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ORIGINAL ARTICLE

# Expression of gingival crevicular fluid markers during early and late healing of intrabony defects after surgical treatment: a systematic review

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## Abstract

**Background** Surgical treatments such as guided tissue regeneration (GTR) and access flap surgery are widely employed for the treatment of intrabony defects. However, little is known regarding the postoperative expression of gingival crevicular fluid (GCF) markers.

**Objective** The aim of this systematic review was to compare the expression of GCF markers following treatment of periodontal intrabony defects with guided tissue regeneration or access surgery. The association of the markers' expression with the clinical outcome was also assessed.

**Methods** An electronic literature search was conducted in MEDLINE, EMBASE, OpenGrey, LILACS and Cochrane Library up to December 2018 complemented by a manual search. Human, prospective clinical studies were identified. The changes from baseline up to 30 days (early healing) and 3 months (late healing) were assessed.

**Results** A total of 164 publications were identified and reviewed for eligibility. Of these, 10 publications fulfilled the inclusion criteria. The included studies evaluated 15 different GCF markers with a follow-up time between 21 and 360 days postoperatively. PDGF, VEGF and TIMP-1 changes were often investigated in the included studies; however, contrasting results were reported. Two studies agreed that both GTR and OFD lead to similar OPG level changes. TGF-β1 is increased early postoperatively, irrespective of the surgical technique employed.

**Conclusion** There is limited evidence available on the expression of GCF markers after surgical interventions of intrabony periodontal defects. However, OPG and TGF-β1 tend to increase early post-operatively, irrespective of the surgical technique employed, irrespective of the surgical technique employed.

**Clinical relevance** More well-designed, powered studies with sampling periods reflecting the regenerative process are needed, and future research should focus on employing standardised protocols for collecting, storing and analysing GCF markers.

**Keywords** Gingival crevicular fluid · Markers · Guided tissue regeneration · Open flap debridement

## Introduction

Periodontitis is a chronic inflammatory disease caused by bacterial biofilm that leads to a progressive destruction of the supporting apparatus of a tooth and eventually to tooth loss. The prevalence of periodontitis, according to the 2009–2010 data from the National Health and Nutrition Examination Survey (NHANES), reaches 46% in US adults [1].

As periodontal disease progresses, it results in bone loss that can be horizontal or vertical or a combination of both. The loss of supporting bone vertically results in the formation of intrabony defects that progressively worsen and are associated with an increased probability of tooth loss [2]. While non-

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surgical periodontal therapy is effective in improving the clinical parameters, such as probing pocket depth (PPD) and clinical attachment levels (CAL) [3], surgical approaches are more effective—in particular for PPD of more than 6 mm [4, 5]. Currently, intrabony defects are identified as sites favourable for periodontal regeneration [6, 7] with the most commonly used techniques being guided tissue regeneration (GTR) and enamel matrix derivatives (EMD) presenting with similar clinical outcomes which are superior to open flap debridement (OFD) and osseous surgery (OS) [8–11].

However, irrespective of the regenerative modality employed for the treatment of intrabony defects, little is known regarding the processes and sequences involved in the periodontal regeneration and consequently, in the postoperative expression of angiogenesis, regeneration and inflammation markers in the gingival crevicular fluid (GCF) that accompany these processes [12]. The expression of such markers postoperatively may define whether the healing process moves towards a regenerative or a reparative direction [12]. Understanding the cellular and biological events in periodontal wound healing can possibly provide useful information in identifying predictable regenerative treatment for the periodontium.

The aim of this systematic review was to investigate the healing patterns of intrabony defects after surgical interventions (GTR, OS, OFD, EMD) by means of angiogenesis, regeneration and inflammation markers detected in the GCF before and early (≤ 30 days) or late (3 months) after the surgical intervention. Furthermore, the association of the expression of the GCF markers with the clinical outcome was investigated.

## Materials and methods

### Protocol and Registration

The present systematic review followed the PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analyses) guidelines [13] (Supplemental Material 1) and was registered with PROSPERO under the ID number CRD42018115794.

### PICO question

The PICO question (patient, intervention, comparison and outcome) formulated was: "In patients with periodontal intrabony defects, does the expression of GCF markers for angiogenesis, regeneration and inflammation differ when treated with GTR employing a membrane and/or bone graft and/or biologics (e.g. EMD) (test group(s)) compared with intrabony defects treated with access surgery [OFD or OS or apically positioned flap (APF)] (control group) early (≤ 30 days) and late (3 months) after the surgical intervention?"

### Eligibility criteria

#### Types of studies

Human, prospective clinical studies assessing the expression of angiogenesis, regeneration and inflammation markers in the GCF were considered. Only studies with at least ten patients per group were included. No language restriction was set.

#### Population

Systemically healthy individuals with chronic periodontitis (CP) with at least one tooth with PPD ≥ 5 mm, CAL and evidence of radiographic bone loss or aggressive periodontitis [14, 15] or periodontitis stages III or IV [16] and contributing a minimum of 1 intrabony defect.

#### Intervention and comparison

Intrabony defects treated with GTR employing a membrane and/or bone graft and/or with biologics (e.g. Emdogain) (test group(s)) and intrabony defects treated with access flap surgery (OFD or OS or APF) (control group). No restriction related to the flap technique (minimally invasive or not) was applied to avoid omitting potentially relevant data. Intrabony defects treated with adjunct growth factors e.g. EMD were included in the test group(s).

### Outcome measures

The primary outcome of this review was the change in the expression of angiogenesis, regeneration and inflammation markers in the GCF during early healing (from baseline up to 30 days) and during late healing (from baseline to at least 3 months postoperatively). Secondary outcomes considered were the association of the expression of GCF markers (early and/or late healing) with the clinical outcome, assessed with the use of surrogate measures such as PPD and/or CAL.

### Information sources and electronic search

An electronic search was conducted by two independent reviewers (VK and GC) in MEDLINE, EMBASE, Cochrane Library, LILACS and OpenGrey for publications up to 10 December 2018. Combinations of controlled terms (MeSH and EMTREE) and keywords were utilised:

("infrabony" or "intrabony" or "infra-bony" or "intra-bony" or "angular defect" or "periodontal defect") and ("guided tissue regeneration" or "GTR" or "periodontal regeneration" or "periodontal surgery" or "open flap debridement" or "OFD" or "access surgery") and ("gingival crevicular fluid"

or "crevicular fluid" or "GCF" or "inflammatory marker" or "marker" or "growth factor" or "inflammatory mediator" or "biomarker")

Additionally, a manual search of periodontology-related journals including Journal of Dental Research, Journal of Clinical Periodontology, Journal of Periodontal Research and the Journal of Periodontology was performed from 2015 to 2018. The list of references in the publications included in this review as well as the list of references in relevant reviews were screened for potential additional publications fulfilling the inclusion criteria.

### Study selection

The search results were initially screened for relevancy by means of title, keywords and abstract, independently and in duplicate by two reviewers (VK, GC). Irrelevant records were excluded at this stage. Any conflict was resolved with discussion. At the second round of screening, the full text of the publications remaining after the first round was assessed for inclusion in this review against the eligibility criteria described previously. The level of agreement between the two reviewers was calculated using Kappa statistics.

### Data collection process/data items

The characteristics of the included publications were extracted by two reviewers (VK, GC). Among the details extracted were study characteristics (authors, journal of publication, year, country), number of patients, their demographics and risk factors (age, gender, smoking), diagnosis, number of intrabony defects, history of non-surgical treatment of the sites and time elapsed, characteristics of the included defects, surgical procedure employed (GTR, OFD), biomaterials used in the test group(s), postoperative care protocol, exposure rate, follow-up period, expression levels of the GCF markers, clinical outcomes (PPD, CAL), details of the methodology employed for the GCF sampling, storage, processing and detection of the markers, information regarding the main study outcome and power calculation of the study. When data from the included studies were missing, the authors of the publication were contacted through email.

### Risk of bias assessment

The risk of bias of the included publications was assessed by the two reviewers independently and in duplicate. For the RCTs included, the quality of the selected publications was assessed according to the Cochrane Collaboration's tool for assessing risk of bias [17]. The selected publications were assessed for seven domains: sequence generation, allocation concealment, blinding of the participants and personnel, blinding of the outcome assessment, incomplete outcome data, free of selective outcome reporting and other sources of bias. For each of the individual domains, studies were classified as low, unclear or high risk of bias. Observational studies were assessed using the MINORS tool [18]. Studies were assessed in 12 items including clarity of the aim, inclusion of consecutive patients, prospective data collection, appropriateness of end points, unbiased assessment of study end points, appropriateness of follow-up time, inclusion of loss to follow-up rate, prospective calculation of the study size, comparable control group, contemporary control groups, baseline equivalence of groups on several factors and adequate statistical analysis. Each study may receive 0–2 points for each item and the total score ranges from 0 to 24 points. Studies with fewer than 16 points are considered of low quality, while high-quality studies need to have a score of greater than or equal to 16.

## Results

### Study selection

The flowchart of the study selection and inclusion process is shown in Fig. 1. The initial search identified 68 MEDLINE, 110 EMBASE, 59 Cochrane database and 1 LILACS titles, with a total of 163 after duplicates' removal. One additional title was identified through hand search for a total of 164 titles. Following the screening of titles and abstracts by the two reviewers, 10 articles qualified for full text screening and all 10 met the inclusion criteria. The kappa value for inter-reviewer agreement was 0.99 at first round and 1.00 at second round.

### Study characteristics

The characteristics of the included studies are presented in Table 1. All 10 included articles were in English. The study samples ranged from 12 [19] to 29 [20] patients. Seven of the included studies were randomised controlled clinical trials [11, 19–24] and the remaining 3 were prospective cohort studies [12, 25, 26].

The characteristics of the included intrabony defects ranged from PD ≥ 5 mm [12, 20, 24–26] to PD ≥ 6 mm [11, 19, 21–23] and accompanying radiographic defect depth ≥ 3 mm [11, 12, 19, 21, 24], ≥ 4 mm [20, 23] or unspecified [22, 25, 26]. The defects included were variations of 1-, 2-, 3-wall defects [19, 24], only 2- or 3-wall defects [11, 21] or non-specified in the majority of the investigations [12, 20, 22, 23, 25, 26].

The types of procedures included in the test group were GTR [11, 12, 21, 22, 24–26] GTR with EMD [19, 23], and minimally invasive surgical technique (MIST) with EMD [20]. Non-resorbable membranes that were removed 6 weeks postoperatively were utilised in 3 studies [12, 25, 26].

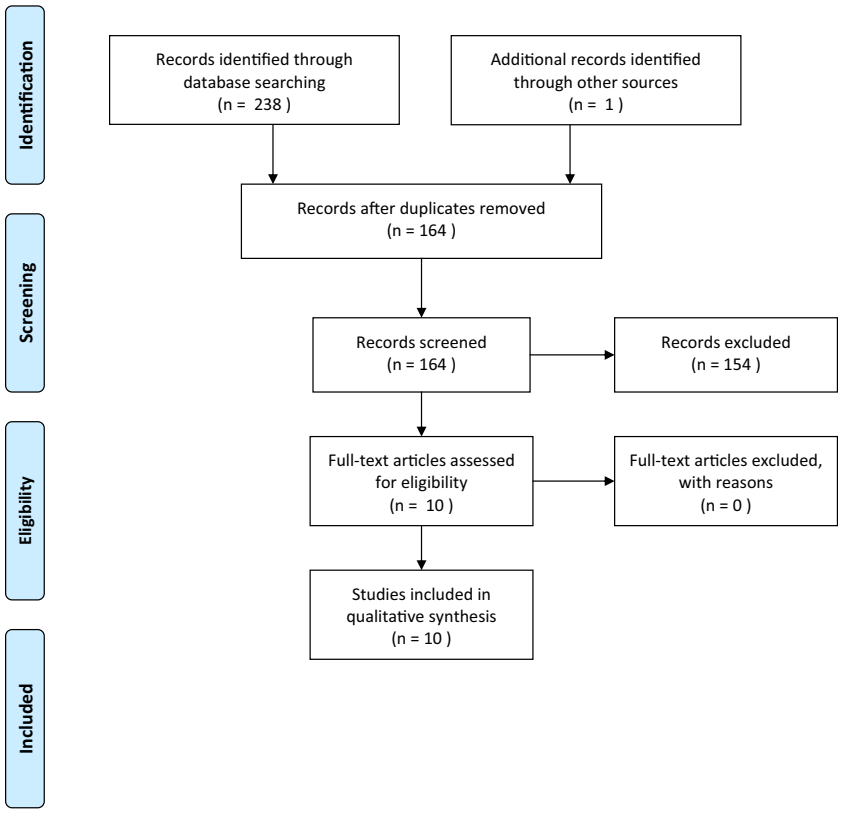


**Fig. 1** PRISMA flow diagram

Five of the included studies utilised Periopaper for the sampling of the GCF [12, 19, 20, 22, 23], 3 studies utilised pre-cut chromatography strips (Whatman 3MM) [24–26] and 2 studies utilised micropipettes [11, 21]. The GCF sampling time presented significant variation across studies, ranging from 30 s [12, 20, 22] to 1 min [23] and 2 min [24–26], while some studies allowed the insertion of the strip or the pipette until 5 μhe pipette until [11, 21] with one study not reporting on the sampling time [19]. Regarding the storage of the samples, great variation was also observed: 2 studies stored their GCF samples at − 80 °C [19, 23], 1 study at − 76 °C [21], 4 studies at − 70 °C [22, 24–26], 1 study at − 26 °C [11] and 2 studies at − 20 °C [12, 20]. Eight of the included studies analysed the GCF samples using enzyme-linked immunosorbent assay (ELISA) [11, 12, 19–21, 23, 25, 26], 1 study used reverse-phase high-performance liquid chromatography with fluorimetric detection [22] and 1 study used multiplex beads assay [24]. Several GCF markers were investigated in the included studies. For facilitating the reader, the GCF markers were categorised under factors related with the healing of the epithelium, the connective tissue, the bone and others, even if some overlap might exist (Table 2).

The follow-up of the expression of GCF markers ranged from 21days [12] to 360 days [24], while the follow-up of the clinical parameters after treatment ranged from 90 [23] to 360 days [24]. However, there was rarely coincidence of the sampling times for the GCF markers with the clinical assessments postoperatively.

Finally, only 2 studies [21, 24] reported the postoperative occurrence of exposures. Gamal et al excluded the exposed sites from the study [21], while Rakmanee et al reported that 13 out of the 18 sites presented exposure of the membrane that was treated either with removal of the membrane (2 sites, classified as major exposure with size > 4 mm) or with administration of antibiotics (2 sites, classified as minor) [24].

## Synthesis of results

The results and conclusions of the individual studies included are presented in Table 2. Due to the significant heterogeneity of the included studies, in relation to the methodology employed, a meta-analysis was not performed.

### GTR

Regarding GTR, 7 studies reported on the expression of GCF markers postoperatively [11, 12, 21, 22, 24–26]. Both Gamal, 2011 [11] and Gamal 2016 [21] employed the same GCF sampling method using a micropipette inserted at 2mm depth in the sulcus and filled with 5μL of GCF. The samples were subsequently stored at -76C and analysed with ELISA. The concentrations of platelet-derived growth factor-BB (PDGF-BB) peaked during the early stages of healing (< 14 days) and decreased to baseline values by 30 days. Similarly, Rakmanee et al employing a different methodology, using pre-cut chromatography strips at the entrance of the gingival crevice for 2 min and stored at − 70C, found again increased PDGF-AB amounts 7 days postoperatively that decreased to baseline levels after 42 days [24].

**Table 1** Characteristics of the included studies. (*RCT*: randomized controlled clinical trial, *M* male, *F* female, *NR* not reported, *PI* plaque index, *FMPS* full mouth plaque score, *FMBS* full mouth bleeding score, *PD* probing depth, *PPD* probing pocket depth, *CAL* clinical attachment level, *BOP* bleeding on probing, *OFD* open flap debridement, *GTR* guided tissue regeneration, *EMD* Emdogain, *MIST* minimally invasive surgical treatment, *OH* oral hygiene instruction, *SRP* scaling and root planing, *BID* two times a day, *TID* three times a day, *d* day, *w* week, *CHX* chlorhexidine, *EGF* epithelial growth factor, *KGF* keratinocyte growth factor, *TGF-β1* transforming growth factor β1, *PDGF* platelet-derived growth factor, *VEGF* vascular endothelial growth factor, *FGF* fibroblast growth factor, *MMP-1* matrix metalloproteinase-1, *MMP-8* matrix metalloproteinase-8, *TIMP-1* metallopeptidase inhibitor-1, Ang-1 angiopoietin-1, *OPG* osteoprotegerin, *OCN* osteocalcin, *BMP-2* bone morphogenetic protein-2, *BMP-7* bone morphogenetic protein-7, *PAF* platelet activating factor, *sICAM-1* soluble intercellular adhesion molecule-1, *sLFA-3* lymphocyte function-associated antigen-3)

| Investigator, year (country) | Study design | Primary unit of analysis | Number of patients completed (m/f), age (mean) | Number of defects | Non-surgical protocol | Characteristics of the defects | Type of procedure | Details of technique |
|---|---|---|---|---|---|---|---|---|
| Agrali et al 2016 (Turkey) | RCT, parallel arm, not blinded | Defect | 12 (6/6), 44.17 | 30 | OH and SRP 8 weeks before surgery | PI < 1, full mouth BOP < 20%, PD ≥ 6 mm, radiographic depth ≥ 3 mm, variation of 1-, 2-, 3-walls included | EMD vs EMD + autograft vs OFD | Sulcular incisions, full-thickness flap reflection buccal and lingual |
| Ribeiro et al 2011 (Brazil) | RCT, parallel arm, blinded examiner | Patient | 29 (NR), 47.10 | 29 | OH and SRP 6 months before surgery | FMPS 16.91% for test and 15.79 for control, FMBS 11.99% for test and 9.33 for control, PD and CAL ≥ 5 mm, BOP(+), radiographic depth ≥ 4 mm and width ≥ 2 mm, number of walls NR | MIST + EMD vs MIST | Minimally invasive technique (MIST) *(Cortellini 2007)*, 4 mg dexamethasone 1 h prior |
| Kuru et al 2004 (UK) | Prospective cohort, examiner blinding NR | Defect | 27 (10/16), 38.1 | 27 | Initial periodontal therapy and reassessment | PI, FMPS, BOP, FMBS NR, PPD and lifetime cumulative attachment loss ≥ 5 mm, radiographic bone loss after initial periodontal therapy, number of walls NR | GTR vs OFD | For GTR: sulcular incisions, full-thickness flap reflection, PTFE membrane removed at 6w<br>For OFD: reverse bevel incisions, full-thickness flap reflection |
| Pellegrini et al 2017 (Italy, USA) | Prospective cohort, multi-centre, examiner blinding NR | Patient | 25 (9/16), NR | 25 | Non surgical periodontal treatment 4–6 months before surgery | FMPS 5.5% for test and 6.2% for control, FMBS 3.4% for test and 3.8% for control, PPD > 5 mm, CAL ≥ 6 mm<br>OFD: ≤ 3 mm intrabony<br>GTR: > 3 mm intrabony, number of walls NR | GTR vs OFD | For GTR: simplified or modified papilla preservation, Ti-dPTFE membrane, removed at 5–6weeks<br>For OFD: modified Widman flap |
| Gamal et al 2011 (Egypt) | RCT, split mouth, blinded examiner | Defect | 12 (NR), 38.2 | 24 | OH + SRP and re-evaluation at 4 weeks | PI 0.3 for test and 0.5 for control groups, matched 2-walled or 3-walled defects, PD > 6 mm, CAL > 4 mm, radiographic depth > 3 mm, premolars/molars | GTR vs OFD | Sulcular incisions, full-thickness flap reflection. For GTR, periosteum pedicle serving as membrane |
| Gamal et al 2016 (Egypt) | RCT, parallel arm, blinded examiner | Defect | 29 (NR), 31.5 | 29 | OH + SRP and re-e valuation at 4 weeks | PI 0.2 for test and control groups, single, 2-walled or 3-walled defect, PD ≥ 6 mm, CAL ≥ 5 mm, radiographic defect ≥ 3 mm, premolars/molars | GTR (occlusive) vs GTR (perforated) vs OFD | Internal bevel incisions, full thickness flap reflections. Occlusive is the standard collagen membrane. The perforated membrane is |

**Table 1** (continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | subject to 1 mm perforations with a pin |
| Keles et al 2006 (Turkey) | RCT, split mouth, blinded examiner | Defect | 15 (6/9), 42.27 | 30 | OH + SRP and re-evaluation at 4–6 weeks | PI 0.65 for test and 0.63 for control, paired, vertical interproximal osseous defects PD ≥ 6 mm, number of walls NR | GTR vs flap surgery | Sulcular incisions, full-thickness flap reflection, for GTR: absorbable polylactide membrane |
| Kuru et al 2005 (UK) | Prospective cohort, examiner blinding NR | Analysis for defect and patient as unit of analysis | 26, (11/24), 39.6 | NR | Non surgical periodontal treatment prior | PI/FMPS and BOP/FMBS NR, PD > 5 mm, lifetime cumulative CAL > 5 mm, radiographic evidence bone loss after initial periodontal therapy, number of walls NR | GTR vs flap surgery | Details of surgical procedures NR, for GTR: ePTFE membrane removed at 6 weeks |
| Okuda et al 2001 (Japan) | RCT, split mouth, double-blind | Patient | 16 (NR), NR | 36 (18 + 18), 2 patients contributed with 2 pairs of defects each | OH + SRP, occlusal adjustment if needed and re-evaluation 6 weeks later | PI 0.28 for test and 0.39 for control, BOP 89% for test and 83% for control, bilateral, one- or two-paired defects, PPD ≥ 6 mm, CAL ≥ 6 mm, osseous defect depth ≥ 4 mm with sounding or radiographically, minimum 2 mm keratinised gingiva, number of walls NR | EMD vs OFD | Sulcular incisions, vertical release incision 1 tooth away, full thickness flap reflection |
| Rakmanee et al 2018 (UK) | RCT, split mouth, blinded examiner | Patient | 16 (NR), NR | 32 | OH + SRP and reassessment 6 weeks later | FMPS 21.4%, FMBS 24.2%, bilateral defects, PPD ≥ 5 mm, radiographic evidence of bone loss ≥ 3 mm, variation of 1-, 2-, 3-walls included | GTR vs access flap | Minimally invasive technique–simplified papilla preservation flap *(Cortellini 1999)* |

| Investigator, year (country) | Postoperative medication | GCF sampling, storage, analysis | GCF markers | GCF follow-up (days) | Clinical follow-up (days) | PD change from baseline to final follow-up | Membrane exposures |
|---|---|---|---|---|---|---|---|
| Agrali et al 2016 (Turkey) | Amoxicillin + potassium clavulanate 1000 mg BID/7d, naproxen sodium 550 mg BID/7 days, 0.12% CHX BID/4 weeks | Periopaper for unspecified time, storage–80 °C, ELISA | TGF-β1 | Baseline, 7, 14, 30, 90, 180 | 180 | For OFD: from 7.6 to 3.2 mm. For EMD: from 8.3 to 3.3 mm. For EMD + autograft: from 7.93 to 3.22 mm. | None reported |
| Ribeiro et al 2011 (Brazil) | Paracetamol every 6 h for 2 days, 0.12% CHX BID for 15 days | Periopaper for 30 sec, storage–20 °C, ELISA | TGF-β1 OPG OCN | Baseline, 15, 90 | 90, 180 | For MIST: from 7.12 to 3.57 mm. For MIST + EMD: from 7.09 to 3.53 mm. | None reported |
| Kuru et al 2004 (UK) | 0.2% CHX BID for 2 weeks | Pre-cut chromatography strips (Whatman 3MM) at crevice entrance for 2 min, storage—− 70 °C, ELISA, pooled samples | T GF-ELas | Baseline, 14, 28, 42, 49, 84, 182 | 180 | For GTR: from 7.73 to 3.33 mm. For OFD: from 7.20 to 3.90 mm. | None reported |

**Table 1** (continued)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Pellegrini et al 2017 (Italy, USA) | Ibuprofen 600 mg pre-op and 6 h later and then if needed, 0.12% CHX TID for 3–4 weeks | Periopaper, 1 mm in crevice, until resistance for 30 sec, storage–20 °C, ELISA | T GF-ELas E-cadherin, EGF, VEGF, FGF-2, MMP-1, TIMP-1, BMP-7, OPG | Baseline, 3 to 5, 7, 14, 21 | 180 | For GTR: from 8.1 to 4.1 mm. For OFD: from 5.6 to 2.9 mm. | No exposure |
| Gamal et al 2011 (Egypt) | Amoxicillin 500 mg TID/7d, 0.12% CHX TID/2 weeks | Micropipette, mesio-facial line angle to maximum depth 2 mm until 5 μm until 5te, mesio-fa− 26 °C, ELISA | PDGF-BB | 2, 3, 7, 14, 30 | 90, 180, 270 | For GTR: from 6.1 to 2.6 mm For OFD: from 5.6 to 4.1 mm. | No exposure |
| Gamal et al 2016 (Egypt) | Amoxicillin 500 mg | Micropipette, mesio-facialline angle to maximum depth 2 mm until 5 μl until 5 μl collected, storage -76 °C, ELISA | PDGF-BB VEGF | 1, 3, 7, 14, 21, 30 | 90, 180, 270 | For GTR (occlusive): from 6.1 to 3.5 mm. For GTR (perforated) from 6.8 to 2.3 mm. For OFD: from 7.1 to 4.5 mm. | 7 patients excluded due to postoperative exposures of the membranes |
| Keles et al 2006 (Turkey) | None | Periopaper, in crevice until mild resistance for 30s, storage -70 °C, reverse-phase high performance liquid chromatography with fluorimetric detection | PAF | Baseline, 42, 84, 168 | 45, 90, 180 | For GTR: 4.5 mm change from baseline to 6 m. For flap surgery: 4.7 mm change from baseline to 6 m. | None reported |
| Kuru et al 2005 (UK) | None | Pre-cut chromatography strips (Whatman 3MM) at entrance of crevice for 2 min, storage −70 °C, ELISA, pooled samples | sICAM-1 LFA-3 | Baseline, 14, 28, 42, 49, 84 | NR | NR | None reported |
| Okuda et al 2001 (Japan) | Cefaclor 750 mg/5 days, 0.12% CHX TID/6 weeks | Periopaper, inserted until resistance for 60 sec, storage -80 °C, ELISA and one-step sandwich enzyme immunoassay | MMP-1 MMP-8 TIMP-1 | Baseline, 14, 28, 84 | 90 | For EMD: from 6.33 to 3.61 mm. For OFD: from 6.22 to 4.28 mm. | NR |
| Rakmanee et al 2018 (UK) | Ibuprofen 600 mg or paracetamol 500 mg, 0.2% CHX BID/6 weeks | Pre-cut chromatography strips (Whatman 3MM) at crevice entrance for 2 min, storage -70 °C, Multiplex Beads Assay | Ang-1 VEGF bFGF BMP-2 OPG TIMP-1 KGF PDGF-AB | Baseline, 3-5, 7, 14, 28, 42, 84, 180, 360 | 180, 360 | For GTR: 2.4 mm change from baseline to 12 m. For access flap: 2.5 mm change from baseline to 12 m. | 13/18 exposures: 2 major (> 4 mm), 2 removals, 2 minor required antibiotics (metronidazole 400 mg TID/2 weeks) |

Furthermore, Rakmanee et al found similar PDGF-AB levels both after GTR and after OFD that were accompanied by a similar clinical response. However, the sites subjected to GTR were associated with high rates of exposure (13/18) that may have significantly affected the regenerative process and thus the clinical response observed.

Furthermore, Rakmanee et al reported that GCF osteoprotegerin (OPG) amounts significantly increased 2–3 days postoperatively and subsequently declined [24]. No significant differences were noted between sites treated with GTR and sites treated with OFD. Pellegrini et al. using Periopaper inserted in the gingival crevice for 30 s found OPG levels to decrease following GTR and OFD; however, no comparison by treatment was reported for the change of the marker [12].

The expression levels of vascular endothelial growth factor (VEGF) were investigated by Rakmanee et al. [24] and Gamal et al. [21]. The former did not detect any significant difference in the change of VEGF GCF levels between sites treated with GTR and sites treated with access surgery using pre-cut chromatography strips [24]. However, the study by Gamal and co-workers, which used micropipettes, found that VEGF concentrations measured statistically significant higher concentrations in defects treated with OFD and GTR using a perforated membrane during the early postoperative period (days 1, 3 and 7) compared to defects treated using the occlusive membrane [21].

Kuru et al. 2004, using pre-cut chromatography strips at the entrance of the gingival crevice for 2 min, found increased transforming growth factor βi (TGF-β1 levels 2 weeks postoperatively, that however were not statistically significant and declined to below baseline levels by 4 weeks [26]. The change in the TGF-β1 levels was similar both after GTR and after OFD and accompanied a similar clinical response 6 months postoperatively.

## EMD

Regarding EMD, 3 studies reported on the expression of GCF markers [19, 20, 23]. Ribeiro et al., using Periopaper in the gingival crevice until resistance was felt and for 30 s, reported that TGF-β1 levels in sites treated with MIST and EMD significantly increased by 15 days postoperatively and the levels decreased after 3 months [20]. Furthermore, the changes for TGF-β1 levels were similar for sites treated with MIST and MIST with EMD and accompanied a similar clinical and radiographic response for both treatments. In contrast, Agrali et al. using Periopaper, inserted in the gingival crevice for unspecified amount of time, reported significantly higher TGF-β1 levels for EMD-treated defects compared with OFD-treated defects 7 and 14 days postoperatively [19]. In the same line, the authors concluded that defects treated with EMD presented a superior clinical and radiographic improvement compared with defects treated with OFD. It is however worth noting that the majority of the defects treated with EMD

**Table 2** Summary of the conclusions of the included studies. (*CAL* clinical attachment level, *PD* probing depth, *PPD* probing pocket depth, *OFD* open flap debridement, *MIST* minimally invasive surgical treatment, *GTR* guided tissue regeneration, *vs* versus, *EGF* epithelial growth factor, *KGF* keratinocyte growth factor, *TGF- βG* transforming growth factor β1, *PDGF* platelet-derived growth factor, *VEGF* vascular endothelial growth factor, *FGF* fibroblast growth factor, *MMP-1* matrix metalloproteinase-1, *MMP-8* matrix metalloproteinase-8, *TIMP-1* metallopeptidase inhibitor-1, *Ang-1* angiopoietin-1, *OPG* osteoprotegerin, *OCN* osteocalcin, *BMP-2* bone morphogenetic protein-2, *BMP-7* bone morphogenetic protein-7, *PAF* platelet activating factor, *sICAM-1* soluble intercellular adhesion molecule-1, *sLFA-3* lymphocyte function-associated antigen-3)

| FACTORS RELATED WITH THE HEALING OF EPITHELIUM | | | | | | |
|---|---|---|---|---|---|---|
| **GCF marker** | **Investigator, Year** | **Is periodontal surgery leading to significant changes in the expression of GCF markers?** | **Is there any significant difference in the expression of GCF markers for intrabony defects treated with GTR vs OFD?** | **Is the study powered for GCF markers, for clinical outcomes or both?** | **Is there any significant difference in the clinical response for intrabony defects treated with GTR vs OFD?** | **Is the expression of GCF markers directly associated with clinically significant changes following treatment?** |
| **E-cadherin** | Pellegrini et al 2017 | No significant change observed for E-cadherin for any group | Not reported | No power calculation reported | Higher percentage of 'better responders' in GTR vs OFD. | No conclusion can be drawn for the association of E-cadherin with the clinical outcome |
| **EGF** | Pellegrini et al 2017 | EGF levels significantly increased post-op in GTR | Not reported | No power calculation reported | Higher percentage of 'better responders' in GTR vs OFD. | No conclusion can be drawn for the association of EGF with the clinical outcome |
| **KGF** | Rakmanee et al 2018 | KGF amounts increased (non-significantly) at 7 days and decreased to baseline levels for GTR and access surgery | No significant differences in KGF amount between GTR and access surgery. | Powered for clinical outcomes | No significant differences for CAL gain, PPD reduction, radiographic bone fill and radiographic defect resolution for GTR or access surgery | The similar expression patterns of KGF accompanied a similar clinical response with GTR and access surgery |

| FACTORS RELATED WITH THE HEALING OF CONNECTIVE TISSUE | | | | | | |
|---|---|---|---|---|---|---|
| **GCF marker** | **Investigator, Year** | **Is periodontal surgery leading to significant changes in the expression of GCF markers?** | **Is there any significant difference in the expression of GCF markers for intrabony defects treated with GTR vs OFD?** | **Is the study powered for GCF markers, for clinical outcomes or both?** | **Is there any significant difference in the clinical response for intrabony defects treated with GTR vs OFD?** | **Is the expression of GCF markers directly associated with clinically significant changes following treatment?** |
| **TGF-β1** | Agrali et al 2016 | GCF volume and TGF-β1 levels increased at 7 days post-op and then decreased to below baseline levels (by 90 days) for both EMD and EMD + autograft | TGF-β1 could not be detected in 41% of OFD, 26% of EMD and 6% of EMD + autograft during the follow up (0 to 180 days). EMD and EMD+autograft showed significantly higher TGF-β1 concentrations at 7 days and TGF-β1 amounts at 14 and 180 days vs OFD. | Powered for clinical outcomes | Clinical and radiographic improvements noted for all groups. EMD and EMD + autograft presented statistical significantly higher CAL gain and radiographic defect fill vs OFD. No significant difference for EMD and EMD + autograft. | The trend for increased TGF-β1 expression observed in EMD and EMD + autograft correlates with a superior clinical response, compared to OFD. |
| | Ribeiro et al 2011 | TGF-β1 levels significantly increased after 15 days and reduced to baseline levels after 3 months for MIST and MIST + EMD. | Similar changes in TGF-β1 levels were observed for both groups, MIST and MIST + EMD | Powered for clinical outcomes | Similar clinical and radiographic improvements were noted for both groups | Similar expression patterns in TGF-β1 accompanied a similar clinical response with MIST and MIST + EMD |
| | Kuru et al 2004 | TGF-β1 levels increased two-fold 2 weeks post-op (not statistically significant), declined to levels lower than baseline after 4 weeks and remained stable until 26 weeks for GTR and conventional flap | TGF-β1 levels were similarly increased for GTR and conventional flap treated sites | Power calculation not reported | No statistically significant differences between GTR and conventional flap noted for clinical parameters 6 months post-op. | Similar expression patterns in TGF-β1 accompanied a similar clinical response with GTR and conventional flap |
| | Pellegrini et al 2017 | TGF-β1 levels were decreased compared to baseline following regeneration surgery | A downward trend was detected only after GTR but not after OFD (non-significant differences between groups) | No power calculation performed | A higher percentage of 'better responders 'in terms of PD and CAL was found in GTR vs OFD. | No conclusion can be drawn for the association of TGF-β1 with the clinical outcome |
| **PDGF** | Gamal et al 2011 | PDGF-BB concentrations peaked during the early post-op days (days 2 and 3) and decreased at 7,14, and 30 days in GTR and OFD | No significant difference was found in PDGF-BB concentrations between GTR with periosteum membrane and OFD sites. | Power calculation not reported | GTR led to statistically significantly higher PPD reduction, CAL gain and intrabony component reduction vs OFD. | No conclusion can be drawn for the association of PDGF levels with the clinical outcome |
| | Gamal et al 2016 | PDGF-BB concentrations at GTR with perforated membranes and OFD sites peaked during the early stages of healing (1-14 days) and then decreased at 21 and 30 days | PDGF-BB levels at GTR with perforated membranes and OFD sites showed statistically significant higher levels than GTR with occlusive membrane at 1, 3, 7, 14 days. PDGF-BB levels decreased gradually at days 21 and 30 in all groups with no significant differences. | Powered for GCF markers and clinical outcomes | GTR with perforated membrane showed a statistically significant improvement in PPD, CAL and intrabony defect vs GTR with occlusive membrane and OFD.<br>GTR with occlusive membrane-treated resulted in significant PPD reduction, CAL gain and reduction of the intrabony defect vs OFD. | No conclusion can be drawn for the association of PDGF levels with the clinical outcome |
| | Rakmanee et al 2018 | PDGF-AB amount increased early post-op (7 days) and decreased to baseline levels after 42 days for GTR and access surgery. | Similar changes for PDGF-AB were observed for GTR and access surgery. | Powered for clinical outcomes | No significant differences for CAL gain, PPD reduction, radiographic bone fill and radiographic defect resolution noted for GTR or access surgery. | Similar expression for PDGF-AB accompanied a similar clinical response after GTR and access surgery |
| **VEGF** | Pellegrini et | VEGF levels increased 3 | Not reported | No power | Higher percentage of | No conclusion can be |

| | | | | | | |
|---|---|---|---|---|---|---|
| | al 2017 | weeks following GTR | | calculation performed | 'better responders 'was found in GTR vs OFD. | drawn for the association of VEGF levels with the clinical outcome |
| | Gamal et al 2016 | VEGF concentrations peaked during the early post-op days (days 1-7) and decreased at 14, 21 and 30 days. | VEGF concentrations were significantly higher for OFD and GTR with perforated membrane during the early post-op days (days 1, 3 and 7) vs GTR with occlusive membrane. | Powered for GCF markers and clinical outcomes | GTR with perforated membrane showed a statistically significant improvement in PPD, CAL and intrabony defect vs GTR with occlusive membrane and OFD.<br>GTR with occlusive membrane showed a significant PPD reduction, CAL gain and intrabony defect reduction vs OFD | No conclusion can be drawn for the association of VEGF levels with the clinical outcome |
| | Rakmanee et al 2018 | In GTR and access surgery, the VEGF amount doubled early post-op (3-5 days) and decreased to baseline levels after 28 days. | No significant difference was detected in the change of VEGF for GTR and access surgery | Powered for clinical outcomes | No significant differences for CAL gain, PPD reduction, radiographic bone fill and defect resolution noted for GTR or access surgery. The total availability of VEGF at 30 days correlated with the clinical changes | Similar expression patterns in the levels of VEGF accompanied a similar clinical response with GTR and access surgery |
| **FGF** | Pellegrini et al 2017 | No significant change was observed for FGF-2 levels after OFD or GTR | Not reported | No power calculation performed | Higher percentage of 'better responders 'was found for GTR vs OFD. | No conclusion can be drawn for the association of FGF with the clinical outcome |
| | Rakmanee et al 2018 | 3-5 days post-operatively, bFGF amounts increased (not statistically significantly), peaked at 7 days and decreased to baseline levels for GTR and access surgery. | No significant differences in the bFGF amount were noted between GTR and access surgery. | Powered for clinical outcomes | No significant differences for CAL gain, PPD reduction, radiographic bone fill and radiographic defect resolution were noted for GTR or access surgery. | Similar expression patterns in the bFGF levels accompanied a similar clinical response with GTR and access surgery |
| **MMP-1** | Okuda et al 2001 | Intragroup analysis showed a significant decrease for MMP-1 from 2 to 4 weeks after surgery.<br>No significant changes noted for MMP-1 levels in the placebo group. | Intergroup analysis showed significantly lower MMP-1 levels at 2, 4 and 12 weeks in EMD vs placebo. | Power calculation not reported | Intragroup differences between baseline and 12 weeks showed a significant decrease in PPD and CAL.<br>No significant intergroup differences noted. | Despite the different expression patterns in the levels of MMP-1, a similar clinical response with EMD and control was noted. |
| | Pellegrini et al 2017 | MMP-1 levels increased in 'worse responders' and remained substantially unchanged in 'better responders'. | Not reported | No power calculation performed | Higher percentage of 'better responders 'was found in GTR vs OFD. | No conclusion can be drawn for the association of MMP-1 levels with the clinical outcome |
| **MMP-8** | Okuda et al 2001 | Intragroup analysis showed a significant increase in MMP-8 for EMD and control significantly increased at 2 weeks. There was a decrease in MMP-8 levels for EMD between 2 and 4 weeks and for both groups between 2 | Intergroup analysis demonstrated significantly lower MMP-8 levels for EMD vs placebo at 4 and 12 weeks | Power calculation not reported | Intragroup differences between baseline and 12 weeks showed a significant decrease in PPD and CAL.<br>No significant intergroup differences were noted. | Despite the different expression patterns in MMP-8 levels, a similar clinical response with EMD and control was noted. |
| **TIMP-1** | Okuda et al 2001 | Both EMD and placebo, presented a significant increase in TIMP-1 | Intergroup analysis demonstrated significantly lower mean | Power calculation not reported | Intragroup differences between baseline and 12 weeks showed a | Despite the different expression patterns in the levels of TIMP-1, a |

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| | | between baseline and 2 weeks. Significant decreases in TIMP-1 levels at 4 and 12 weeks when compared to 2 weeks were noted for both groups. | levels of TIMP-1 at 4 weeks for EMD vs placebo | | significant decrease in PPD and CAL. No significant intergroup differences were noted. | similar clinical response with EMD and control was noted. |
| | Pellegrini et al 2017 | Following GTR, a trend for increased TIMP-1 levels was observed | Not reported | No power calculation performed | Higher percentage of 'better responders 'was found for GTR vs OFD. | No conclusion can be drawn for the association of TIMP-1 levels with the clinical |
| | Rakmanee et al 2018 | 3-5 days post-op, TIMP-1 amounts increased significantly for GTR, access flap or control sites. At 7 days, TIMP-1 amount decreased to baseline levels for GTR, while access surgery sites presented reduced levels after 14 days | No significant differences in TIMP-1 amount were noted for GTR and access surgery. | Powered for clinical outcomes | No significant differences for CAL gain, PPD reduction, radiographic bone fill and radiographic defect resolution were noted for GTR or access surgery. | Similar expression patterns in levels of TIMP-1 accompanied a similar clinical response with GTR and access surgery |
| **Ang-1** | Rakmanee et al 2018 | 3-5 days post-op, Ang-1 amounts increased significantly, peaked at 7 days and decreased to baseline levels for GTR. For access flap, Ang-1 amount increased by 7 days and declined to baseline levels. | No significant differences in Ang-1 amount between GTR and access surgery. | Powered for clinical outcomes | No significant differences for CAL gain, PPD reduction, radiographic bone fill and radiographic defect resolution were noted between GTR and access surgery. | Similar expression patterns in the levels of Ang-1 accompanied a similar clinical response with GTR and access surgery |

| **FACTORS RELATED WITH THE HEALING OF BONE** | | | | | | |
|---|---|---|---|---|---|---|
| **GCF marker** | **Investigator, Year** | **Is periodontal surgery leading to significant changes in the expression of GCF markers?** | **Is there any significant difference in the expression of GCF markers for intrabony defects treated with GTR vs OFD?** | **Is the study powered for GCF markers, for clinical outcomes or both?** | **Is there any significant difference in the clinical response for intrabony defects treated with GTR vs OFD?** | **Is the expression of GCF markers directly associated with clinically significant changes following treatment?** |
| **OPG** | Ribeiro et al 2011 | Significant increases for OPG noted after 15 days, but no differences observed after 3 months | Similar changes in OPG levels observed for both groups, MIST and MIST + EMD | Powered for clinical outcomes | Similar clinical and radiographic improvements were noted for both groups | Similar expression patterns in the levels of OPG accompanied a similar clinical response with MIST and MIST + EMD |
| | Rakmanee et al 2018 | OPG amount significantly increased (2-fold) 3-5 days post-op for GTR and declined. A non-significant increase was noted for access surgery. | Similar OPG levels were observed for both groups, GTR and access surgery, that were significantly higher than healthy control sites | Powered for clinical outcomes | No significant differences for CAL gain, PPD reduction, radiographic bone fill and radiographic defect resolution were noted for GTR and access surgery. | Similar expression patterns in the levels of OPG accompanied a similar clinical response with GTR and access surgery |
| | Pellegrini et al 2017 | OPG levels were decreased after GTR and OFD. | Not reported | No power calculation performed | Higher percentage of 'better responders 'was found in GTR vs OFD. | No conclusion can be drawn for the association of OPG levels with the clinical outcome. |
| **OCN** | Ribeiro et al 2011 | No significant changes were noted for OCN levels up to 3 months post-op | No significant changes for OCN levels were observed for any group. | Powered for clinical outcomes | Similar clinical and radiographic improvements were noted for both groups | No conclusion can be drawn for the association of OCN levels with the clinical outcome. |
| **BMP-2** | Rakmanee et al 2018 | 3-5 days post-op, BMP-2 amounts increased (significantly for GTR), | No significant differences in the BMP-2 amount between GTR | Powered for clinical outcomes | No significant differences for CAL gain, PPD reduction, radiographic | Similar expression patterns in the levels of BMP-2 accompanied a similar |
| | | peaked at 7 days and decreased to baseline levels for GTR and access surgery | and access surgery were noted. | | bone fill and radiographic defect resolution were noted between defects treated with GTR or access surgery. | clinical response with GTR and access surgery |
| **BMP-7** | Pellegrini et al 2017 | BMP-7 levels tended to increase for 'better responders' (by means of PD and CAL) and to decrease for 'worse responders'. | Not reported | No power calculation performed | Higher percentage of 'better responders' was found for GTR vs OFD. | No conclusion can be drawn for the association of BMP-7 levels with the clinical outcome |

| OTHER GCF FACTORS | | | | | | |
|---|---|---|---|---|---|---|
| **GCF marker** | **Investigator, Year** | **Is periodontal surgery leading to significant changes in the expression of GCF markers?** | **Is there any significant difference in the expression of GCF markers for intrabony defects treated with GTR vs OFD?** | **Is the study powered for GCF markers, for clinical outcomes or both?** | **Is there any significant difference in the clinical response for intrabony defects treated with GTR vs OFD?** | **Is the expression of GCF markers directly associated with clinically significant changes following treatment?** |
| **PAF** | Keles et al 2006 | Significant decreases in PAF levels for GTR and flap surgery were noted at 6, 12 and 24 weeks post-operatively compared to pre-surgery | No significant differences in PAF levels between the study groups pre-operatively and at 6, 12 and 24 weeks postoperatively were noted | Power calculation not reported | Both treatment modalities significantly reduced the PPD and improved the CAL. No significant differences were observed between the groups. | Similar expression patterns in the levels of expression of PAF accompanied a similar clinical response with GTR and flap surgery |
| **sICAM-1** | Kuru et al 2005 | For sites treated with GTR and conventional flap, the total amount of sICAM-1 increased during the initial 2-4 weeks (non-significantly) and then reduced at a lower level than baseline up to 12 weeks | No significant differences in sICAM-1 amount between the groups were noted | Power calculation not reported | Not reported | No conclusion can be drawn for the association of the sICAM-1 levels with the clinical outcome. |
| **sLFA-3** | Kuru et al 2005 | For sites treated with GTR and conventional flap, the total amount of sLFA-3 significantly increased at 2 weeks and thereby returned to baseline levels | No significant differences in sLFA-3 amount between the groups were noted | Power calculation not reported | Not reported | No conclusion can be drawn for the association of the sLFA-3 levels with the clinical outcome. |

| | No significant difference between | | Significant difference between | | Data is Inconclusive |
|---|---|---|---|---|---|
| | groups | | groups | | |

were localised at anterior teeth, compared with the majority of the sites treated with OFD that were localised at molar teeth.

Okuda et al. described an increase for matrix metalloproteinase-8 (MMP-8) and metallopeptidase inhibitor 1 (TIMP-1) GCF levels 2 weeks postoperatively for defects treated with EMD and OFD that thereafter declined, more dramatically for EMD-treated defects [23]. MMP-1 levels significantly decreased from 2 to 4 weeks postoperatively for defects treated with EMD [23].

Ribeiro et al. using Periopaper in the gingival crevice for 30 s concluded that OPG levels increase 15 days postoperatively and similar changes are noted after both MIST and MIST with EMD [20]. Interestingly, Rakmanee et al. concluded that the OPG amount significantly increased 2–3 days postoperatively and subsequently declined with no significant differences between sites treated with GTR and OFD [24]. Consequently, similar changes were noted for the OPG levels after MIST and MIST with EMD or after GTR and access flap. Furthermore, both studies reported similar clinical and radiographic improvements for both treatment groups [20, 24]; thus, the similar expression patterns in the levels of expression of OPG accompanied a similar clinical response, irrespective of the surgical technique employed.

### GTR and EMD

Regarding the combination of GTR and EMD, Agrali et al. reported on the levels of TGF-β1. The combination-treated defects, similarly distributed to anterior and posterior teeth, presented similar changes in the TGF-β1 levels as the EMD-treated defects in the first 2 postoperative weeks [19]. The similar changes of TGF-β1 levels accompanied a similar clinical response for EMD and EMD with GTR, that was superior to OFD. However, as discussed previously the majority of the defects treated with OFD were localised in posterior teeth.

### OFD

Finally, regarding OFD alone, there is agreement between two investigations that an increase in TGF-β1 levels is initially observed, accompanied by a return to baseline levels by 14 days

[19, 26]. For PDGF, conflicting results are presented; Gamal and co-workers reported an initial increase and a decrease by 7 days postoperatively to below baseline levels [11]. In contrary, another investigation from the same group reported a decrease for the PDGF levels that continued until 30 days postoperatively [21]. Rakmanee et al. noted an increase in the PDGF amount after OFD that continued until 3 months postoperatively [24].

For the remaining markers and for more detail regarding the expression of the investigated GCF markers after the surgical treatments, the reader is referred to the detailed Table 2.

### Risk of bias assessment

The risk of bias assessment is presented in Fig. 2 and Table 3. Seven of the included studies (RCTs) were assessed using the Cochrane Collaboration tool [11, 19–24]. Four of the seven studies were of low risk of bias in all but one domain [11, 20, 22, 24], two were of low risk of bias in five domains [21, 24] and one [19] was of high risk of bias. The remaining three studies [12, 25, 26] were prospective cohort studies and were assessed using the MINORS tool. These studies were rated with 16 to 18, indicating high quality of the included studies.

## Discussion

This systematic review identified 15 GCF markers expressed after surgical treatment of intrabony defects (GTR, OS, OFD). For 7 of those, most of which are related with the healing of connective tissue, TGF-β1, PDGF, VEGF, FGF, MMP-1, TIMP-1 and OPG, data was available from more than one investigation. While for the majority of factors a definitive conclusion cannot be reached, robust suggestions can be drawn regarding the OPG levels in regenerative surgeries. In two investigations, employing different GCF sampling and storing techniques, the OPG levels after MIST or MIST with EMD and after GTR or access flap similarly increased within the two postoperative weeks and thereafter declined [20, 24]. Furthermore, both studies reported similar clinical and radiographic improvements; thus, the similar expression patterns of OPG likely accompanied a similar clinical response. OPG acts as a soluble decoy receptor, binding to the receptor activator of nuclear factor-kappa B (RANKL) and inhibiting the osteoclastogenic action [27]. Therefore, OPG has been identified as a critical factor in bone formation and the regulation of bone resorption.

The finding of this review however comes in contrast with a human polymerase chain reaction (PCR) study assessing the

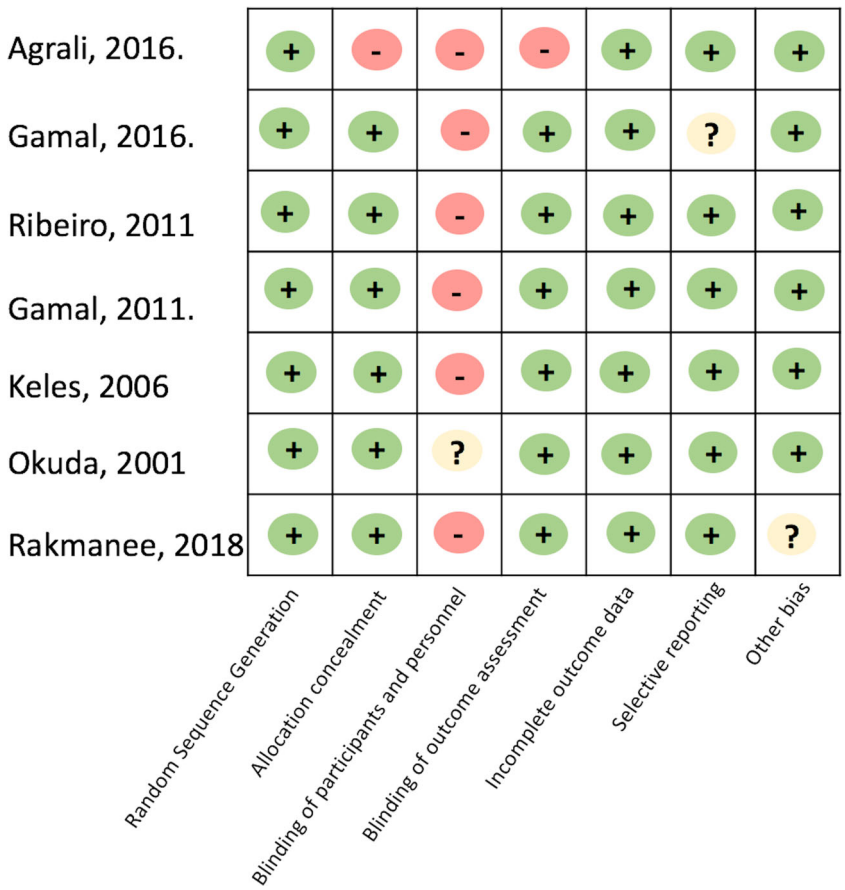


**Fig. 2** Risk of bias assessment of RCTs using the Cochrane Collaboration tool

**Table 3** Risk of bias assessment of observational studies using the MINORS tool. Studies were assessed in 12 items and can receive 0–2 points for each for a total score ranging from 0 to 24 points. Studies with fewer than 16 points are considered of low quality while high quality studies need to have a score of greater than or equal to 16

| Minors tool | Kuru L, J Periodontol. 2005 | Kuru L, J Clin Periodontol. 2004 | Pellegrini, J Periodont Res 2017 |
|---|---|---|---|
| A clearly stated aim | 2 | 2 | 2 |
| Inclusion of consecutive patients | 0 | 0 | 0 |
| Prospective collection of data | 2 | 2 | 2 |
| End points appropriate to the aim of the study | 2 | 2 | 2 |
| Unbiased assessment of the study end point | 0 | 0 | 0 |
| Follow-up period appropriate to the aim of the study | 2 | 2 | 2 |
| Loss to follow-up < 5% | 2 | 2 | 1 |
| Prospective calculation of the study size | 0 | 0 | 0 |
| An adequate control group | 2 | 2 | 2 |
| Contemporary groups | 2 | 2 | 2 |
| Baseline equivalence of groups | 0 | 2 | 1 |
| Adequate statistical analyses | 2 | 2 | 2 |
| Total | 16 | 18 | 16 |

gene modulation 21 days following treatment of intrabony defects with either GTR with an expanded polytetrafluorethylene (ePTFE) membrane or flap surgery [28]. Among others, OPG mRNA levels were significantly higher in GTR sites, compared with access flap sites. Furthermore, in an investigation of the gene expression profile of cells derived from GTR subjected defects (regenerating-tissue derived cells–RTCs), a differential and highlighted expression of the gene encoding OPG (*TNFRSF11B*) was found compared with matched periodontal ligament mesenchymal cells (PLCs) [29]. These contrasting results may be due to the high rate of exposures (13/18 GTR sites) in the study by Rakmanee et al. that may have significantly affected the regenerative process and thus the OPG expression [24]. In addition, the Ribeiro et al. investigation, as most of the included studies, was not powered for GCF and could therefore lack statistical power to detect differences in the expression levels between treatments [20].

Interestingly, Okuda et al. found that the use of EMD in intrabony defects resulted in an early postoperative increase for MMP-8 and TIMP-1, followed by an accelerated return to baseline levels, when compared to OFD [23]. This highlighted reduction may associate with an EMD-induced accelerated pattern of wound healing and resolution of inflammation moving towards regeneration rather than repair.

With respect to PDGF, three isoforms exist (AA, AB, BB). Two studies included in this review [11, 24] demonstrated that GTR and access flap lead to similar changes: an initial increase of PDGF-BB [11] and PDGF-AB [24] during the early healing period (up to 7 days), accompanied by a decrease to baseline levels. Two PDGF receptors exist, the PDGF-Rα and the PDGF-Rβ who binds PDGF-AB with low and PDGF-BB with high affinity [30]. In contrast with the included in this review studies, a significant upregulation of PDGF-Rβ in regenerating periodontal tissues has been observed [31] suggesting that the ligands are involved in the early cascade of events involved in regeneration.

Furthermore, in the only study powered for GCF markers [21], perforated PTFE membranes were shown to result in significantly higher VEGF during the early healing period (1, 3 and 7 days) when compared to occlusive membranes. In an animal model of GTR using porcine extracellular matrix (ECM), membrane cells recruited early postoperatively into the membrane compartment result in highlighted expression of, among other factors, VEGF at the RNA level. The VEGF expression was significantly highlighted 3 days postoperatively and thereby decreased by 28 days [32]. This VEGF upregulation, along with other regenerative molecules, early postoperatively in the mRNA and the protein level, may suggest that the membrane itself acts as a bioactive compartment guiding the regenerative process and not solely as an active barrier.

TGF-β1 is a connective tissue cell signalling protein that plays a critical role in several stages of wound healing, as it promotes the mitogenic activity of gingival and periodontal ligament cells and the upregulation of extracellular matrix components [33, 34]. With regards to TGF-β1, the existing literature is conflicting. The studies included in this review studies suggest that the clinical and radiographic outcome may be related to the TGF-β1 level changes [20, 26]. Ribeiro et al and Kuru et al showed that GTR or OFD and MIST or EMD treatments exhibited similar TGF- β1 increase early post-operatively, as well as similar clinical and radiographic improvements [20, 26]. However, the significant clinical and radiographic improvement following EMD in Agrali's study was associated with a significant TGF- β1 increase for EMD at anterior teeth,

in contrast with OFD at mainly posterior teeth [19]. When OFD was employed alone for the treatment of intrabony defects, two investigations agreed that an increase in TGF-β1 levels is initially observed, accompanied by a return to baseline levels by 14 days [19, 26]. In the same line, in an immunocytochemistry investigation in biopsies, a highlighted increase was noted for TGF-β1 receptor in regenerating periodontal tissues (6 weeks), while the receptor was almost undetectable in healthy tissues [31]. The highlighted receptor presence in regenerating tissues may suggest that the corresponding TGF-β1 plays a pivotal role in the early healing.

As it became evident, an important limitation was that only one of the ten included studies [21] was powered to detect significant differences in the GCF markers, whereas the remaining nine studies were either powered for the clinical outcomes or did not report any power calculation. Furthermore, inclusion of intrabony defects with varying number of defect walls does not allow for meaningful conclusions as regeneration is more likely to occur in 3-walled defects and to be accompanied by a different array of GCF markers compared to a 1-wall defect. In addition, reportedly, a large variation across investigations was observed in the methodology employed for the GCF sampling, storage and detection. These variations would introduce confounders if a meta-analysis was attempted. For example, the sampling methods (Periopaper, micropipette), the duration of collection (30 s, 2 min, until a specific volume is collected), the depth of strip insertion (entrance of the pocket or full depth), the storage (temperature) or the preparation of the samples (processing individual or pooled samples) introduce variations that would affect the conclusions drawn and their generalisability. Furthermore, it becomes imperative that more well-designed, powered studies with sampling periods reflecting the regenerative process are needed. Future investigations should employ standardised protocols for GCF sampling, processing and storage.

In conclusion:

There is limited evidence available on the expression of markers of angiogenesis, regeneration and inflammation in the GCF in the early and late healing after surgical interventions of intrabony periodontal defects

OPG is increased early postoperatively, irrespective of the surgical technique employed

A trend is noted for TGF-β1 increase early postoperatively, irrespective of the surgical technique employed. A highlighted increase is noted after use of EMD at anterior teeth that may relate with an improved clinical outcome.

More well-designed, powered studies with sampling periods reflecting the regenerative process are needed

Future research should focus on employing standardised protocols for collecting, storing and analysing GCF markers and establishing adequate statistical power to reach conclusions that may shed light in the biological events involved in the early periodontal wound healing and thus facilitate the development of predictable regenerative treatments

## 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.

**Informed consent** For this type of study, formal consent is not required.



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