Title

Recombinant Factors for Periodontal Intrabony Defects: A Systematic Review and Network Meta-Analysis of Preclinical Studies

Authors:

Shahnawaz Khitmatgar[2,3,#,*], Saurav Panda[1,2,#], Mohit Das[1], Heber Arbildo-Vega[4,5], Massimo Del Fabbro[2,6]

[#]Both the authors have equal contribution

Affiliation:

[1] Department of Periodontics and Oral Implantology, Institute of Dental Sciences, Siksha O Anusandhan (Deemed to be) University, Bhubaneswar, Odisha, India – 751003

[2] Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy

[3] Nitte (Deemed to be University), AB Shetty Memorial Institute of Dental Sciences, Department of Oral Biology and Genomic Studies, Mangalore, India.

[4] Department of General Dentistry, Dentistry School, Universidad San Martín de Porres, Chiclayo 14012, Peru

[5] Department of General Dentistry, Dentistry School, Universidad Particular de Chiclayo, Chiclayo 14012, Peru

[6] IRCCS Istituto Ortopedico Galeazzi, Milano, Italy.

Corresponding Author:

Professor Massimo Del Fabbro

Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy

Email: massimo.delfabbro@unimi.it

Abstract

The use of bioactive agents combined with osteoconductive scaffolds for the regeneration of periodontal intrabony defects has been the subject of intensive research in the past 20 years. Most studies reported that such agents, used in different concentrations, doses and combined with various scaffolds, might promote periodontal tissue regeneration, but evidence for the most effective combination of such agents is lacking. The objective of this study was to rank the different combinations of recombinant human-derived growth and differentiation factors with/without scaffold biomaterial in the treatment of periodontal intrabony defects, through network meta-analysis of pre-clinical studies. The systematic review and network meta-analysis protocol was registered on the PROSPERO Systematic Review database with reference number: CRD42021213673. Relevant published articles were obtained after searching five electronic databases. A specific search strategy was followed by using keywords related to intrabony defects, regenerative materials, scaffolds and recombinant factors, and animal studies. All pre-clinical studies used for periodontal regeneration were included. The primary outcomes were: regeneration of junctional epithelium (mm), new cementum, connective tissue attachment, percentage of new bone formation (%), bone area (mm^2), bone volume density (g/cm^3) and bone height (mm) data was extracted. The analysis was carried out using network meta-analysis methods, i.e. illustrating network plots, contribution plots, predictive and confidence interval plot, surface under the cumulative ranking (SUCRA), multidimensional scale ranking and net funnel plots using STATA IC statistical software. An SYRCLE's tool for assessing risk of bias was used for reporting risk of bias among individual studies. A total of N=24 for qualitative and N=21 studies for quantitative analysis published till 2020 were included. The cumulative total number of animals included in the control and test groups were N=162 and N=339, respectively. The duration of the study was between 3 and 102 weeks. rhBMP-2 ranked higher in SUCRA as the agent associated with the best performance for bone volume density. rhGDF-5/TCP ranked best in the bone area (mm2), rhPDGF-BB/Equine ranked best in bone height (mm), rhBMP-2 ranked best in the percentage of new bone fill, rhBMP-2/ACS ranked best in new cementum formation, and rhGDF-5/b-TCP/PLGA ranked best in connective tissue attachment and junctional epithelium. There were no adverse effects identified in the literature that could affect the different outcomes for regeneration in intrabony defects. Various recombinant factors are effective in promoting the regeneration of both soft and hard tissue supporting structures of the periodontium. However, when considering different outcomes, different agents, associated or not with biomaterials, ranked best. Keeping into account the limited transferability of results from animal studies to the clinical setting, the choice of the most appropriate formulation of bioactive agents may depend on clinical needs and purpose.

Key Words

rhPDGF; recombinant human growth factor; Network Meta-analysis; Systematic review; Biomaterial; Bioactive agents; Scaffold; bone regeneration; intrabony defects; Periodontal regeneration

Introduction

One of the most important factors for periodontal regeneration is the mechanical stabilisation of blood clot (1,2). Effective stabilisation of blood clot would facilitate all the steps of the healing process, optimising the interaction among the various factors involved in the healing process. Although conventional flap techniques in many cases may be effective in reducing the existing periodontal defect, the introduction of bioactive agents and biomaterials in the last decades represented a breakthrough to enhance periodontal regeneration, especially in the most severe cases (3). Several natural or synthetic materials like enamel matrix proteins (EMD), collagen membranes and, more recently, recombinant human (rh) growth and differentiation factors such as platelet-derived growth factor (PDGF), Insulin growth factor-1 (IGF-1), Transforming growth factor- β (TGF-β), and bone morphogenetic protein-2 (BMP-2), have been used over the years. Many experimental studies proved the efficacy of such bioactive agents in tackling the challenges of most types of periodontal defects, by promoting periodontal healing, especially when combined with osteoconductive scaffolds (4,5). However, these factors have been used in different concentrations, doses and combined with different scaffolds, which makes difficult to understand if there is some combination that works better than others do.

Darby & Morris (2013) and Khoshkam et al (2015) aimed to conduct meta-analysis on rh factors for periodontal regeneration and intrabony defects, respectively (6,7). Conventional pairwise meta-analysis may determine the efficacy/effectiveness of any given bioactive agents against control, but no hierarchy of such agents can be determined, in the absence of direct comparison. Network meta-analyses (NMA) may address this limitation, allowing to establishing a ranking of different treatments, based on both direct and indirect comparisons. Therefore, pairwise meta-analysis and network meta-analysis serve different purposes (8,9). NMA would provide directions for future preclinical studies by helping researchers by providing an updated information and minimizing cost incurred while conducting such researches. Currently, there are no published NMAs on rh factors in combination with scaffolding biomaterial and bioactive agents, attempting to demonstrate the most effective combination for periodontal regeneration procedures. Therefore, our aim was to systematically search for published pre-clinical studies that investigated the effect of recombinant human growth and differentiating factors with/without scaffolding biomaterials for the regeneration of periodontal intrabony defects, and perform a comprehensive network meta-analysis to determine the best combination for periodontal regeneration.

Methods

We followed the guidance from the PRISMA (Preferred Reporting Items of Systematic Reviews and Network Meta-Analyses) Checklist to report this systematic review (10) and SYRCLE guidelines were followed to report the risk of bias (ROB) (11). The Network Meta-analysis protocol was registered on the PROSPERO database with registration number: CRD42021213673

Review Question

What is the effect of recombinant human-derived growth and differentiating factors and other bioactive agents with/without scaffold biomaterial in the treatment of intrabony defects in terms of parameters related to periodontal regeneration?

Inclusion Criteria

The PICOS question was used to determine the population, intervention, comparator, outcomes and study design in our review.

Population (P)

All animal models that included experimental intrabony defects and have used recombinant human-derived growth and differentiating factors and bioactive agents with/without scaffold biomaterial were included in the analysis.

Intervention/exposure (I)

Recombinant human-derived growth and differentiating factors and bioactive agents with/without scaffold biomaterial were used for regenerative treatment of experimental intrabony periodontal defects.

Comparators/Control (C)

The same intervention without bioactive agents.

Outcome Measures (O)

The outcome measures like bone area (mm2), Bone density, Bone Height (mm), Connective tissue attachment, new cementum formation and junctional epithelium with mean and SD or mean and SD differences will be extracted. Histological and histomorphometric data of new bone formation (%) (mean values, SD) will be included. The studies which reported only median, ranges, relative changes or no SD values and 95% confidence interval were excluded from the analysis.

Study design (S)

Pre-clinical studies in which intrabony defects have been created, with any follow-up duration were included in the review. The in vitro studies, laboratory studies, and all clinical studies like experimental and observational studies, were excluded from the review.

Other limitations applied

Further specific inclusion criteria were:

1. The defect in the animal model consisted of a horizontal bone defect involving tooth from the bottom to the fornix of the furcation.

2. Notches on the internal root surfaces created at the level of the reduced bone.

Search strategy

The strategy for screening included articles was illustrated in prisma flow chart (Figure 1). MEDLINE, EMBASE, Web of Science, Scopus, Cochrane Central Library were searched from their inception to till date. A range of text words and indexed terms related to "intrabony defects" "growth factors" "Recombinant" "Bioactive agents" and "Animal studies" "Preclinical Studies" were searched. The search strategy includes ((((((intra-bony defect) OR (intrabony defect)) OR (intrabony defects)) OR (periodontal defect)) OR (intraosseous defects)) AND ((((((((((((recombinant human platelet-derived growth factor) OR (rhpdgf)) OR (PDGF)) OR (rhPDGF)) OR (recombinant human fibroblast growth factor)) OR (rhFGF)) OR (insulin growth factor)) OR (IGF)) OR (Vascular endothelial growth factor)) OR (VEGF)) OR (recombinant human growth/differentiation factor)) OR (GDF)) OR (Recombinant human bone))) AND ((((((((((Cats) OR (cats)) OR (feline cats)) OR (feline)) OR (((((Dogs) OR (dog)) OR (dogs)) OR (canine)) OR (canines))) OR (((Guinea Pigs) OR (guinea pig)) OR (guinea pigs))) OR (((((((Mice) OR (mice)) OR (mice model)) OR (mouse)) OR (mouse model)) OR (mus)) OR (murine))) OR (((Primates) OR (non-human primate)) OR (non-human primates))) OR (((((((((Rabbits) OR (rabbit)) OR (rabbits)) OR (lagomorpha)) OR (Rats)) OR (rat)) OR (rats)) OR (rodent)) OR (rodents))) OR ((((Sheep) OR (sheep)) OR (ovis)) OR (ovine))) OR (((((Swine) OR (swine)) OR (pig)) OR (pigs)) OR (porcine))). The reference lists of studies meeting the inclusion criteria were searched to identify additional relevant studies. Two researchers (SK, SP) screened references for eligibility independently. Study authors were contacted to obtain relevant missing data if necessary.

The published articles retrieved using the search strategy and those included from the additional sources were screened independently by two review authors to identify studies that potentially meet the inclusion criteria. The full text of selected studies was retrieved and independently assessed by eligible members of the review. Any disagreement between the two authors was discussed with the third author/reviewer. A standardised PRISMA flow chart was used to summarise the selection process.

Data extraction

Extracted data include year, author, study id, type of animal model used, number and type of animals, number of defects, number of osseous walls, defect depth, healing, period, healing type, and histologic results.

The histological outcome measures that determine the success and failure in treating intrabony defect included: long junctional epithelium (LJE), epithelium down growth covering the tooth treated surface, connective tissue attachment, new cementum with inserting collagen fibres on the treated root surface but not in contact with opposing new bone, connective tissue adhesion, connective tissue contact with the root without apparent cementum formation, regeneration new cementum, new inserting fibres, new bone composing a new PL structure, osseous repair, a new bone filling opposite tooth surface leading to defect filling.

Histomorphometric data included new cementum (NC), new bone (NB) formation and collagen fibres (CF). In addition, linear measurements (in mm) of the junctional epithelium (JE) on the tooth surface

was presented as a percentage of defect depth resolution. Missing data were requested from to study authors.

The studies selected for full analysis underwent extraction of the following data;

1. Junctional Epithelium
2. New Cementum formation
3. Bone Area (mm^2)
4. Bone Volume Density (g/cm^3)
5. Bone Height (mm)
6. Connective Tissue attachment levels
7. Amount of bone fill (%) after surgery (Histology, Histomorphometric or Radiographic)

Risk of bias (ROB) / quality assessment

SYRCLE guidelines were followed to report the risk of bias (ROB). The ROB was assessed by two independent reviewers, and the discrepancies will be resolved by discussion and in consultation with a third reviewer. The studies were graded under high, uncertain or low risk, based on subjective bias such as randomisation and blinding, outcome measures, statistical methods, experimental animals used, experimental procedures and results.

Data Synthesis

For pairwise meta-analysis, the overall effect size was estimated using STATA 16.1 version. A generic inverse variance random-effects model was used to pool the mean difference (MD) with a 95% confidence interval (CI) on continuous outcomes. It is anticipated that the units of the outcome measures used across studies may not be consistent, and therefore it is likely that the effects were to be reported as standardised mean differences (SMD) rather than MD. A random-effects model was used as it incorporates heterogeneity both within and between studies (Higgin and Green, 2011). E.g. heterogeneity between studies' follow-up length was accounted for by the use of a random-effects model. An overall effect size with 0.2-0.5 was regarded as small, 0.5-0.8 as moderate and more than 0.8 as large (Cohen, 1988). In the case of missing or incomplete data, we contacted study authors to obtain the required data.

Network Meta-analysis

The data suitable for NMA were used to generate a network geometry plot, where nodes indicate the number of subjects involved in specific intervention and edges represents the direct comparison between pairs of treatment. The contribution plot helps in identifying the large and small contribution of evidence in a network. A significant inconsistency formed in direct and indirect loops and suggesting

that they do not differ significantly was reported by inconsistency plot. It is essential to know the results of future clinical studies and expected to lie. Therefore, a predictive and confidence interval plot was generated. The surface under the cumulative ranking curves (SUCRA) expresses the percentage of effectiveness/safety each treatment has compared to an 'ideal' treatment always ranked first without uncertainty (Salanti, 2011). Also, the mean rank, which is the mean of the distribution of the ranking probabilities, was reported. The multidimensional ranking was used to rank the materials based on their dissimilarity between the two treatments. Net funnel plots are most commonly used to report publication bias that helps in assessing small study effects. The grading of direct, indirect and network evidence was based on A GRADE Working Group approach for rating the quality of treatment effect estimates from network meta-analysis (12).

Heterogeneity

Heterogeneity between study was assessed using the I^2 statistic, which describes the percentage of variation across studies that is due to heterogeneity rather than chance. Rules of thumb for interpretation of this statistic suggest that I^2 >30% equates to moderate heterogeneity, I^2 >50% equates to substantial heterogeneity, and I^2 >75% equates to considerable heterogeneity (Higgin and Green, 2011). For all I^2 values about 50%, we investigated sources of heterogeneity.

Results

A total of 24 pre-clinical studies were included for qualitative analysis. These include Lee et.al 2020 (22); Bae et.al 2018 (17); Ogawa et.al 2016 (18); Bizenjima et.al 2014 (19); Du bing et.al 2014 (20); Park JC et.al 2012 (23); Lee et.al 2012 (24); Leknes et.al 2012 (25); Nevins et.al 2012 (26); Oortgiesen et.al 2012 (27); Emerton et.al 2011 (28); Min et.al 2011 (29); Markapoulou et.al 2011 (30); Irokowa et.al 2010 (31); Kwon et.al 2010 (32); Lee et.al 2010 (33); Shirakata et.al 2010 (34); Oi et.al 2009 (35); Qahash et.al 2007 (36); Blumenthal et.al 2002 (37); Choi et.al 2002 (38); Giannobile et.al 1998 (39); Giannobile et.al 1996 (40); Giannobile et.al 1994 (41) (Figure 2). In the control group, a total of n=157 defects were assessed, and in the test group n=352 defects were assessed. The majority of the studies were funded (i.e. n=19). The duration of studies was between 3 and 102 weeks. The mean intrabony defect size for the control and test group was 4.81±0.86mm and 4.64±0.26mm, respectively. A majority of studies included beagle dogs intrabony defect models (n=128) (Figure 2). The outcomes that determined the successful hard and soft tissue regeneration were junctional epithelium, new cementum, percentage of new bone fill, a bone area in mm^2, bone volume density, bone height in mm, connective tissue formation. The inconsistency plot was not possible, as there were no loops formed between the interventions.

Junctional Epithelium

The network geometry plot illustrates the network of interventions in junction epithelium outcomes using nodes and edges (Suppl. Fig.1). The evidence of direct contribution was by all the interventions (100%) and indirect contribution equally by all interventions (50%). The evidence flow from the entire network was 16.7% from all the interventions (Suppl. Fig.2). The risk of bias among interventions between Con Vs bFGF was low, and Con Vs rhGDF-5/b-TCP was high. All other interventions were found to be a

moderate risk of bias (Suppl. Fig.3). rhBMP-2/CaSO4, bFGF, rhGDF-5/b-TCP and rhGDF-5+b-TCP/PLGA would perform best in future clinical studies as demonstrated in predictive interval plot (Suppl. Fig.4). rhGDF-5+b-TCP/PLGA was ranked higher in SUCRA ranking (Figure 2) and found to be the most dissimilar material as illustrated in multidimensional scale ranking (MDS) (Suppl. Fig.5). The net funnel plots illustrating the publication bias for JE outcome (Suppl.Fig.6).

Connective Tissue Attachment

The most common comparison was between Con Vs IGF-1, as illustrated in the network geometry plot (Suppl. Fig.7). The contribution plot and the ROB for each comparison were illustrated in Suppl.Fig.8 and 9. Osteogenic protein-1, IGF-1, rhBMP-2/ACS, rhBMP-2/CaSO4 predicted to perform better in future in clinical studies (Suppl. Fig.10). rhGDF-5+b-TCP/PLGA was ranked higher in SUCRA ranking and projected as the best material in bone regeneration in intrabony bone defects (Figure 5). b-FGF was ranked higher in the MDS plot as being the most dissimilar material in connective tissue attachment (Suppl. Fig.11).

New Cementum

The most common comparison was between Con Vs rhBMP-2, Con Vs rhGDF-5 and Con Vs rhGDF-5+b-TCP/PLGA (Suppl. Fig.12). The risk of bias was moderate between Con Vs rhGDF-5 and Con Vs rhGDF-5+b-TCP/PLGA and high risk of bias for Con Vs rhBMP-2 (Suppl. Fig.14). The contribution of evidence flow was equally distributed among each intervention (Suppl. Fig.13). rhBMP-2/ACS, rhBMP-2/CaSO4, rhGDF-5/b-TCP, and rhGDF-5+b-TCP/PLGA would be likely to perform better in future clinical studies as illustrated in predictive interval plot (Suppl. Fig.15). rhBMP-2/ACS was ranked higher in SUCRA ranking, followed by rhGDF-5/b-TCP (Figure 5). rhGDF-5+b-TCP/PLGA was the least dissimilar material, and rhBMP-2/CaSO4 was ranked higher than most dissimilar material in new cementum regeneration in MDS ranking (Suppl. Fig.16).

Percentage of New Bone Fill

The network plot as illustrated in Suppl.Fig.17. rhBMP-2 was ranked higher in SUCRA ranking (Figure 4), and rhBMP-2 was most likely to perform better in future clinical studies as illustrated in predictive interval plot followed by larger particle size rhPDGF-BB, β-TCP/FGF-2 and rhBMP-2+bdECM (Suppl. Fig.18). VEGF+nHA+coral and rhBMP-2/ACS was the most dissimilar material and ranked highest in MDS ranking (Suppl. Fig.19).

Bone Area (mm^2)

The most common comparator was between Con Vs rhGDF-5, Con Vs rhBMP-2/ACS and Osteogenic protein-1 (Suppl. Fig.20). The contribution plot was illustrated in Suppl.Fig.21 and all rh factors equally contributed towards direct, indirect and mixed evidence. The ROB was illustrated in Supple.Fig.22. rhGDF-5/TCP recombinant factors were most likely to perform better in future clinical studies and ranked higher in performance in the amount of area in bone regeneration in the treatment of intrabony defects (Suppl. Fig.23). rhBMP-2/ACS was found to be the most dissimilar material as illustrated in MDS ranking (Suppl. Fig.24).

Bone Volume density (g/cm^3)

The network geometry plot as illustrated in Suppl.Fig.25. The contribution plot as illustrated in Suppl.Fig. 26 and ROB in Suppl.Fig.27. PCL/b-TCP/bdECM/BMP was the highest-ranked recombinant bioactive bone regenerative agent under SUCRA ranking (Figure 4). Since there were not many studies that demonstrated bone volume density, it was not possible to predict which rh factor would perform better in future clinical studies (Suppl.Fig.28). PCL/b-TCP/bdECM/BMP ranked higher in MDS ranking, suggestive of the most dissimilar rh factor among the other agents (Suppl. Fig.29).

Bone Height (mm)

The network geometry plot illustrates Con Vs PDGF-bb/IGF-1, and IGF-1 was the most common comparison. rhPDGF-BB/Equine was ranked higher in SUCRA ranking and more likely to perform better in future clinical studies. IGF-1 and rhPDGF-BB/b-TCP was the most dissimilar material as illustrated in multidimensional scale ranking (Supple.Fig.30).

Sycryl Assessment Risk of Bias of Individual studies

Lee et al.2020; Du Bing et al.2014; Park JC et al.2012; Markapoulou et al.2011; Shirakata et al.2010; Oi et al.2009; Qahash et al.2007; Choi et al.2002 were found to be a high risk of bias and N=7 studies found to be moderate risk and remaining studies categorised into the low risk of bias (Figure 3).

Discussion

Growth factors (GF's) are naturally occurring soluble substance in humans that are heterogeneous polypeptides and have pleiotropism, redundant property. GF's act on receptors and have the ability to proliferate, heal wounds and cellular differentiation in a biological environment. There are different classes of growth factors, which are based on tissue of origin. For example, fibroblast growth factor (FGF), nerve growth factor (NGF), vascular endothelial growth factor (VEGF), insulin derived growth factor (IDGF), platelet-derived growth factors (PDGF), to name a few. Several different types of delivery models have been tested for PDGF in periodontal models. Some of them include recombinant human-derived growth (rhPDGF). These are mitogenic, chemotactic and has an effect on periodontal tissue regeneration. This has been used in combination with various bioactive agents with/without scaffolds in the regeneration of periodontal fibres, cementum, alveolar bone, perivascular cells and endothelial cells with no evidence of fibrosis (17-19). The injury to periodontal supporting structures causes increased expression of PDGF receptors and though, to increase the regenerative ability of rhPDGF (20).

Since there is a number of combinations of rh growth and differentiation factors used in studies that were applied for regeneration of bone in intrabony defects, it is difficult to know which is the most effective rh factors in different outcomes. Network meta-analysis has bridged this gap by comparing multiple different treatments simultaneously in a single analysis by combining the mixed, direct and indirect evidence from the randomised controlled clinical trials (RCT's) (42). Our results from this analysis have found that rhBMP-2 in bone volume density (BV/TV), rhGDF-5/TCP in the bone area (mm2) (BA), rhPDGF-BB/Equine in bone height (mm), rhBMP-2 in the percentage of bone fill, rhBMP-2/ACS in new cementum, rhGDF-5+b-TCP/PLGA in connective tissue attachment (CT) and rhGDF-

5+b-TCP/PLGA in the junctional epithelium (JE) were the most effective agents in the regeneration of supporting structures. When we look at the results of the SUCRA ranking, rhGDF-5+b-TCP/PLGA was effective in soft tissue (CT and JE) regeneration, and rhBMP-2 is effective was hard tissue regeneration (BA and BV) (Figure 4&5).

One of the main objective for conducting pre-clinical studies before human studies were to identify and pick the most effective bioactive agents and biomaterial. Hence prevent attrition rates, adverse events and be cost-effective in clinical studies. Due to unpredictability about the behaviour of material or biologic, the accidental selection of poorly characterised biologics and biomaterial is higher. From our present review, we found around twenty-four pre-clinical studies. Similarly, we have found twelve clinical studies which looked at the regenerative potential of rh factors in the treatment of intrabony defects. From both types of studies, it is clear that rh factors accelerate the regenerative potential of cells in the periodontium. Due to variations in the type of rh factors and combinations, different rh factors have ranked best in varying clinical outcomes. Having said that, rhGDF-5+β-TCP/PLGA and rhPDGF-BB+β-TCP most frequently ranked best in performance in respective pre-clinical and clinical studies.

It is commonly observed in pre-clinical studies that reproducibility is low because of a high prevalence of bias and suboptimal research practices. The majority of the times, it is observed that the pre-clinical studies are too small, which results in false negative and false positive observations. In our review, eight studies were at high risk of bias and seven were rated as the moderate risk of bias (Figure 3). These questions were translating pre-clinical evidence into clinical practices. However, if we look at the animal models, the majority of studies was carried on the most effective animal model for periodontal regeneration, i.e. beagle dogs (21). This is also suggestive that the results of rh factors being effective in the regeneration of periodontal supporting structures could be translated into clinical practice. To decide which rh factors should be used, we need more evidence from clinical studies with significant power.

A different formulation was used in the literature and those include; 20µg/ml of rhGDF-5/b-TCP; 188 mg/defect (high dose) of rhGDF-5; 37 mg/defect (medium) rhGDF-5; 18 mg/defect (Low) of rhGDF-5; 0.75mg/g of IGF-1; 2.5mg/g of PDGF-BB; 7.5mg/g of PDGF-BB/IGF-I; 1.0 and 2.0 of rhGDF-5/g bTCP; 200µg and 500 µg of rhBMP-2 of rhBMP-2. Since the formulations where highly heterogeneous it was not feasible to conduct pair-wise meta-analysis.

Quality of Evidence

The results of our study found that recombinant factors were effective in treating intrabony defects. Twenty-four pre-clinical studies were included and judged at moderate and high risk of bias. It was challenging, however, to grade the evidence for effectiveness due to diverse groups of rhGFs formulations used in each individual study. Therefore, well-designed pre-clinical studies with randomisation and blinding are needed to provide recommendations on the most effective rhGFs for periodontal intrabony defects regeneration.

We concluded that rhGDF-5+b-TCP/PLGA was effective in soft tissue (CT and JE) regeneration, and rhBMP-2 is effective for hard tissue regeneration (BA and BV) and ranked highest in performance, as

demonstrated in the SUCRA ranking. These rhGFs are more likely to perform better in future clinical studies. Although we have estimated rankings and performance of rhGF's in combination with bioactive agents and scaffolds, we do not have enough evidence to recommend a specific rhGF for different outcomes in treating intrabony defects. Therefore, well-designed pre-clinical studies with a minimum of three groups, which include control and two different rhGF's along with bioactive agents and scaffolds, would provide clinicians with more information for considering bioactive agents in their clinical practice.

Conclusion

Various recombinant factors are effective in promoting the regeneration of both soft and hard tissue supporting structures of the periodontium. Results from animal studies are extremely valuable to understanding the effectiveness and the mechanisms of action of the agents that could be used in the treatment of human diseases, but a limited transferability to the clinical setting still exists. The present NMA showed that when considering different outcomes related to periodontal regeneration, different factors, associated or not with biomaterials, ranked best. Therefore, the choice of the most appropriate combination and formulation of bioactive agents and scaffolds for periodontal regeneration may depend on clinical needs and purpose.

Conflict of Interests

None

Acknowledgement

None

Authors contribution

Sourav Panda has contributed to conception, design, data acquisition and interpretation, drafted and critically revised the manuscript. Shahnawaz Khijmatgar has contributed to conception, design, data acquisition and interpretation, performed all statistical analyses, drafted and critically revised the manuscript. Mohit Das has contributed to conception, design, and critically revised the manuscript. Herber Isaac Arbildo-Vega has contributed to conception, design, and critically revised the manuscript Massimo Del Fabbro has contributed to conception, design, data acquisition and interpretation, drafted and critically revised the manuscript. All authors discussed the results and contributed to the final manuscript and all of the authors gave their final approval and agree to be accountable for all aspects of the work.

Support of Funds/Sponsor

None

References

1. Ausenda F, Rasperini G, Acunzo R, Gorbunkova A, Pagni G. (2019). New perspectives in the use of biomaterials for periodontal regeneration. Materials.12(13):2197.doi: 10.3390/ma12132197
2. Pellegrini G, Pagni G, Rasperini G. (2012). Surgical approaches based on biological objectives: GTR versus GBR techniques. International journal of dentistry.2013:521547. doi: 10.1155/2013/521547.
3. Tavelli L, Ravidà A, Barootchi S, Chambrone L, Giannobile WV. (2012). Recombinant Human Platelet-Derived Growth Factor: A Systematic Review of Clinical Findings in Oral Regenerative Procedures. JDR Clin Trans Res. 6(2):161-173. doi: 10.1177/2380084420921353
4. Donos N, Dereka X, Calciolari E. (2019). The use of bioactive factors to enhance bone regeneration: A narrative review. Journal of Clinical Periodontology. 46:124-61. doi: 10.1111/jcpe.13048
5. Trombelli L, Farina R. (2008). Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration. Journal of clinical periodontology. 35:117-35. doi: 10.1111/j.1600-051X.2008.01265.x.
6. Darby IB, Morris KH. (2013). A systematic review of the use of growth factors in human periodontal regeneration. Journal of periodontology. 84(4):465-76. doi: 10.1902/jop.2012.120145.
7. Khoshkam V, Chan HL, Lin GH, Mailoa J, Giannobile WV, Wang HL, Oh TJ. (2015). Outcomes of regenerative treatment with rh PDGF-BB and rh FGF-2 for periodontal intra-bony defects: a systematic review and meta-analysis. Journal of Clinical Periodontology. 42(3):272-80. doi: 10.1111/jcpe.12354
8. Chaimani A, Caldwell DM, Li T, Higgins JP, Salanti G. (2019). Undertaking network meta-analyses. Cochrane handbook for systematic reviews of interventions. 23:285-320. doi.org/10.1002/9781119536604.ch11
9. Dias S, Caldwell DM. (2019). Network meta-analysis explained. Arch Dis Child Fetal Neonatal Ed. 104(1):F8-F12. dx.doi.org/10.1136/archdischild-2018-315224
10. Hutton B, Salanti G, Caldwell DM, Chaimani A, Schmid CH, Cameron C, Ioannidis JP, Straus S, Thorlund K, Jansen JP, Mulrow C, Catalá-López F, Gøtzsche PC, Dickersin K, Boutron I, Altman DG, Moher D. (2015). The PRISMA Extension Statement for Reporting of Systematic Reviews Incorporating Network Meta-analyses of Health Care Interventions: Checklist and Explanations. Ann Intern Med. 162(11):777-784. doi: 10.7326/M14-2385
11. Hooijmans CR, Rovers MM, De Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW.(2014). SYRCLE's risk of bias tool for animal studies. BMC medical research methodology. 14(1):1-9. doi: 10.1186/1471-2288-14-43.

12. Puhan, M.A., Schünemann, H.J., Murad, M.H., Li, T., Brignardello-Petersen, R., Singh, J.A., Kessels, A.G. and Guyatt, G.H., 2014. A GRADE Working Group approach for rating the quality of treatment effect estimates from network meta-analysis. *Bmj*, 24;349:g5630. doi: 10.1136/bmj.g5630.

13. Yamakawa H, Muraoka N, Miyamoto K, Sadahiro T, Isomi M, Haginiwa S, Kojima H, Umei T, Akiyama M, Kuishi Y, Kurokawa J. (2015). Fibroblast growth factors and vascular endothelial growth factor promote cardiac reprogramming under defined conditions. Stem cell reports. 8;5(6):1128-42. 8;5(6):1128-1142. doi: 10.1016/j.stemcr.2015.10.019

14. Kim SG, Zhou J, Solomon C, Zheng Y, Suzuki T, Chen M, Song S, Jiang N, Cho S, Mao JJ.(2012). Effects of growth factors on dental stem/progenitor cells. Dental Clinics. 1;56(3):563-75. doi: 10.1016/j.cden.2012.05.001

15. Hallman M, Thor A. (2008). Bone substitutes and growth factors as an alternative/complement to autogenous bone for grafting in implant dentistry. Periodontology 2000. 47(1):172-92. doi: 10.1111/j.1600-0757.2008.00251.x.

16. Hollinger JO, Hart CE, Hirsch SN, Lynch S, Friedlaender GE. (2008). Recombinant human platelet-derived growth factor: biology and clinical applications. JBJS. 1;90(Supplement_1):48-54. doi: 10.2106/JBJS.G.01231.

17. Bae EB, Park KH, Shim JH, Chung HY, Choi JW, Lee JJ, Kim CH, Jeon HJ, Kang SS, Huh JB (2018). Efficacy of rhBMP-2 Loaded PCL/*β*-TCP/bdECM Scaffold Fabricated by 3D Printing Technology on Bone Regeneration. Biomed Res Int. 27;2018:2876135. doi: 10.1155/2018/2876135.

18. Ogawa K, Miyaji H, Kato A, Kosen Y, Momose T, Yoshida T, Nishida E, Miyata S, Murakami S, Takita H, Fugetsu B, Sugaya T, Kawanami M. (2016). Periodontal tissue engineering by nano beta-tricalcium phosphate scaffold and fibroblast growth factor-2 in one-wall infrabony defects of dogs. J Periodontal Res. 51(6):758-767. doi: 10.1111/jre.12352.

19. Bizenjima T, Seshima F, Ishizuka Y, Takeuchi T, Kinumatsu T, Saito A. (2015). Fibroblast growth factor-2 promotes healing of surgically created periodontal defects in rats with early, streptozotocin-induced diabetes via increasing cell proliferation and regulating angiogenesis. J Clin Periodontol. 42(1):62-71. doi: 10.1111/jcpe.12324.

20. Du B, Gao Y, Deng Y, Zhao Y, Lai C, Guo Z, Rong M, Zhou L. (2015). Local delivery of rhVEGF165 through biocoated nHA/coral block grafts in critical-sized dog mandible defects: a histological study at the early stages of bone healing. Int J Clin Exp Med. 15;8(4):4940-53.

21. Kantarci A, Hasturk H, Van Dyke TE.(2015). Animal models for periodontal regeneration and peri-implant responses. Periodontol 2000.68(1):66-82. doi: 10.1111/prd.12052.

22. Lee J, Yun J, Kim KH, Koo KT, Seol YJ, Lee YM. (2020). Periodontal Regeneration Using Recombinant Human Bone Morphogenetic Protein-2 and a Bilayer Collagen Matrix. J Craniofac Surg. 31(6):1602-1607. doi: 10.1097/SCS.0000000000006517.

23. Park JC, Wikesjö UM, Koo KT, Lee JS, Kim YT, Pippig SD, Bastone P, Kim CS, Kim CK. (2012). Maturation of alveolar bone following implantation of an rhGDF-5/PLGA composite

into 1-wall intra-bony defects in dogs: 24-week histometric observations. J Clin Periodontol. 39(6):565-73. doi: 10.1111/j.1600-051X.2012.01868.x.

24. Lee JS, Wikesjö UM, Park JC, Jang YJ, Pippig SD, Bastone P, Choi SH, Kim CK. (2012). Maturation of periodontal tissues following implantation of rhGDF-5/β-TCP in one-wall intra-bony defects in dogs: 24-week histological observations. J Clin Periodontol.39(5):466-74. doi: 10.1111/j.1600-051X.2012.01862.x

25. Leknes KN, Yang J, Qahash M, Polimeni G, Susin C, Wikesjö UM. (2013). Alveolar ridge augmentation using implants coated with recombinant human growth/differentiation factor -5 (rhGDF-5). Radiographic observations. Clin Oral Implants Res. 24(11):1185-91. doi: 10.1111/j.1600-0501.2012.02564.x.

26. Nevins M, Nevins ML, Karimbux N, Kim SW, Schupbach P, Kim DM. (2012). The combination of purified recombinant human platelet-derived growth factor-BB and equine particulate bone graft for periodontal regeneration. J Periodontol.83(5):565-73. doi: 10.1902/jop.2011.110298.

27. Oortgiesen DA, Walboomers XF, Bronckers AL, Meijer GJ, Jansen JA.(2014). Periodontal regeneration using an injectable bone cement combined with BMP-2 or FGF-2. J Tissue Eng Regen Med. 8(3):202-9. doi: 10.1002/term.1514.

28. Emerton KB, Drapeau SJ, Prasad H, Rohrer M, Roffe P, Hopper K, Schoolfield J, Jones A, Cochran DL. (2011). Regeneration of periodontal tissues in non-human primates with rhGDF-5 and beta-tricalcium phosphate. J Dent Res. 90(12):1416-21. doi: 10.1177/0022034511423665.

29. Min CK, Wikesjö UM, Park JC, Chae GJ, Pippig SD, Bastone P, Kim CS, Kim CK. (2011). Wound healing/regeneration using recombinant human growth/differentiation factor-5 in an injectable poly-lactide-co-glycolide-acid composite carrier and a one-wall intra-bony defect model in dogs. J Clin Periodontol. 38(3):261-8. doi: 10.1111/j.1600-051X.2010.01691.x

30. Markopoulou CE, Dereka XE, Vavouraki HN, Pepelassi EE, Mamalis AA, Karoussis IK, Vrotsos IA. (2011). Effect of rhTGF-β1 combined with bone grafts on human periodontal cell differentiation. Growth Factors. 29(1):14-20. doi: 10.3109/08977194.2010.533663

31. Irokawa D, Ota M, Yamamoto S, Shibukawa Y, Yamada S. (2010). Effect of β tricalcium phosphate particle size on recombinant human platelet-derived growth factor-BB-induced regeneration of periodontal tissue in dog. Dent Mater J. 29(6):721-30. doi: 10.4012/dmj.2010-033.

32. Kwon DH, Bisch FC, Herold RW, Pompe C, Bastone P, Rodriguez NA, Susin C, Wikesjö UM.(2010). Periodontal wound healing/regeneration following the application of rhGDF-5 in a beta-TCP/PLGA carrier in critical-size supra-alveolar periodontal defects in dogs. J Clin Periodontol. 37(7):667-74. doi: 10.1111/j.1600-051X.2010.01569.x.

33. Lee JS, Wikesjö UM, Jung UW, Choi SH, Pippig S, Siedler M, Kim CK. (2010). Periodontal wound healing/regeneration following implantation of recombinant human growth/differentiation factor-5 in a beta-tricalcium phosphate carrier into one-wall intrabony defects in dogs. J Clin Periodontol. 37(4):382-9. doi: 10.1111/j.1600-051X.2010.01544.x.

34. Shirakata Y, Taniyama K, Yoshimoto T, Miyamoto M, Takeuchi N, Matsuyama T, Noguchi K. (2010). Regenerative effect of basic fibroblast growth factor on periodontal healing in two-wall intrabony defects in dogs. J Clin Periodontol. 37(4):374-81. doi: 10.1111/j.1600-051X.2010.01539.x.

35. Oi Y, Ota M, Yamamoto S, Shibukawa Y, Yamada S.(2009). Beta-tricalcium phosphate and basic fibroblast growth factor combination enhances periodontal regeneration in intrabony defects in dogs. Dent Mater J. 28(2):162-9. doi: 10.4012/dmj.28.162

36. Qahash M, Hardwick WR, Rohrer MD, Wozney JM, Wikesjö UM. (2007). Surface-etching enhances titanium implant osseointegration in newly formed (rhBMP-2-induced) and native bone. International Journal of Oral & Maxillofacial Implants. 1;22(3).

37. Blumenthal NM, Koh-Kunst G, Alves ME, Miranda D, Sorensen RG, Wozney JM, Wikesjö UM. (2002). Effect of surgical implantation of recombinant human bone morphogenetic protein-2 in a bioabsorbable collagen sponge or calcium phosphate putty carrier in intrabony periodontal defects in the baboon. J Periodontol. 73(12):1494-506. doi: 10.1902/jop.2002.73.12.1494.

38. Choi SH, Kim CK, Cho KS, Huh JS, Sorensen RG, Wozney JM, Wikesjö UM. (2002). Effect of recombinant human bone morphogenetic protein-2/absorbable collagen sponge (rhBMP-2/ACS) on healing in 3-wall intrabony defects in dogs. J Periodontol. 73(1):63-72. doi: 10.1902/jop.2002.73.1.63.

39. Giannobile WV, Ryan S, Shih MS, Su DL, Kaplan PL, Chan TC.(1998). Recombinant human osteogenic protein-1 (OP-1) stimulates periodontal wound healing in class III furcation defects. J Periodontol.69(2):129-37. doi: 10.1902/jop.1998.69.2.129.

40. Giannobile WV, Hernandez RA, Finkelman RD, Ryan S, Kiritsy CP, D'Andrea M, Lynch SE.(1996). Comparative effects of platelet-derived growth factor-BB and insulin-like growth factor-I, individually and in combination, on periodontal regeneration in Macaca fascicularis. J Periodontal Res.31(5):301-12. doi: 10.1111/j.1600-0765.1996.tb00497.

41. Giannobile WV, Finkelman RD, Lynch SE. (1994). Comparison of canine and non-human primate animal models for periodontal regenerative therapy: results following a single administration of PDGF/IGF-I. J Periodontol. 65(12):1158-68. doi: 10.1902/jop.1994.65.12.1158.

42. Zafari Z, Thorlund K, FitzGerald JM, Marra CA, Sadatsafavi M. (2014). Network vs. pairwise meta-analyses: a case study of the impact of an evidence-synthesis paradigm on value of information outcomes. Pharmacoeconomics. 1;32(10):995-1004. doi: 10.1007/s40273-014-0179-1.

Figures

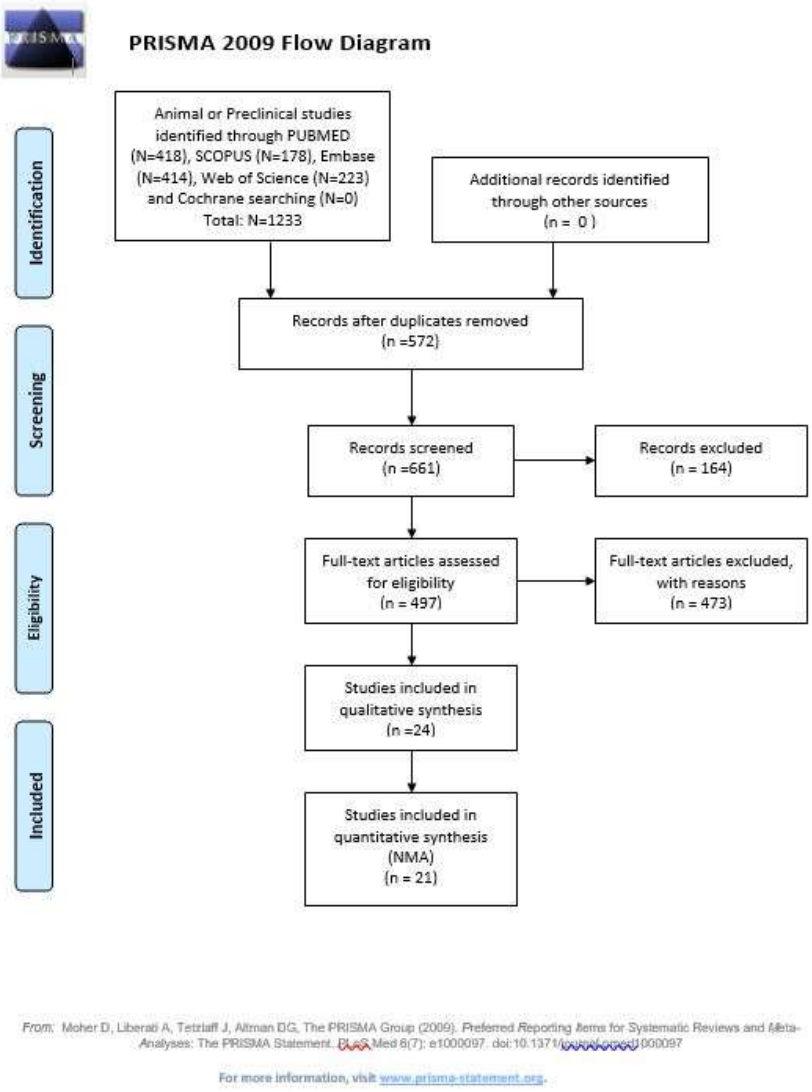

Figure 1: PRISMA Flow Chart

Sl.No	Author	Country	Sponsor/funding	Bioactive agent	Biomaterial Scaffold	Total (N) included	Total Assessed (n)	Control (n)	Control Assessed (n)	Test (n)	Test Assessed (n)	Histology
1	Lee et al 2020	China	SNUDH Research Fund	rhBMP-2	Bilayer collagen matrix	18 beagle dogs	18	6	6	12	12	Yes
2	Bae et al 2018	Korea	National Research Foundation of Korea	rhBMP-2 (5µg)	llularized extracellular matrix (bdE	28 Sprague-Dawley rats	28	7	7	21	21	yes
3	Ogawa et al 2016	Japan		FGF-2	nano-b-TCP/collagen scaffold	4 Female Beagle Dogs	NR	NR	NR	NR	NR	Yes
4	Bizenjima et al 2014	Japan		rhFGF-2	NR	25 Male Wilstar rats(split mouth)	25	25	20	20	21	Yes
5	Du Bing et al 2014	China	Guangdong Provincial Stomatological Hospital	VEGF	HA	2 Beagle dogs(split mouth)	8	4	4	4	4	yes
6	Park JC et al 2012	Korea	Scil Technology GmbH	rhGDF-5	PLGA membrane	10 male Beagle dogs(split mouth)	40	10	10	30	30	Yes
7	Lee et al 2012	Korea	National Research Foundation of Korea	rhGDF-5	β-TCP	9 young adult beagle dogs	9	4	4	5	5	Yes
8	Leknes et al 2012	Norway	Nobel Biocare AB, Go¨ teborg, Sweden	rhGDF-5	None	12 mongrel dogs	24	6	6	6	18	Yes
9	Nevins et al 2012	USA	Osteohealth, Shirley, New York	rhPDGF-BB	β-TCP	9	36	12	12	24	24	yes
10	Oortgiesen et al 2012	Netherlands	Dutch technology foundation STW	rhBMP-2,rhFGF-2								
11	Emerton et al 2011	USA	Medtronic, Inc	rhGDF-5	β-TCP	32	32	8	8	24	24	Yes
12	Min et al 2011	Korea	Scil Technology GmbH	rhGDF-5	PLGA membrane	15	15	5	5	15	15	Yes
13	Markapoulou et al 2011	Greece	Self Funded	rhTGF-β-1	FDBA	4	4			4	4	No
14	Irokowa et al 2010	Japan	Self funded	PDGF-BB	L-TCP	36	36	12	12	24	24	Yes
15	Kwon et al 2010	USA	Scil Technology GmbH, Martinsried, Germany.	rhGDF-5	β-TCP/PLGA composite	10	10	5	5	5	5	Yes
16	Lee et al 2010	Korea	Scil Technology GmbH, Martinsried, Germany.	rhGDF-5	β-TCP	30	30	10	10	20	20	Yes
17	Shirakata et al 2010	Japan	Ministry of Education, Science, Sports and Culture, Japan	bFGF, PDGF	β-TCP	16	16	4	4	12	12	Yes
18	Oi et al 2009	Japan	Self funded	FGF-2	β-TCP	15	15	5	5	10	10	Yes
19	Qahash et al 2007	USA	University of Minnesota School of Dentistry	rh BMP-2	PTFE membrane	8	8	4	4	4	4	
20	Blumenthal et al 2002	USA	Genetics Institute/Wyeth-Ayerst Research, Cambridge, Massachusetts	rhBMP-2	Collagen membrane	20	20	4	4	16	16	Yes
21	Blumenthal et al 2002	Korea	Self funded	rhBMP-2	Collagen membrane	4	4	1	1	3	3	Yes
22	Giannobile et al 1998	USA	Creative BioMolecules, Inc.	OP-1	Collagen membrane	70	70	20	20	50	50	Yes
23	Giannobile et al 1996	USA	Institute of Molecular Biology, Inc.	IGF-1, PDGF-BB	Collagen membrane	10	10	10	10	30	30	Yes
24	Giannobile et al 1994	USA	Institute of Molecular Biology, Inc	PDGF-BB/IGF-1	Collagen membrane	NR						Yes

Figure 2: Characteristics table for included studies

Sl.No	Publication Year	Author	Randomisation	Allocation Bias	Performance Bias	Blinding	Attrition Bias	Selective reporting bias	Overall Bias
1	2020	Lee et al	Yes	Unclear	Unclear	Yes	High	Unclear	High
2	2018	Bae et al	Yes	Low	Unclear	Yes	Low	Unclear	Moderate
3	2014	Du Bing et al	Yes	Low	Low	Yes	High	Unclear	High
4	2012	Park JC et al	Yes	Low	Unclear	yes	High	Unclear	High
5	2012	Lee et al	Yes	Low Low	Low	Yes	Low	Low	Low
6	2012	Leknes et al	Yes	Low Low	Low	Yes	Low	Unclear	Moderate
7	2012	Nevins et al	Yes	Low	Low	yes	Low	Unclear	Moderate
8	2011	Emerton et al	Yes	Low	Low	Yes	Low	Unclear	Moderate
9	2011	Min et al	Yes	Low	Low	Yes	Low	Low	Low
10	2011	Markapoulou et al	No	High	Unclear	No	High	Unclear	High
11	2010	Irokowa et al	Yes	Low Low Low	Low	Yes	Low	Low	Low
12	2010	Kwon et al	Yes	Low	Unclear	Yes	Low	Unclear	Moderate
13	2010	Lee et al	Yes	Low	Unclear	Yes	Low	Unclear	Moderate
14	2010	Shirakata et al	Yes	Unclear	Low	Yes	High	Unclear	High
15	2009	Oi et al	Yes	Low	Low	Yes	Low	High	High
16	2007	Qahash et al	Yes	Unclear	High	Yes	High	High	High
17	2002	Blumenthal et al	Yes	Low	Low	Yes	Low	Low	Low
18	2002	Choi et al	Yes	Low	Unclear	Yes	High	Low	High
19	1998	Giannobile et al	Yes	Low	Unclear	Yes	Low	Low	Moderate
20	1996	Giannobile et al	Yes	Low	Low	Yes	Low	Low	Low
21	1994	Giannobile et al	Yes	Low	Low	Yes	Low	Low	Low

Figure 3: SYRCLE's tool for assessing risk of bias **for individual studies**

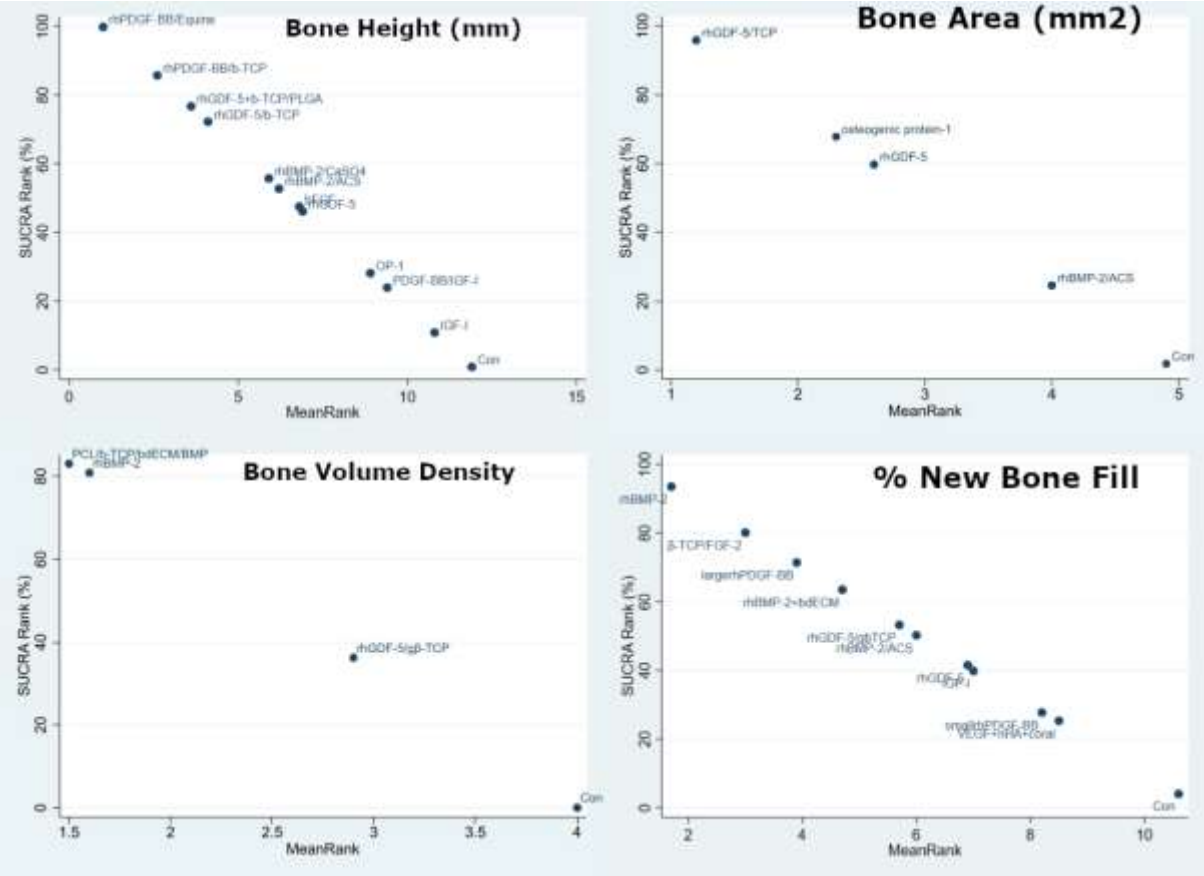

Figure 4: Surface Under the Cumulative Ranking (SUCRA) for different outcomes in bone regeneration in intrabony bone defects.

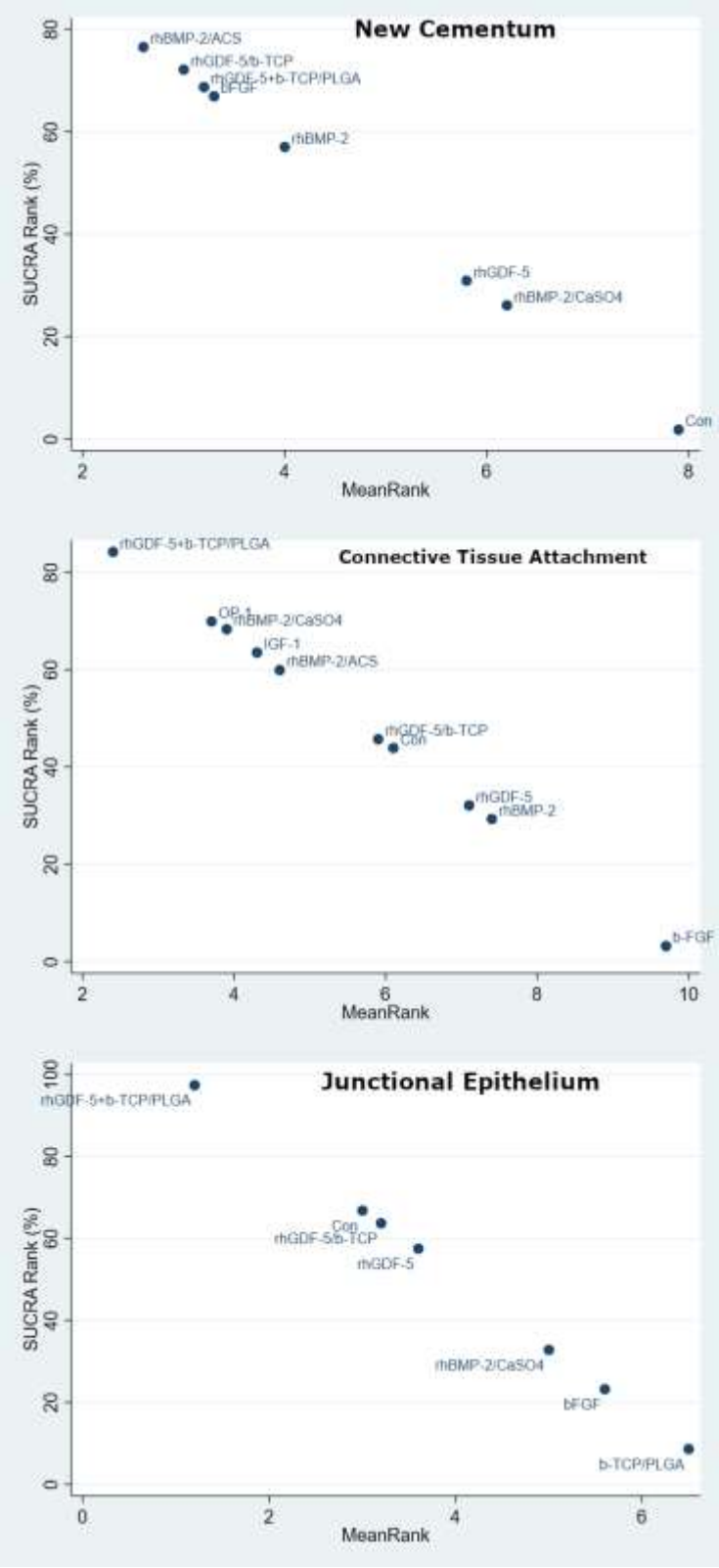

Figure 5: Surface Under the Cumulative Ranking (SUCRA) for different outcomes in periodontal tissue regeneration in intrabony bone defects.