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REVIEW

Effect of EDTA root conditioning on the outcome of coronally advanced flap with connective tissue graft: a systematic review and meta-analysis

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Abstract

Objectives CAF in combination with a connective tissue graft (CTG) is considered the technique of choice for treating gingival recessions (GRs). Among the many recognized factors that can affect the outcomes, the use of chemical agents has been proposed. The effect of EDTA, as a commonly used agent, remains controversial. Therefore, the aim of this review was to assess the efficacy of EDTA root conditioning when combined to CAF + CTG.

Material and methods A literature search was conducted to identify randomized clinical trials (RCTs) that performed CAF + CTG with and without EDTA for root coverage procedures. The following outcomes were assessed: recession reduction (Rec Red), complete root coverage (CRC), keratinized tissue gain (KT gain), clinical attachment level changes (CAL gain), and changes in pocket depth (PD changes).

Results Fourteen RCTs (575 GRs) were included and analyzed. Six articles were included in the EDTA group, with 8 in the non-EDTA group. Meta-analyses revealed statistically significant differences for the outcomes of Rec Red (3.68 mm versus 3.07 mm), CAL gain (4.15 mm versus 3.07 mm), and PD changes (− 0.44 mm versus 0.27 mm) in favor of the EDTA group, while outcomes of CRC (odds ratio of 1.15) and KT gain (1.98 mm versus 1.62 mm) were not significantly different.

Conclusions Limited evidence is available when evaluating the effectiveness of EDTA root conditioning with CAF + CTG. However, the adjunct application of EDTA with CAF + CTG appears to be beneficial.

Clinical relevance The adjunct application of EDTA may provide benefits when performing root coverage procedure via CAF + CTG.

Keywords Gingival recession · Root coverage · Subepithelial connective tissue graft · Mucogingival surgery · Ethylenediaminetetraacetic acid (EDTA) · Meta-analysis

Introduction

The efficacy of mucogingival procedures for the treatment of gingival recessions (GRs) has been extensively investigated [1, 2]. Among the available techniques, the coronally advanced flap (CAF) is considered to be the most common surgical approach for root coverage [3, 4]. Anatomical factors that influence the treatment outcome of CAF have been studied; these include but are not limited to the following: interproximal attachment loss [5], dimension of the papillae [6], gingival thickness [7], and many others [8, 9]. Furthermore, others have examined the impact of different CAF designs (full or split thickness [10], envelope or with vertical incisions [11], with triangular or trapezoidal papillae [12]) on complete root coverage (CRC) and esthetic outcomes.

Among the investigated variables that can affect the outcomes of CAF, treatment of the exposed root surface (mechanical and/or chemical) has been of fundamental importance in root coverage procedures [13]. The mechanical treatment of the root has been suggested for smoothing out irregularities, reducing its convexity, removing possible caries, and minimizing cementum toxicity [14, 15], while the use of chemical agents has been recommended for removing

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the smear layer produced by mechanical instrumentation [14], decontaminating the root surface from endotoxin and bacteria [16], exposing the collagen fibers within the root matrix, and thus, facilitating the re-attachment of the connective tissue [17, 18]. Chemical agents such as citric acid, fibronectin, ethylenediaminetetraacetic acid (EDTA), tetracycline, and fibrin glues have been among the most investigated agents in the literature [13].

Citric acid was introduced in the early 1970s with the aim of chemically removing the remaining layer of debris after mechanical instrumentation along with endotoxins from the root surface [19, 20]. Later on, tetracycline was proposed for its antibacterial and chelating properties and was thought to enhance the attachment of gingival fibroblasts to the root surface [21]. Fibronectin is assumed to mediate the formation of a new attachment between fibroblasts and the root surface as its application was found capable of enhancing cellular proliferation and promoting faster healing [22]. However, unlike other acidic agents, due to its neutral pH and biocompatibility, the use of EDTA has been advocated as it lacks the necrotizing effect on the surrounding tissues [23]. It has been reported that it is capable of promoting early cell and tissue colonization [24] while exposing a vast number of intact collagen fibers [25], and simultaneously decreasing gingival recession, probing depth, and the formation of long junctional epithelium [25].

Despite initial promising results supporting the use of EDTA [26, 27], others have failed to find significant improvements [16, 28], rendering its use in periodontal surgery controversial. Interestingly, EDTA has slowly gained popularity among clinicians due to its quick and easy application [29]. Nonetheless, evidence supporting its clinical use remains largely lacking [29, 30]. In this regard, it is necessary to exercise caution when interpreting results of existing systematic reviews when considerable heterogeneity exists among their included studies. Similarly, readers should be aware of conclusions based on reviews that include different surgical procedures, different flap designs, and various materials and chemical agents [29–32]. In 2003, the AAP consensus report by Mariotti [29] concluded that the use of root surface modifiers such as citric acid, tetracycline, and EDTA provides no benefit in regeneration for patients with chronic periodontitis. Nevertheless, only three articles utilizing EDTA as root modifiers were selected and analyzed (2 for regenerative procedures [26, 33], and 1 for subgingival debridement [34]). Oliviera and Muncinelli conducted a systematic review, examining the effect of root surface biomodification on root coverage procedures, concluding that due to lack of evidence, root surface biomodification is not recommended [31]. Inclusion of different flap designs (free gingival graft, CAF + CTG, semilunar flap), different chemical agents (citric acid, EDTA), and the use of lasers, in addition to the lack of a statistical analysis, constituted the main drawbacks of this article.

The effect of root biomodification in the treatment of gingival recessions with only CAF + CTG was investigated by Karam et al. [30] where, once more, no additional clinical benefits were found to justify its use. However, only one article in the review had utilized EDTA [27]. In a more recent meta-analysis, Liu et al. [32] attempted to evaluate periodontal surgery outcomes by only including articles in which EDTA was used. However, the meta-analysis included three studies [26–28] with different surgical procedures (1 with semilunar coronally advanced flap, 1 CAF + CTG, and 1 for periodontal regeneration of intrabony defects).

The limited number of studies included in the past reviews and/or the combination of different surgical procedures and chemical agents does not provide an accurate resolution to the question whether periodontal plastic/regenerative surgery can benefit from EDTA root conditioning. And while CAF in combination with a connective tissue graft (CTG) should be the investigated approach for determining the advantages of a chemical agent, as it is considered the gold standard for the treatment of GRs [1], the effectiveness of chemical agents when combined with CAF + CTG has not been determined yet. Thus, it is reasonable to assume that whether EDTA, the most widely used chemical agent, can improve the clinical outcomes or not should be tested in a setting where the GRs are treated with the same surgical technique (CAF) and the same graft material (CTG) and where only one chemical agent is used. Therefore, the aim of this meta-analysis is to assess the efficacy of EDTA chemical root conditioning during CAF + CTG treatment.

Material and methods

Study registration

The review protocol was registered and allocated the identification number (CRD42018086208) in the PROSPERO International Prospective Register of Systematic Reviews hosted by the National Institute for Health Research, University of York, Centre for Reviews and Dissemination.

Patient, intervention, comparison, outcome (PICO) question

This systematic review utilized the Preferred Reporting Items Systematic review and Meta-Analyses (PRISMA) statement and checklist [35], as well as the patient, intervention, comparison, outcomes (PICO) method.

P: Patients receiving a CTG for single or multiple gingival recession (GR) defects classified as Miller I and II or RT1 [36, 37]
I: CAF together with an autologous CTG for the treatment of GR defects

C: Treatment of GRs with either CAF + CTG + EDTA or CAF + CTG
O: Recession reduction (Rec Red), complete root coverage (CRC), keratinized tissue (KT) gain, clinical attachment level (CAL) gain, and probing depth (PD) reduction

Information sources

Electronic and manual literature searches were carried out by two independent reviewers (SB, LT) using the databases MEDLINE (PubMed) and EMBASE. Potential articles were examined in full text by two reviewers (SB, LT) independently, and the articles' eligibility for this review was confirmed after discussion. Disagreements were resolved by consulting with an additional investigator (AR).

Screening process

The MEDLINE/PubMed search was performed on October 3, 2017, using the following strategy:

- ((“connective tissue”[MeSH Terms] OR (“connective”[All Fields] AND “tissue”[All Fields]) OR “connective tissue”[All Fields]) AND (“transplants”[MeSH Terms] OR “transplants”[All Fields] OR “graft”[All Fields])) AND (“humans”[MeSH Terms] AND English[lang] AND jsubsetd[text])
- (coronally[All Fields] AND positioned[All Fields] AND (“surgical flaps”[MeSH Terms] OR (“surgical”[All Fields] AND “flaps”[All Fields]) OR “surgical flaps”[All Fields] OR “flap”[All Fields])) AND english[Language] AND (“humans”[MeSH Terms] AND jsubsetd[text])
- (coronally[All Fields] AND advanced[All Fields] AND (“surgical flaps”[MeSH Terms] OR (“surgical”[All Fields] AND “flaps”[All Fields]) OR “surgical flaps”[All Fields] OR “flap”[All Fields])) AND (“humans”[MeSH Terms] AND English[lang] AND jsubsetd[text])

The EMBASE search was completed on October 3, 2017, using the following strategy:

- ‘english’:la AND coronally AND advanced AND flap AND connective AND tissue AND graft) AND ‘human’/de AND ‘article’/it

The search on the Cochrane Oral Health Group Trials Register was performed on October 3, 2017, using the following strategy:

- “Gingival Recession” [Search All Text] AND “Root Coverage” [Search All Text]

Furthermore, a manual search through periodontics-related journals, including *Journal of Dental Research*, *Journal of Clinical Periodontology*, *Journal of Periodontology*, *Journal of Periodontal Research*, and *International Journal of Periodontics & Restorative Dentistry*, from January 2012 to October 2017, was performed. The references of all the articles were reviewed in full text to identify all other available articles. Finally, previous systematic reviews investigating root coverage procedures for gingival recessions were also screened for article identification recession [1, 2, 38–54]. Moreover, when necessary, authors were contacted to obtain further information regarding the harvesting approach.

Design of the included studies and eligibility criteria

In this systematic review, only prospective randomized controlled clinical trials (RCTs), for the treatment of single or multiple gingival recessions with CAF, of at least 1-year duration, were considered. Either the test or control treatment group of each selected RCT was included in the article if the following inclusion criteria were met:

- The use of a CTG without adjunct biologic agents
- Follow-up ≥ 12 months
- Miller class I and/or II
- CTG placement completely underneath the flap without exposure
- Root coverage procedure performed in a single surgery

Correspondingly, articles were excluded based on the following criteria:

- Two-step surgery
- Follow-up < 1 year
- CAF + CTG combined with enamel matrix derivatives (EMD), platelet-rich plasma (PRP), or other biologic agents
- Different flap design from the conventional CAF (i.e., CAF without de-epithelialization of the papillae, CAF where CTG was left exposed, CAF positioned at CEJ without overcorrection of the flap, CAF without releasing incisions, etc.) [55, 56]
- Recruitment of patients from a previously published article
- Cohort studies, case-control studies, retrospective studies, case series, case reports

Quality and risk of bias assessment Two authors (SB, LT) independently evaluated all the included RCTs using the Cochrane Risk of Bias Tool for Randomized Controlled Trials [57], by addressing the following questions: (1) appropriate population size; (2) definitions of inclusion and

exclusion; (3) presence of randomization; (4) methods of allocation concealment; (5) masking of examiners; (6) incomplete outcome data adequately addressed; and (7) free of suggestion of selective outcome reporting. The potential risk of bias was categorized as low if a study provided detailed information about all parameters above. Moderate risk was considered if a study failed to provide information on only one of the parameters, whereas if a study showed missing information of > 2 parameters, the study was categorized as having a high risk of bias.

Statistical analysis (Planned methods for meta-analysis)

All statistical meta-analyses were performed using the metafor statistical package in the Rstudio software environment (Rstudio Version 1.1.383, Rstudio, Inc., MA, USA). For continuous outcomes of Rec Red, KT gain, CAL gain, and PD reduction, inverse variance weighted mean values (WMV) with 95% confidence intervals (CI) were calculated by utilizing the obtained means and standard deviations, and further analyzed for the EDTA and the non-EDTA studies to form EDTA and non-EDTA models. The results from the EDTA and non-EDTA models were then combined, and differences between the two groups (subtraction of non-EDTA from the EDTA value (EDTA − non − EDTA)) were estimated using the rma function. For every outcome, a forest plot was produced to visualize the WMV with CI and to demonstrate their difference and compare both groups. A $p < 0.05$ was considered significant. Furthermore, in order to make a statistically significant evaluation of the differences between the EDTA and non-EDTA studies, baseline characteristics of both groups were also explored.

For binary outcomes of CRC, log odds and log odds ratios were calculated for and between each group (EDTA and non-EDTA) and results were summarized with forest plots. The logs were then exponentiated to obtain odds and odds ratios (OR). Additionally, baseline characteristics of all continuous outcomes were explored to signify the presence of significant difference between the two groups. Lastly, for assessment of heterogeneity among selected studies, the Chi-square (X^2) and the I^2 statistics test were used, and funnel plots were produced to visualize possible publications bias.

Results

Study selection

Search results based on the PRISMA guidelines are depicted in Fig. 1. A total of 575 recessions treated with CAF + CTG in 14 randomized clinical trials (6 test, 8 control) were included in the present systematic review. Among the total number of GRs, 261 were treated with CAF + CTG + EDTA [58–62] and 314 were treated with CAF + CTG without the adjunct use of chemical root biomodification [63–70].

Study characteristics

Study design and study population

Among the 14 RCTs selected for analysis, seven trials allowed for the participation of patients that smoked up to 10 cigarettes per day [60–62, 65, 67, 68, 70], while the rest excluded smokers from participating [58, 59, 63, 64, 66, 69, 71]. Nine trials focused on the treatment of localized single GRs [58–61, 64, 66, 67, 70, 71], whereas five treated both single and multiple GRs [62, 63, 65, 68, 69].

All included studies had treated both Miller class I and II GRs except one which only included patients with Miller class II GRs [58]. Additionally, 10 trials treated GRs on both jaws [58, 59, 63–65, 67–71], while three only treated maxillary GRs [61, 62, 66], and one focused on GRs on the mandible [60]. Mechanical treatment of the root by means of curettes was performed in 11 trials [60–63, 65–71]. Two studies also utilized chisel and finishing burs [58, 59]; however, in one article, information regarding mechanical instrumentation was missing [64].

All trials had a final follow-up of 12 months except two [62, 64] which provided a longer follow-up of 5 years; however, the data from the 12-month period was extracted to maintain uniformity. Lastly, in two studies, the surgeries were performed in private practices [58, 59], while the rest of the procedures were carried out in university settings.

The general characteristics of the included studies are outlined in Table 1.

Quality assessment

The results of bias risk assessment for the included RCTs, using the Cochrane Risk of Bias Tool, are summarized in Data S2. Nine trials were considered to have a low risk of bias [60–63, 65–69], and five were considered to have a moderate risk of bias [58, 59, 64, 70, 71].

Meta-analysis

Data from the included trials were extracted and organized into tables to condense an overview of the reported clinical parameters. Characteristics of the intervention, root surface treatment, and clinical outcomes are summarized in Table 2.

All trials reported data on Rec Red, CRC, KT, CAL, and PD except one study [64] that did not report on PD

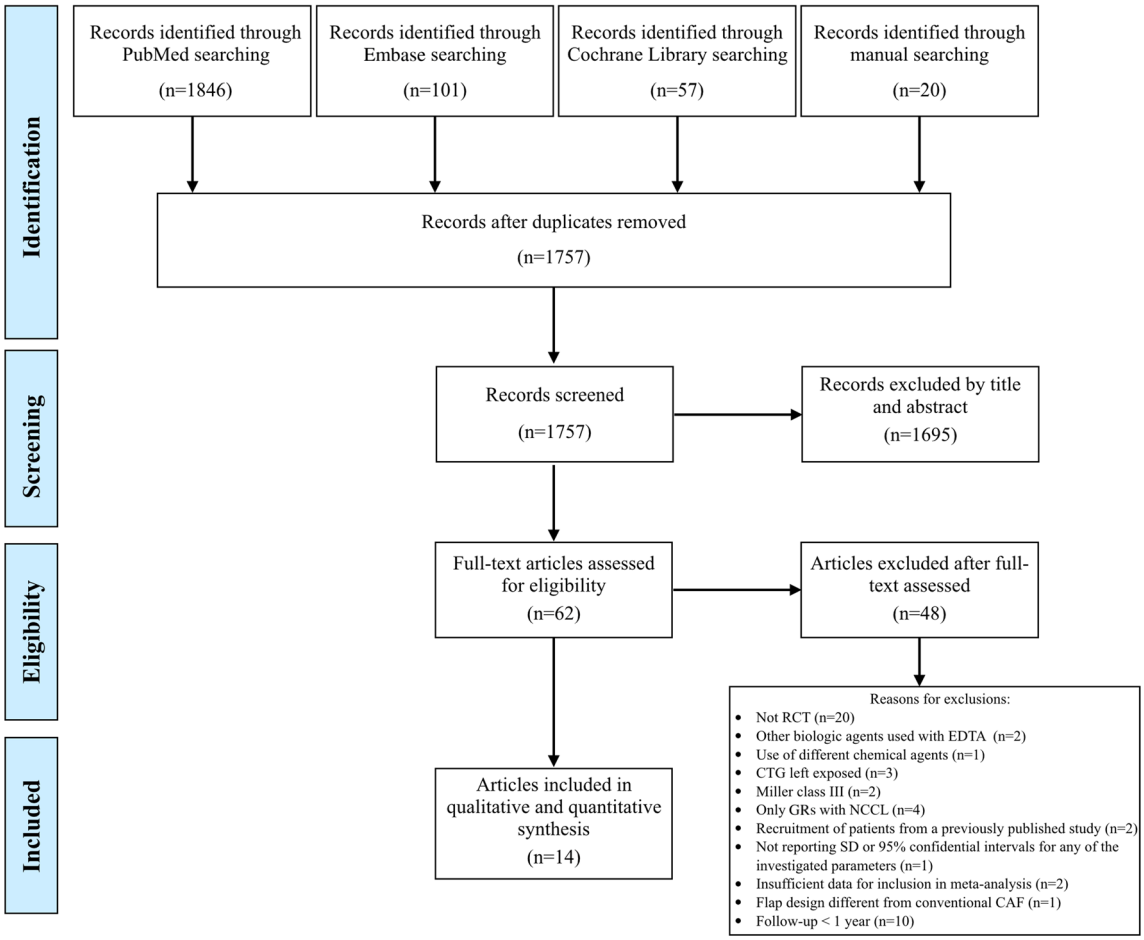

Fig. 1 PRISMA flowchart

and CAL, and two trials [59, 70] that did not specify the CRC values. The statistical analyses are described below.

Recession reduction

Baseline characteristics of recession defects in both groups did not reveal a statistically significant difference when initial recession depths were explored. The estimated difference between the initial values between the two groups was 0.2 mm (95% CI [− 0.36, 0.77]) ($p = 0.47$) (Data S3A, S3B, S3C). At 12 months, the WMV for Rec Red was 3.68 mm (95% CI [3.41, 3.95]) for the EDTA and 3.07 mm (95% CI [2.69, 3.45]) for the non-EDTA studies (Fig. 2a, b) with an estimated difference of 0.61 mm (95% CI [0.08, 1.14]) ($p = 0.016$) between the combined WMV of both test and control groups (Fig. 2c). Heterogeneity among the trials was almost equally observed in both treatment groups ($I^2 = 72.34\%$, $p < 0.01$ for the EDTA, and $I^2 = 70.94\%$, $p < 0.01$ for the non-EDTA group), as further illustrated by the funnel plots (Data S4A, S4B).

Complete root coverage

The log odds of CRC for both groups are summarized in forest plots (Fig. 2d, e). At the final follow-up, the analysis of included trials did not show a statistically significant difference between the EDTA and non-EDTA groups (Fig. 2f). When the odds of obtaining CRC were calculated, the value for EDTA studies was 3.43 (95% CI [1.71, 6.82]), and that for the non-EDTA studies was 2.97 (95% CI [1.51, 5.87]), with an odds ratio of 1.15 (95% CI [0.6, 2.22]) which was not statistically significant ($p = 0.7$). As demonstrated by funnel plots (Data S4C, S4D), a moderate heterogeneity was observed among both treatment groups ($I^2 = 57.08\%$, $p < 0.01$ for the EDTA, and $I^2 = 58.07\%$, $p < 0.01$ for the non-EDTA group).

Keratinized tissue gain

The KT height at baseline for EDTA and non-EDTA studies is summarized in forest plots (Data S3D, S3E). The estimated difference between the two groups was − 0.13 (95% CI [− 0.79, 52]) which was not statistically significant ($p = 0.6$) (Data S3F). At follow-up, the WMV for the EDTA and non-

Table 1 General overview of the included randomized clinical trials

Study or Subgroup	Follow-up	Patients, no. of recessions treated with CAF + CTG (*N*)	Mean age (years)	Systemic and periodontal status, smoking habits	Recession type	Location	Site, setting, and funding
Zucchelli et al. [66] (Control)	12 months	Patients, $n = 15$ Recessions, $n = 15$	18–35	Healthy, non-smoking patients FMPS < 20% FMBS < 15%	Single GRs Miller classes I and II	Maxilla (incisor, canine, premolar)	Italy, university, NR
Zucchelli et al. [66] (Test)	12 months	Patients, $n = 15$ Recessions, $n = 15$	18–35	Healthy, non-smoking patients FMPS < 20% FMBS < 15%	Single GRs Miller classes I and II	Maxilla (incisor, canine, premolar)	Italy, university, NR
Nemcovsky et al. [70]	12 months*	Patients, $n = 70$ Recessions, $n = 40$	39 ± 12.2	Healthy, smoking ≤ 10 cigarettes/day	Single GRs Miller classes I and II	Maxilla and mandible (incisor, canine, premolar)	Israel, university, university
Zucchelli et al. [68] (DGG)	12 months	Patients, $n = 25$ Recessions, $N = 50$	34.7 ± 6	Healthy, smoking ≤ 10 cigarettes/day FMPS < 20% FMBS < 15%	Single and multiple GRs Miller classes I and II	Maxilla and mandible (incisor, canine, premolar)	Italy, university, self
Zucchelli et al. [68] (SCTG)	12 months	Patients, $n = 25$ Recessions, $n = 50$	34.7 ± 6	Healthy, smoking ≤ 10 cigarettes/day FMPS < 20% FMBS < 15%	Single and multiple GRs Miller classes I and II	Maxilla and mandible (incisor, canine, premolar)	Italy, university, self
Zucchelli et al. [67]	12 months	Patients, $n = 25$ Recessions, $n = 25$	33.6 ± 5.8	Healthy, smoking ≤ 10 cigarettes/day FMPS < 20% FMBS < 15%	Single GRs Miller classes I and II	Maxilla and mandible (molar)	Italy, university, self
Roman et al. [65]	12 months	Patients, $n = 21$ Recessions, $n = 34$	31 ± 8.5	Healthy, smoking ≤ 10 cigarettes/day FMPS ≤ 20%	Single and multiple GRs Miller classes I and II	Maxilla and mandible (incisor, canine, premolar, molar)	Romania, university, government
Kuis et al. [64]	12 months*	Patients, $n = 37$ Recession, $n = 57$	31.1	Healthy, non-smoking patients FMPS < 20%	Single GRs Miller classes I and II	Maxilla and mandible (incisor, canine, premolar, molar)	Croatia, university, government
Azaripour et al. [63]	12 months	Patients, $n = 20$ Recessions, $n = 29$	38.6 ± 12.8	Healthy, non-smoking patients FMPS < 15% FMBS < 15%	Single and multiple GRs Miller classes I and II	Maxilla and mandible (incisor, canine, premolar, molar)	Germany, university, university
Gobbato et al. [69]	12 months	Patients, $n = 50$ Recessions, $n = 49$	27.6 ± 6	Healthy, non-smoking patients FMPS < 15% FMBS <15%	Single and multiple GRs Miller classes I and II	Maxilla and mandible (incisor, canine, premolar)	Italy, university, self
McGuire and Nunn, [58]	12 months	Patients, $n = 20$ Recessions, $n = 20$	44.9 ± 11.6	Healthy, non-smoking patients FMPS < 15%	Single GRs Miller class II	Maxilla and mandible (incisor, canine, premolar)	USA, private practice, company
McGuire and Scheyer, [59]	12 months	Patients, $n = 25$ Recessions, $n = 25$	43.7 ± 12.2	Healthy, non-smoking patients	Dehiscence type recessions	Maxilla and mandible (incisor, canine, premolar)	USA, private practice, partially by a company

Table 1 (continued)

Study or Subgroup	Follow-up	Patients, no. of recessions treated with CAF + CTG (*N*)	Mean age (years)	Systemic and periodontal status, smoking habits	Recession type	Location	Site, setting, and funding
Rasperini et al. [71]	12 months	Patients, $n = 30$ Recession, $n = 30$	35.5 ± 9.2	Healthy, non-smoking patients FMPS ≤ 15% FMBS ≤ 20%	Single GRs Miller classes I and II	Maxilla and mandible (incisor, canine, premolar)	Italy, university, NR
Zucchelli et al [60] (Control)	12 months	Patients, $n = 25$ Recessions, $n = 25$	NR	Healthy, smoking ≤ 10 cigarettes/day FMPS < 15% FMBS < 15%	Single GRs Miller classes I and II	Mandible (incisor)	Italy, university, self
Zucchelli et al [61] (Test)	12 months	Patients, $n = 25$ Recessions, $n = 25$	NR	Healthy, smoking ≤ 10 cigarettes/day FMPS < 15% FMBS < 15%	Single GRs Miller classes I and II	Mandible (incisor)	Italy, university, self
Zucchelli et al [61] (Control)	12 months	Patients, $n = 30$ Recessions, $n = 60$	NR	Healthy, smoking ≤ 10 cigarettes/day FMPS < 15% FMBS < 15%	Single GRs Miller classes I and II	Maxilla	Italy, university, self
Zucchelli et al [61] (Test)	12 months	Patients, $n = 30$ Recessions, $n = 60$	NR	Healthy, smoking ≤ 10 cigarettes/day FMPS < 15% FMBS < 15%	Single GRs Miller classes I and II	Maxilla	Italy, university, self
Zucchelli et al [62]	12 months*	Patients, $n = 25$ Recessions, $n = 76$	33.2 ± 7.4	Healthy, smoking ≤ 10 cigarettes/day FMPS < 15% FMBS < 15%	Single and multiple GRs Miller classes I and II	Maxilla (incisor, canine, premolar)	Italy, university, self

GRs, gingival recessions; *FMPS*, Full Mouth Plaque Score; *FMBS*, Full Mouth Bleeding Score; *NR*, not reported

*Longer follow-up results presented by the article however for the purposes of maintaining uniformity data from the 12-month follow-up were used for analysis

Table 2 General characteristics of the intervention and results

Study	Mechanical instrumentation	EDTA application	Suture removal	Rec Red ± SD (mm)	CRC (%)	KT gain ± SD (mm)	PD red ± SD (mm)	CAL gain ± SD (mm)
Zucchelli et al. [66] (Control)	Curette	No	2 weeks	3.6 ± 0.72	80	3.3 ± 0.72	0.5 ± 0.51	3.1 ± 0.74
Zucchelli et al. [66] (Test)	Curette	No	2 weeks	3.9 ± 0.71	86.7	2.3 ± 0.59	0.1 ± 0.25	3.9 ± 0.7
Nemcovsky et al. [70]	Curette	No	10–12 days	4 ± 1.18	NR	2.7 ± 1.3	0 ± 0.88	4 ± 1.24
Zucchelli et al. [68] (DGG)	Curette	No	2 weeks	3.4 ± 0.81	84	2.12 ± 0.52	0.2 ± 0.5	3.2 ± 0.71
Zucchelli et al. [68] (SCTG)	Curette	No	2 weeks	3.08 ± 0.7	72	1.92 ± 0.74	0.12 ± 0.56	3.02 ± 0.71
Zucchelli et al. [67]	Curette	No	2 weeks	4.04 ± 1.02	48	0.96 ± 0.73	0.8 ± 0.82	4.84 ± 1.43
Roman et al. [65]	Curette	No	2 weeks	2.91 ± 1.67	70.7	1.56 ± 1.22	− 0.04 ± 0.8	2.95 ± 2.08
Kuis et al. [64]	NR	No	2 weeks	2.54 ± 0.81	93	1.34 ± 1.81	NR	NR
Azaripour et al. [63]	Curette	No	2 weeks	2.3 ± 1.2	96.6	0.36 ± 0.6	− 0.1 ± 1.03	2.44 ± 1.43
Gobbato et al. [69]	Curette	No	7 days	2.95 ± 0.75	52	1.68 ± 1.10	0 ± 0.64	2.92 ± 0.95
McGuire and Nunn, [58]	Chisel, finishing burs, and curette	Yes	7 days	4.01 ± 0.73	79	1.56 ± 1.31	− 0.09 ± 1.21	4.1 ± 1.9
McGuire and Scheyer, [59]	Chisel, finishing burs, and curette	Yes	NR	3.17 ± 0.37	NR	1.2 ± 1.8	0.23 ± 0.9	2.95 ± 1
Rasperini et al. [71]	Curette	Yes	8 days	3.6 ± 1.5	47	2 ± 1.5	− 0.1 ± 0.1	3.5 ± 1.5
Zucchelli et al [60] (Control)	Curette	Yes	2 week	3.08 ± 1.12	48	2.2 ± 1.77	− 1.56 ± 1	4.6 ± 1.41
Zucchelli et al., [60] (Test)	Curette	Yes	2 week	3.68 ± 1.11	88	1.56 ± 1.1	− 1.6 ± 0.96	5.24 ± 1.76
Zucchelli et al [61] (Control)	Curette	Yes	2 weeks	3.8 ± 0.92	80	2.5 ± 0.73	0.2 ± 0.54	3.6 ± 1.06
Zucchelli et al [61] (Test)	Curette	Yes	2 weeks	3.66 ± 0.96	83	2.17 ± 0.59	0.16 ± 0.53	3.36 ± 0.78
Zucchelli et al [62]	Curette	Yes	2 weeks	3.02 ± 0.6	86.8	1 ± 0.44	− 0.04 ± 0.26	3.01 ± 0.62

NR, not reported; *Rec Red*, recession reduction; *CRC*, complete root coverage; *KT*, keratinized tissue; *CAL*, clinical attachment level; *SD*, standard deviation

EDTA groups was 1.98 mm (95% CI [1.59, 2.37]), and 1.62 mm (95% CI [1.09, 2.15]), respectively (Fig. 3a, b). The estimated difference between the test and control groups was 0.36 mm (95% CI [− 0.22, 0.94]) which lacked statistical significant ($p = 0.2$) (Fig. 3c). Substantial heterogeneity was observed among both treatment groups ($I^2 = 72.28\%$, $p < 0.01$ for the EDTA, and $I^2 = 76.76\%$, $p < 0.01$ for the non-EDTA group) which is demonstrated by funnel plots (Data S4E, S4F).

Clinical attachment level changes

The difference between both groups at baseline was 0.22 mm (95% CI [−0.58, 1.02]) ($p = 0.4$) (Data S4A, S4B, S4C). At 12 months follow-up, the WMV for the EDTA and non-EDTA group was 4.15 mm (95% CI [3.61, 4.69]) and 3.07 mm (95% CI [2.61, 3.53]), respectively (Fig. 3d, e). The difference between the combined WMVs was 1.08 mm (95% CI [0.33, 1.83]) which was statistically significant ($p = 0.015$) (Fig. 3f). As displayed by the asymmetry in the funnel plots (Data S4G, S4H), considerable heterogeneity was noticed among both EDTA and non-EDTA treatment groups ($I^2 = 74.23\%$, $p < 0.01$; and $I^2 = 72.93\%$, $p < 0.01$, respectively).

Changes in pocket depth

The difference at baseline in PD measurements between the two groups was 0.13 mm (95% CI [− 0.32, 0.58]) that lacked statistical significance ($p = 0.3$) (Data S4D, S4E, S4F). However, at 1 year, the difference between the WMV for PD reduction of the EDTA (− 0.44 mm, 95% CI [−0.97, 0.09]) and non-EDTA groups (0.27 mm, 95% CI [0.10, 0.44]) was statistically significant ($p = 0.021$) and was estimated at − 0.71 mm (95% CI [− 1.22, − 0.2]) (Fig. 4a, b, c). Considerable heterogeneity among the EDTA ($I^2 = 79.4\%$, $p < 0.01$) and moderate homogeneity ($I^2 = 61.16\%$, $p < 0.01$) among the non-EDTA treatment groups were noticed (Data S4I, S4J).

Table 3 summarizes the analyzed results.

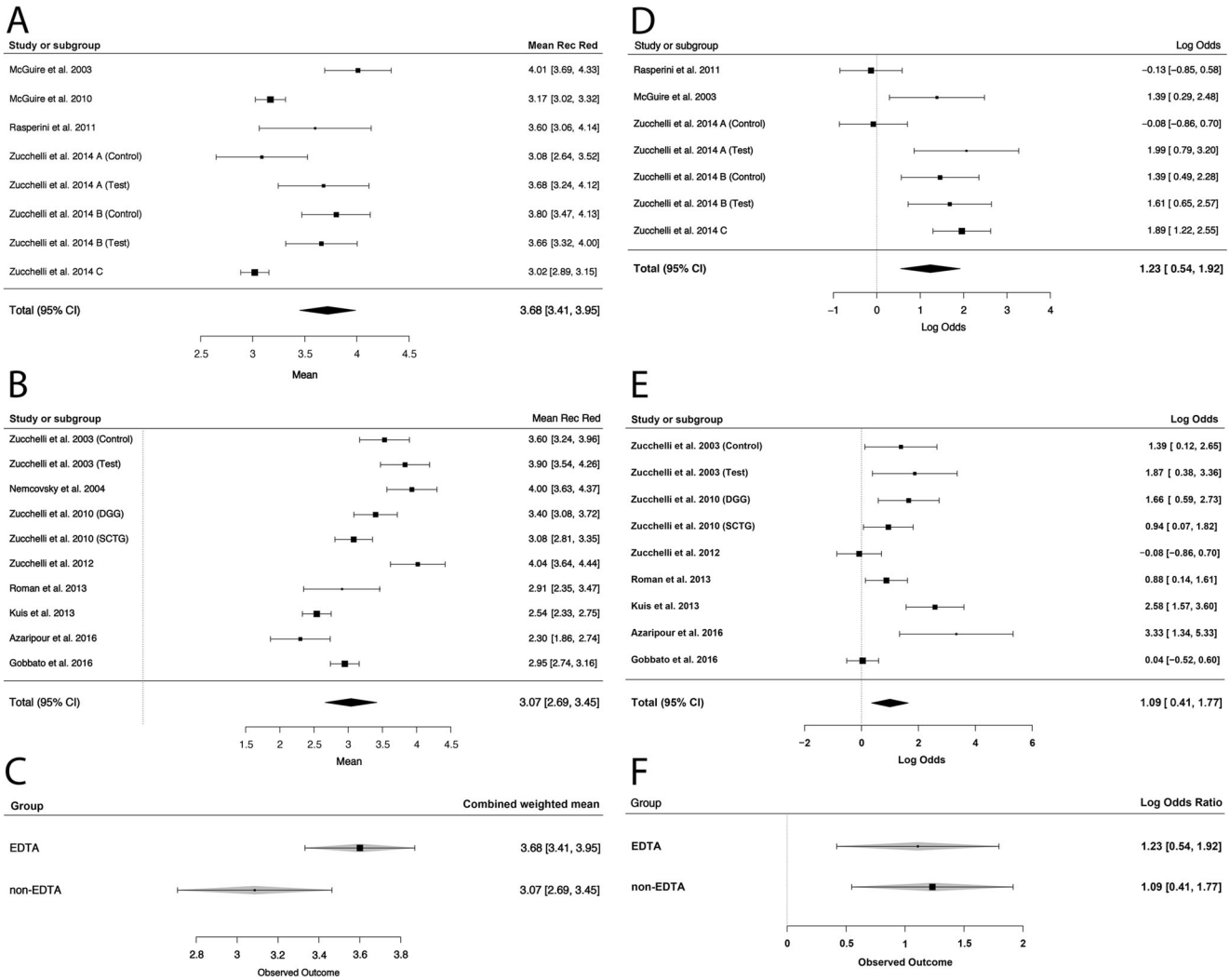

Fig. 2 Meta-analysis and comparison of WMV for the EDTA and non-EDTA groups for Rec Red and CRC. **a** Rec Red of EDTA studies. **b** Rec Red of non-EDTA studies. **c** Comparison of both groups for Rec Red. **d** CRC of EDTA studies. **e** CRC of non-EDTA studies. **f** Comparison of both groups for CRC

Discussion

The present systematic review was designed to answer the question whether EDTA root surface biomodification enhances the treatment outcomes of CAF + CTG. The results of the meta-analysis demonstrated that EDTA combined with CAF + CTG could significantly enhance Rec Red, PD reduction, and CAL gain which are also ultimate goals of root coverage procedures [56, 72]. In particular, the EDTA group showed a mean Rec Red of 3.68 mm compared to 3.07 mm in the non-EDTA group ($p < 0.01$). The reasons for this significant difference are open to speculations.

The healing and revascularization of connective tissue grafts have been histologically evaluated, demonstrating that the attachment between the graft and the root is mainly composed of a combination of long junctional epithelium and connective tissue attachment, with connective tissue fibers parallel to the root surface [73]. Given its properties of exposing dentinal collagen fibers [74–76] and favoring the migration and the attachment of fibroblasts to the root surface [77–79], it may be assumed that EDTA can enhance the attachment of connective tissue fibers to the root. A possible explanation of the higher Rec Red in the EDTA group might be the early cellular colonization and improved stability of the bond between the fibrin of the blood clot and the root surface during the first stage of healing following the application of EDTA [80].

The higher CAL gain in the EDTA group compared to the control group (4.15 mm vs 3.07 mm) is in line with a previous study demonstrating the efficacy of EDTA root conditioning in reducing the CAL when used in regenerative therapy [26]. However, care should be taken in interpreting these results as another study failed to demonstrate a significant effect of EDTA on CAL gain following flap surgery [33].

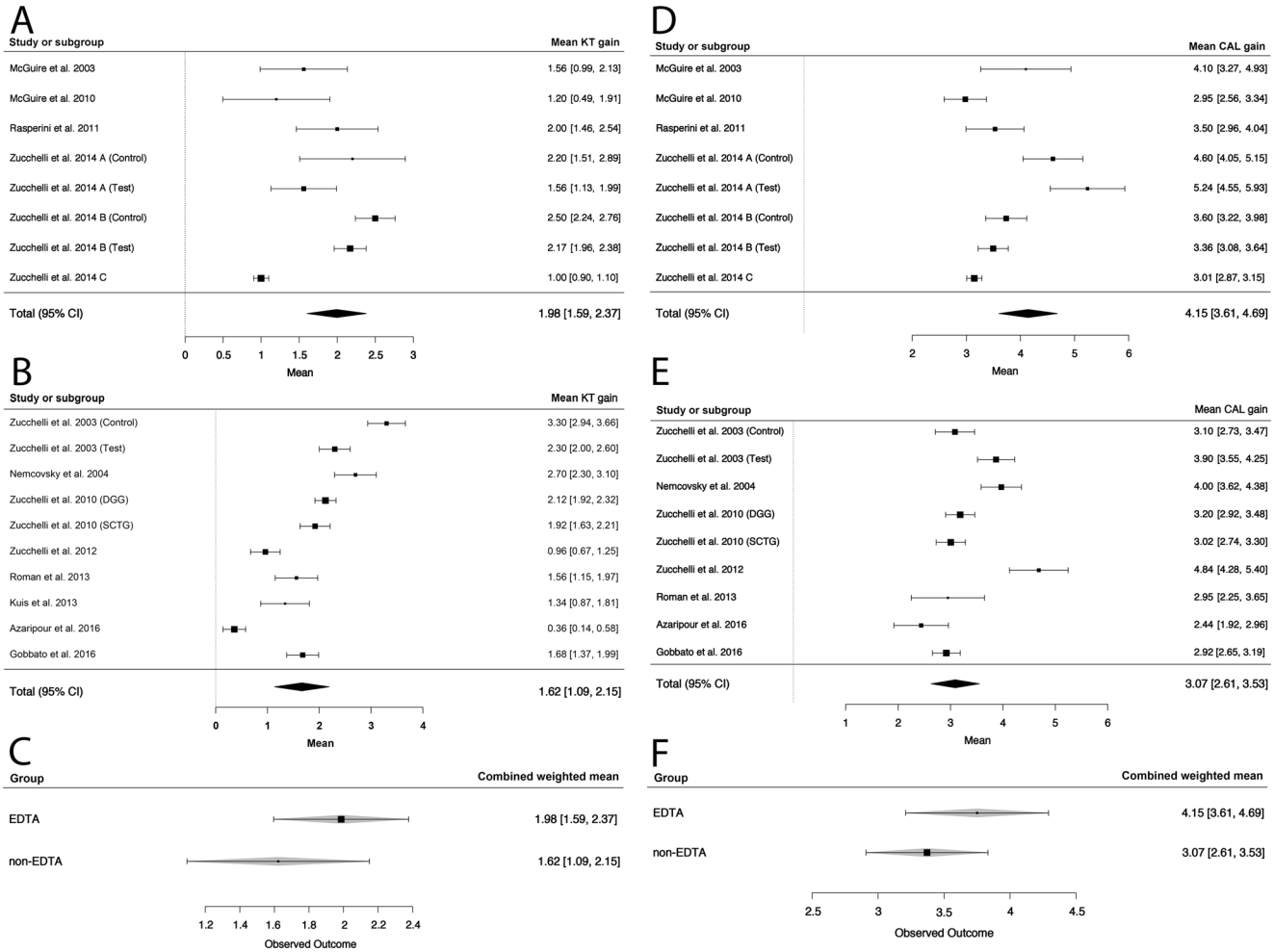

Fig. 3 Meta-analysis and comparison of WMV for the EDTA and non-EDTA groups for KT gain and CAL gain. **a** KT gain for EDTA studies. **b** KT gain for non-EDTA studies. **c** KT gain comparison of both groups. **d** CAL gain for EDTA studies. **e** CAL gain for non-EDTA studies. **f** CAL gain comparison of both groups

A recent animal study demonstrated the efficacy of EDTA root surface conditioning in minimizing blood clot retraction and thus the microgap with the root surface during the first stage of healing [81]. Since the stability of blood clot plays a key role in the healing process [82, 83], these findings may reinforce the assumption of the beneficial effects of EDTA root conditioning on the CAF + CTG outcome.

Furthermore, in an in vitro study investigating the early root surface colonization by fibroblasts, Kasaj et al. found that when applied on the root, EDTA alone or in combination with enamel matrix protein was able to increase fibroblast size, proliferation, and density compared to control (scaling and root planing alone). Moreover, root surfaces previously demineralized with EDTA seem to stimulate morphologic alteration of fibroblasts that show a close adhesion to the root with long extensions of their cytoplasmic processes [24]. These in vitro findings may suggest that a root surface chemically conditioned with EDTA may be considered a more biocompatible environment for cell proliferation and attachment than mechanical root conditioning [24, 80].

It is worthy to mention that the only clinical trial designed to investigate the outcomes of CAF with and without the application of EDTA includes the limitations of having a small sample size, EDTA application being prior to flap elevation, lack of flap advancement beyond the CEJ, and leaving the CTG partially exposed with a relatively short follow-up [27].

The present meta-analysis is based on data extracted from arms of RCTs with at least 12 months follow-up. The reason behind this is the evidence that the evaluation of clinical parameters such as the position of the gingival margin and the amount of KT should be performed medium to long term [84, 85]. Furthermore, the selection of CAF + CTG is dictated by several advantages that render this procedure the gold standard technique for root coverage [1, 52]. Primarily, early

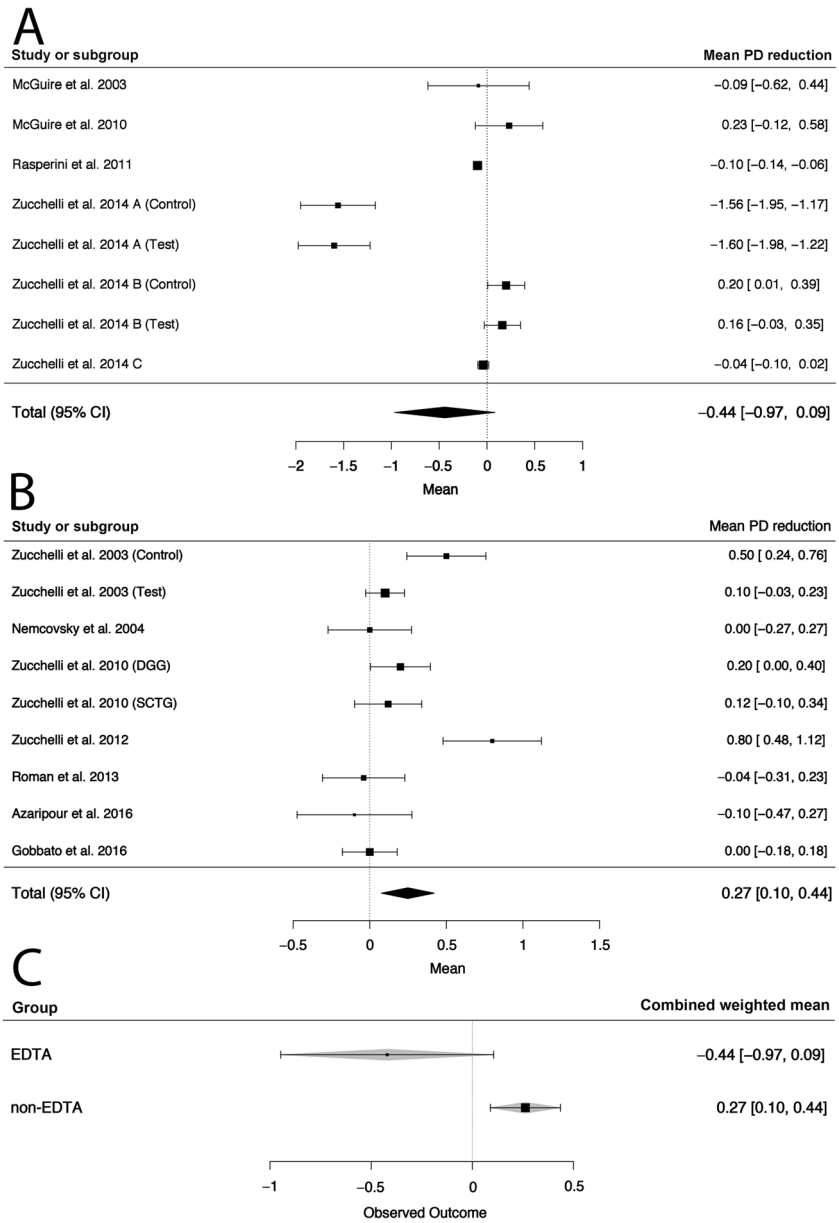

Fig. 4 Meta-analysis and comparison of WMV for the EDTA and non-EDTA groups for PD reduction. **a** EDTA studies. **b** Non-EDTA studies. **c** Comparison of both groups

wound healing seems to be improved when a CTG is interposed between the wound bed and the flap [86], especially when CAF is prepared with a split-full-split design [87]. Next, it has been suggested that CTG can help in stabilizing the flap in the desired coronal position during the first healing phase, increasing the chances of a complete root coverage [86]. Additionally, it has been shown that CTG can increase the marginal soft tissue thickness, which is positively correlated to higher root coverage [88]. Another advantage of an augmented marginal gingival thickness is the tendency to migrate coronally over time, increasing the percentage of root coverage [84, 85, 89], which was termed creeping attachment, and follows autologous free gingival grafts or a connective tissue graft [90, 91].

The authors are aware of the limitations of the present study. The insufficient clinical studies comparing root coverage outcomes with and without EDTA may prevent drawing definitive conclusions on its efficacy. Furthermore, due to the lack of a RCT investigating CAF + CTG with and without EDTA, the present meta-analysis was conducted by selecting the arms of an RCT which performed the root coverage procedure by using a CTG and the CAF technique and comparing it to the combined values of arms of randomized control trials that used same procedure (CAF + CTG) with the adjunct application of EDTA as a root biomodification agent. In simple terms, essentially, the statistical analysis was similar to running a two-sample *t* test and

Table 3 Summarized results of the investigated outcomes for both EDTA and non-EDTA groups

Criteria	Weighted mean values (95% CI)		Estimated difference (EDTA−nonEDTA) (95% CI) *p* value
	EDTA group	non-EDTA group	
Rec Red	3.68 mm (95% CI [3.41, 3.95])	3.07 mm (95% CI [2.69, 3.45])	0.61 mm (95% CI [0.08, 1.14]) $p = 0.01$*
KT gain	1.98 mm (95% CI [1.59, 2.37])	1.62 mm (95% CI [1.09, 2.15])	0.36 mm (95% CI [− 0.22, 0.94]) $p = 0.2$
CAL gain	4.15 mm (95% CI [3.61, 4.69])	3.07 mm (95% CI [2.61, 3.53])	1.08 mm (95% CI [0.33, 1.83]) $p = 0.01$*
PD reduction	− 0.44 mm (95% CI [−0.97, 0.09])	0.27 mm (95% CI [0.10, 0.44])	− 0.71 mm (95% CI [− 1.22, − 0.2]) $p = 0.021$*
	Odds	Odds	OR (95% CI), *p* value
CRC	3.43 (95% CI [1.71, 6.82])	2.97 (95% CI [1.51, 5.87])	1.15 (95% CI [0.6, 2.22]) $p = 0.7$

Rec Red, recession reduction; *CRC*, complete root coverage; *KT*, keratinized tissue; *CAL*, clinical attachment level; *PD*, pocket depth; *CI*, confidence interval; *OR*, odds ratio

*Statistical significance

estimating the difference between the two groups (by creating separate meta-analyses for each subset and comparing the two). It is understandable that several confounding factors, such as different operators, the CTG harvesting approach, and single or multiple GRs, may have affected the results.

Ultimately, it can be speculated from our findings that EDTA may be beneficial in improving the overall clinical outcomes when CAF + CTG is performed. Particularly, given the relatively low cost and short time consumption, the application of EDTA could be considered. However, further RCTs are required for assessing these results and the effectiveness of this agent on the root surface when performing CAF + CTG.

Indication for further research

- RCTs based on CONSORT guidelines
- Increase the number of RCTs investigating the root coverage following the application of EDTA and flap (test group) compared to the flap alone (control group)
- Increase the number of RCTs investigating recession coverage procedures following the adjunct use of EDTA after CAF + CTG (test group) compared to CAF + CTG alone (control group)

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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