

o *Secondary outcome variables*. The above-mentioned comparison also demonstrated statistically significantly greater reduction in probing pocket depth at the end of the 12 mo follow-up period (relative to baseline values), but no statistically significant change in gingival recession depth (REC). Furthermore, no statistically significant differences between the two groups were demonstrated with regard to changes in radiographical parameters, namely the amount of hard tissue fill within the intraosseous defects and the amount of crestal osseous resorption. No tooth loss and no postoperative complications or adverse events were reported for both therapeutic modalities.

Platelet-rich plasma combined with xenografts and other regenerative materials —

• Platelet-rich plasma combined with bovine-derived porous bone mineral (BM; experimental group) vs. BM alone (control group; 38; Tables 3 and 6).

o *Primary outcome variable* (*change in clinical attachment level*). When compared with the use of BM alone, the addition of PRP to BM resulted in statistically significantly higher clinical attachment level gain (intergroup dif-

Table 3. Main characteristics of selected split-mouth randomized controlled clinical trials

Study	Study groups	Experimental interventions	Control interventions	Follow-up (months)
Hanna *et al*. 2004 (38)	13 patients: 8 females and 5 males; age 37–74 years. In each site group, 13 defects.	PRP + BM	BM	6
Keles *et al*. 2006 (39)	15 patients: 7 females and 8 males; age 39.1 ± 7.4 (29–51) years. In each site group, 15 defects.	PP + PAM	BG + PAM	6
Harnack *et al*. 2008 (40)	22 patients: 12 females and 10 males[a]; age 47.6 ± 12.3 (32–71) years[a]. In each site group, 22 defects[a].	PRP + β-TCP	β-TCP	6

[a] Information retrieved after contact with the corresponding author of the study.

Abbreviations: β-TCP, β-tricalcium phosphate; BG, bioactive glass; BM, bovine-derived porous bone mineral; PAM, polylactic acid membrane; PP, platelet pellet; and PRP, platelet-rich plasma.

Table 4. Method of platelet-rich plasma preparation in all (parallel group and split-mouth) selected randomized controlled clinical trials

Study	Centrifuge	Steps of centrifugation (frequency, duration)	Platelet count (baseline/treatment)	Concentration of growth factors (baseline/treatment)	Activator(s) of coagulation
Parallel group randomized controlled clinical trials					
Okuda *et al*. 2005 (31)	Heraeus Labofuge 300[a,b]	Two (2400 r.p.m., 10 min & 3600 r.p.m., 15 min)	Not recorded directly; reference was made to a previous study (41)[b] Baseline: $257 \times 10^3/\mu L \pm 46 \times 10^3/\mu L$[b] Treatment: $709 \times 10^3/\mu L \pm 216 \times 10^3/\mu L$[b]	Not recorded directly; reference was made to a previous study (41)[b] Baseline: PDGF-AB, 51.8 ± 33.4 ng/mL; TGF-β1, 41.6 ± 11.4 ng/mL[b] Treatment: PDGF-AB, 182.0 ± 75.5 ng/mL[b]; TGF-β1, 140.9 ± 53.5 ng/mL[b]	0.1 g of sodium alginate[c]
Demir *et al*. 2007 (32)	Heraeus Christ Medifuge[b,d]	Two (3000 r.p.m., 10 min & 3600 r.p.m., 10 min; or 200 *g*, 10 min & 250 *g*, 10 min)[b]	Baseline: $189 \times 10^3/\mu L \pm 37 \times 10^3/\mu L$[b]. Treatment: $680 \times 10^3/\mu L \pm 103 \times 10^3/\mu L$[b]	Not recorded[b]	0.3 mL of 0.025 M $CaCl_2$, mixed with blood harvested from the surgical site
Döri *et al*. 2007 (33) & Döri *et al*. 2007 (34) & Döri *et al*. 2008 (35) & Döri *et al*. 2008 (36)	Curasan PRP kit[e]	Two (2400 r.p.m., 10 min & 3600 r.p.m., 15 min; or 547 *g*, 10 min & 1231 *g*, 15 min)[b]	Not recorded directly; reference was made to a previous study (42)[b] Baseline: $212.4 \times 10^3/\mu L \pm 39.6 \times 10^3/\mu L$[b]. Treatment: $2519.6 \times 10^3/\mu L \pm 834.3 \times 10^3/\mu L$[b]	Not recorded directly; reference was made to a previous study (42)[b] Baseline: PDGF-AB, 10.2 ± 4.5 ng/mL[b] TGF-β1, 12.8 ± 5.2 ng/mL[b] Treatment: PDGF-AB, 295.2 ± 142.7 ng/mL[b] TGF-β1, 499.8 ± 388.6 ng/mL[b]	Sterine saline solution with 10% $CaCl_2$, mixed with 100 U/mL sterile bovine thrombin
Piemontese *et al*. 2008 (37)	SmartPReP[f]	Two (2400 r.p.m., 10 min & 3600 r.p.m., 15 min)	Not recorded[b]	Not recorded[b]	1 mL of 10% $CaCl_2$, mixed with 1000 United States Units of topical thrombin
Split-mouth randomized controlled clinical trials					
Hanna *et al*. 2004 (38)	SmartPReP[f]	Two (2400 r.p.m., 10 min & 3600 r.p.m., 15 min)[b]	Baseline: $256 \times 10^3/\mu L$ to $373 \times 10^3/\mu L$ Treatment: not recorded[b]	Not recorded[b]	1 mL of 10% $CaCl_2$, mixed with 1000 United States Units of topical thrombin[g]
Keles *et al*. 2006 (39)	Not reported[b]	Not reported[b]	Baseline: not reported[b] Treatment: $67{,}768 \times 10^3/\mu L \pm 11{,}514 \times 10^3/\mu L$	Not reported[b]	10% $CaCl_2$ at 1:10 ratio (v/v)
Harnack *et al*. 2008 (40)	Curasan PRP kit[e]	Two (3169 r.p.m., 10 min & 4725 r.p.m., 15 min; or 900 *g*, 10 min & 2000 *g*, 15 min)[b]	Not recorded[b]	Not recorded[b]	Blood harvested from the surgical site[b]

[a] Heraeus Labofuge 300; Kendro Laboratory Products, Osterrode, Germany.
[b] Information retrieved (or not retrieved) after contact with the corresponding author of the study.
[c] Alto, Kaigen Inc., Osaka, Japan.
[d] Heraeus Christ Medifuge; Heraeus, Stuttgart, Germany.
[e] Curasan PRP kit; Curasan AG, Kleinostheim, Germany.
[f] SmartPReP; Harvest Technologies Corp., Plymouth, MA, USA.
[g] Thrombin-JMI; GenTrac, Inc., Middletown, WI, USA.

Table 5. Main outcomes of selected parallel group randomized controlled clinical trials

Study	Gain in clinical attachment level (mean ± SD in mm)	Reduction in probing pocket depth (mean ± SD in mm)
Okuda *et al*. 2005 (31)	PRP + HA, 3.4 ± 1.7 Saline + HA, 2.0 ± 1.2 ($p < 0.001$)	PRP + HA, 4.7 ± 1.6 Saline + HA, 3.7 ± 2.0 ($p < 0.05$)
Demir *et al*. 2007 (32)	PRP + BG, 3.13 ± 0.46 BG, 2.86 ± 0.42 ($p > 0.05$)	PRP + BG, 3.60 ± 0.51 BG, 3.28 ± 0.45 ($p > 0.05$)
Döri *et al*. 2007 (33)	PRP + BM + e-PTFE, 4.7 ± 1.1 BM + e-PTFE, 4.6 ± 0.8 ($p > 0.05$)	PRP + BM + e-PTFE, 5.5 ± 1.2 BM + e-PTFE, 5.7 ± 1.2 ($p > 0.05$)
Döri *et al*. 2007 (34)	PRP + BM + COL, 4.5 ± 1.1 BM + COL, 4.6 ± 1.1 ($p > 0.05$)	PRP + BM + COL, 5.5 ± 1.3 BM + COL, 5.5 ± 1.7 ($p > 0.05$)
Döri *et al*. 2008 (35)	PRP + β-TCP + e-PTFE, 4.1 ± 0.7 β-TCP + e-PTFE, 3.9 ± 0.9 ($p > 0.05$)	PRP + β-TCP + e-PTFE, 5.8 ± 0.6 β-TCP + e-PTFE, 5.4 ± 0.7 ($p > 0.05$)
Döri *et al*. 2008 (36)	PRP + BM + EMD, 4.8 ± 1.3 BM + EMD, 5.0 ± 0.9 ($p > 0.05$)	PRP + BM + EMD, 5.8 ± 1.8 BM + EMD, 5.9 ± 1.3 ($p > 0.05$)
Piemontese *et al*. 2008 (37)	PRP + DFDBA, 3.6 ± 1.8 Saline + DFDBA, 2.4 ± 2.2 ($p < 0.001$)	PRP + DFDBA, 4.6 ± 1.3 Saline + DFDBA, 3.5 ± 1.9 ($p < 0.05$)

Abbreviations: β-TCP, β-tricalcium phosphate; BG, bioactive glass; BM, bovine-derived porous bone mineral; COL, collagen membrane; DFDBA, demineralized freeze-dried bone allograft; EMD, enamel matrix protein derivative; e-PTFE, expanded polytetrafluoroethylene membrane; HA, hydroxyapatite; and PRP, platelet-rich plasma.

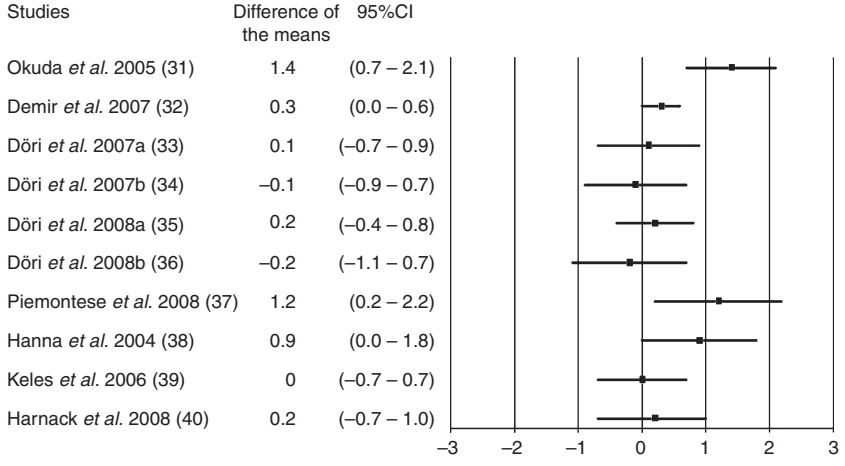

Fig. 1. Graphical presentation of the primary outcome variable (clinical attachment level change) as a difference of the mean values between test and control group in each selected randomized controlled clinical trial. Confidence intervals (95% CI) of the difference of the means are also presented.

ference of mean change in clinical attachment level for the deepest sites, 0.9 mm; 95% CI, 0.0 (zero) to 1.8 mm; Fig. 1) at the end of the 6 mo follow-up period for the buccal ($p = 0.041$), lingual ($p = 0.014$) and deepest sites ($p = 0.026$).

o *Secondary outcome variables*. The above-mentioned comparison also demonstrated statistically significantly higher probing pocket depth reduction at the end of 6 mo follow-up period, but no statistically significant change in REC. No tooth loss and no postoperative complications and adverse events were reported for both therapeutic modalities.

- Platelet-rich plasma combined with BM and GTR with expanded polytetrafluoroethylene (e-PTFE, non-resorbable) membranes (experimental group) vs. only the combination of BM and e-PTFE membranes (control group; 33; Tables 2 and 5).

o *Primary outcome variable* (*change in clinical attachment level*). When compared with the use of the combination of BM and e-PTFE membranes, the addition of PRP to this combination provided no statistically significant ($p > 0.05$) additional improvement in clinical attachment level (intergroup difference of mean change in clinical attachment level, 0.1 mm; 95% CI, −0.7 to 0.9 mm; Fig. 1) at the end of the 12 mo follow-up period.

o *Secondary outcome variables*. The above-mentioned comparison also demonstrated no statistically significant additional improvements in probing pocket depth and REC at the end of 12 mo follow-up period. No tooth loss was reported. The postoperative healing was uneventful for both therapeutic modalities, but minor exposure of the coronal portion of the e-PTFE membrane was observed in the fifth week in four cases treated with the adjunctive use of PRP and in five cases treated without further addition of PRP.

- Platelet-rich plasma combined with BM and GTR with collagen (resorbable) membranes (experimental group) vs. only the combination of BM and collagen membranes (control group; 34; Tables 2 and 5).

o *Primary outcome variable* (*change in clinical attachment level*). When compared with the use of the combination of BM and collagen membranes, the addition of PRP to this combination

provided no statistically significant ($p > 0.05$) additional improvement in clinical attachment level (intergroup difference of mean change in clinical attachment level, −0.1 mm; 95% CI, −0.9 to 0.7 mm; Fig. 1) at the end of the 12 mo follow-up period.

o *Secondary outcome variables*. The above-mentioned comparison also demonstrated no statistically significant additional improvements in probing pocket depth and REC at the end of the 12 mo follow-up period. No tooth loss was reported. The postoperative healing was uneventful for both therapeutic modalities; membrane exposure was observed in three cases treated with the adjunctive use of PRP and in four cases treated without further addition of PRP, but the exposed parts of the membranes disintegrated and no side-effects occurred.

- Platelet-rich plasma combined with BM and enamel matrix protein derivative (EMD; experimental group) vs. only the combination of BM and EMD (control group; 36; Tables 2 and 5).

o *Primary outcome variable* (*change in clinical attachment level*). When compared with the use of the combination of BM and EMD, the addition of PRP to this combination provided no statistically significant ($p > 0.05$) additional improvement in clinical attachment level (intergroup difference of mean change in clinical attachment level, −0.2 mm; 95% CI, −1.1 to 0.7 mm; Fig. 1) at the end of the 12 mo follow-up period.

o *Secondary outcome variables*. The above-mentioned comparison also demonstrated no statistically significant additional improvements in probing pocket depth and REC at the end of the 12 mo follow-up period. No tooth loss was reported. The postoperative healing was uneventful for both therapeutic modalities; a slight wound dehiscence, without exposing particles of the graft, was observed in the third week in two cases treated with the adjunctive use of PRP and in three cases treated without further addition of PRP, but all dehiscences epithelialized within a few days and no side-effects occurred.

Platelet-rich plasma combined with alloplastic and other regenerative materials —

- Platelet-rich plasma combined with hydroxyapatite (experimental group) vs. the combination of hydroxyapatite and saline as placebo (control group; 31; Tables 2 and 5).

o *Primary outcome variable* (*change in clinical attachment level*). When compared with the use of hydroxyapatite and a non-therapeutic substance (saline), the addition of PRP to hydroxyapatite resulted in statistically significantly higher clinical attachment level gain ($p < 0.001$, intergroup difference of mean change in clinical attachment level, 1.4 mm; 95% CI, 0.7–2.1 mm; Fig. 1) and vertical relative clinical attachment level gain ($p < 0.001$) at the end of the 12 mo follow-up period.

o *Secondary outcome variables*. The above-mentioned comparison also demonstrated statistically significantly higher probing pocket depth reduction at the end of the 12 mo follow-up period. At 12 mo, no statistically significant differences between the two treatment modalities existed with regard to changes in REC and radiographical intrabony defect depth. No tooth loss and no postoperative complications or adverse events were reported for both therapeutic modalities. Soft tissue response was characterized as 'excellent' for both treatments; an objective or subjective evaluation of aesthetics was not performed.

- Platelet-rich plasma combined with bioactive glass (experimental group) vs. bioactive glass alone (control group; 32; Tables 2 and 5).

o *Primary outcome variable* (*change in clinical attachment level*). When compared with the use of bioactive glass alone, the addition of PRP to bioactive glass provided no statistically significant ($p > 0.05$) additional improvement in clinical attachment level (intergroup difference of mean change in clinical attachment level, 0.3 mm; 95% CI, 0 (zero) to 0.6 mm; Fig. 1) at the end of the 9 mo follow-up period.

o *Secondary outcome variables*. The above-mentioned comparison also demonstrated no statistically significant additional improvements in probing pocket depth and REC, as well as intrasurgically measured intrabony defect depth and crestal osseous resorption at the end of the 9 mo follow-up period. No tooth loss was reported, and the postoperative healing was uneventful for both therapeutic modalities.

- Platelet-rich plasma combined with β-tricalcium phosphate (β-TCP; experimental group) vs. β-TCP alone (control group; 40; Tables 3 and 6).

o *Primary outcome variable* (*change in clinical attachment level*). When compared with the use of β-TCP alone, the addition of PRP to β-TCP provided no statistically significant ($p > 0.05$) additional improvement in clinical attachment level (intergroup difference of the medians of change in clinical attachment level, 0.2 mm; 95% CI, −0.7 to 1.0 mm; Fig. 1; mean values of change in clinical attachment level were not reported in this study and therefore the intergroup difference of the medians of change in clinical attachment level are depicted in Fig. 1 instead of the intergroup difference of the means of change in clinical attachment level), as well as no statistically significant ($p > 0.05$) additional improvement in the relative attachment level (RAL) at the 6 mo surgical re-entry.

o *Secondary outcome variables*. The above-mentioned comparison also demonstrated no statistically significant additional improvement in the intrasurgically recorded vertical depth of the intraosseous periodontal defect at the 6 mo surgical re-entry. No tooth loss and no postoperative complications and adverse events were reported for both therapeutic modalities. Furthermore, as revealed by an early healing index, no significant differences in postsurgical early healing, within the initial four postoperative weeks, were observed between the two therapeutic modalities. The experimental and the control group were possibly not comparable at the baseline of the study with regard to the values of certain clinical outcome variables (clinical attachment level and probing pocket depth), as well as the sole radiographical variable (radiographical defect depth). Hence, a direct comparison between the two study groups seems to be difficult with respect to alterations

Table 6. Main outcomes of selected split-mouth randomized controlled clinical trials

Study	Gain in clinical attachment level	Reduction in probing pocket depth
Hanna *et al.* 2004 (38)	Mean ± SD in mm: PRP + BM, 3.23 ± 1.16 buccally, 3.31 ± 0.85 lingually and 3.15 ± 0.99 at the deepest sites; BM, 2.07 ± 1.11 buccally, 2.53 ± 1.12 lingually and 2.31 ± 1.18 at the deepest sites ($p = 0.041$ buccally, $p = 0.014$ lingually and $p = 0.026$ at the deepest sites)	Mean ± SD in mm: PRP + BM, 3.50 ± 1.76 buccally, 3.53 ± 1.56 lingually and 3.54 ± 1.20 at the deepest sites; BM, 1.90 ± 1.18 buccally, 2.69 ± 1.10 lingually and 2.53 ± 0.96 at the deepest sites ($p = 0.012$ buccally, $p = 0.010$ lingually and $p = 0.033$ at the deepest sites)
Keles *et al.* 2006 (39)	Mean ± SD in mm: PP + PAM, 4.1 ± 0.7 BG + PAM, 4.1 ± 1.2 ($p > 0.05$)	Median (Min–Max) in mm: PP + PAM, 4 (3–6) BG + PAM, 4 (3–7) ($p > 0.05$)
Harnack *et al.* 2008 (40)	Median in mm: PRP + β-TCP, 0.28 β-TCP, 0.13 (No statistical test was performed for this intergroup comparison)[a]	Median in mm: PRP + β-TCP, 0.8 β-TCP, 0.4 (No statistical test was performed for this intergroup comparison)[a]

[a]After contact with the corresponding author of the study.
Abbreviations: β-TCP, β-tricalcium phosphate; BG, bioactive glass; BM, bovine-derived porous bone mineral; Max, maximum value; Min, minimum value; PAM, polylactic acid membrane; PP, platelet pellet; and PRP, platelet-rich plasma.

in clinical attachment level, probing pocket depth and radiographical defect depth.

- Platelet-rich plasma combined with β-TCP and GTR with e-PTFE membranes (experimental group) vs. only the combination of β-TCP and e-PTFE membranes (control group; 35; Tables 2 and 5).

o *Primary outcome variable* (*change in clinical attachment level*). When compared with the use of the combination of β-TCP and e-PTFE membranes, the addition of PRP to this combination provided no statistically significant ($p > 0.05$) additional improvement in clinical attachment level (intergroup difference of mean change in clinical attachment level, 0.2 mm; 95% CI, −0.4 to 0.8 mm; Fig. 1) at the end of the 12 mo follow-up period.

o *Secondary outcome variables*. The above-mentioned comparison also demonstrated no statistically significant additional improvements in probing pocket depth and REC at the end of the 12 mo follow-up period. No tooth loss was reported. The postoperative healing was uneventful for both therapeutic modalities, but minor exposure of the coronal portion of the e-PTFE membrane was observed in the fourth to sixth week in seven cases treated with the adjunctive use of PRP and in nine cases treated without further addition of PRP. Chlorhexidine gel and rinses were used twice per day with the aim of preventing bacterial infection, until membranes were removed.

Platelet-rich plasma combined only with GTR —

- Platelet pellet combined with GTR using polylactic acid (resorbable) membranes (experimental group) vs. the combination of polylactic acid membranes and bioactive glass (control group; 39; Tables 3 and 6).

o *Primary outcome variable* (*change in clinical attachment level*). When compared with the use of the combination of polylactic acid membranes and bioactive glass, the addition of platelet pellet to polylactic acid membranes provided no statistically significant ($p > 0.05$) additional improvement in clinical attachment level (intergroup difference of mean change in clinical attachment level, 0.0 (zero); 95% CI, −0.7 to 0.7 mm; Fig. 1) at the end of the 6 mo follow-up period.

o *Secondary outcome variables*. The above-mentioned comparison also demonstrated no statistically significant additional improvements in probing pocket depth, REC and radiographical alveolar bone level at the end of the 6 mo follow-up period. No tooth loss was reported and no information was provided on postoperative healing.

Quality assessment of selected RCTs (Table 7)

The results provided by the independent and duplicate quality assessment of all selected RCTs by two reviewers (S.K. and N.M.), prior to and after contact with the corresponding author of each study, are summarized in Table 7. The proportion of inter-reviewer agreement (18,19) was 90% for quality criteria A and B and 100% for the remaining quality criteria (C–G). The κ score (17–19) was 0.861 for quality criterion A, 0.750 for quality criterion B and 1.000 for the remaining quality criteria (C–G). Based on proposed interpretations of the magnitude of κ score (18,19), its value for all quality criteria (A–G) was ≥0.75 and therefore could be considered to represent an excellent level of agreement beyond chance.

Overall, based on proposed definitions of degrees of risk of bias (low, moderate and high) (20), the risk of bias was estimated to be moderate for the vast majority (31,33–36,38,39) of RCTs selected, except two (32,40), in which the risk of bias was regarded as high, because more than one quality criterion was not met, and another RCT (37), that fulfilled all quality criteria and therefore entailed a low risk of bias (Table 7).

Meta-analysis

Owing to considerable discrepancies (high heterogeneity) among the RCTs selected (primarily different combinations of PRP with other therapeutic bioactive agents/procedures and limited quantity of available data), no

Table 7. Quality assessment of all (parallel group and split-mouth) selected randomized controlled clinical trials, before and after contact with their corresponding author (scorings formed after contact have been placed in parentheses)

Study	A (0–3)	B (0–3)	C (0–1)	D (0–1)	E (0–2)	F1 (0–2)	F2 (0–2)	G (0–2)	Estimated risk of bias
Parallel group randomized controlled clinical trials									
Okuda *et al.* 2005 (31)	3[a]	2 (2)[b]	1[a]	1[a]	2[a]	1 (2[a])[b]	0 (0)[b]	2[a]	Moderate
Demir *et al.* 2007 (32)	0 (0)[b]	2 (2)[b]	1[a]	1[a]	2[a]	2[a]	0 (0)[b]	2[a]	High
Döri *et al.* 2007 (33)	2 (2)[b]	2 (3[a])[b]	1[a]	1[a]	2[a]	2[a]	0 (2[a])[b]	2[a]	Moderate
Döri *et al.* 2007 (34)	2 (2)[b]	3[a]	1[a]	1[a]	2[a]	2[a]	2[a]	2[a]	Moderate
Döri *et al.* 2008 (35)	2 (2)[b]	2 (3[a])[b]	1[a]	1[a]	2[a]	2[a]	0 (2[a])[b]	2[a]	Moderate
Döri *et al.* 2008 (36)	2 (2)[b]	3[a]	1[a]	1[a]	1 (2[a])[b]	2[a]	2[a]	2[a]	Moderate
Piemontese *et al.* 2008 (37)	3[a]	3[a]	1[a]	1[a]	2[a]	2[a]	2[a]	2[a]	Low
Split-mouth randomized controlled clinical trials									
Hanna *et al.* 2004 (38)	1 (1)[b]	3[a]	1[a]	1[a]	2[a]	2[a]	2[a]	2[a]	Moderate
Keles *et al.* 2006 (39)	0 (1)[b]	2 (3[a])[b]	1[a]	1[a]	2[a]	2[a]	0 (2[a])[b]	2[a]	Moderate
Harnack *et al.* 2008 (40)	0 (0)[b]	0 (3[a])[b]	1[a]	1[a]	1 (1)[b]	1 (2[a])[b]	2[a]	2[a]	High

[a] The maximum possible score has been achieved.
[b] After contact with the corresponding author of the study.
Criteria: A, sample size calculation and adequacy; B, randomization and allocation concealment method; C, clear definition of inclusion/exclusion criteria; D, completeness of follow-up (specified reasons for withdrawals and dropouts in each study group); E, experimental and control groups comparable at study baseline for important prognostic factors; F1, presence of examiner masking; F2, presence of operator masking; and G, appropriate statistical analysis.

meta-analysis could be performed. For the same reasons, it was deemed not meaningful to carry out any subgroup analyses on the RCTs selected (e.g. subgroup analyses for smokers/non-smokers).

Discussion

The present systematic review evaluated any available information in the dental literature in any language up to and including September 2008, derived exclusively from RCTs and addressing the focused question, 'What is the efficacy, with respect to clinical, radiographical and patient-centred outcomes, of combinations of PRP with other therapeutic bioactive agents/procedures, compared with the efficacy of the same agents/procedures without the adjunctive use of PRP in the therapy of periodontal intraosseous defects in patients with chronic periodontitis and without systemic diseases that could potentially influence the outcome of periodontal therapy?'

Summary of main results

Overall, as revealed mainly by the primary outcome variable (change in clinical attachment level) and, to a more limited extent, by the most important secondary outcome variable (change in probing pocket depth) selected in this systematic review (Tables 5 and 6), most of the RCTs selected (32–36,39,40) demonstrated that the addition of PRP to certain regenerative materials, namely bioactive glass (32), β-TCP (40), BM and e-PTFE membranes (33), β-TCP and e-PTFE membranes (34), BM and collagen membranes (35) and BM and EMD (36), failed to confer statistically significant additive benefits in the therapy of periodontal intraosseous defects. However, according to other RCTs (31,37,38), such adjunctive positive outcomes may result from other combinations of PRP, namely together with BM (38), DFDBA (37) and hydroxyapatite (31).

These results should not necessarily be regarded as conflicting, because the selected RCTs have examined combinations of PRP with different regenerative materials and, owing to the diversity of therapeutic modalities, no antitheses exist among the RCTs. Instead, it seems reasonable to suggest that the specific selection of regenerative materials combined with PRP is possibly important. Given the limited amount of data currently available, this hypothesis has to be evaluated by additional RCTs on the use of each specific combination of PRP. Another interesting speculation, requiring thorough evaluation in the future, is that when PRP is combined with many regenerative materials (already established to be efficacious) at the same time, its adjunctive beneficial effects might be masked by the significant regenerative outcomes provided by these materials. A third**,** equally valid, explanation for differences among the results of selected RCTs might be that in the case of an heterogeneous sample of studies with limited sample sizes, the role of chance would be expected to divide results into those suggesting a significant added efficacy of PRP and those not supporting such an added efficacy.

The use of PRP was demonstrated by all selected RCTs (31–40) to be entirely safe, without causing complications or adverse events; postoperative healing was uneventful in all RCTs. An association between the use of PRP as an adjunct to regenerative procedures and the incidence of the exposure of non-resorbable (33,35) and resorbable (34) barrier membranes or bone grafts (36) has never been demonstrated. Unfortunately, the vast majority of the selected RCTs provided no information on whether the adjunctive use of PRP was associated with improved aesthetics, a substantially higher progression/rate of soft and hard tissue healing or improved clinical handling/management properties of the combinations of PRP with various materials. A split-mouth RCT (38) reported an acceleration of early healing phenomena, since the PRP-treated sites demonstrated at 1 wk a

lower degree of clinical inflammation and swelling and higher density in appearance compared with the contralateral control sites. It should be remarked, however, that this clinical observation was not documented by specially selected secondary variables in this study (38), i.e. it was not supported by scientific data. In contrast, another split-mouth RCT (40) reported no significant differences in postsurgical early healing, within the initial four postoperative weeks, between PRP-treated and non-PRP-treated sites.

Overall completeness and applicability of evidence

The focused question concentrated solely on the adjunctive use of PRP in the therapy of periodontal intraosseous defects. Literature search revealed that up to and including September 2008 no data at all, derived from specially designed RCTs with appropriate control group, existed with regard to the exclusive (individual) use of PRP in the treatment of such defects. Only RCTs on chronic periodontitis patients were examined in this systematic review, whereas no RCTs had been conducted on aggressive periodontitis patients up until September 2008.

The RCTs selected (31–40) encompass a wide range of types of interventions by reporting on several potential combinations of PRP with other therapeutic bioactive agents/procedures. Therefore, the evidence acquired is relevant to the review focused question to a high extent, suggesting a high external validity/generalizability/applicability of evidence. The interventions described in the selected RCTs fit to a high degree into the context of current clinical periodontal practice.

Quality of evidence

All RCTs (31–40) selected in this systematic review (both parallel group and split-mouth) correctly included patient-based analyses. Almost all RCTs (both parallel group and split-mouth) generally demonstrated appropriate methodology with regard to definition of inclusion/exclusion criteria, report of reasons for patient withdrawals/dropouts, presence of comparable study groups at study baseline, masking and methods of statistical analysis (Table 7). However, in certain RCTs a sample size calculation had not been performed at all before their initiation (32,39,40) and in other RCTs randomization and allocation concealment methods were not clearly adequate (31,32).

In relation to split-mouth RCTs, the risk of carry-over (i.e. the situation in which the effects of an intervention given in one period persist into a subsequent period, thus interfering with the effects of a different subsequent intervention) has to be examined. The statistical methods to demonstrate carry-over are not adequate and therefore the estimation of carry-over inevitably has to be subjective to a great extent. In split-mouth RCTs selected in this systematic review (38–40), the risk of carry-over could be regarded as low, because the interventions (therapy of periodontal intraosseous defects with various bioactive materials/procedures) were not in neighbouring sites and resulted in too strictly localized effects (tissue formation around each single tooth), without influencing each other.

The number of RCTs and therefore the amount of data available for each specific combination of PRP with other therapeutic bioactive agents/procedures is low, suggesting that the currently acquired evidence could be regarded as limited in quantity. Owing to the limited amount of RCTs existing, the consistency of their results cannot be evaluated and no robust conclusions may be drawn regarding their objective(s). The obtained evidence seems to be weak to allow the recommendation of a specific protocol in clinical practice for the adjunctive use of PRP in the therapy of periodontal intraosseous defects. Overall, the internal validity of the evidence might be judged as moderate.

Potential biases in the review process and their impact on the results and conclusions

A comprehensive literature search is necessary for the identification of the maximum number of RCTs and, furthermore, for the minimization of selection bias for the RCTs identified. It has been documented (43,44) that the exclusive use of electronic data sources may not be sufficient and provide fewer RCTs than those that would have been retrieved by the use of several data sources. In the present systematic review, extensive electronic and manual searches were undertaken and, furthermore, other data sources were used, particularly contact with experts, that provided a significant amount of missing or ambiguous data. These strategies contributed to the collection of all relevant RCTs and data and the prevention of potential selection bias.

In view of the relatively recent introduction of PRP in the field of Clinical Periodontology, a number of aspects of selection bias might be anticipated to be acting, which would tend to provide a more favourable impression of the efficacy of the adjunctive use of PRP, such as the so-called reporting or publication bias and particular types of publication bias, including time-lag bias and language bias. These aspects of potential bias need to be further considered.

Publication bias is the type of selection bias caused by the selective availability of data (i.e. the identification of only a subset of all relevant available data) and arises when the likelihood of identifying studies is related to the results of those studies (45–47). The publication of research may depend on its results, and in certain cases studies revealing that an intervention is not effective are not published (45–47). Therefore, systematic reviews failing to identify unpublished research could overestimate the true effectiveness of the intervention examined, owing to a publication bias (45–47). According to recommendations in the literature (48), in the present systematic review an effort was carried out to include the so-called 'grey literature' (literature not formally published), as a means to minimize the risk of introducing publication bias. In this systematic review, contact with experts was used as a means of improving access to unpublished data.

The probability of identifying a study may be affected by its results, even if the study has already been published (49,50). Language bias is a type of publication bias arising from the preferential publication of studies without significant results in languages other than English, resulting in a reduced probability of identifying such studies (49,50).

In the present systematic review, an advantage of the literature search performed was that no language limitations were imposed, since it is generally recommended (15,51–53), although it is not obligatory, that systematic reviews evaluate publications in any language, in order to include all available material and concomitantly limit the effect of publication/language bias (49,50). It is also of interest to note that limiting the search to US National Library of Medicine *PubMed* and *Cochrane CENTRAL* might involve a tendency to identify English language journals. The use of additional databases, such as *EMBASE* and *LILACS*, could possibly provide a more comprehensive non-English language search.

Time-lag bias is a type of publication bias arising from the fact that studies with striking positive (statistically significant) results are more likely to be terminated earlier than initially intended and/or published earlier, whereas studies with negative (statistically non-significant) findings are more likely to be delayed in publication (54). This type of bias might erroneously lead to the conclusion that a new intervention is effective (54). In the present systematic review, the possibility of time-lag bias has to be considered, particularly because only a limited number of RCTs (31–40) were finally selected (54).

In summary, conceptually, publication bias and specific types of publication bias (language bias and time-lag bias) might possibly have influenced the results and conclusions of this systematic review towards an overestimation of the efficacy of the adjunctive use of PRP.

Agreement/disagreement with previous systematic and non-systematic reviews

A systematic review (12) on all clinical applications of PRP in Dentistry reported evidence 'for beneficial effects of PRP in the treatment of periodontal defects.' However, a more recent conventional (not systematic) review (13) reported contrasting results, ranging from a significant added efficacy of the adjunctive use of PRP to no effect. The present systematic review also demonstrated significant additive effects in certain cases and no such effects in other cases.

Implications for future research

An important issue is whether parallel group or split-mouth design of an RCT is the most appropriate for the evaluation of the efficacy of the adjunctive use of PRP in periodontal intraosseous defects. Parallel group design is certainly the most appropriate design and is strongly recommended for the correct statistical comparison of the primary (change in clinical attachment level) and the main secondary outcome variables (changes in probing pocket depth, gingival recession, clinical and/or radiographical bone level etc.) between the experimental and the control group, because these comparisons would not be affected by patient factors. However, split-mouth design may exhibit some advantages as well, because it allows the comparison of certain secondary outcome variables (aesthetics, progression/rate of soft and hard tissue healing, postoperative complications, adverse events etc.) within the same patient and thus unaffected by patient factors. The use of unclear or mixed (parallel group and split-mouth) design is, nonetheless, certainly improper.

Future studies evaluating the adjunctive use of PRP in the therapy of periodontal intraosseous defects should pay particular attention to the selection of an appropriate control group, since certain RCTs (21–23,28,30), otherwise well-designed studies, were excluded from this systematic review, owing to inappropriate control group (as regards the focused question examined in this systematic review; Table 1). A control group may be considered appropriate when it contains the same therapeutic materials/procedures as those employed in at least one experimental group, differing only in that PRP is not added in the control group, whereas it is used as an adjunct in the experimental group(s).

Since sample size calculation had not been performed in the majority of RCTs selected (Table 7) before their initiation, it is not easy to estimate whether their sample sizes were adequate or not. It may be recommended that future studies perform and report sample size calculation.

Future RCTs are encouraged to provide more information on aesthetics and rate of wound healing as secondary outcome variables, since such data were missing from the majority of RCTs selected (31–37,39).

The follow-up periods of the selected RCTs ranged from 6 (38–40) to 12 mo (31,33–37). Therefore, RCTs with longer periods of follow-up (preferably long-term data) are required, in order to evaluate whether the potential additive clinical effects of PRP are ephemeral or not. This issue is important, because it has been postulated that although PRP exerts a direct influence upon only the initial phase of osseous healing, physiological mechanisms still continue to promote osseous repair at an enhanced and accelerated level throughout the entire period of osseous maturation (12,55).

As deduced from Table 4, a substantial heterogeneity among RCTs selected exists with regard to various parameters of PRP preparation and application, which can partly account for the difference in results reported on the efficacy of the adjunctive use of PRP in the therapy of periodontal intraosseous defects. Therefore, consensus on an appropriate methodology for PRP preparation seems to be required before animal and human studies evaluate the efficacy of PRP (56).

The use of PRP (either adjunctive or individual) in the therapy of periodontal intraosseous defects is a relatively recently introduced clinical application, requiring many well-designed RCTs and additional systematic reviews to be adequately

documented; the present systematic review primarily provided the basic requirements for the correct design and conduction of the impending RCTs.

Conclusions

General conclusions

- Most RCTs selected generally demonstrate appropriate methodology with regard to the majority of quality criteria.
- However, most of studies selected are lacking sample size calculation, and in certain RCTs randomization and allocation concealment methods are not clearly adequate.
- The selected RCTs differ in their design with regard to therapeutic bioactive agents/procedures combined with PRP for the therapy of periodontal intraosseous defects.
- The amount of data currently available for each combination of PRP with other therapeutic bioactive agents/procedures could be regarded as limited.
- Publication bias and its specific types, language bias and time-lag bias, might possibly lead to an overestimation of the efficacy of the adjunctive use of PRP.

Specific conclusions

- The clinical use of PRP is an entirely safe procedure, causing no adverse events or postoperative complications.
- Diverse outcomes (positive and negative) have been reported for the efficacy of PRP combined with various therapeutic bioactive agents/procedures, reflecting the limited and heterogeneous data available and possibly suggesting that the specific selection of agents/procedures combined with PRP could be important.

Implications for research and clinical practice

- Randomized controlled clinical trials should include an appropriate (concurrent with the experimental group) non-PRP control group and longer follow-up periods.
- Consensus on an appropriate methodology for PRP preparation seems to be required.
- A specific protocol for the clinical use of PRP cannot be recommended at present.

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