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Title: Cell-based therapies for alveolar bone and periodontal regeneration: a systematic review.

Short title: Cell-based therapies for periodontal regeneration

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Abstract

Introduction: Current regenerative strategies for alveolar bone and periodontal tissues are effective and well adopted. These are mainly based on the use of a combination of synthetic/natural scaffolds and bioactive agents, obviating the incorporation of cells. However, there are some inherent limitations associated with traditional techniques and we hypothesised that the use of cell-based therapies as part of comprehensive regenerative protocols may help overcome these hurdles to enhance clinical outcomes.

Methods: We conducted a systematic review of human controlled clinical trials investigating the clinical and/or histological effect of the use of cell-based therapies for alveolar bone and periodontal regeneration and explored the translational potential of the different cell-based strategies identified in the included trials.

Findings: A total of sixteen studies (11 RCTs, 5 CCTs) were included for data synthesis and qualitative analysis with meta-analyses performed when appropriate. The results suggest a clinical benefit from the use of cell therapy. Improved outcomes were shown for alveolar ridge preservation, lateral ridge augmentation and periodontal regeneration. However, there was insufficient evidence to identify best-performing treatment modalities amongst the different cell-based techniques.

Interpretation: In light of the clinical and histological outcomes, we identify extraction socket and challenging lateral and vertical bone defects requiring bone block grafts as strong candidates for the adjuvant application of mesenchymal stem cells (MSCs). Given the complexity, invasiveness and costs associated with techniques which include “substantial manipulation’ of tissues and cells, their additional clinical benefit when compared to ‘minimal manipulation’ must be elucidated in future trials.

Introduction

Outcomes in alveolar bone and periodontal regeneration are largely dependent on the biological and the material characteristics of scaffolds and the availability, recruitment and activation of cells and biomolecules in the injured area during healing. A large number of reviews have assessed the outcome of bone regeneration techniques either before or at the time of implant placement and following maxillary sinus augmentation¹⁻⁹, including Cochrane systematic reviews^{10,11}. The efficacy of different surgical techniques and biomaterials for periodontal regeneration has also been extensively documented^{12,13}. These therapies are relatively simple and highly cost-effective and, as a result, the use of autologous bone and/or bone substitutes, resorbable and non-resorbable membranes and different commercially available bioactive products are now routine practice across the globe. While proven effective, there is also evidence that there is large degree of variability on the outcomes achieved. Failures and high complication rates are common in general dental practice in larger horizontal and vertical alveolar bone defects because the augmentation procedures are highly technique-sensitive. Furthermore, the use of slow resorbing bone biomaterials may reduce the amounts and quality of newly formed bone in the augmented area. These issues delay treatment delivery significantly, especially for the replacement of teeth with dental implants shortly after tooth extraction. The addition of “smart” bioactive agents and cell-based approaches to bone scaffolds and membranes may

help overcoming some of these limitations. The opportunity lies on helping surgeons' activity through advanced but simple and fast protocols of tissue engineering.

The primary aim of this systematic review is to assess the efficacy of cell therapies on clinical and histological outcomes for alveolar bone and periodontal regeneration. We will focus on the use of these innovative approaches in human clinical studies investigating specifically ridge preservation, horizontal and vertical alveolar bone augmentation, sinus augmentation techniques and periodontal regenerative surgeries. The results will be stratified according to the defect type but also with regards to the source of MSCs and the techniques for manipulation of tissue samples and cells. As a secondary outcome, we aim to investigate the translational potential outside of a hospital/university setting of the different cell-based strategies identified in the included controlled clinical trials.

Methods

The protocol was registered on the PROSPERO database: ID CRD42019121119 (www.crd.york.ac.uk/PROSPERO).

Objectives

- To investigate the evidence for the effect of cell-based therapies as adjuncts to surgery for the regeneration of alveolar bone / periodontium.
- Critically appraise the validity, methodology and outcomes of the included studies.
- Identify and appraise the clinical applicability of emerging cell-based techniques for alveolar bone/periodontal regeneration.

Types of studies

The review included prospective controlled clinical studies in human adults (18 years of age or above) evaluating the effect of surgical treatment with or without the adjunctive use of cell-based therapies for the treatment of alveolar bone / periodontal defects assessing changes in alveolar bone dimensions / periodontal clinical measurements. Secondary outcomes were assessed only for those studies reporting data on the primary outcomes. A minimum of six weeks follow-up post-surgically for alveolar ridge preservation, three

months follow-up for lateral/vertical ridge and sinus augmentation and twelve months for periodontal regeneration studies was required. Studies were stratified according to the defect model and each group was analysed independently: a) ridge preservation (ARP) b) lateral and/or vertical ridge augmentation (GBR) c) sinus augmentation (SINUS) d) periodontal regeneration (PERIO). Studies investigating the use of cell-therapies to reconstruct large defects due to maxillectomies were excluded. Case reports and/or case series, retrospective clinical studies and publications reporting the outcomes of in vitro studies or preclinical (animal) studies only were excluded.

Determination of outcome measures

The primary outcome was dependent on the defect model: postsurgical alterations in the alveolar bone defect size, based upon direct/indirect clinical and/or radiographic measurements were considered as primary outcomes. See the Supplementary files for all primary and secondary outcomes selected according to the defect type.

Search strategy

A highly sensitive search was conducted. Electronic databases (MEDLINE, EMBASE, LILACS) were searched using a string of medical subject heading and free-text terms. OpenGrey was searched for unpublished, grey literature. The search strategy was first designed for the MEDLINE database and then modified appropriately for the other databases (see Supplementary files). There was no language or publication date restrictions. Reference lists of all studies included for full text screening and previous reviews were searched for missing records. A manual search for the period from December 2013 to May 2019 was completed for a number of scientific journals (see Supplementary files). Further details regarding study selection, data extraction and analysis, quality assessment and the processes followed for data synthesis can be found in the Supplementary files.

Findings

Search (See Figure 1.)

A total of 5,053 potentially eligible records were returned from the electronic searches. A further 39 publications were found through the manual search of the selected journals.

Following deletion of duplicates, a total of 4741 records were available for initial title/abstract screening, after which the full-text of 74 publications were retrieved. After assessment of the full papers, a further 56 records were excluded. Therefore, a total of 18¹⁴⁻³¹ publications corresponding to 16 original studies were included for qualitative analysis. One of the included studies reported follow-up data from a previously published study³⁰ while another one was a correction of a previous report³¹. The reason for exclusion of all studies which were not included following full text assessment was documented (see Supplementary files). Agreement between examiners after full-text screening was excellent (Kappa score: 0.896).

There was a total of eleven randomised controlled (RCTs)^{16-20,24-29} and five non-randomised controlled original trials^{14,15,21-23} included for qualitative analysis. All RCTs incorporated a parallel design while CCTs included reports with either a parallel or a “split mouth” design. Chief characteristics of the included studies stratified according to defect type can be found in the Supplementary files. Outcomes were assessed through an array of different methods. In alveolar bone regeneration studies (ARP, GBR, SINUS) the method of measurement of the alveolar ridge dimensions (width and/or height) varied between studies and ranged from direct linear measurements measured in situ with a probe to linear and volumetric changes assessed radiographically (Orthopantomogram (OPG) and Cone Beam Computed Tomography (CBCT)). Furthermore, analysis of core bone biopsies via histology, microCT and Synchrotron X-ray Holotomography was also reported. The biopsy assessments mainly quantified new bone formation and relative ratios of new bone, graft remnants and marrow space. Secondary outcomes frequently reported included complication rates but only two studies reported other clinically relevant assessments such as need for additional bone grafting at the time of implant placement and amount of additional bone graft needed (16,17). For periodontal regeneration, outcome measures included Clinical Attachment gain (CAL), probing depth reduction (PD), Recession (REC) and defect bone fill (BF).

We identified cell-based therapies according to the type of MSCs harvested and the donor tissue. Seven studies reported the use of bone marrow stem cells (BMSCs) harvested from posterior iliac crest aspiration^{16-21,25,26}, two studies investigated the performance of dental pulp stem cells (DPSCs) harvested from the dental pulp of extracted teeth^{14,28}, five of the included publications involved the harvesting of periosteum-derived stem cells (PdSCs) from gingival connective tissue samples^{15,21,22,24,29}, one report produced periodontal ligament-derived stem cells (PdISCs) “cell sheets”²⁷, and another publication used the adipose-stromal vascular fraction from adipose tissue samples obtained via lipo-aspiration²³. In line with the definition from the European Medicines Agency (EMA) Regulation (EC) no 1394/2007³², we further sub-categorised the techniques in another two groups: 1) “minimal manipulation” of tissues/cells or 2) ‘substantial manipulation’ otherwise (see Table 1). More details about the cell-scaffold combinations and the specifics of the different cell-based harvesting techniques used in all the included publications are presented in the Supplementary files.

Risk of bias and methodological quality

For the ROBIN-I assessment of non-randomised clinical trials, five out of the six studies included were considered to be at a high risk of bias. Amongst the RCTs assessed with the Cochrane RoB 2.0 tool, two studies showed a low risk of bias, three exhibited some methods which raised some concerns about the risk of bias and six reported systematic errors in design, adherence or reporting which translated in a high risk of bias. Figure 2. displays the results of the bias and methodological quality assessment for all studies (for further information, see Supplementary files).

Narrative synthesis and quantitative analysis

The reader is referred to the extensive tables in the Supplementary files for a comprehensive summary of clinical, histological and other relevant outcome data reported in the included studies categorised according to defect type. A brief synthesis is presented below with Forest plots of all meta-analysis carried out presented in Figure 3.

Alveolar Ridge Preservation

Due to significant differences in study characteristics, it was not possible to complete meta-analysis of effects size for the studies reporting on the use of stem cells for alveolar ridge preservation; a short narrative synthesis is therefore presented.

Of the four original reports included¹⁴⁻¹⁷, two were CCTs^{14,15}. D' Aquino and co-workers¹⁴ reported statistically significant improvements in radiographic ridge regeneration (%) twelve months after extraction. Another study by D'Aquino and co-workers¹⁵ showed that the percentage of resorption of the alveolar ridge was less for the test group than the control group both in the vertical (*Mean diff* 36.5% $p < 0.001$) and the horizontal (*Mean diff* 38.33% $p < 0.001$) dimensions 45-90 days after ridge preservation surgery. Another two RCTs reported on the use of BMSCs^{16,17}. In one of the studies, significant differences in % of radiographic bone height were shown at six weeks (*Mean diff* 23.6% (6.02-41.09) $p = 0.01$) but the benefit of the cell-based therapy was lost at twelve weeks (*Mean diff* 5.4% (-12.11, 22.95) $p = 0.28$) (16). Histological analyses showed that there was a non-statistically significant increase in the % of Bone Tissue Area/Tissue Area at the test sites compared to control at six weeks (*Mean diff* 13.9% (-5.03-33.2) $p = 0.09$) and no difference at twelve weeks (*Mean diff* 0.2% (-19.1% 19.4) $p = 0.49$)¹⁶. The second RCT reported that at six months after tooth extraction, there were no differences between test and control in direct linear measurements of alveolar bone height but the sites treated with cell-therapy showed significantly less horizontal ridge reduction (T $1.14 \text{ mm} \pm 0.87 \text{ mm}$ C $2.46 \pm 0.4 \text{ mm}$, $p = 0.014$) with the test group losing on average $13.61 \pm 12.5\%$ and the control group $31.35 \pm 11.88\%$ of the original ridge width ($p = 0.006$)¹⁷. The percentage of mineralised bone in the core bone biopsies was similar in both groups (T $45.47 \pm 7.21\%$ C $42.87 \pm 11.33\%$, $p = 0.36$) (17). Both studies reported in the number of cases requiring further bone grafting at the time of implant placement and reported a lesser need for additional grafting procedures in cell-based treated sites compared to control.

Guided Bone Regeneration (GBR): Lateral Ridge Augmentation

Two studies reporting radiographic horizontal bone width linear measurements outcomes^{19,20} and three¹⁸⁻²⁰ studies reporting percentage of newly formed bone histologically were included in separate meta-analysis in the GBR category. Random effects meta-analysis of the studies reporting alveolar bone width showed a mean extra 1.13 mm alveolar bone gain (95%CI -1.13, 3.65), four to six months post-surgery, favouring cell-based therapies with considerable heterogeneity ($I^2 = 84.9\%$). The combined estimate for the three studies reporting data for percentage of mineralised tissue histologically was 12.76% (95%CI 2.15, 23.36; $I^2 = 0.0\%$). The three RCTs reported no complications or adverse events.

Sinus augmentation

Three CCTs and three RCTs reported data for radiographic changes in alveolar ridge dimension. However due to discrepancies between studies, only two studies^{25,26} evaluating increase in bone volume in the augmented area were pooled for random-effects meta-analysis. The studies reported contradicting results and, as a result, a non-significant mean volumetric bone gain of 0.13 mm³ (95%CI -0.55, 0.81) in favour of the stem cell groups with substantial heterogeneity ($I^2 = 58.3\%$, $p=0.122$) was observed. This data is in line with the outcomes of the other four studies with an adequate control groups²¹⁻²⁴ showing no differences in alveolar ridge vertical or volumetric gain between cell-based approaches and positive controls without cell endorsement (see Supplementary files).

Histologically, two studies^{23,26} reported similar findings: there were no significant differences between groups with or without cell therapy for new bone formation. Furthermore, two studies^{23,25} reported a non-significant higher bone volume fraction within the newly regenerated bone as assessed by microCT analysis. The publications showed no complications or significant adverse events with the exception of one study that reported one injury of the inferior alveolar nerve when harvesting autogenous bone and two other infections of the bone donor site.

Periodontal regeneration

For studies aiming to achieve periodontal regeneration, one of the RCTs did not report clinical measurements beyond three months and did not report the standard deviations of the differences in radiographic bone fill at twelve months post-op²⁷. For this reason, two studies^{28,29} assessing CAL, PD, REC and BF twelve months after surgery were included in separate meta-analysis. The combined estimate for the two studies showed significant benefits of using cell therapy for periodontal regeneration in terms of clinical attachment gain (1.33 mm, 95%CI 0.47, 2.19; $I^2=0.0\%$) and radiographic defect bone fill (2 mm, 95%CI 1.41, 2.58; $I^2=0.3\%$) with no observed heterogeneity and a non-significant improvement in PD (0.91 mm, 95%CI -0.05, 1.88; $I^2=50.2\%$) and REC (0.36 mm, 95%CI -0.30, 1.02; $I^2=18.1\%$). This positive effect is further illustrated by one of the studies reporting CAL gain as a % of the original defect, showing 83.5% (± 31.7) defect resolution in cell-grafted sites compared to 55.0% (± 21.9) in control sites²⁹. Finally, the other study included in the meta-analysis demonstrated a significantly higher percentage of sites showing CAL gain >4mm (73.3% vs 28.6%) and less sites with probing pocket depths >6mm (0% vs 14.3%) for the defects treated with cell techniques²⁸.

Interpretation

Overall quality, strength and consistency of evidence

The low number of included studies does not come as a surprise given that only recently the application of cell-based therapies for regeneration of alveolar bone and periodontal tissues has been reported. We focused our research question around RCTs and CCTs which could contribute clinical outcome measures and not only histology analysis. For this reason, the secondary histology outcomes reported in this review were limited due to many studies reporting only histology analyses being excluded.

The CCTs included in this review were considered to be at a high risk of bias^{14,15,21-23}. On the other hand, the RCTs comprised a somehow less biased dataset with five out of the eleven original reports showing low levels of bias^{16,25,27-29}. Furthermore, there was a great degree of variability on study design as well as many different cell populations and scaffold combinations, making the test and control groups not homogenous as a whole. The overall

estimates from the meta-analysis, albeit they represent the best-available evidence, should be interpreted with caution.

Clinical applicability: beyond the Hospital/University setting

There were virtually no complications and no severe adverse events across all of the included studies in all the different defect categories, demonstrating the safety of this treatment modality. Although there was insufficient data to draw any conclusions regarding best cell-based treatment strategies, an appraisal of the clinical applicability of the different techniques identified through the systematic search is warranted. Our assessment of the different techniques will appraise aspects which may be relevant for widespread adoption later on the translational pathway. We hope this will help identifying techniques which may be prioritised in future research.

The studies¹⁴⁻¹⁷ assessing changes after grafting of the alveolar ridge following tooth extraction suggest that ARP should be a targeted intervention to demonstrate the clinical potential of cell-based therapy for alveolar bone regeneration. The clinical strategy after a tooth is extracted most often involves the decision to place a dental implant with a type 1 / type 2 placement³³ leaving the ridge to heal undisturbed. The placement of slow-resorbing bone substitutes unnecessarily lengthens the waiting period before a dental implant can be inserted⁹. In the current review, limited data suggests that significant improvements may be obtained on reducing ridge resorption and improving bone quality within the first 6 weeks of healing when progenitor cells are endorsed in fast-resorbing collagen sponges and grafted on “extraction socket” defects. The additional benefit provided by cell-therapy may justify the associated costs of (some of) the techniques by reducing the need of major grafting while shortening treatment times. Similar conclusions may be drawn for lateral ridge augmentation of large horizontal defects. On the other hand, sinus augmentation procedures seem less appropriate for this technique²¹⁻²⁶. A marginal or no effect was reported both for clinical and histology outcomes. This is perhaps related to the fact that the maxillary sinus defect is, by nature, a spontaneous-healing model³⁴. Although excellent outcomes were shown in the

periodontal regeneration studies, the outcomes in the control groups cannot be considered comparable to what is expected from gold standard therapy according to previous research. Therefore, further research comparing this technique to Guided Tissue Regeneration or well-proven bioactive agents such as Emdogain® is needed to elucidate whether or not there is merit on pursuing this application further. Both in sinus augmentation surgery and periodontal regeneration, it is evident that a wide range of techniques have proven to be largely successful on achieving predictable and highly successful outcomes and, hence, it may be difficult to ever find a large enough 'perceived clinical benefit' which could justify the cost and operational needs of cell-based therapies. An exception may be the use of cost-effective "minimal" manipulation techniques such as the one described by Ferraroti and co-workers²⁸. The regeneration of tissues depends mainly on three key players: scaffolds, cells and biomolecules coming together to orchestrate a series of spatial-temporal events which result in a pattern of healing that resembles the original components, structure and function of the lost tissues. There were significant differences between the techniques appraised in this review on the use of scaffolds and "biologics". For instance, there was a wide range of different scaffolds which were used as "carriers" for the cells. When the studies are stratified according to defect type, it becomes apparent that the scaffolds were selected according to the biomechanical and biological requirements of the defects which were being reconstructed and not for their ability to improve cell attachment and/or behaviour. For this reason, we will focus the discussion on the cell harvesting and manipulation steps.

We based our "clinical applicability" appraisal on domains like those described in Roger's diffusion of innovation theory³⁵, see Table 1. This theory has been previously tailored and implemented for analysis of key elements influencing the wide-spread adoption of new technologies/techniques in healthcare³⁶ and dentistry³⁷. Stem cells can be categorised in four different groups depending on their source³⁸: a) embryonic tissues b) foetal tissues c) postnatal tissues and d) induced pluripotent stem cells. Embryonic stem cells have the highest proliferative rate and are able to differentiate into any of the three embryonic germ layers

(ectoderm, mesoderm and endoderm) and can therefore be a source for almost all cell types within the human body. However, their consideration for use is affected by ethical issues since their isolation involves the use of human embryos³⁸. In the future the use of induced pluripotent stem cells (iPS cells)³⁹ would circumvent these ethical concerns. Another alternative currently available is the recruitment of postnatal stem cells, more widely known as mesenchymal stem cells (MSCs) derived broadly from nearly every organ and tissue. Compared to embryonic stem cells and iPS cells, MSCs have restricted differentiation potential and proliferative rates, showing great variability depending on the tissue source³⁸. Although the term stem cell therapy is widely used to describe the use of any of the cell populations above or even to also include therapies using cells further committed in the differentiation pathway, it seems more appropriate to use more focused terms which provide a better description of the cell population being applied providing a better description of their differentiation potential (i.e., tissue-specific cell therapy). We identified therapies involving five distinct cell populations: BMSCs obtained from BMA^{16, 17-20, 26}, DPSCs from the dental pulp of extracted teeth^{14, 28}, PdSCs^{15, 21, 22, 24, 29} from gingival connective tissue samples, PdISCs²⁷ from extracted teeth and A-SVF²³ from adipose tissue.

We consider the invasiveness of the harvesting procedure as one of the factors potentially affecting adoption. The techniques involving DPSCs and PdISCs harvesting require the extraction of a tooth. This invasive procedure would only be appropriate in cases where extractions are already scheduled, limiting its applicability. The harvesting of A-SVF requires a liposuction under general anaesthesia and is therefore considered highly invasive. Bone marrow aspirates were carried out under local anaesthesia and, in some cases, conscious sedation and were rated as moderately invasive. The less invasive technique was the harvesting of gingival connective tissue from intra oral sources. The clear advantage of this technique is that tissues are readily available. Besides their invasiveness, the need of a BMA or lipo-suction adds an extra layer of complexity due to the need to contact and organise specialists outside of the dental office while also adding significant costs.

After harvesting, the cells were either ‘minimally’ or “substantially” modified before application in the surgical site. The rationale for cell expansion is based on the fact that only a small fraction of the cells within the donor tissues are characterised as MSCs. Billions of cells can be generated from as little as 1 ml of bone marrow aspirate following *ex vivo* expansion^{38,40}. On the other hand, despite low cell yields, “minimal manipulation’ of the source tissue will include cells and matrix proteins other than MSCs which may also play a key role for tissue regeneration. Furthermore, there is not known threshold above or below which cell yield is relevant for improving clinical outcomes. It has recently been suggested that we should “move away from autograft-based therapy” and that “the preferred therapeutic strategy moving forward is the use of *ex vivo* expanded MSC preparations rather than whole bone marrow aspirate transplantation or autografts that contain limited MSC numbers diluted within a heterogeneous population of blood/ immune cells”³⁸. *Ex vivo* cell expansion is a complex process that requires significant investment in terms of time, resources and capital and there is currently no evidence to support an additional clinical benefit compared to “minimal’ manipulation cell therapies, at least for the management of the alveolar bone defects most commonly found in day to day practice. It is also evident, albeit only from the limited number of clinical trials available, that techniques requiring “minimal” manipulation of tissues and cells have shown clinical and histology benefits in all the defect models reviewed, casting doubts on the “absolute need” for cell expansion. Furthermore, cell survival *in vivo* following transplantation may be short-lived⁴¹. It has been suggested that MSCs effects go beyond their own innate ability to survive and differentiate into tissue forming cells but lies within their ability to “secrete bioactive factors that are immunomodulatory and trophic”⁴². The use of cellular therapies may act in the clinical context by one of either two broad mechanisms: 1) Cell Replacement – whereby transplanted (generally autologous cells) successfully are accepted to the local host site and then begin the process of tissue regeneration of the local defect; and/or: 2) Cell Empowerment – that mechanism whereby cells (either autologous or allogeneic) display a more transient effect in the local area to release factors that may promote the regenerative response⁴³. Whether or not *ex vivo* cell

expansion is a pre-requisite for the success of cell-based therapies is an important question, one that should be answered through future well-designed randomised controlled clinical trials.

The time that lapsed from cell harvesting to application in “substantially manipulated” therapies ranged from 12 days to over 6 weeks^{14, 16, 21, 22, 25, 27, 29}. Furthermore, cell expansion requires GMP grade laboratories and personnel. A publication of a report of 3 cases using these techniques showed that the GMP level laboratory preparatory costs to the Finnish healthcare system were the equivalent of £8900 per patient⁴⁴. In addition to these elevated expenses, the need of significant external resources and the high complexity of the interventions, make these therapies impractical outside of Hospital/University settings. Amongst the “minimally manipulated” techniques, A-SVF obtained through processing of adipose samples using an automated processing unit (Celution©, Cytori Therapeutics, USA) is the most invasive, expensive and complex alternative followed by the use of BMAC obtained through processing with a commercially available system (Harvest Terumo BCT© , Terumo Medical do Brasil, Brazil) after iliac crest aspiration. A viable alternative may be the use of a commercially available tissue disaggregator (Rigenera©, Human Brain Wave, Italy) able to filter progenitor cells with a size of 50 micron from micrograft tissue samples (i.e. adipose tissue, dental pulp, periosteum)⁴⁵. The Rigenera© system presented clinical data in four studies investigating all defect models covered in this review. Using this device, DPSCs and PdSCs were obtained from dental pulp tissue and gingival connective tissue samples showing promising results.

Outstanding questions

Based on a limited number of studies, cell-based therapies have the potential to improve outcomes of regenerative treatment for the reconstruction of alveolar bone and periodontal tissues. We identified extraction socket defects and challenging lateral and vertical bone defects requiring bone block grafts as ideal candidates for the adjuvant application of MSCs. This review showed that there is insufficient evidence to identify best-performing treatment

modalities amongst the different cell-based techniques described in the included publications. This research synthesis has also highlighted a high risk of bias in most of the studies and the need for well-designed randomised clinical controlled trials including the gold standard of treatment as a positive control. Given the complexity, invasiveness and costs associated with techniques which include “substantial manipulation’ of tissues and cells, their clinical benefit when compared to ‘minimal manipulation’ must be elucidated in future trials. Further research evaluating the clinical effect and cost-effectiveness of simple, fast and economical methods for cell harvesting and processing is warranted.

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Table 1. Key features of cell harvesting and manipulation techniques described in the included controlled trials.

Cells	Source	Harvesting Invasiveness	Manipulation (Proprietary system)	Defect/Studies		Study type / RoB	External resources	Time	Cost	Clinical outcome	Histology outcomes
BMSCs	Bone Marrow Aspirate	Moderate Iliac crest punch LA /Conscious sedation	Substantial (Replicell®)	ARP / SINUS /	Kaigler et al. 2013 Kaigler et al. 2015	RCT / SC RCT / SC	Multiple Hematologist and GMP lab	>12 days	Very High	Superior / Large* No difference	Enhanced* Similar
			Minimal (BMAC) ¹ (Harvest Terumo®) ² (BMAC®)	ARP / GBR /	Pelegrine et al. 2010 Correa et al. 2013 ¹ Da Costa et al. 2011 Pelegrine et al. 2016 ² Sauerbier et al. 2011 ²	RCT / High RCT / High RCT / High RCT / High RCT / High	Hematologist	Hour(s)	High	Superior / Large^ No difference Superior / Modest No difference Superior / modest	Similar Similar Enhanced** Similar Poorer
DPSCs	Dental pulp, extracted teeth	High, not readily available	Substantial (Rigenera®)	ARP /	D'Aquino et al. 2009	CCT / High	GMP lab	21 days	Very High	Superior/ Modest	Enhanced ²
			Minimal (Rigenera®)	PERIO /	Ferraroti et al. 2018	RCT / Low	None	Minutes	Very Low	Superior / Large	-
PdSCs	Periosteum, Gingival connective tissue sample	Very low	Minimal (Rigenera®)	ARP / SINUS /	D'Aquino 2016 Ceccarelli et al. 2018	CCT / High RCT / high	None	Minutes	Very Low	Superior / Modest -	- -
			Substantial (Cell sheets)	SINUS / PERIO /	Nagata et al. 2012 Ogawa et al. 2016 Yamamiya et al. 2008	CCT / High CCT / High RCT / SC	GMP lab	>6 weeks	Very High	No difference No difference Superior / Large	Enhanced ² - -
PdISCs	Periodontal ligament, extracted teeth	High, not readily available	Substantial (Cell sheets)	PERIO /	Chen et al. 2016	RCT / Low	GMP lab	4-5 weeks	Very High	Superior / Modest	-
A-SVF	Adipose tissue, Lipo-aspiration	High, liposuction / GA	Minimal (Celution®)	SINUS /	Prins et al 2016	CCT / SC	Plastic surgeon	Hour(s)	High	No difference	Similar

BMSCs, bone marrow stem cells; DPSCs, dental pulp stem cells; PdSCs, periosteum derived stem cells; PdISCs, periodontal ligament stem cells; A-SVF, adipose stromal vascular fraction; LA, local anesthetic; GA, general anesthetic; TRCs, tissue repair cells; BMAC, bone marrow aspirate concentrate; ARP, alveolar ridge preservation; LRA, lateral ridge augmentation; SINUS, sinus augmentation PERIO, periodontal regeneration; RCT, randomised controlled clinical trial; CCT, controlled clinical trial; SC, some concern; GMP, good manufacturing practice; Mod; Moderate; RoB, risk of bias;

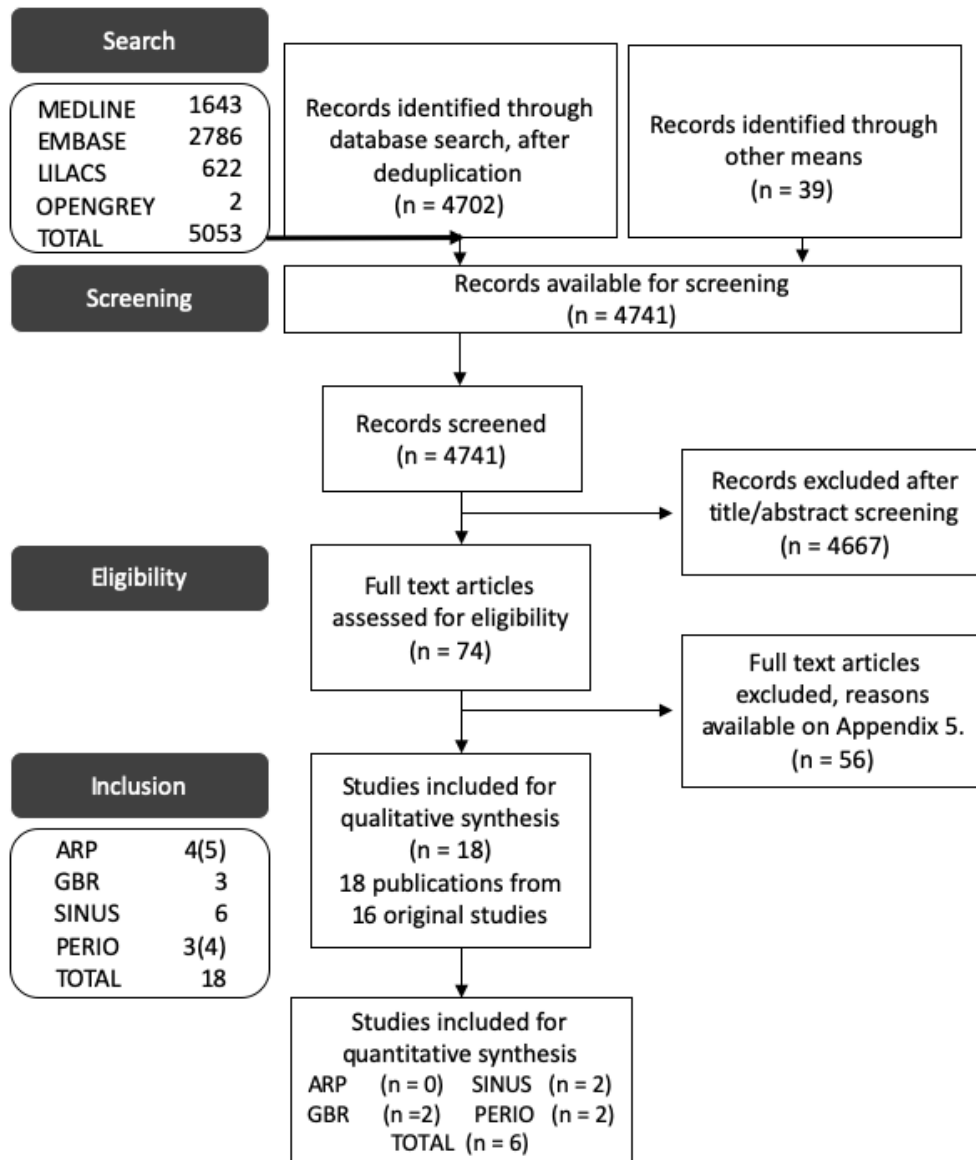
*At 6 weeks (see discussion); ** At 6 months; ^Alveolar thickness loss.² Described in text but without quantitative outcome measurement.

Replicell® (Astrom Biosciences, USA) is a commercial automated cell processing unit for isolation of a mixed population of CD90+ MSCs, hematopoietic SCs, inflammatory and endothelial cells (TRCs).

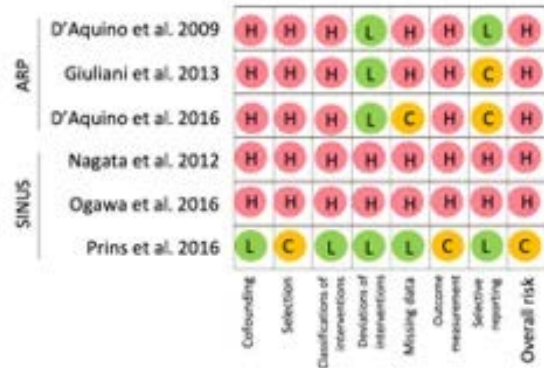
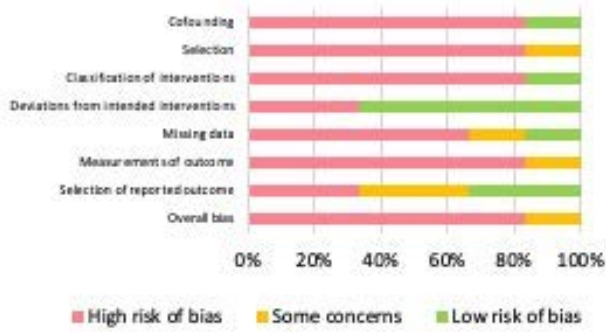
Harvest Terumo BCT® (Terumo Medical do Brasil, Brazil) is a commercially available system to obtain BMAC including a centrifuge (SmartPrep2®) and a processing kit.
BMAC® System (Harvest Technologies, USA) is a commercially available system to obtain BMAC which includes a Harvest BMAC process disposable, 11- and 15-gauge aspiration needles.
Rigenera® (Human Brain Wave, Italy) is a commercial tissue disaggregator able to filter and select progenitor cells with a size of 50 micron from micrograft samples (adipose tissue, dental pulp, periosteum...).

Celution® is an automated processing unit for the standardized extraction, washing, and concentration of autologous adipose-derived SVF. Highlighted in grey: techniques requiring substantial manipulation.

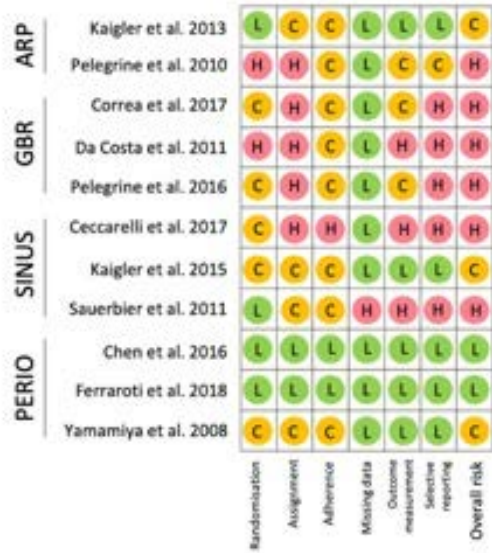
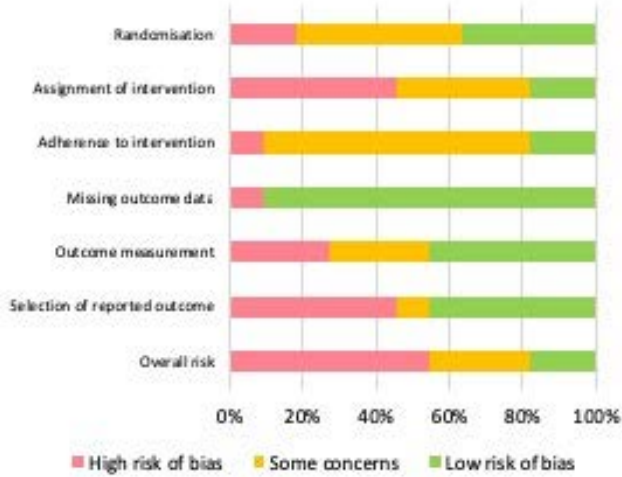
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

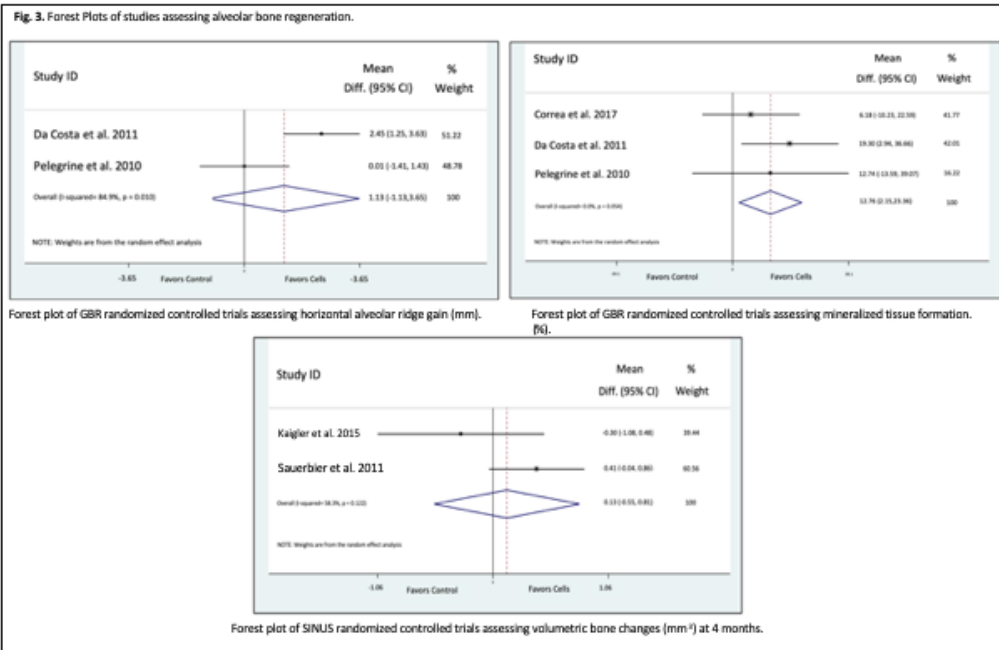


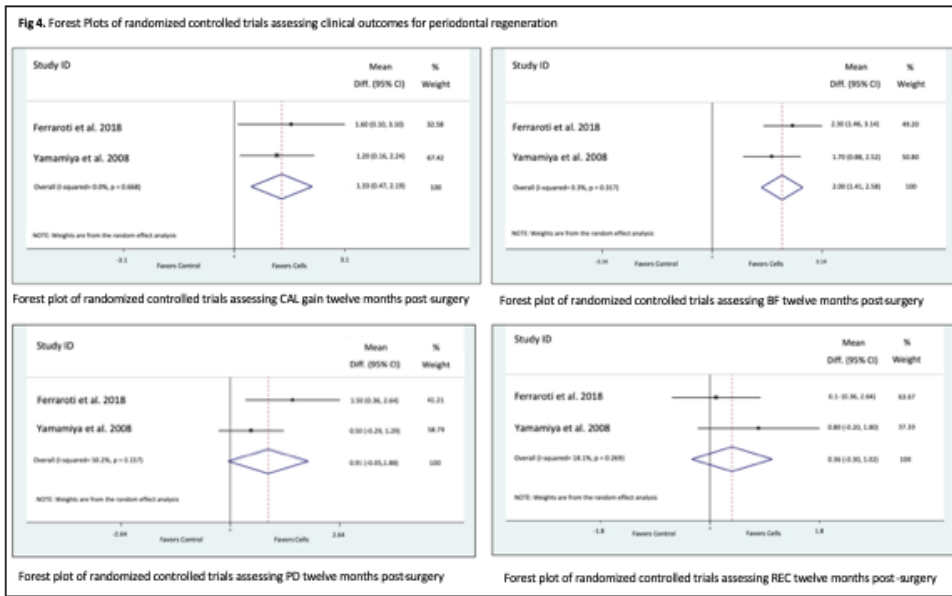
ROBIN-I Non-randomised trials



RoB 2.0 Randomised Trials

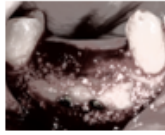








There is a **short-term benefit** of cell therapy for **alveolar ridge preservation**. This additional effect seems to be lost at longer follow-up periods.



For **alveolar ridge augmentation**, there was an **additional gain** of alveolar ridge width of 1.33 mm four to six months post-surgery, in sites regenerated using cell techniques.



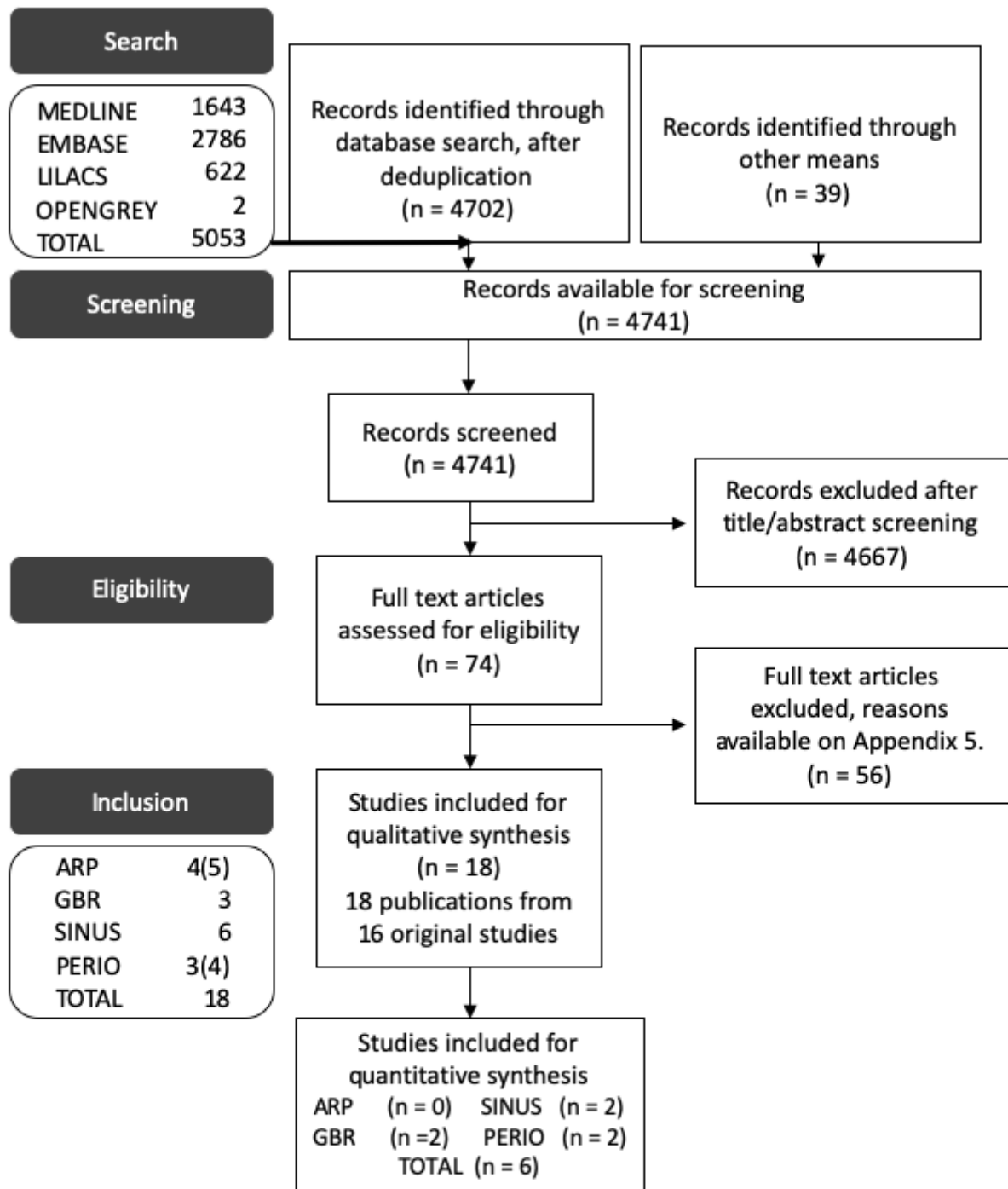
The use of cell-based techniques for **periodontal regeneration** resulted in a **significant improvement in clinical attachment gain** (1.33 mm additional CAL gain) and **radiographic bone fill** (2 mm)

There were **no** significant **clinical** or **histological benefits** for **sinus** augmentation.

○ Unresolved questions:

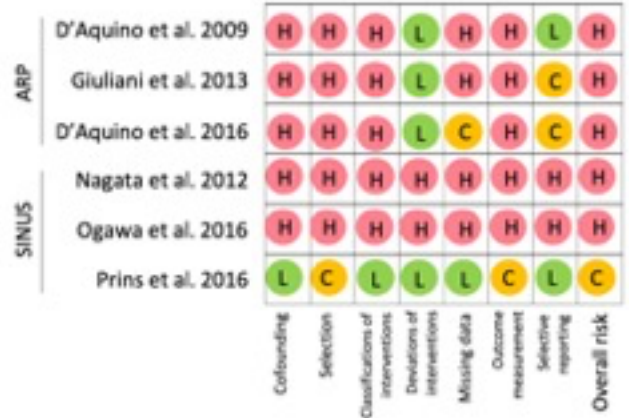
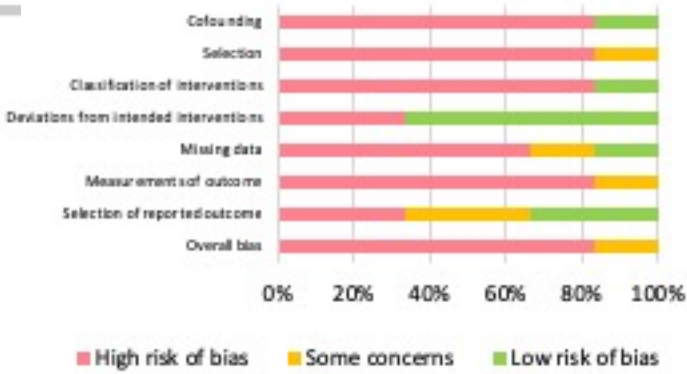
Which cell therapies are more effective on achieving improved clinical outcomes?

Benefit of '**Substantial manipulation**' of tissues and cells vs '**minimal manipulation**'?

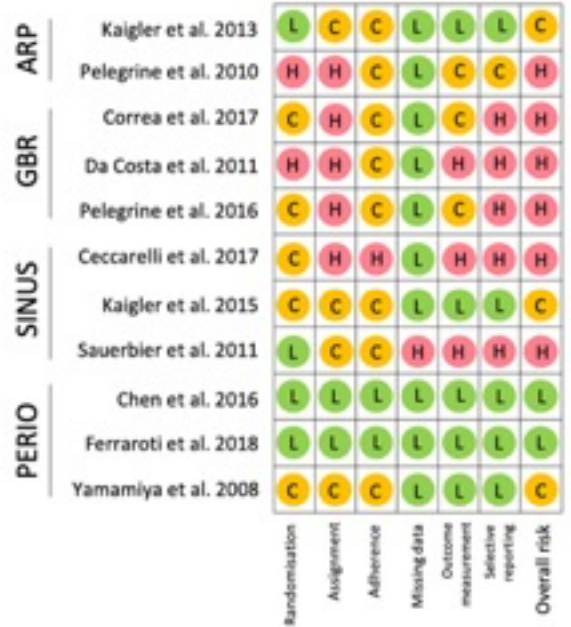
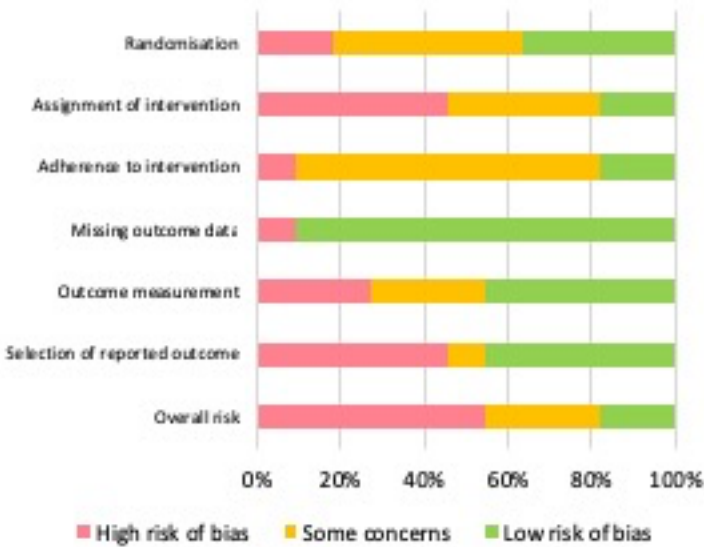


SCT3_12612_Federico Moreno Fig 1 TOP (1).tiff

ROBIN-I Non-randomised trials

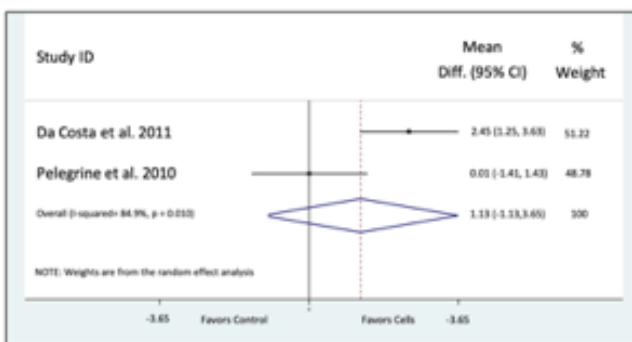


RoB 2.0 Randomised Trials

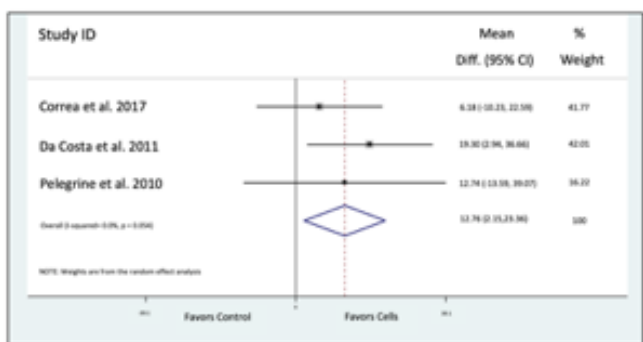


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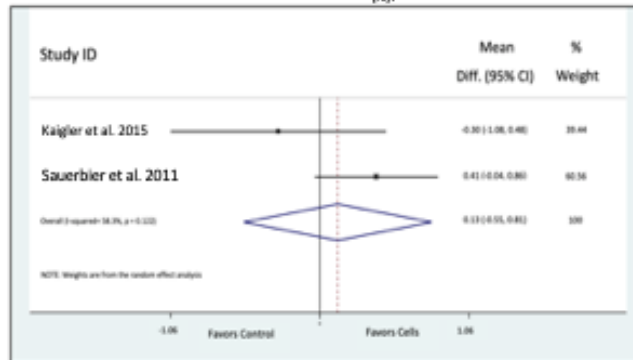
Fig. 3. Forest Plots of studies assessing alveolar bone regeneration.



Forest plot of GBR randomized controlled trials assessing horizontal alveolar ridge gain (mm).



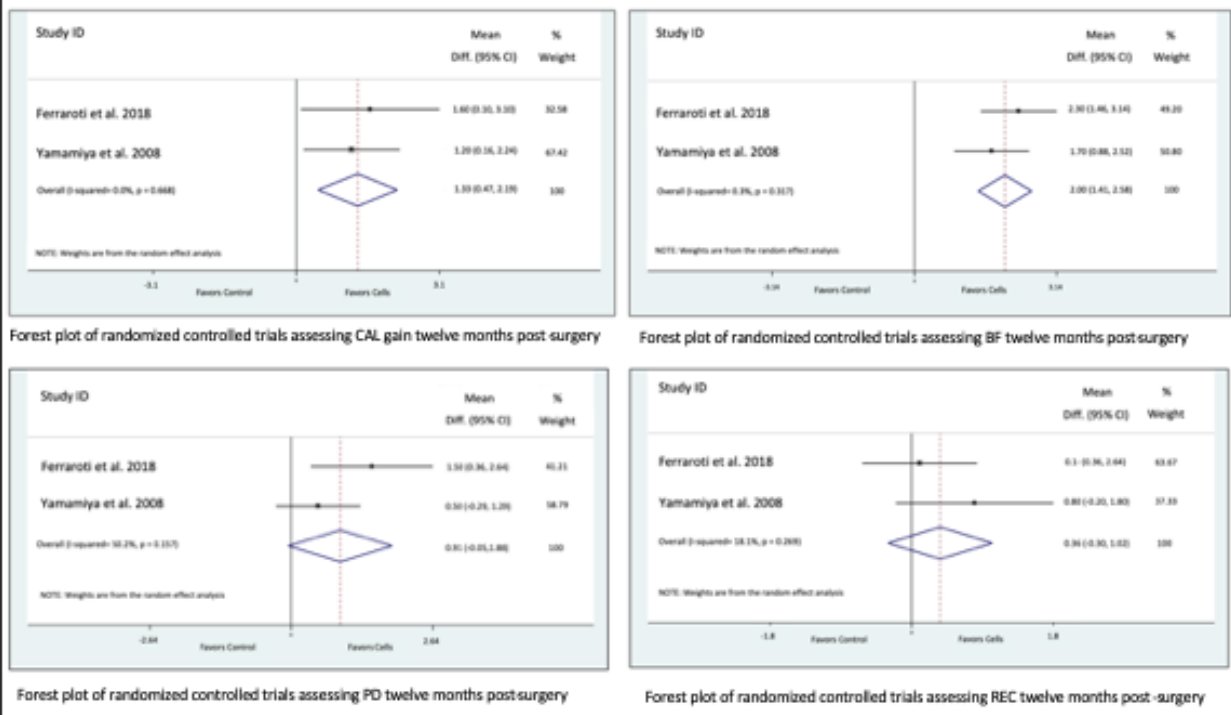
Forest plot of GBR randomized controlled trials assessing mineralized tissue formation. [6].



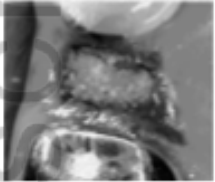
Forest plot of SINUS randomized controlled trials assessing volumetric bone changes (mm³) at 4 months.

SCT3_12612_Federico Moreno Fig 3 TOP.tiff

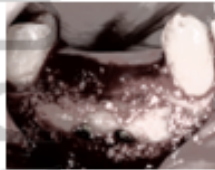
Fig 4. Forest Plots of randomized controlled trials assessing clinical outcomes for periodontal regeneration



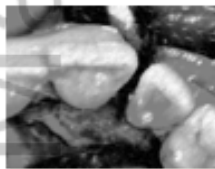
SCT3_12612_Federico Moreno Fig 4 TOP.tiff



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