Long-term Clinical Performance of Regeneration versus Conservative Surgery in the Treatment of Infra-bony Defects: A Systematic Review

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Abstract

Aims: To determine the differences in the long-term clinical outcomes between Regeneration (REG) and Conservative Surgery (CS) in infra-bony defects.

Materials and Methods: Three databases were searched [PubMed, Medline and Embase] up to April 2019. Following screening, 17 studies were included. Randomized Controlled Clinical Trials, Controlled Clinical Trials and retrospective studies with long-term clinical observations (≥ 24-months) were selected. After subgrouping the studies regarding the grafting material and the used flap, meta-analysis was performed for different outcomes [clinical attachment level gain (CALGain), probing pocket depth reduction (PPDRed), recession increase (RECInc) and bone fill (BF)] at different follow-ups (24-, 36-, 48- to 60- and 120- to 240-months).

Results: The time-related meta-analysis favoured REG at every interval for every outcome. In subgroup analysis, enamel matrix derivative (EMD) performed significantly better for both CALGain [24- ($p<0.0001$), 36- ($p=0.02$) and 60-months ($p<0.00001$)] and PPDRed [24- ($p=0.0004$), 36- ($p=0.003$) and 60-months ($p<0.00001$)]. For Ceramic Grafts (CGs), CALGain at 48-months ($p<0.00001$) and PPDRed at 24- ($p=0.0006$), 36- ($p<0.00001$) and 48-months ($p<0.00001$) follow-up showed better results.

Conclusion: The better outcomes from REG using EMD or CGs can be maintained for a longer duration, suggesting a potential longevity of the occurred healing.

Keywords: Periodontal Regeneration, Guided Tissue Regeneration, Enamel Matrix Derivative,

Introduction

Deep infra-bony defects have long been considered a clinical challenge and are frequent anatomical sequelae to periodontitis (Papapanou and Tonetti, 2000). Treatments for infra-bony defects range from non-surgical (scaling and root planing) to surgical treatment such as flap surgery, osseous resective surgery and periodontal regeneration (REG) (Pagliaro *et al.*, 2008).

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Infra-bony defects can be conservatively treated by different surgical techniques. Conservative surgery (CS) comprises of different surgical techniques [open flap debridement (OFD), minimal soft tissue resective approaches and Modified Widman flaps (MWF) aimed at conserving interdental soft tissues) meant to gain root surface access for accomplishing elimination of residual plaque/calculus with no active removal of bone and mostly no resection of soft tissues (Graziani *et al.*, 2012). In most clinical studies, CS has been used as the control when assessing regenerative procedures in infra-bony

defects. Although results are not better than REG, considerable advantages for CS have been noted (Needleman *et al.*, 2005). Also, depending upon the flap design such as papilla preservation flap (PPF), the clinical efficacy of CS may considerably differ (Graziani *et al.*, 2012).

Periodontal REG is defined as the *de novo* reconstitution or reproduction of an injured or lost part to re-establish the architecture as well as function of the periodontium (AAP, 2001). Periodontal REG is effective in the treatment of one-, two- and three-wall or combined infra-bony defects (Cortellini and Tonetti, 2015). Systematic Reviews (SRs) of Randomized Controlled Clinical Trials (RCTs), as well as animal and human histologic studies, supported the significance of guided tissue regeneration (GTR) (Nyman *et al.*, 1982; Needleman *et al.*, 2006), bone replacement grafts (BRGs) (Rosen *et al.*, 2000; Reynolds *et al.*, 2003), enamel matrix derivative (EMD) (Hammarström *et al.*, 1997; Esposito *et al.*, 2009; Koop *et al.*, 2012) and combination therapy of the above-mentioned techniques (Trombelli and Farina, 2008; Tu *et al.*, 2012; Iorio-Siciliano *et al.*, 2014) in periodontal REG.

Though conventional methods use barrier membranes enabling progenitor periodontal ligament cells to selectively repopulate the root surfaces, the effectiveness of bioactive agents is based primarily on mitogenic and chemotactic effects on the periodontal ligament and alveolar bone cells (Trombelli and Farina, 2008).

A relevant question with respect to REG is whether or not the achieved outcomes can be maintained over an extended time period. As suggested by the growing amount of evidence, REG outcomes may be maintained over time leading to long-term retention of teeth with deep baseline infra-bony defects (Cortellini and Tonetti, 2004; Nygaard-Østby *et al.*, 2010).

Clinical studies on infra-bony defect regeneration have reported positive outcomes after 5-years (Sculean *et al.*, 2001; Zucchelli *et al.*, 2002; Tonetti *et al.*, 2002; Sculean *et al.*, 2004; Eickholz *et al.*, 2007; Cortellini and Tonetti, 2011) and after 6- to 7-years (Stavropoulos and Karring, 2004), however paucity of data is available for longer follow-ups (Cortellini and Tonetti, 2004; Pretzl *et al.*, 2008; Sculean *et al.*, 2008; Cortellini *et al.*, 2017) and the majority of studies did not use CS as the control. A systematic review on the clinical performance of CS in infra-bony defects has been recently published by Graziani *et al.* in 2012.

There are several studies that have compared short-term results (< 24-months follow-up period) of REG versus CS in infra-bony defects (Sculean *et al.*, 2001; Zucchelli *et al.*, 2002; Tonetti *et al.*, 2002; Cortellini and Tonetti, 2011), but very few studies comparing long-term (≥ 24-months follow-up period) clinical outcomes (Sculean *et al.*, 2008; Cortellini *et al.*, 2017).

Therefore, this review compares periodontal REG with CS in infra-bony defects to investigate any comparable differences in terms of clinical attachment level gain (CALGain), probing pocket depth reduction (PPDRed), recession increase (RECInc) and bone fill (BF) as observed in RCTs, CCTs or retrospective studies with long-term observation.

Materials and Methods

This SR followed the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement (Moher *et al.*, 2009).

Literature Search

The PICO framework [P (Patient): Infra-bony defects, I (Intervention): Regenerative periodontal surgery (GTR, BRGs, EMD and combination therapy), C (Comparison): CS and O (Outcome): CALGain, PPDRed, RECInc and BF] was used to design the research question "What is the difference between long-term clinical performance of periodontal REG versus CS in the treatment of infra-bony defects?".

Relevant articles complying with the eligibility criteria were searched up to April 2019 using the following electronic databases: PubMed (NLM), Medline (Ovid) and Embase (Ovid).

Key terms used for search were:

P: "intrabony defect" OR "intra bony defect" OR "intra-bony defect" OR "infrabony defect" OR "infra bony defect" OR "infra-bony defect" OR "intraosseous" OR "intra osseous" OR "intra-osseous" AND

I: "periodontal regeneration" OR "regenerative periodontal surgery" OR "barrier membrane" OR "guided tissue regeneration" OR GTR OR "bone graft" OR "bone substitute" OR "bone mineral" OR "bone replacement graft" OR BRG OR "xenograft" OR "autograft" OR "enamel matrix protein" OR "enamel matrix derivative" OR EMD OR "emdogain" OR "amelogenin" AND

C: "surgical flap" OR "periodontal pocket surgery" OR "access surgery" OR "conservative surgery" OR "modified widman flap" OR "open flap debridement" OR OFD OR "modified papilla preservation flap" OR MPPF OR "simplified papilla preservation flap" OR SPPF AND

O: "long-term" AND "clinical attachment level" OR CAL OR "periodontal pocket depth" OR PPD OR "bone fill" AND (Clinical Trial[ptyp] AND Humans [Mesh] AND English[lang]).

A manual search was performed to integrate the retrieved batch of studies on "Journal of Clinical Periodontology, Journal of Periodontology, Journal of Periodontal Research" and "Periodontology 2000".

Literature Selection

Inclusion Criteria

- RCTs or CCTs comparing GTR versus OFD, BRG versus OFD, EMD versus OFD and combination therapy (GTR+BRG versus OFD or GTR+EMD versus OFD or BRG+EMD versus OFD).
- Studies with a mean follow-up period of at least 24-months or more.
- Defects with pocket depth ≥5 mm and/or Infra-bony defect depth ≥3 mm.
- Studies in English language and conducted on humans.

Exclusion Criteria

- RCTs or CCTs comparing GTR+BRG with GTR and GTR+BRG with BRG.
- RCTs or CCTs comparing GTR+EMD with GTR and GTR+EMD with EMD.
- RCTs or CCTs comparing EMD+BRG with BRG, EMD+BRG with EMD and EMD+BRG with GTR.
- Studies on furcation and supra-osseous (horizontal) defects.
- Studies reporting histological data, conducted on animals and in in-vitro.
- Case series, case reports and secondary research (reviews or SRs).

Literature Screening Stages

Following the search on databases, a systematic screening of the retrieved articles was done in three phases, comprising screening of titles, abstracts and full-texts according to the inclusion and exclusion criteria. All reports were screened independently by two reviewers (MSS and FP) and the inter-agreement score was recorded by the Cohen Kappa score (McHugh, 2012). Any discrepancy between the two reviewers was resolved via discussion with a third reviewer (MA). All studies meeting the inclusion criteria underwent the validity assessment.

Outcome Measures

The outcome measures included were: CALGain (mm), PPDRed (mm), RECInc (mm) and BF (mm).

Quality Assessment

The RCTs were evaluated for quality by Jadad Score (Jadad *et al*., 1996) and Cochrane risk of bias tool (Sterne *et al*., 2017). The inter-agreement K score was recorded by a blinded screening and scoring of the included papers (Landis and Koch, 1977).

Data Extraction

Using a standard protocol, the data collected from studies as authors, publication year, study design, treatment given (test and control group), participants (number, gender, mean age), number of infra-bony defects, defect location, use of antibiotics, follow-up (months) and the outcome values (Mean±SD) were recorded in a tabular form (Table 1).

Data Synthesis

To summarize and compare studies, outcome data were displayed as a weighted mean difference (WMD). For continuous outcomes, mean differences and 95% confidence intervals were used to summarize the data for each study. Forest plots were created to illustrate the effects of different studies and the global estimation. Review Manager (RevMan) version 5.3. for MacOs from Cochrane collaboration was used for all analyses. Statistical significance has been set as a p value <0.05.

The statistical heterogeneity among studies has been assessed in two different ways: Cochran's Q statistical test and I^2 test (Higgins *et al*., 2003). A random-effects model was adopted due to the hypothesis of a population of studies with possible variations.

Publication bias

Publication bias was evaluated, if any, using a funnel plot and Egger's linear regression model (Egger *et al*., 1997).

Results

Study Selection

From an original yield of 1509 titles and 62 abstracts, 12 studies were selected at the end of the screening process. Moreover, a bibliography hand searching incorporated an additional eight full-text articles, resulting in total 20 full-text articles available for evaluation. Three studies were excluded, and a total of 17 studies were selected for the analysis (Figure 1).

From the 17 included studies, 14 studies were RCTs, two studies were CCTs and one study was longitudinal evaluation of a clinical trial. An overview of the study incorporation with study evidence and their characteristics is shown in Table 1. Appendix A reveals search tracking and Appendix B reveals authors and reasons for exclusion after full-text evaluation.

To test the extent of inter-agreement between the two reviewers, Cohen's Kappa Statistics was used (McHugh, 2012). Its value lies between −1 and 1, where 1 is the perfect agreement, 0 is exactly what would be expected by chance and negative values indicate agreement less than chance, that is, potential systematic disagreement. The calculated score of Cohen's Kappa statistic $\varkappa$ was 0.81, which according to the commonly cited scale for interpretation of Kappa statistic (Landis and Koch, 1977) indicates a substantial good and reliable agreement between the involved reviewers.

Table 1. Table of Included Studies - Summary of findings presented in studies

Author/Year	Study Type	Treatment	Participants/No. of Defects/ Defects Location (Max./ Mand.)	Antibiotics (Y/N)	Follow-up (Months)	Primary Outcomes (mm) Mean ± SD				Secondary Outcomes
						O	T	C	SS	
(Yukna *et al.*, 1989) A	Longitudinal, single-centre, follow-up, CCT	T: OFD + Durapatite ceramic alloplastic implants C: OFD	6 patients (out of 8) completed the study/ 94 (T: 62, C: 32)/ NR	N	24	CALGain	1.6 ± 1.9	1.1 ± 2.2		NR
						PPDRed	3.5 ± 1.4	2.8 ± 1.6		
						RECInc	1.8 ± 1.9	1.8 ± 1.9		
						BF	NR	NR		
(Yukna *et al.*, 1989) B					36	CALGain	1.4 ± 2.0	1.3 ± 2.0		NR
						PPDRed	2.8 ± 1.6	1.7 ± 1.9		
						RECInc	1.4 ± 2.2	0.5 ± 1.9		
						BF	NR	NR		
(Yukna *et al.*, 1989) C					48	CALGain	1.3 ± 2.0	0.8 ± 2.3		NR
						PPDRed	3.0 ± 1.5	1.8 ± 2.2		
						RECInc	1.7 ± 1.8	1.2 ± 1.6		
						BF	NR	NR		
(Yukna *et al.*, 1989) D					60	CALGain	1.1 ± 2.2	0.5 ± 2.2		NR
						PPDRed	2.8 ± 1.9	1.4 ± 2.2		
						RECInc	1.7 ± 2.0	1.1 ± 1.8		
						BF	NR	NR		
(Nery *et al.*, 1990) A	Prospective, multi-centre, RCT	T1: Ceramic C: OFD	101 patients (out of 137) completed the study/ 150 (T1: 71, T2: 38, C: 41)/ NR	N	36	CALGain	T1: 1.0 ± 0.3	C: 0.9 ± 0.2	*NS*	The PI and GI increased steadily, but there were statistically significant differences between the treatment groups.
						PPDRed	NR	NR		
						RECInc	NR	NR		
						BF	T1: 1.2 ± 0.3	C: 1.4 ± 0.3	*NS*	
(Nery *et al.*, 1990) B		T2: Bone C: OFD			36	CALGain	T2: 0.4 ± 0.4	C: 0.9 ± 0.2	*NS*	
						PPDRed	NR	NR		
						RECInc	NR	NR		
						BF	T2: 0.4 ± 0.4	C: 1.4 ± 0.3	*NS*	

Table 1 continued overleaf.....

Table 1. Table of Included Studies - Summary of findings presented in studies continued....

(Galgut *et al.*, 1992) A	Prospective, single-centre, RCT	T: OFD + Ceramic HAp implant C: OFD	10 patients/ 117 (T: 58, C: 59)/ NR	N	24	CALGain PPDRed RECInc BF	3.19 ± 1.33 4.70 ± 1.28 1.51 ± 0.69 NR	2.79 ± 1.43 4.07 ± 1.22 1.28 ± 1.16 NR	*NS* *NS* *NS*	The bleeding and plaque scores were evaluated (Data not shown).
(Galgut *et al.*, 1992) B					36	CALGain PPDRed RECInc BF	3.21 ± 0.82 4.90 ± 0.92 1.69 ± 0.07 NR	2.66 ± 1.05 4.21 ± 1.06 1.55 ± 1.04 NR	*NS* *NS* *NS*	
(Galgut *et al.*, 1992) C					48	CALGain PPDRed RECInc BF	3.27 ± 1.16 5.00 ± 0.81 1.74 ± 0.59 NR	2.24 ± 0.96 4.20 ± 0.83 1.95 ± 1.03 NR	*p=0.058* *p<0.05* *NS*	
(Heijl *et al.*, 1997)	Prospective, multi-centre, split-mouth, RCT	T: MWF + EMD C: MWF + Placebo (PGA)	26 patients (out of 33) completed the study/ 54 (T: 27, C: 27)/ 30/38	Y	36	CALGain PPDRed RECInc BF	2.2 ± 1.1 3.1 ± 1.0 NR 2.6 ± 1.7	1.7 ± 1.3 2.3 ± 1.1 NR 0 ± 0.7	*p<0.01* *p<0.001* *p<0.001*	Local bleeding and plaque scores were maintained at or below 10% throughout the study period.
(Zetterström *et al.*, 1997)	Prospective, parallel, multi-centre, CCT	T: MWF + EMD C: MWF	66 patients (out of 140) completed the study/ 66 (T: 45, C: 21)/ NR	Y	36	CALGain PPDRed RECInc BF	2.9 ± 1.7 3.8 ± 1.8 NR 2.4 ± 1.4	2.2 ± 1.4 3.2 ± 2.0 NR 0.0 ± 1.1	*p<0.05* *p<0.05* *p<0.001*	NR
(Sculean *et al.*, 2004) A	Parallel, follow-up, RCT	T1: OFD + EMD C: OFD	42 patients (out of 56) completed the study/ 42 (T1: 11, T2: 11, T3: 10, C: 10)/ NR	Y	60	CALGain PPDRed RECInc BF	T1: 2.9 ± 1.6 T1: 4.3 ± 1.7 T1: 1.3 ± 0.7 NR	C: 1.3 ± 1.2 C: 2.7 ± 1.2 C: 1.7 ± 0.5 NR	*NS* *NS* *NS*	The FMPS and BoP showed no significant differences between the treatment groups.
(Sculean *et al.*, 2004) B		T2: OFD + GTR C: OFD			60	CALGain PPDRed RECInc BF	T2: 2.7 ± 0.9 T2: 3.9 ± 1.6 T2: 1.2 ± 1.0 NR	C: 1.3 ± 1.2 C: 2.7 ± 1.2 C: 1.7 ± 0.5 NR	*NS* *NS* *NS*	

Table 1 continued overleaf.....

Table 1. Table of Included Studies - Summary of findings presented in studies continued....

(Sculean *et al.*, 2004) C		T3: OFD + EMD + GTR C: OFD			60	CALGain PPDRed RECInc BF	T3: 2.6 ± 0.7 C: 1.3 ± 1.2 T3: 4.0 ± 1.0 C: 2.7 ± 1.2 T3: 1.5 ± 0.7 C: 1.7 ± 0.5 NR NR	*NS* *NS* *NS*	
(Francetti *et al.*, 2004)	Prospective, parallel, single-centre, RCT	T: SPPF + EMD C: SPPF	22 patients (out of 24) completed the study/ 22 (T: 11, C: 11)/ NR	Y	24	CALGain PPDRed RECInc BF	4.29 ± 1.38 2.71 ± 0.76 4.86 ± 1.95 3.00 ± 1.15 NR NR 3.44 ± 1.18 1.84 ± 0.53	*p<0.05* *NS* *p<0.05*	FMBS and FMPS showed no significant differences between the treatment groups (Data not shown).
(Francetti *et al.*, 2005)	Prospective, multi-centre, parallel, RCT	T: SPPF + EMD with EDTA C: SPPF	110 patients (out of 153) completed the study/ 137 (T: 82, C: 55)/ 107/88	Y	24	CALGain PPDRed RECInc BF	3.51 ± 2.0 2.51 ± 2.1 4.02 ± 1.96 3.51 ± 1.47 NR NR 3.18 ± 2.16 2.40 ± 1.58	*p<0.01* *NS* *NS*	No statistical difference was found between the T and C group at any time frame for either FMBS or FMPS (Data not shown).
(Sakallıoğlu *et al.*, 2007)	Prospective, single-centre, parallel, RCT	T: OFD + GTR C: OFD	27 patients (out of 31) completed the study/ 28 (T: 15, C: 13)/ 15/13	Y	36	CALGain PPDRed RECInc BF	3.27 ± 1.71 1.57 ± 1.05 3.37 ± 1.10 2.27 ± 0.84 0.2 ± 1.24 0.7 ± 0.83 2.7 ± 1.24 1.5 ± 1.58	*p<0.05* *p<0.05* *NS* *p<0.05*	Full-mouth PI and GI were evaluated (Data not shown).
(Sculean *et al.*, 2007)	Prospective, parallel, RCT	T: OFD + NBM + GTR C: OFD	19 patients (out of 28) completed the study 19 (T: 10, C: 9)/ NR	Y	60	CALGain PPDRed RECInc BF	3.7 ± 1.1 1.4 ± 0.7 4.8 ± 1.6 3.3 ± 1.4 1.1 ± 1.2 2.0 ± 0.8 NR NR	*p<0.01* *NS* *NS*	The FMPS and BoP showed no statistically significant difference between the treatment groups.
(Sculean *et al.*, 2008) A	Parallel, follow-up, RCT	T1: OFD + EMD C: OFD	38 patients (out of 56) completed the study/ 38 (T1: 10, T2: 10, T3: 9, C: 9)/ NR	Y	120	CALGain PPDRed RECInc BF	T1: 2.9 ± 1.5 C: 1.8 ± 1.15 T1: 3.6 ± 1.5 C: 3.5 ± 1.4 T1: 0.7 ± 1.2 C: 1.7 ± 1.1 NR NR	*p<0.05* *NS* *NS*	The FMPS and BoP showed no significant differences between the treatment groups

Table 1 continued overleaf.....

Table 1. Table of Included Studies - Summary of findings presented in studies continued....

(Sculean *et al.*, 2008) B		T2: OFD + GTR C: OFD			120	CALGain	T2: 2.8 ± 1.4	C: 1.8 ± 1.15	*p<0.05*	
						PPDRed	T2: 3.4 ± 1.3	C: 3.5 ± 1.4	*NS*	
						RECInc	T2: 0.6 ± 1.3	C: 1.7 ± 1.1	*NS*	
						BF	NR	NR		
(Sculean *et al.*, 2008) C		T3: OFD + EMD + GTR C: OFD			120	CALGain	T3: 2.9 ± 1.30	C: 1.8 ± 1.15	*p<0.05*	
						PPDRed	T3: 3.5 ± 1.35	C: 3.5 ± 1.4	*NS*	
						RECInc	T3: 0.6 ± 1.0	C: 1.7 ± 1.1	*NS*	
						BF	NR	NR		
(Nickles *et al.*, 2009) A	Prospective, single-centre, split-mouth, RCT	T: OFD + GTR C: OFD	15 patients (out of 16) completed the study/ 35 (T: 18, C: 17)/ 12/23	N	120	CALGain	2.85 ± 2.24	3.65 ± 3.36	*NS*	The PI and SBI showed no statistically significant difference between the treatment groups.
						PPDRed	4.15 ± 2.47	4.40 ± 2.84	*NS*	
						RECInc	NR	NR		
						BF	1.30 ± 3.47	2.15 ± 2.15	*NS*	
(Nickles *et al.*, 2009) B	Prospective, single-centre, parallel, RCT				120	CALGain	2.89 ± 2.12	3.41 ± 2.75	*NS*	The PI and SBI showed no statistically significant difference between the treatment groups.
						PPDRed	4.25 ± 2.44	4.41 ± 2.37	*NS*	
						RECInc	NR	NR		
						BF	1.69 ± 2.91	2.03 ± 2.30	*NS*	
(Chambrone *et al.*, 2010)	Prospective, single-centre, RCT	T: OFD + EMD C: OFD	10 patients (out of 10) completed the study/ 38 (T: 19, C: 19)/ NR	N	24	CALGain	5.69 ± 1.96	5.24 ± 1.55	*NS*	The PI and GI showed no statistically significant difference between the groups.
						PPDRed	4.21 ± 0.97	3.28 ± 1.15	*p=0.03*	
						RECInc	1.02 ± 1.4	0.69 ± 1.4	*NS*	
						BF	NR	NR		
(Kurhańska-Flisykowska *et al.*, 2012)	Prospective, single-centre, split-mouth, CCT	T: OFD + EMD C: OFD	28 patients (out of 28) completed the study/ 56 (T: 28, C: 28)/ NR	N	24	CALGain	0.8 ± 1.68	0.5 ± 1.44	*NS*	NR
						PPDRed	2.67 ± 1.78	2.5 ± 1.67	*NS*	
						RECInc	NR	NR		
						BF	NR	NR		

Table 1 continued overleaf.....

Table 1. Table of Included Studies - Summary of findings presented in studies continued....

(Bhutda and Deo, 2013)	Prospective, single-centre split-mouth, RCT	T: OFD + EMD with EDTA 24% C: OFD + EDTA 24%	15 patients (out of 15) completed the study/ 30 (T: 15, C: 15)/ 0/30	Y	60	CALGain PPDRed RECInc BF	3.18 ± 0.87 3.84 ± 1.05 0.66 ± 0.01 3.20 ± 0.63	1.60 ± 0.54 1.92 ± 0.35 0.32 ± 0.52 1.30 ± 0.68	*p<0.05* *p<0.05* *NS* *NS*	The mean PI and PBI remained low throughout the study.
(De Leonardis and Paolantonio, 2013) A	Prospective, single-centre, split-mouth, RCT	T1: SPPF/MPPT + EMD + HAp/β-TCP C: SPPF/MPPT	34 patients (out of 36) completed the study/ 102 (T1: 34, T2: 34, C: 34)/ NR	Y	24	CALGain PPDRed RECInc BF	T1: 3.63 ± 0.91 T1: 4.25 ± 0.63 T1: 0.63 ± 0.42 T1: 3.35 ± 0.80	C: 1.40 ± 1.13 C: 2.38 ± 1.01 C: 1.01 ± 0.46 C: 0.23 ± 0.55	*p<0.001* *p<0.001* *p<0.001* *p<0.001*	FMPS and FMBS remained <20% throughout the entire study (data not shown).
(De Leonardis and Paolantonio, 2013) B		T2: SPPF/MPPT + EMD C: SPPF/MPPT			24	CALGain PPDRed RECInc BF	T2: 2.95 ± 0.74 T2: 3.76 ± 0.74 T2: 0.80 ± 0.39 T2: 2.61 ± 0.49	C: 1.40 ± 1.13 C: 2.38 ± 1.01 C: 1.01 ± 0.46 C: 0.23 ± 0.55	*p<0.001* *p<0.001* *p<0.01* *p<0.001*	

Table 1 continued overleaf.....

Table 1. Table of Included Studies - Summary of findings presented in studies continued....

(Cortellini *et al.*, 2017) A	Parallel, follow-up, RCT	T1: MPPT + Titanium e-PTFE C: MWF	41 patients (out of 45) completed the study/ 39 (T1: 14, T2: 13, C: 12)/ 31/8	Y	240	CALGain	T1: 5 ± 2.6	C: 0.6 ± 2.95	FMBS and FMPS remained stable over the 240M follow-up period.
						PPDRed	T1: 5.4 ± 1.7	C: 2.8 ± 2.35	
						RECInc	NR	NR	
						BF	NR	NR	
(Cortellini *et al.*, 2017) B		T2: OFD + e-PTFE C: MWF			240	CALGain	T2: 3.6 ± 2.25	C: 0.6 ± 2.95	
						PPDRed	T2: 4.6 ± 1.65	C: 2.8 ± 2.35	
						RECInc	NR	NR	
						BF	NR	NR	

C (Control group), O (Outcome), T (Test group), β-TCP (β-tricalcium phosphate), BoP (Bleeding on Probing), CALGain (Clinical attachment level gain), CCT (Controlled clinical trial), EDTA (Ethylenediamine-tetra acetic acid), EMD (Enamel matrix derivatives), e-PTFE (Expanded-polytetrafluoroethylene), FMBS (Full-mouth bleeding score), FMPS (Full-mouth plaque score), GI (Gingival Index), GTR (Guided tissue regeneration), HAp (Hydroxyapatite), MPPT (Modified papilla preservation technique), MWF (Modified Widman flap), NBM (Natural bone mineral), NR (Not reported), NS (Not significant), OFD (Open flap debridement), PBI (Papillary bleeding index), PGA (Propylene glycol alginate), PI (Plaque index), PPDRed (Probing pocket depth reduction), RCT (Randomized controlled clinical trial), RECInc (Recession increase), SPPF (Simplified papilla preservation flap), SD (Standard deviation), SS (Statistical significance), SBI (Sulcus bleeding index)

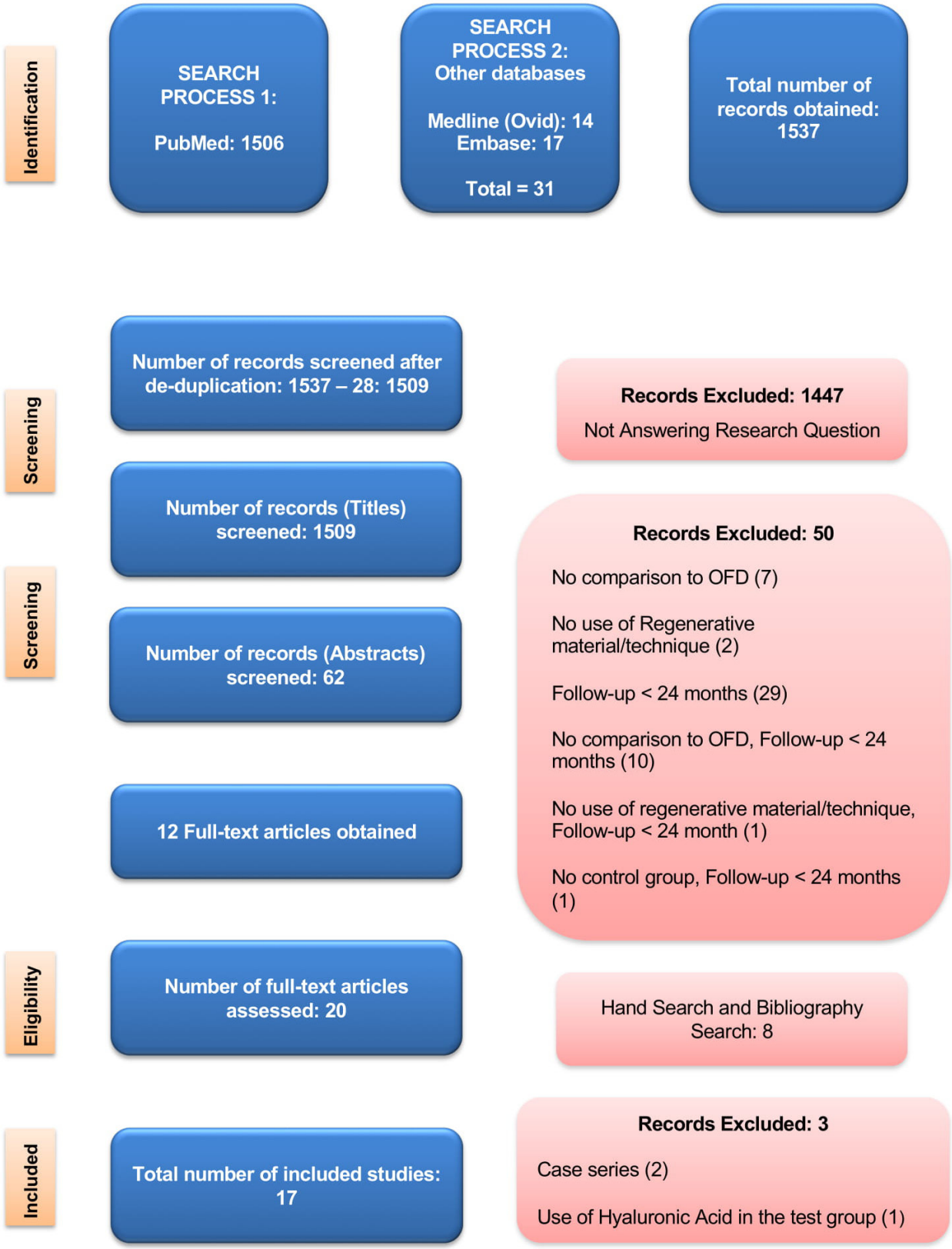

Figure 1. Search Strategy

Categorization of Studies

The included studies were categorized in different groups (Table 2) with the purpose to make them comparable and to solve the heterogeneity. This was done according to the type of regenerative material, flap design, changes in pocket depth, use of anti-microbials, the involved arch (data could not be assessed as most of the studies used anti-microbials and most did not report defect location) and follow-up timeline.

Quality Assessment

From 17 studies, eight studies were categorized as unclear risk (Yukna *et al*., 1989; Nery *et al*., 1990; Galgut *et al*., 1992; Francetti *et al*., 2005; Sakallıoğlu *et al*., 2007; Nickles *et al*., 2009; Chambrone *et al*., 2010; Cortellini *et al*., 2017), followed by six studies classified as low risk (Heijl *et al*., 1997; Sculean *et al*., 2004; Francetti *et al*., 2004; Sculean *et al*., 2007; Sculean *et al*., 2008; De Leonardis and Paolantonio, 2013), whereas the remaining three studies were evaluated to be of high risk (Zetterström *et al*., 1997; Kurhańska-Flisykowska *et al*., 2012; Bhutda and Deo, 2013) (Figure 2). Although a strict quality appraisal screening was done on the retrieved articles, the decision to include all of them was made as some older papers were fundamental to provide data for the long-term observation.

Overall Time Related Meta-analysis

Outcomes (CALGain, PPDRed, RECInc and BF) were evaluated according to follow-up periods, such as 24-, 36-, 48- to 60- and 120- to 240-months.

24-months

A statistically significant difference between the two groups was found (favouring REG) for CALGain, PPDRed and BF (mean=1.04 mm, p=0.0003; mean=1.00 mm, p<0.00001 and mean=2.02 mm, p<0.00001 respectively. For the RECInc analysis, statistically insignificant difference was found between the two groups with a mean of -0.11 mm (p=0.41) (Figure 3).

36-months

For CALGain (mean=0.32 mm, p=0.12) and RECInc (mean=0.15 mm, p=0.62) analysis, no statistically significant difference was seen between two groups. For PPDRed and BF analysis, better results for REG were seen than CS with a mean of 0.80 mm, p<0.00001 and 0.92 mm, p=0.04 respectively (Figure 4).

48- to 60-months

Favourable results were found for REG in terms of CALGain and PPDRed with mean values of 1.29 mm, p<0.00001 and 0.96 mm, p= 0.0002 respectively, which were statistically significant. However, no statistically significant difference between the two groups was seen for RECInc analysis (mean=0 mm, p=0.96) (Figure 5).

120- to 240-months

CALGain and RECInc analysis were found to be statistically significant between the two groups (favouring REG) with a mean difference of 1.26 mm (p=0.02) and -1.07 mm (p=0.0004) respectively. Whereas for the PPDRed (mean=0.56 mm, p=0.18) and BF (mean=-0.57 mm, p=0.38) analysis, no statistically significant difference was noted (Figure 6).

Heterogeneity Assessment

To assess within-study or between study variability, heterogeneity was evaluated. The I^2 statistics showed a substantial heterogeneity at majority of follow-up time periods, therefore, a subgroup analysis was done on the basis of regenerative materials. It was possible to elaborate the meta-analysis only for EMD and Ceramic Grafts (CGs)+OFD groups.

Meta-analysis for EMD

The EMD analysis was done at 24-, 36- and 60-months follow-up for CALGain and PPDRed (Figure 7).

24-months

An additional CALGain of 1.04 mm (p<0.0001) and PPDRed of 0.92 mm (p=0.0004) was demonstrated for the EMD group compared to CS. Five trials each were included in these analyses.

36-months

A mean difference of 0.58 mm (p=0.02) and 0.75 mm (p=0.003) was seen in terms of CALGain and PPDRed respectively, favouring EMD. Two trials were included in both the analyses.

60-months

CALGain and PPDRed at 60-months follow-up analysis was found to be statistically significant between EMD and CS (favouring EMD) with a mean difference of 1.58 mm (p<0.00001) and 1.87 mm (p<0.00001) respectively. Two trials each were included in both the analyses.

Meta-analysis for CGs+OFD

The CGs+OFD analysis was performed at 24-, 36- and 48-months follow-up for CALGain and PPDRed (Figure 8).

24-months

CALGain was found to be statistically insignificant between the two groups with a mean difference of 0.42 mm (p=0.06). However, PPDRed analysis showed statistically significant difference between the two treatments, favouring CGs+OFD group (mean=0.65 mm, p<0.0006). In each analysis, two trials were included.

Table 2. Categorization of studies according to regenerative materials, flap designs and PPDRed

Regenerative Material							
GTR		BRGs		EMD		Combination Therapy	
Study	**Treatment/Follow-up (months)**	**Study**	**Treatment/Follow-up (months)**	**Study**	**Treatment/Follow-up (months)**	**Study**	**Treatment/Follow-up (months)**
Sculean *et al.*, 2004 B	GTR + OFD vs. OFD/60	Yukna et al., 1989	Durapatite ceramic alloplastic implants + OFD vs. OFD/24, 36, 48, 60	Heijl *et al.*, 1997	EMD + MWF vs. MWF + placebo (PGA)/36	Sculean *et al.*, 2004 C	EMD + GTR + OFD vs. OFD/60
Sakallıoğlu *et al.*, 2007	GTR + OFD vs. OFD/36	Nery et al., 1990	Ceramic + OFD vs. OFD/36 Autogenous bone + OFD vs. OFD/36	Zetterström *et al.*, 1997	EMD + MWF vs. MWF/36	Sculean *et al.*, 2007	GTR + NBM + OFD vs. OFD/60
Sculean *et al.*, 2008 B	GTR + OFD vs. OFD/120	Galgut et al., 1992	Ceramic HAp implant + OFD vs. OFD/24, 36, 48	Sculean *et al.*, 2004 A	EMD + OFD vs. OFD/60	Sculean *et al.*, 2008 C	EMD + GTR + OFD vs. OFD/120
Nickles *et al.*, 2009	GTR + OFD vs. OFD/120			Francetti *et al.*, 2004	EMD + SPPF vs. SPPF/24	De Leonardis and Paolantonio, 2013 B	EMD + HAp/β-TCP + SPPF or MPPT vs. SPPF or MPPT/24
Cortellini *et al.*, 2017	Titanium e-PTFE + MPPT vs. MWF/240 e-PTFE + OFD vs. MWF/240			Francetti *et al.*, 2005	EMD + SPPF vs. SPPF/24		
				Sculean *et al.*, 2008 A	EMD + OFD vs. OFD/120		
				Chambrone *et al.*, 2010	EMD + OFD vs. OFD/24		
				Kurhańska-Flisykowska *et al.*, 2012	EMD + OFD vs. OFD/24		
				Bhutda and Deo, 2013	EMD with EDTA 24% + OFD vs. OFD + EDTA 24%/60		
				De Leonardis and Paolantonio, 2013 A	EMD + SPPF or MPPT vs. SPPF or MPPT/24		

Table 2 continued overleaf....

Table 2. Categorization of studies according to regenerative materials, flap designs and PPDRed continued...

Flap Design							
OFD		**MWF**		**PPFs**		**Two or more different flap designs**	
Study	**Treatment/Follow-up (months)**	**Study**	**Treatment/Follow-up (months)**	**Study**	**Treatment/Follow-up (months)**	**Study**	**Treatment/Follow-up (months)**
Yukna *et al.*, 1989	OFD + Durapatite ceramic alloplastic implants vs. OFD/24, 36, 48, 60	Heijl *et al.*, 1997	MWF + EMD vs. MWF + Placebo (PGA)/36	Francetti *et al.*, 2004	SPPF + EMD vs. SPPF/24	Cortellini *et al.*, 2017	MPPT + Titanium e-PTFE vs. MWF/240 OFD + e-PTFE vs. MWF/240
Nery *et al.*, 1990	OFD + Ceramic vs. OFD/36 OFD + Autogenous Bone vs. OFD/36	Zetterström *et al.*, 1997	MWF + EMD vs. MWF/36	Francetti *et al.*, 2005	SPPF + EMD vs. SPPF/24		
Galgut *et al.*, 1992	OFD + Ceramic HAp implant vs. OFD/24, 36, 48			De Leonardis and Paolantonio, 2013	SPPT or MPPT + EMD + HAp/β-TCP vs. SPPF or MPPT/24 SPPF or MPPT + EMD vs. SPPF or MPPT/24		
Sculean *et al.*, 2004	OFD + EMD vs. OFD/60 OFD + GTR vs. OFD/60 OFD + EMD + GTR vs. OFD/60						
Sakallıoğlu *et al.*, 2007	OFD + GTR vs. OFD/36						
Sculean *et al.*, 2007	OFD + NBM + GTR vs. OFD/60						
Sculean *et al.*, 2008	OFD + EMD vs. OFD/120 OFD + GTR vs. OFD/120 OFD + EMD + GTR vs. OFD/120						
Nickles *et al.*, 2009	OFD + GTR vs. OFD/120						

Table 2 continued overleaf....

Table 2. Categorization of studies according to regenerative materials, flap designs and PPDRed continued...

Chambrone *et al.*, 2010	OFD + EMD vs. OFD/24
Kurhańska-Flisykowska *et al.*, 2012	OFD + EMD vs. OFD/24
Bhutda and Deo, 2013	OFD + EMD with EDTA 24% vs. OFD + EDTA 24%/60

PPDRed	For studies with ≥ 24 months but < 60 months follow-up						For studies with ≥ 60 months follow-up					
	GTR	BRGs	EMD	Combination Therapy	CS		GTR	BRGs	EMD	Combination Therapy	CS	
PPDRed	Treatment Groups [Frequency Distribution (n)] (%)											
< 2 mm	0	0	0	0	2 (100%)	2 (7%)	0	0	0	0	2 (100%)	2 (9.6%)
≥ 2 - ≤ 4 mm	1 (6%)	3 (17.6%)	4 (23.5%)	0	9 (52.9%)	17 (58.6%)	2 (18.2%)	1 (9.1%)	2 (18.2)	2 (18.2%)	4 (36.3%)	11 (52.4%)
> 4 mm	0	3 (30%)	3 (30%)	1 (10%)	3 (30%)	10 (34.4%)	4 (50%)	0	1 (12.5%)	1 (12.5%)	2 (25%)	8 (38%)
						n= 29						n= 21

β-TCP (β-tricalcium phosphate), BRGs (Bone replacement grafts), CS (Conservative surgery), EDTA (Ethylenediamine-tetra acetic acid), EMD (Enamel matrix derivative), e-PTFE (Expanded-polytetrafluoroethylene), GTR (Guided tissue regeneration), HAp (Hydroxyapatite), MPPT (Modified papilla preservation technique), MWF (Modified Widman flap), NBM (Natural bone mineral), OFD (Open flap debridement), PGA (Propylene glycol alginate), SPPF (Simplified papilla preservation flap)

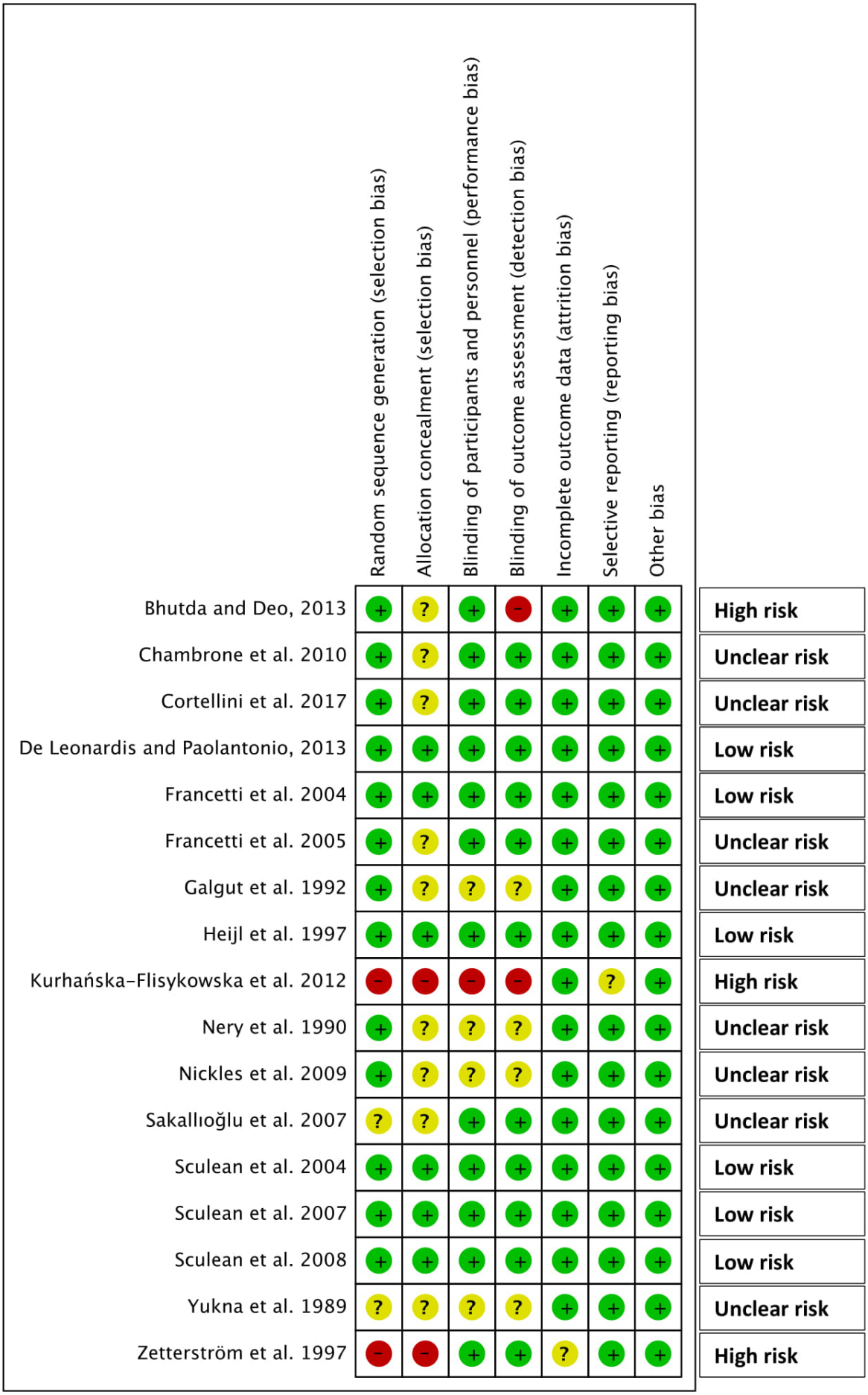

Figure 2. Tabular representation of risk of bias in individual studies; Green: Low risk, Yellow: Unclear risk, Red: High risk

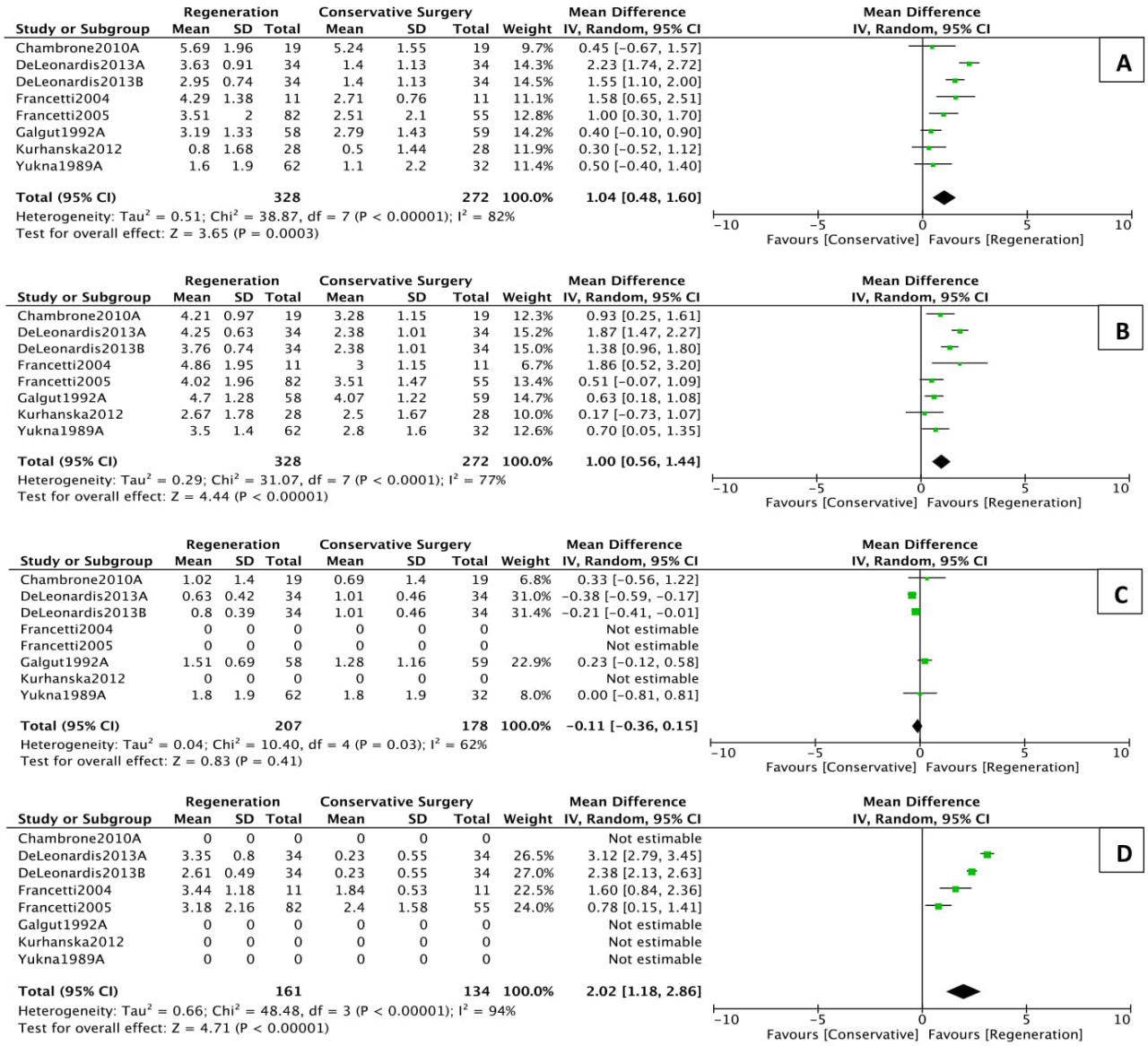

Figure 3. Forest plot showing A) CALGain B) PPDRed C) RECInc and D) BF at 24-months

36-months

There was no statistically significant difference seen in terms of CALGain with a mean difference of 0.26 mm (p=0.15) while there was regarding PPDRed analysis (mean=0.76 mm, p<0.00001). Three trials were included in the CALGain analysis and two trials for PPDRed.

48-months

An additional CALGain of 0.95 mm (p<0.00001) and PPDRed of 0.84 mm (p<0.00001) was seen for the CGs+OFD group than CS. Two trials each were included in both the analyses.

Discussion

To best of our knowledge, no review has been published yet comparing long-term results between periodontal REG and CS in infra-bony defects. Although the vast majority of RCTs and SRs with short-term observation have demonstrated better results of periodontal REG than CS in terms of CALGain and PPDRed in the treatment of infra-bony defects, the focus of this review was to analyse whether the same results are true in the long-term. The aim was to provide a reliable evidence-based research for the use of periodontal REG in the treatment of infra-bony defects to maintain the attachment levels, the bone levels and the pocket length reduction for long period of time and subsequently to address any future research on the topic. In the time related meta-analysis, a significant level of heterogeneity was encountered so in order to reduce and solve it, a subgroup analysis was performed.

Overall Time-Related Meta-analysis

For most of the outcomes and time periods, the results were in favour of REG techniques. This was outlined by all the outcomes (CALGain, PPDRed, BF and less RECInc). For CALGain and PPDRed, three out of four follow-up periods showed a statistical significance

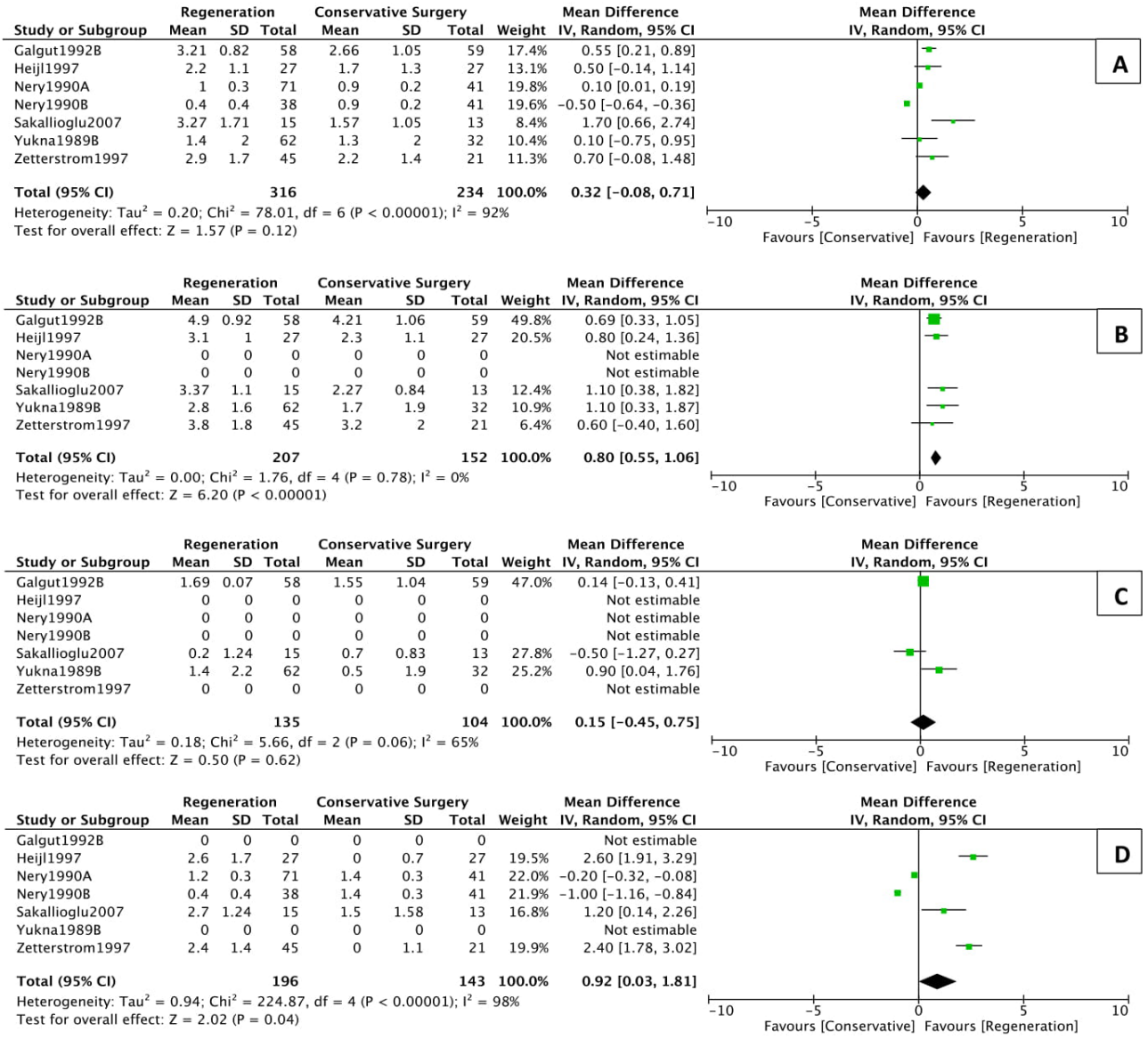

Figure 4. Forest plot showing A) CALGain B) PPDRed C) RECInc and D) BF at 36-months

between the two groups favouring REG. For RECInc, only one out of four follow-up periods favoured REG. This can be attributed to the fact that potentially the pattern of healing in REG might have been improved in the last period due to formation of new attachment compared to a long junctional epithelium. These results are consistent with other SRs demonstrating better results for 1) GTR than OFD group in terms of CALGain (mean=1.22 mm; $p<0.001$) and PPRed (mean=1.21 mm; $p<0.001$) (Needleman *et al.*, 2006), 2) BRGs than OFD in terms of CALGain (mean=0.55 mm; $p<0.05$) and PPDRed (mean=0.30 mm; $p<0.05$) (Reynolds *et al.*, 2003) and 3) EMD than OFD in terms of CALGain (mean=1.1 mm; $p<0.05$) and PPDRed (mean=0.9 mm; $p<0.05$) (Esposito *et al.*, 2009).

Subgroup Analysis

When assessing the performance of several regenerative materials/techniques, the subgroup analysis was only possible for EMD and the CGs+OFD category, as a complete set of data and a substantial homogeneity was recorded.

The heterogeneity encountered in the time-related meta-analysis (I^2=98%) was reduced by the use of subgroup categorization, although not ideally (I^2=69%).

The use of EMD was proven to be more effective than CS, producing a better CALGain of 1.04 mm, 0.58 mm and 1.58 mm at 24-, 36- and 60-months follow-up respectively, and an effective PPDRed of 0.92 mm, 0.75 mm and 1.87 mm at 24-, 36- and 60-months follow-up respectively. This finding is consistent with a SR in which EMD has been proved to perform much better than CS and showed a better CALGain (mean=1.30 mm; $p<0.05$) (Koop *et al.*, 2012). The results of the present study demonstrated that short-term clinical outcomes achieved with EMD can be safely maintained for a longer period assuming a strict adherence supportive periodontal therapy.

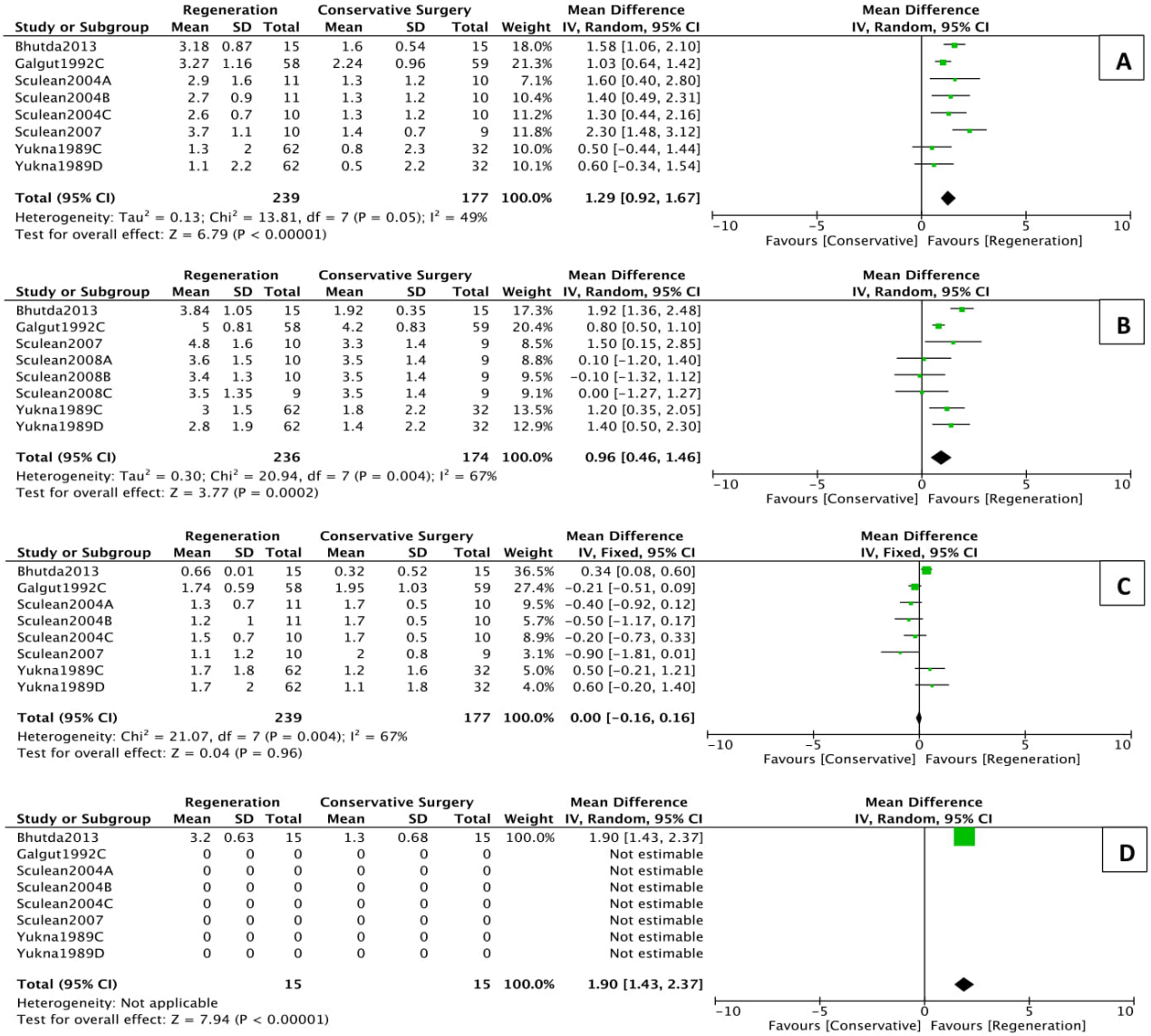

Figure 5. Forest plot showing A) CALGain B) PPDRed C) RECInc and D) BF at 48- to 60-months

The comparison focused on CGs+OFD showed again a better performance of REG than CS in terms of CALGain and PPDRed in all the observations, but with a stronger significance set at 48-months (mean=0.95 mm). These results agree with a previous review that supported the use of CGs as an adjunct to CS alone [CALGain (mean=0.78 mm; $p<0.003$) and PPDRed (mean=0.42 mm; $p=0.03$)] (Reynolds *et al.*, 2003).

Although the actual study had suggested a better clinical behaviour of regenerative techniques compared to CS, the latter cannot be neglected as a favourable treatment option in periodontal cases. A SR demonstrated that a conservative surgical treatment of infra-bony defect appears to be associated with the improvement of periodontal clinical parameters as well as high tooth retention rate (Graziani *et al.*, 2012), as in many occasions it may represent a meaningful therapeutic option when a regenerative treatment is not feasible for several reasons.

Further the clinical performance can vary considerably according to the type of surgical flap adopted (as encountered with papilla preservation flaps) and the expected healing type. In the classic pocket reduction techniques (i.e. access flaps with no preservation of the inter-dental tissues), the lack of primary wound closure and the subsequent blood clot instability has been associated histologically with a repair pattern. Interestingly, when the access flaps were performed with the inter-dental tissue preservation a greater CALGain combined with smaller recessions were seen, as they advocate that a primary intention healing as well as the greater wound stability could lead to better outcomes independently from grafting. The vascular stability within the papillary area ensured by PPFs determines a higher blood clot stability in the inter-proximal area and hence a more favourable infra-bony defect healing (Retzepi *et al.*, 2007) comparable to the outcomes of regenerative treatment (Trombelli *et al.*, 2010; Cortellini and Tonetti, 2011). A robust support to this finding is

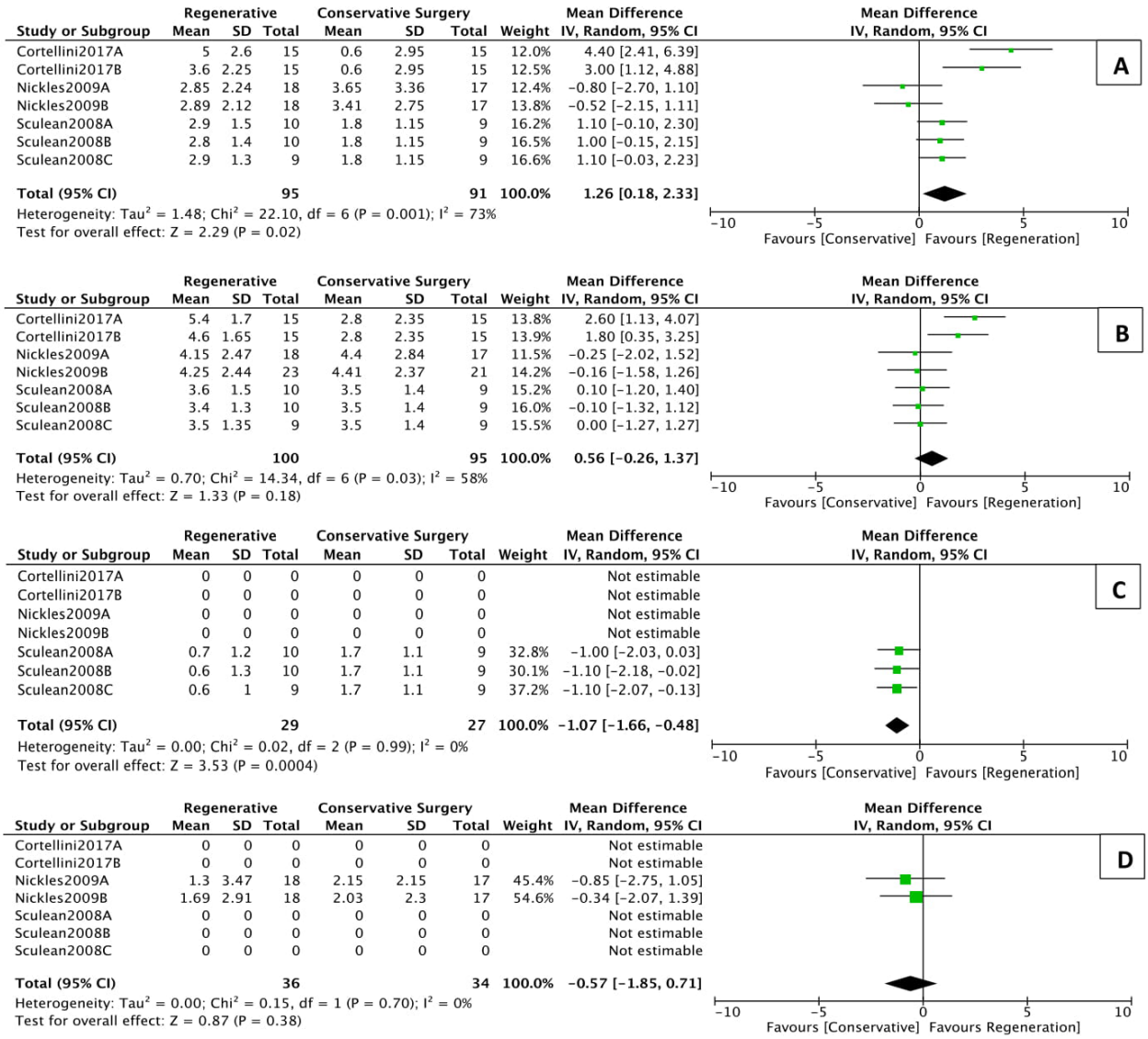

Figure 6. Forest plot showing A) CALGain B) PPDRed C) RECInc and D) BF at 120- to 240-months

provided by Tu et al., who evaluated an overall better clinical performance of infra-bony defects healing over a 15-year period, as the use of papilla preservation flaps was introduced (Tu *et al.*, 2008). Therefore new RCTs (short and long-term) comparing these two modalities are advocated to determine if the standard of CS requires the adoption of a PPF design.

The use of antibiotics in the trials can be a confounding factor as they can influence the early healing process. To overcome this, future studies may need to separate antibiotics use with no antibiotics use to assess the effect of intervention. Because the majority of the included studies have used the antibiotics, no further analysis could be done in this review. Secondly, the involved arch might have an influence on wound healing due to alveolar bone density and loss pattern. However, the absence of data categorised by defect location and the amount of pooled outcomes didn't allow to the present review to infer any conclusion about it.

Limitations of the Present Review

There were some limitations encountered in the present study.

1. Qualitative Assessment: Three out of the 17 included studies scored one as reported by the Jadad appraisal. Their inclusion due to the paucity of data didn't affect the meta-analysis outcomes.
2. Heterogeneity: There was a great amount of heterogeneity in the overall time-related meta-analysis, which was encountered using the sub-group meta-analysis
3. Missing Data: No meta-analysis could be done for other REG therapies such as GTR, BRGs (except CGs) and combination therapy for the outcomes at different long period follow-ups due to lack of homogeneity and missing or not reported data.

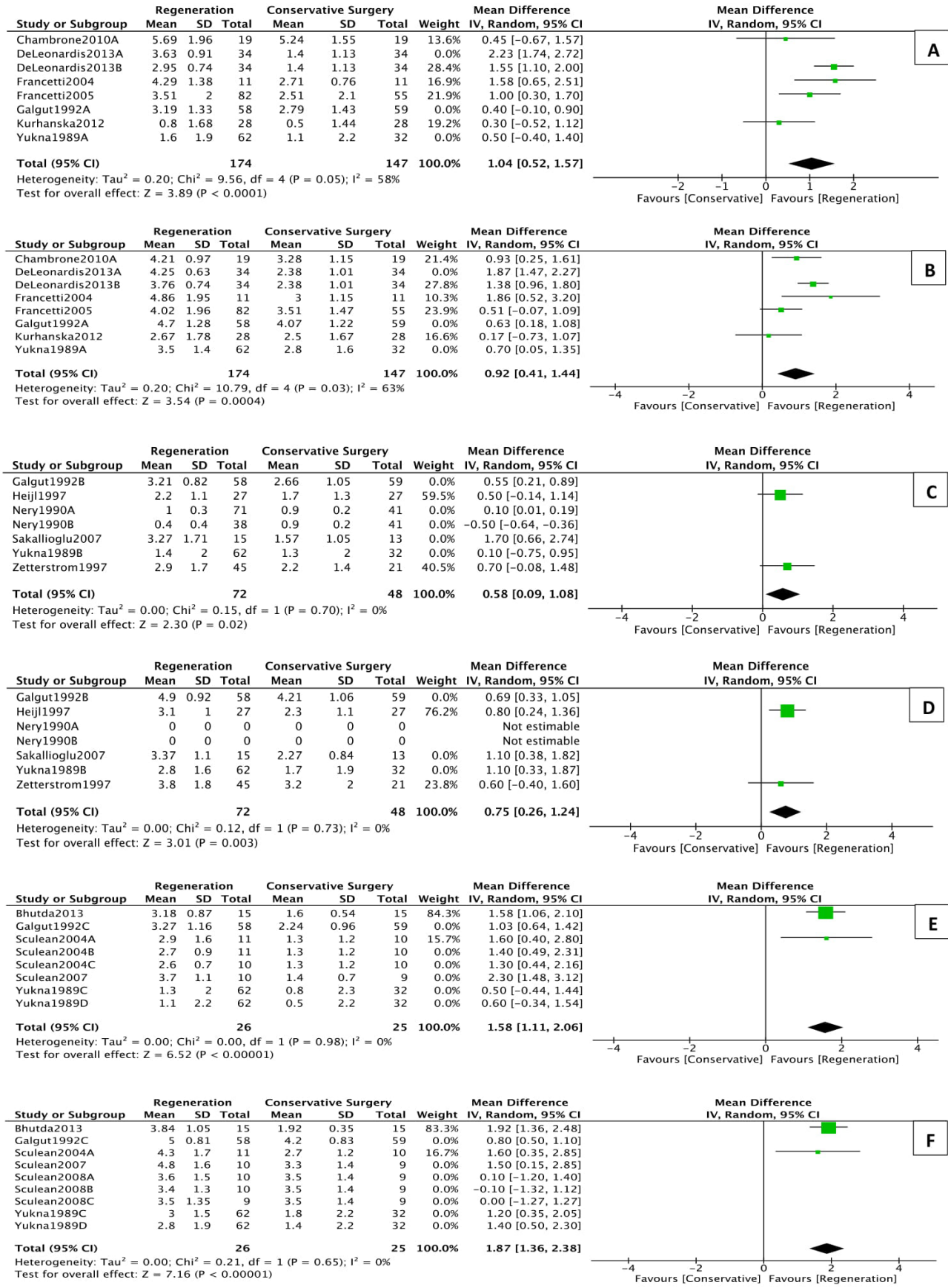

Figure 7. Forest plot showing A) CALGain and B) PPDRed at 24-months, C) CALGain and D) PPRed at 36-months, and E) CALGain and F) PPDRed at 60-months for EMD

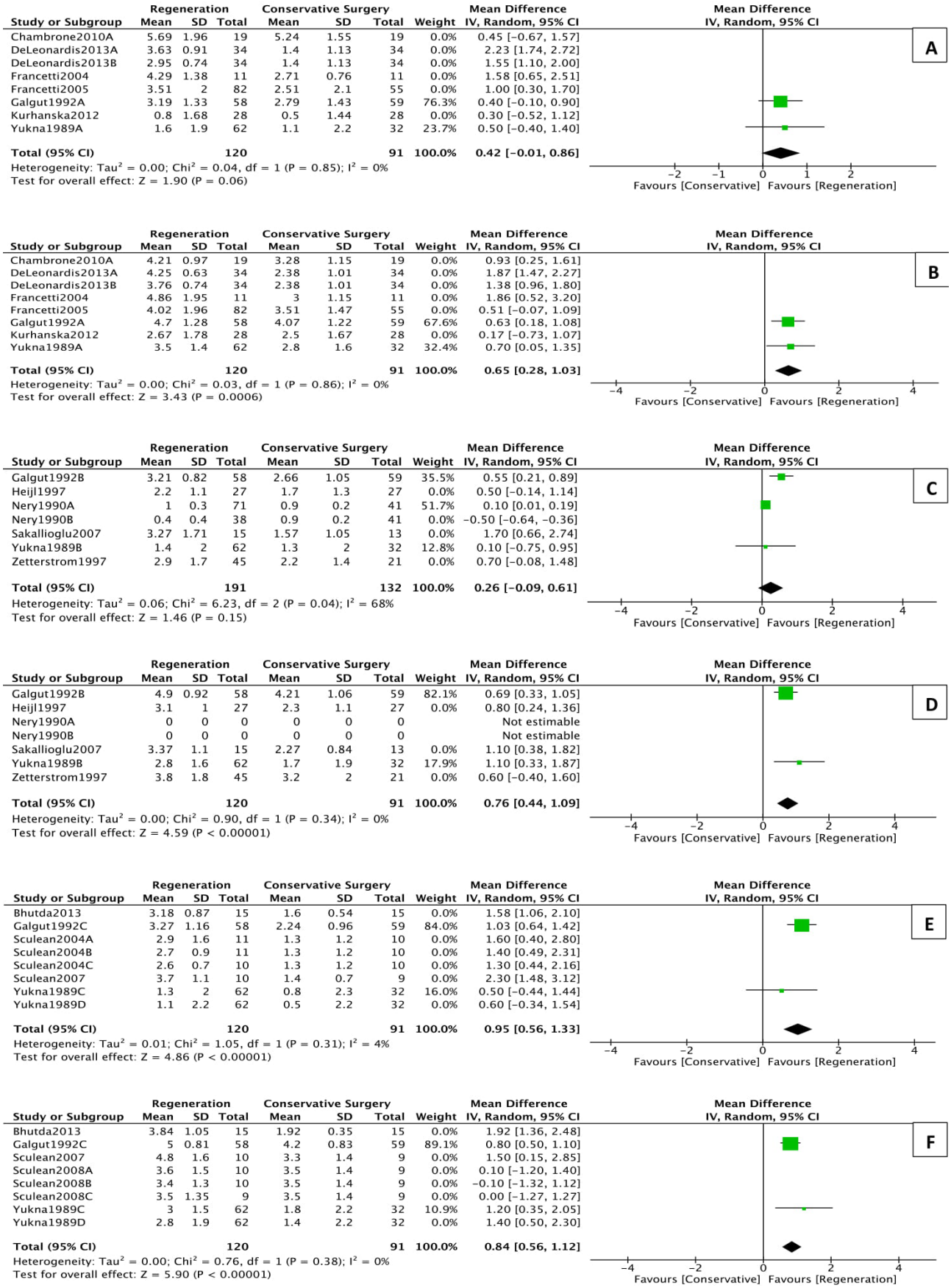

Figure 8. Forest plot showing A) CALGain and B) PPDRed at 24-months, C) CALGain and D) PPRed at 36-months, and E) CALGain and F) PPDRed at 48-months for CGs+OFD

4. Not Removable Confounding Factors: The use of antibiotics and the defect location were not reported properly in the included studies. A potential bias due to the involved arch, or to the benefits of peri-operative antimicrobials couldn't be removed from the analysis.

Future Research/Recommendations

For the future research, more long-term randomized studies (>120-months long preferably) are needed comparing REG therapy versus CS to check the long-term stability of the achieved results in relation to the different healing pattern.

Conclusion

Within the limitations of this study, several conclusions can be drawn: REG demonstrated better long-term clinical outcomes than CS irrespective of the materials/techniques. In particular EMD used in the regenerative approach can display better clinical outcomes throughout the short and the long-term period, while the combination of CGs showed better performance at the follow-up period of 48-months. Further long-term clinical trials are needed to determine: the efficacy of GTR, BRGs and combination therapy against CS in infra-bony defects (preferably >120-months follow-up), the effectiveness of the alternative use of CS in infra-bony defects whenever REG is not possible and the comparison among the performance of different regenerative materials or techniques.

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Appendix A: Search Tracking

Data Source		Limits				Duplicates Results			
Database	**Keywords**	**English Only**	**Time Period Search**	**Publications Type**	**Other Limits**	**Items Found**	**Internal Duplicates**	**External Duplicates**	**New**
PubMed (NLM)	"intrabony defect" OR "intra bony defect" OR "intra-bony defect" OR "infrabony defect" OR "infra bony defect" OR "infra-bony defect" OR "intraosseous" OR "intra osseous" OR "intra-osseous" AND "periodontal regeneration" OR "regenerative periodontal surgery" OR "barrier membrane" OR "guided tissue regeneration" OR GTR OR "bone graft" Or "bone substitute" OR "bone mineral" OR "bone replacement graft" OR BRG OR "xenograft" OR "autograft" OR "enamel matrix protein" OR "enamel matrix derivative" OR EMD OR "emdogain" OR "amelogenin" AND "surgical flap" OR "periodontal pocket surgery" OR "access surgery" OR "conservative surgery" OR "modified widman flap" OR "open flap debridement" OR OFD OR "modified papilla preservation flap" OR MPPF OR "simplified papilla preservation flap" OR SPPF AND "long-term" AND "clinical attachment level" OR CAL OR "periodontal pocket depth" OR PPD OR "bone fill" AND (Clinical Trial[ptyp] AND Humans[Mesh] AND English[lang])	Yes	-	Clinical Trials	Humans	1506	0	-	1506
Medline (Ovid)	"intrabony defect" OR "intra bony defect" OR "intra-bony defect" OR "infrabony defect" OR "infra bony defect" OR "infra-bony defect" OR "intraosseous" OR "intra osseous" OR "intra-osseous" AND "periodontal regeneration" OR "regenerative periodontal surgery" OR "barrier membrane" OR "guided tissue regeneration" OR GTR OR "bone graft" Or "bone substitute" OR "bone mineral" OR "bone replacement graft" OR BRG OR "xenograft" OR "autograft" OR "enamel matrix protein" OR "enamel matrix derivative" OR EMD OR "emdogain" OR "amelogenin" AND "surgical flap" OR "periodontal pocket surgery" OR "access surgery" OR "conservative surgery" OR "modified widman flap" OR "open flap debridement" OR OFD OR "modified papilla preservation flap" OR MPPF OR "simplified papilla preservation flap" OR SPPF AND "long-term" AND "clinical attachment level" OR CAL OR "periodontal pocket depth" OR PPD OR "bone fill"	-	-	-	-	14	0	12	2

Appendix A continued overleaf

Appendix A: Search Tracking continued

Embase (Ovid)	"intrabony defect" OR "intra bony defect" OR "intra-bony defect" OR "infrabony defect" OR "infra bony defect" OR "infra-bony defect" OR "intraosseous" OR "intra osseous" OR "intra-osseous" AND "periodontal regeneration" OR "regenerative periodontal surgery" OR "barrier membrane" OR "guided tissue regeneration" OR GTR OR "bone graft" OR "bone substitute" OR "bone mineral" OR "bone replacement graft" OR BRG OR "xenograft" OR "autograft" OR "enamel matrix protein" OR "enamel matrix derivative" OR EMD OR "emdogain" OR "amelogenin" AND "surgical flap" OR "periodontal pocket surgery" OR "access surgery" OR "conservative surgery" OR "modified widman flap" OR "open flap debridement" OR OFD OR "modified papilla preservation flap" OR MPPF OR "simplified papilla preservation flap" OR SPPF AND "long-term" AND "clinical attachment level" OR CAL OR "periodontal pocket depth" OR PPD OR "bone fill"	-	-	-	-	17	2	14	1
		Total				1537	2	26	1509

Appendix B: Reasons for exclusion after full-text analysis

Authors	Reasons for Exclusion
(Yukna and Yukna, 1998) (Yukna *et al.*, 2002)	Case series
(Briguglio *et al.*, 2013)	Use of hyaluronic acid in the test group