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Cell-based therapies for the surgical treatment of periodontal intrabony defects: a systematic review

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Abstract. – OBJECTIVE: The aim of the study is to investigate the efficacy of cell-based therapy in the surgical treatment of periodontal intrabony defects.

MATERIALS AND METHODS: PRISMA guidelines were followed, and the study protocol was regis-tered in PROSPERO. Electronic and hand searches were carried out on electronic databases and major international journals of periodontology. All randomized clinical trials (RCTs) comparing cell-based therapies combined with surgery to surgery alone for the treatment of periodontal intrabony defects were considered. Quality assessment was performed using the Cochrane Risk of Bias Tool for randomized clinical trials (RoB 2). Quantitative evaluation of data was performed by meta-analysis.

RESULTS: Five hundred twenty-eight records were initially screened and 5 RCTs fulfilling the eligibility criteria were included. Periodontal ligament stem cells, dental pulp stem cells, periosteum-derived stem cells, gingival fibroblasts and their associated stem cells were used in combination with different surgical techniques to treat intrabony periodontal defects. Meta-analysis showed a statistically significant effect in favor of cell-based groups for clinical attachment level gain (p=0.004), with a difference in means of 1.7 mm (95% CI 0.5; 2.9). This was replicated for intrabony defect depth reduction (p=0.006), with a difference in means of 1.3 (95% CI 0.4; 2.3).

CONCLUSIONS: Cell-based therapies have been positively applied for the surgical treatment of intrabony periodontal defects with promising results. However, the results obtained should be interpreted with caution due to the low number of available RCTs, the study design heterogeneity, and the limited extension of the follow-up.

Key Words:
Cell-based therapy, Stem cells, Periodontitis, Intrabony defects, Surgical therapy, Systematic review, Meta-analysis.

Introduction

Periodontitis is a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus[1]. It is the leading cause of tooth loss in adults[2]. Severe periodontitis is found in 5-20% of middle-aged (35-44 years) adults in Europe and up to 40% of older people (65-74 years)[3].

Periodontal treatment aims to control the infection and arrest the progression of the disease. Supragingival and subgingival instrumental therapy by hand or powered instruments is the first phase of periodontal treatment[4]. With a re-evaluation visit, the clinician can ascertain if additional therapies are required, including surgical treatment[5], aiming to eliminate residual pockets through a conservative, resective or regenerative approach. Periodontal regenerative therapy is indicated only in selected cases, particularly for the treatment of periodontal intrabony defects[6]. When the bottom of a periodontal defect is located apically compared to the crestal bone peak, it is defined as intrabony[7]. Different periodontal regenerative techniques exist, including guided tissue regeneration (GTR) and induced tissue regeneration (ITR)[8]. The first one is based on the application of a membrane that excludes the cells of supra-crestal soft tissues and allows the proliferation of deep periodontal tissues[9]. The second one is based on the use of enamel matrix derivative (EMD), a protein matrix derived from porcine tooth buds, mainly composed of amelogenin. EMD gel is locally applied in periodontal defects and promotes the proliferation of cementum and periodontal ligament cells, while inhibiting epithelial proliferation[10].

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Recently, a new type of regenerative, cell-based therapy has been proposed in periodontal therapy[11].

Cell therapies aim to repair the mechanisms underlying disease initiation and progression. Multiple cell types can be utilized in such treatments, including stem, progenitor, or primary cells. The most common are mesenchymal stem cells (MSCs). Once harvested, isolated, and amplified, cells can be intravenously injected or directly transplanted into the injured organ to induce tissue repair[12]. The application fields for this type of therapy are various: type 1 diabetes mellitus[13], stroke[14], or liver diseases[15]. In literature, the safety of this kind of therapy has been deeply discussed, but such an innovative approach seems to be safe, since no association with toxicity, organ system complications, infections, or malignancy was found[16].

Cell-based approaches have shown the ability to regenerate periodontal tissues in different animal models[17-20]. Pre-clinical results are encouraging, showing a positive impact of cell-therapy on periodontal tissue regeneration, although the results are influenced by the type of cells and scaffolds used[21].

Initial evidence of the efficacy of using oral stem cell populations for the surgical therapy of periodontal defects has recently been provided[22].

The purpose of this systematic review and meta-analysis is to investigate the effects of cell-based therapies in the surgical treatment of periodontal defects by the analysis of the available randomized controlled trials (RCT).

Materials and Methods

The study protocol of the present systematic review and meta-analysis was registered in PROSPERO (CRD42021233504). The search strategy used was based on the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines (http://www.prisma-statement.org)[23].

Focused Question

The focused question for this systematic review was: "What is the efficacy and the supporting evidence of cell-based therapies in combination with any surgical approach for the treatment of periodontal defects compared to surgery alone in patients affected by periodontitis?". Clinical questions were formulated according to the PICO framework for evidence-based practice[24] comprising: patients affected by periodontitis (P), surgical periodontal cell-based therapies as intervention (I), compared to surgical therapy alone or with placebo as control (C), clinical attachment level (CAL) or relative attachment level (RAL) gain and intrabony defect depth (IBD) reduction (or bone fill) as investigated outcomes (O).

Search Strategy

A literature search was carried out in January 2021 by two independent and calibrated reviewers in PubMed (MEDLINE), EMBASE, ClinicalTrials.gov, Researchgate, and the Cochrane Central Register of Controlled Trials (CENTRAL). The authors used an ad-hoc search string: "(stem OR mesenchymal OR stromal OR MSC OR "cell sheet" OR "cell culture" OR "cell therapy" OR "cell-based therapy" OR "tissue based therapy" OR "tissue engineering") AND ("periodontal regeneration" OR "periodontal tissue regeneration" OR "intrabony defects" OR "infrabony defects" OR "intraosseous defects" OR "furcation defects" OR "periodontal defects" OR "intrabony periodontal defects" OR "infrabony periodontal defects" OR "guided tissue regeneration" OR "induced tissue regeneration" OR "bone fill") AND (random OR randomized OR randomly OR randomization)". A hand search was also conducted on the major international journals of periodontics. The reference lists of all studies identified to be relevant to the subject were scanned for possible additional studies.

Two independent reviewers (G.C. and A.I.) screened the title and abstract of studies identified by the search strategy and, in case of disagreements on the selection process, a consensus was achieved through discussion. For abstracts meeting the eligibility criteria or not providing sufficient data, the full texts were carefully read and analyzed for inclusion and data extraction. The inter-examiner agreement was verified by kappa coefficient, and any discrepancy resolved via discussion.

All RCTs with a test group assessing the efficacy of cell-based therapies combined with surgery for the treatment of periodontal defects compared to surgery alone were considered. In particular, articles were screened, and full-text reading and data extraction were performed when the following selection criteria were fulfilled.

Inclusion Criteria:

- Study design: RCTs with both parallel-group and split-mouth design;
- At least 6-month follow-up;
- Patients with periodontitis diagnosis;

– Intervention group should use cell-based therapy as a sole adjunct to surgical periodontal therapy;
– The control group should comprise surgical periodontal therapy alone or associated with placebo;
– The outcome should include at least one periodontal measurement, such as CAL or RAL gain, IBD reduction, or bone fill.

Exclusion Criteria:

– Non-RCT studies;
– Animal studies;
– Letters;
– Reviews;
– Conference abstracts.

No language or publication date restrictions were applied. In case of doubtful or incomplete data, the author responsible for the work was contacted. After analysis of the selected studies, clinical data were extracted by two independent reviewers (P.P. and L.N.).

Primary outcomes were considered: CAL (or RAL) gain and IBD reduction (or bone fill).

Secondary outcomes were considered: probing depth (PD) reduction, gingival recession (REC or GR) increase, or gingival marginal position (GMP), bleeding on probing (BOP), plaque index (PI), and gingival index (GI).

Mean changes from the baseline for the measured outcomes and their standard deviations were recorded when available. The following information for each study was also registered: study design, number of patients/defects, gender, age, study groups, type of defect, type of cells and source, application method and protocol, follow-up, outcomes evaluated, method of evaluation, and conclusions.

Risk of Bias (Quality) Assessment

The quality of the included studies was assessed using the Cochrane Risk of Bias Tool for randomized clinical trials (RoB 2) (updated on 22 August 2019)[25] by two independent calibrated examiners (G.C. and L.G.) to ensure agreement on the scoring system. Each study was judged as at low, high, or with some concerns risk-of-bias, based on five domains: (1) bias arising from the randomization process; (2) bias due to deviations from intended interventions; (3) bias due to missing outcome data; (4) bias in the measurement of the outcome; (5) bias in the selection of the reported result.

Strategy for Data Synthesis

Data were descriptively presented and, when possible, a meta-analysis was performed on primary and secondary outcomes to compare treatment results. Data were displayed as a difference in means, with 95% confidence intervals. The study-specific estimates were pooled using the random effects model if significant heterogeneity was found. Forest plots were created to illustrate the effects of the different studies and global estimation. Comprehensive Meta-analysis software (Biostat Inc., North Dean Street Englewood, NJ, USA) was used to perform all analyses. Statistical significance was defined as a p-value < .05.

The statistical heterogeneity among the included studies was evaluated using Cochrane's Q-test, with significance set at $p < 0.1$, and the I^2 test with a >75% value corresponding to high heterogeneity. If the meta-analysis contained sufficient trials to make a visual inspection of the plot meaningful (ten trials minimum), funnel plots were considered as a tool for assessment of publication bias.

Results

Study Selection

One-hundred ninety-three items in MEDLINE/PubMed, 122 items in Embase, and 213 in other sources were found after the initial search. After duplicates and items with no data available were removed, 498 records remained. After screening titles and abstracts for inclusion/exclusion criteria, 489 studies were excluded, and 9 studies remained. After full-text assessment, four studies were excluded. Those of Dhote et al 2015[26] and Shalini & Vandana 2018[27] were excluded because cell-therapy was not the only difference between test and control groups. Sali & Pauline George 2016[28] did not use living cells but freeze-dried preparations. Finally, the study of Hernández-Monjaraz et al 2020[29] was excluded because it did not report study outcomes of interest (CAL or IBD).

Finally, 5 RCTs published between 2008 and 2020 were included in this systematic review (Figure 1). A high level of agreement was found between the reviewers at both screening stages (K=0.91).

Study Characteristics

The number of participants ranged from 20[30,31] to 30 patients[32,33]. The age of participants ranged

from 25 to 70 years old. All patients were systemically healthy (or with controlled chronic diseases) and non-smokers; they suffered from periodontitis and had intrabony defects eligible for surgical treatment. All the RCTs had a parallel group design. A total of 140 intrabony defects were treated in the included RCTs.

The effect of periodontal ligament stem cells was assessed in 2 studies[30,32], dental pulp stem cells were used in 1 study[34], periosteum derived stem cells in another RCT[33] and gingival fibroblasts and their associated stem cells in another study[31]. Two studies[30,32] evaluated the adjunctive effects of periodontal ligament stem cells associated with a slow resorption xenograft, and one of these[32] also covered the graft with a collagen membrane both in the test and control groups. Yamamiya et al[33] used periosteum-derived stem cell sheets in association with platelet-rich plasma (PRP) and porous hydroxyapatite. Abdal-Wahab et al[31] investigated the effects of gingival fibroblasts and their associated stem cells with collagen membrane and β-calcium triphosphate (β-TCP). Ferrarotti et al[34], in their study, used a minimally invasive surgical technique (MIST) and a collagen sponge with (test) or without (control) dental pulp micro-grafts. The main characteristics of the selected studies are described in Table I.

Clinical parameters such as CAL gain and PD reduction were evaluated in all studies. BOP was evaluated in one study[33]. Ferrarotti et al[34] considered full-mouth bleeding score (FMBS). Abdal-wahab et al[31] and Yamamiya et al[33] evaluated GI. Plaque index (PI), FMPS, or full mouth plaque index (FMPI) were evaluated in almost all studies. Gingival recession or gingival margin position were reported in 4 studies[30,32-34].

Radiographic parameters, such as IBD (or bone fill), measured as the radiographic distance between the bone crest and the bottom of the bone defect, or bone defect depth (BDD), measured as the distance from the cement-enamel junction (CEJ) of the tooth and the bottom of the defect, or the distance CEJ-ABC (alveolar bone crest) were measured in 4 studies[31-34]. The clinical and radiographic parameters were reported in Table II.

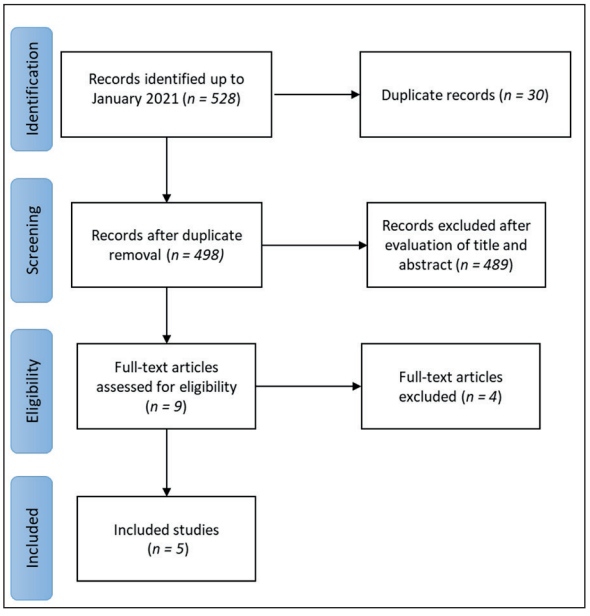

Figure 1. Flow diagram of sources selection process.

Risk of Bias and Power of Analysis

The overall risk of bias for each study included is presented in Figure 2. Three of the assessed RCTs fulfilled all criteria with a low risk of bias[30,32,34]. Two studies showed an unclear (some concerns) risk of bias[31,33].

Meta-Analysis

Only data at 6- and 12-month follow-up were considered for meta-analysis.

Four studies were included in the quantitative analysis of CAL gain, PD reduction[30,31,33,34], and IBD reduction[31-34], while three studies[30,33,34] were included in the analysis of REC increase.

Since high data heterogeneity was found, a random effect model was preferred in all cases.

Due to the limited number of included studies, additional investigations regarding publication bias or sensitivity analysis were not performed.

Regarding CAL gain (Figure 3a), three studies[31,33,34] showed a statistically significant effect size in favor of cell-based therapies groups. In contrast, only one study[30] showed no statistically significant differences between test and control groups. The overall effect size was in favor of cell-based therapies in a statistically significant way (p=0.004) with a difference in means of 1.7 mm (95% CI 0.5; 2.9), and a significant data heterogeneity (Q 13.5, p=0.004; I^2 77.9%).

Two of the four studies included in the meta-analysis of PD reduction showed a statistically significant effect size in favor of cell-based therapies[31,34] and two[30,33] did not show statistically significant inter-group differences (Figure 3b). An overall effect size in favor of cell-based therapies, although not statistically significant (p=0.067), was found, with a difference in means of 1.4 mm (95% CI -0.1; 3.0) and a significant data heterogeneity (Q 32.3, p=0.000; I^2 90.7%).

Table I. Study populations.

Authors, year	Pats/ defs	Gender (M/F)	Age	Test	Control	Cells	Source of cells	Invasiveness	Manipulation	External resources	Time	Cost
Yamamiya et al 2008[33]	30/30	2/28	55.8 (mean) ± 9.1 (SD)	PRP + HA gran-ules +	PRP + HA gran-ules HCP sheets	PdSC	Harvested periosteum	High (periosteum samples; 25 mm^2)	Substantial	Cell culture lab	6-7 w	High
Chen et al 2016[32]	30/41	8/33	18-65	BBM + collagen membrane + PDLSC sheets	BBM + collagen membrane	PDLSC	Extracted teeth	High (low only for scheduled extractions)	Substantial	Cell culture lab	4-5 w	High
Ferrarotti et al 2018[34]	29/29	14/15	36-69	Collagen sponge + DPSCs	Collagen sponge	DPSC	Extracted teeth	High (low only for scheduled extractions)	Minimal	None (tissue disaggregator)	Minutes	Medium (device cost)
Sánchez et al 2020[30]	20/20	14/6	25-70	XBS + PDLMSC	XBS	PDLMSC	Extracted teeth	High (low only for scheduled extractions)	Substantial	Cell culture lab	Not specified (weeks)	High
Abdal-Wahab et al 2020[31]	20/20	9/11	32-50	β-TCP + collagen membrane + HGF/GMSC	β-TCP + collagen membrane	HGF/ GMSC	Gingival biopsies	Moderate (low only for scheduled gingival surgery)	Substantial	Cell culture lab	2-3 w	High

Pats/defs: number of patients/defects; PRP: platelet-rich plasma; HA: hydroxyapatite; HCP: human cultured periosteum; PdSC: periostium-derived stem cells; PDLSC: periodontal ligament stem cell; BBM: bovine bone mineral; DPSC: dental pulp stem cell; OFD: open flap debridement; XBS: xenogeneic bone substitute; PDLMSC: periodontal ligament-derived mesenchymal stem cells; β-TCP: β-calcium triphosphate; HGF/GMSC: gingival mesenchymal stem cell.

Table II. Clinical and radiographic parameters at 6 and 12 months.

Authors, year	Method of evaluation	Test	Control	Additional effects	Adverse effects
Yamamiya et al[33] 2008	Periodontal probe; periapical standardized radiographs	*12 m* CALgain: 4.1 ± 1.2 PDred: 2.9 ± 0.4 RECinc: 1.3 ± 1.3 IBDred: 0.4±0.9	*12 m* CALgain: 5.3 ± 1.5 PDred: 3.3 ± 1.0 RECinc: 2.1 ± 1.4 IBDred: 1.7 ± 1.2	CALgain, IBDred significant difference	None
Chen et al[32] 2016	Periodontal probe; standardized periapical radiographs	*6 m* IBDred: 4.61 ± 1.87 *12 m* IBDred: 4.49 ± 2.03	*6 m* IBDred: 5.11 ± 1.53 *12 m* IBDred: 4.80 ± 1.41	Not statistically significant	None
Ferrarotti et al[34] 2018	Periodontal probe; standardized periapical radiographs	*6 m* CALgain:5.4 ± 1.2 PDred:3.5 ± 0.8 RECinc:1.9 ± 1.2 IBDred:2.7 ± 0.8 *12 m* CALgain: 5.5 ± 1.1 PDred: 3.4 ± 0.9 RECinc: 2.1 ± 1.3 IBDred: 2.5 ± 0.7	*6 m* CALgain:6.6 ± 1.3 PDred:4.6 ± 1.0 RECinc:2.0 ± 1.1 IBDred:4.1 ± 0.9 *12 m* CALgain: 6.5 ± 1.2 PDred: 4.5 ± 1.0 RECinc: 2.0 ± 1.2 IBDred: 4.0 ± 0.8	PDred, CAL, IBDred significant difference	None
Sánchez et al[30] 2020	Electronic pressure-sensitive periodontal probe	*12 m* CALgain: 9.44 ± 2.35 PDred: 4.33 ± 1.00 RECinc: 5.33 ± 2.29	*12 m* CALgain: 9.10 ± 2.18 PDred: 4.70 ± 2.11 RECinc: 4.40 ± 1.35	Not statistically significant	None
Abdal-Wahab[31] et al 2020	Periodontal probe; CBCT	*6 m* CALgain: 2.30 ± 1.16 PDred: 3.10 ± 0.88 IBDred: 3.14 ± 1.33	*6 m* CALgain: 4.20 ± 1 PDred: 5.20 ± 0.8 IBDred: 1.91 ± 0.16	PDred, CALgain, IBDred significant difference	None

CALgain: clinical attachment level gain; PDred: probing depth reduction; RECinc: gingival recession increase; IBDred: intrabony defect depth reduction. CBCT: Cone-beam Computed Tomography.

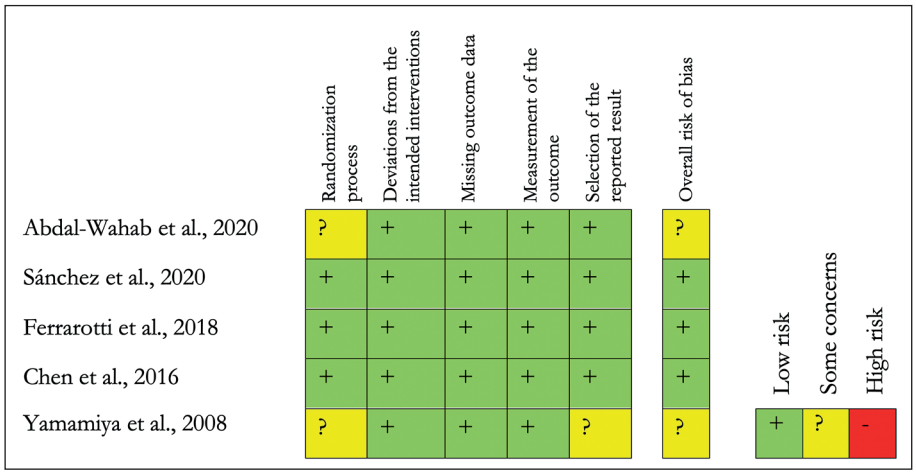

Figure 2. Risk of Bias assessment of the included RCTs.

Regarding REC increase, three studies showed a tendential, although insignificant, effect estimate in favor of control therapies[30,33,34] (Figure 3c). The overall effect estimate followed the same trend with an insignificant (p=0.277) difference in means in favor of control of -0.3 mm (95% CI -0.8; 0.2) and insignificant data heterogeneity (Q 1.3, p=0.5; I^2 0%).

Regarding IBD reduction, three studies[31,33,34] showed a statistically significant effect estimate in favor of cell-based therapies, while only one study showed no differences between groups[32] (Figure 3d). The overall effect was in favor of cell-based therapies in a statistically significant way (p=0.006) with a difference in means of 1.3 (95% CI 0.4; 2.3) and a significant data heterogeneity (Q 21.7, p=0.000; I^2 86.2%).

Discussion

The present systematic review and meta-analysis focused on the effects of cell-based therapies in the surgical treatment of intrabony periodontal defects. In order to include studies with the highest level of evidence, only RCTs were considered. Furthermore, all the studies in which the cell-based therapy was not the only difference between test and control groups were excluded. This was important in order to minimize confounding factors and discriminate the real adjunctive effect of cell-based therapies.

Periodontal regeneration is defined as the formation of new cementum, alveolar bone, and a functional periodontal ligament on a root surface previously affected by periodontitis[8]. The regeneration of deep periodontal tissues can be achieved through different techniques, such as GTR[35] or ITR *via* EMD application[36]. Both techniques improved long term results in terms of PD reduction and CAL gain compared to other periodontal therapies[37,38]. However, periodontal regenerative surgery still presents certain limits, since there is a lack of evidence for its efficacy in the regeneration of suprabony defects or the supracrestal component associated with intrabony defects[39]. Consequently, efforts aiming to improve the results of such techniques are being carried out continuously.

Tissue engineering is a therapeutic approach in regenerative medicine that aims to induce new functional tissue regeneration via the synergistic combination of cells, biomaterials, and/or growth factors[40]. Cell-based therapy has also been proposed in periodontology, in order to improve the results of regenerative surgical techniques, using different sources of stem cells and biomaterials/biological mediators. Stem cells are undifferentiated cells with the potential of proliferating and differentiating in several cell types with specific functions[41]. MSCs are post-natal stem cells isolated from a great variety of tissues[41]. Compared to embryonic stem cells, MSCs have a lower proliferation and differentiation potential depending on their tissue source[42]. Among the studies included in the present review, periodontal ligament[30,32], dental pulp[34], periosteum[33], and gingival tissue[31] were used as a source of MSCs.

A meta-analysis was performed with 6- and 12-month follow-up data from the included studies for four outcomes: PD reduction, CAL gain,

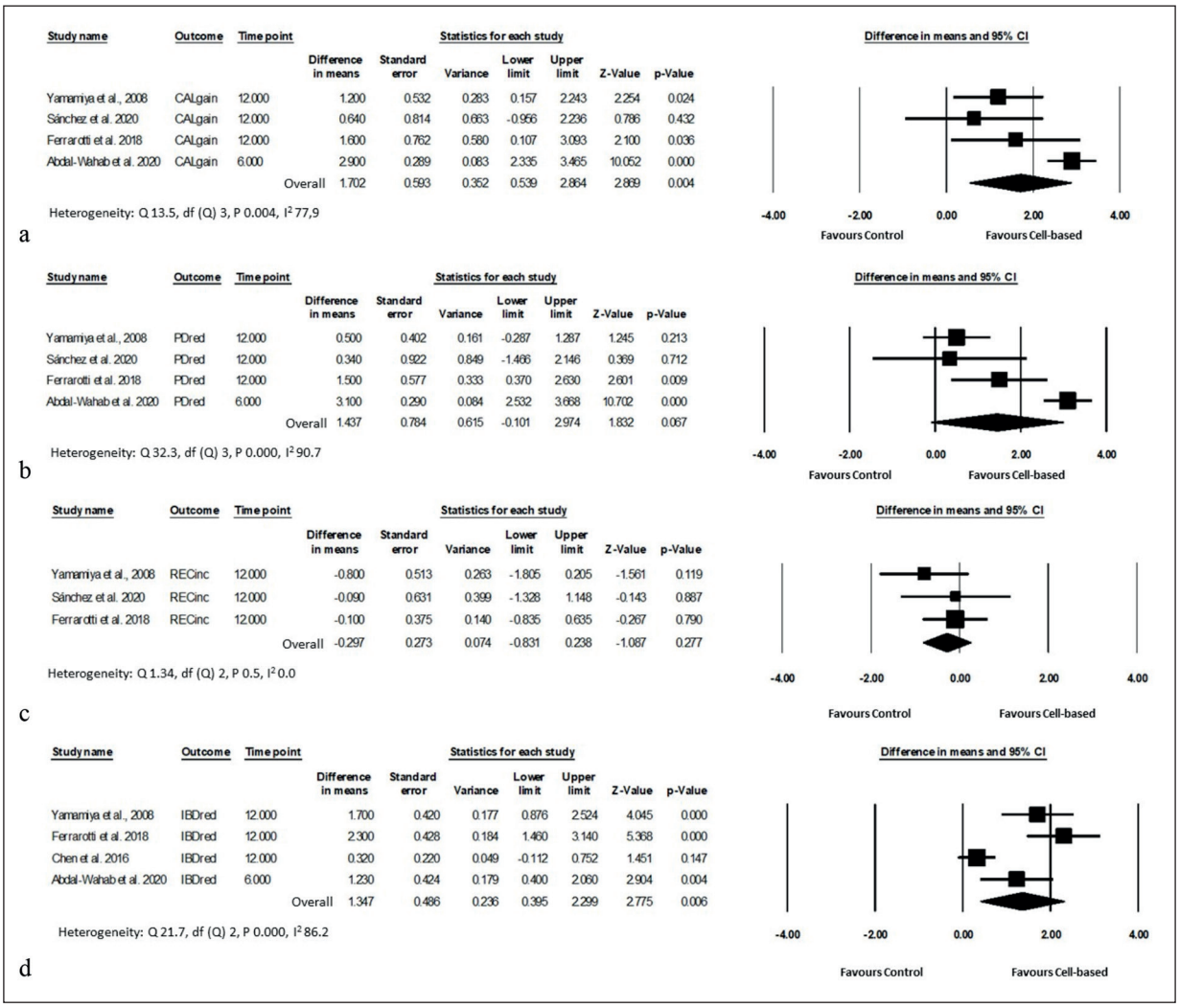

Figure 3. Forest plot and heterogeneity test for RCTs assessing 6- and 12-month CAL gain (**a**), PD reduction (**b**), REC increase (**c**), and IBD reduction (**d**) using cell-based therapy compared to any other surgical technique alone. Overall effect and effect for each study are presented.

REC increase and IBD reduction. Such a quantitative analysis showed an overall effect size favoring cell-based therapies in a statistically significant way only for CAL gain and IBD reduction, although with high heterogeneity.

The overall quality of the RCTs included in the present systematic review was moderate-high, since only 3 of 5 studies showed a low risk of bias. However, the marked heterogeneity found in the study design and the different types of cells used impose some caution in data interpretation, also in consideration of the small number of available studies. A balanced sex distribution was reported by most of the analyzed studies. Patients were healthy (or with controlled chronic diseases), and the periodontal defects were all intrabony ones. Smoking was considered an exclusion criterion in all the RCTs, due to its negative effects on periodontal healing[8].

No study reported adverse effects, indicating a relative safety of cell-based therapies applied to periodontal surgical therapy. However, the maximum follow-up of the RCTs was 12 months, and longer follow-up periods are recommended to better evaluate possible long term adverse events.

The application of a cell-based approach showed positive effects in the surgical treatment of intrabony periodontal defects, although the isolation of stem cells requires tissue harvesting. Such a procedure has intrinsic invasiveness that can vary based on the source of cells and the withdrawal method. Four studies included in the

present review used cells obtained from extracted teeth. Chen et al[32] and Sanchez et al[30] used periodontal ligament stem cells, while Ferrarotti et al[34] used dental pulp stem cells. In this case, teeth selected for cell harvesting need to be already scheduled for extraction, limiting the availability of the stem cell source. Similarly, Abdal-Wahab et al[31] withdrew cells from gingival tissue, during a scheduled periodontal surgery. In contrast, the withdrawal procedure of a 25 cm^2 periosteum sample adopted by Yamamiya et al[33] was done during a dedicated harvesting surgical procedure with an increased invasiveness. When utilizing stem cells, the need for external resources for cell culture and amplification has to be considered. These procedures can require several weeks, influencing the overall treatment costs and duration. An alternative to external facilities may be the use of a commercially available tissue disaggregator (Rigenera®, Human Brain Wave, Italy). Ferrarotti et al[34] used such a device to filter progenitor cells from dental pulp micrografts through a 50 μm microgrid. An accurate evaluation of the cost-benefit ratio must be made, both from a biological and an economic point of view also in consideration of satisfactory results already obtainable with currently used techniques in daily clinical practice.

Other review articles focused on stem cells and cell-based therapies in periodontal regenerative surgery[11,43]. However, the number of the included studies in those papers is lower compared to the present review.

Despite the promising results of the present systematic review, some limitations must be considered for a comprehensive interpretation, such as the paucity, the heterogeneity and the short follow-up of the available studies, together with the pooling of different follow-up time points in the meta-analysis. Further research is necessary to evaluate the clinical benefit of these techniques compared with traditional, non-cell-based techniques and to investigate alternative, simple, rapid, and economical methods for cell harvesting and manipulation.

Conclusions

Cell-based therapies have been positively applied for the surgical treatment of intrabony periodontal defects with promising results. Quantitative analysis of data from the available studies showed a significant difference in terms of CAL gain and IBD reduction in favor of cell-based therapies compared to control treatments. However, these results must be interpreted with caution due to the limited number of available studies, the study design heterogeneity, and the limited follow-up extension. Furthermore, the effectiveness of such therapies is severely restricted by high costs, lengthy duration, complexity of procedures and invasiveness. Larger RCTs with an appropriate design and longer follow-up are required to better evaluate positive effects of cell-based therapy in the surgical treatment of periodontal intrabony defects. In addition, more appropriate strategies to enhance the effective applicability in clinical practice should be found.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, Flemmig TF, Garcia R, Giannobile WV, Graziani F, Greenwell H, Herrera D, Kao RT, Kebschull M, Kinane DF, Kirkwood KL, Kocher T, Kornman KS, Kumar PS, Loos BG, Machtei E, Meng H, Mombelli A, Needleman I, Offenbacher S, Seymour GJ, Teles R, Tonetti MS. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol 2018; 89: S173-S182.
2) Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005 ; 366: 1809-1820.
3) World Health Organization. World Oral Health Report 2018/2019. https://www.euro.who.int/en/health-topics/disease-prevention/oral-health/data-and-statistics (accessed March 15, 2021).
4) Sanz M, Herrera D, Kebschull M, Chapple I, Jepsen S, Beglundh T, Sculean A, Tonetti MS; EFP Workshop Participants and Methodological Consultants. Treatment of stage I-III periodontitis-The EFP S3 level clinical practice guideline. J Clin Periodontol 2020; 47 (Suppl 22): 4-60.
5) Italian society of Periodontology decisional tree. https://www.sidp.it/media/tazz32a.pdf (accessed March 15, 2021).
6) Cortellini P, Tonetti MS. Clinical concepts for regenerative therapy in intrabony defects. Periodontol 2000. 2015; 68: 282-307.
7) Goldman HM, Cohen DW. The Infrabony Pocket: Classification and Treatment. J Periodontol 1958; 29: 272–291.
8) Reynolds MA, Kao RT, Camargo PM, Caton JG, Clem DS, Fiorellini JP, Geisinger ML, Mills

MP, Nares S, Nevins ML. Periodontal regeneration - intrabony defects: a consensus report from the AAP Regeneration Workshop. J Periodontol 2015; 86: S105-S107.

9) Scantlebury TV. 1982-1992: A Decade of Technology Development for Guided Tissue Regeneration. J Periodontol. 1993; 64 Suppl 11S: 1129-1137.

10) Guida L, Annunziata M, Carinci F, Di Feo A, Passaro I, Oliva A. In vitro biologic response of human bone marrow stromal cells to enamel matrix derivative. J Periodontol 2007; 78: 2190-2196.

11) Moreno Sancho F, Leira Y, Orlandi M, Buti J, Giannobile WV, D'Aiuto F. Cell-Based Therapies for Alveolar Bone and Periodontal Regeneration: Concise Review. Stem Cells Transl Med 2019; 8: 1286-1295.

12) Buzhor E, Leshansky L, Blumenthal J, Barash H, Warshawsky D, Mazor Y, Shtrichman R. Cell-based therapy approaches: the hope for incurable diseases. Regen Med 2014; 9: 649-672.

13) Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 2008; 26: 443-452.

14) Li Y, Chen J, Chen XG, Wang L, Gautam SC, Xu YX, Katakowski M, Zhang LJ, Lu M, Janakiraman N, Chopp M. Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. Neurology 2002; 59: 514-523.

15) Almeida-Porada G, Zanjani ED, Porada CD. Bone marrow stem cells and liver regeneration. Exp Hematol 2010; 38: 574-580.

16) Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, Granton J, Stewart DJ; Canadian Critical Care Trials Group. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. PLoS One 2012; 7: e47559.

17) Kawaguchi H, Hirachi A, Hasegawa N, Iwata T, Hamaguchi H, Shiba H, Takata T, Kato Y, Kurihara H. Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. J Periodontol 2004; 75: 1281-1287.

18) Akizuki T, Oda S, Komaki M, Tsuchioka H, Kawakatsu N, Kikuchi A, Yamato M, Okano T, Ishikawa I. Application of periodontal ligament cell sheet for periodontal regeneration: a pilot study in beagle dogs. J Periodontal Res 2005; 40: 245-251.

19) Liu Y, Zheng Y, Ding G, Fang D, Zhang C, Bartold PM, Gronthos S, Shi S, Wang S. Periodontal ligament stem cell-mediated treatment for periodontitis in miniature swine. Stem Cells 2008; 26: 1065-1073.

20) F awzy El-Sayed KM, Paris S, Becker ST, Neuschl M, De Buhr W, Sälzer S, Wulff A, Elrefai M, Darhous MS, El-Masry M, Wiltfang J, Dörfer CE. Periodontal regeneration employing gingival margin-derived stem/progenitor cells: an animal study. J Clin Periodontol 2012; 39: 861-870.

21) Gaubys A, Papeckys V, Pranskunas M. Use of Autologous Stem Cells for the Regeneration of Periodontal Defects in Animal Studies: a Systematic Review and Meta-Analysis. J Oral Maxillofac Res 2018; 9: e3.

22) Hynes K, Menicanin D, Gronthos S, Bartold PM. Clinical utility of stem cells for periodontal regeneration. Periodontol 2000 2012; 59: 203-227.

23) Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol 2009; 62: 1006-1012.

24) Schardt C, Adams MB, Owens T, Keitz S, Fontelo P. Utilization of the PICO framework to improve searching PubMed for clinical questions. BMC Med Inform Decis Mak 2007; 7: 16.

25) Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, Cates CJ, Cheng HY, Corbett MS, Eldridge SM, Emberson JR, Hernán MA, Hopewell S, Hróbjartsson A, Junqueira DR, Jüni P, Kirkham JJ, Lasserson T, Li T, McAleenan A, Reeves BC, Shepperd S, Shrier I, Stewart LA, Tilling K, White IR, Whiting PF, Higgins JPT. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ 2019; 366: l4898.

26) Dhote R, Charde P, Bhongade M, Rao J. Stem Cells Cultured on Beta Tricalcium Phosphate (β-TCP) in Combination with Recombinant Human Platelet-Derived Growth Factor - BB (rh-PDGF-BB) for the Treatment of Human Infrabony Defects. J Stem Cells 2015; 10: 243-254.

27) Shalini HS, Vandana KL. Direct application of autologous periodontal ligament stem cell niche in treatment of periodontal osseous defects: A randomized controlled trial. J Indian Soc Periodontol 2018; 22: 503-512.

28) Sali DD, Pauline George J. Demineralized Freeze Dried Bone Allograft With Amniotic Membrane in the Treatment of Periodontal Intrabony Defects - 12 Month Randomized Controlled Clinical Trial. J Periodontol 2016: 1-18.

29) Hernández-Monjaraz B, Santiago-Osorio E, Ledesma-Martínez E, Aguiñiga-Sánchez I, Sosa-Hernández NA, Mendoza-Núñez VM. Dental Pulp Mesenchymal Stem Cells as a Treatment for Periodontal Disease in Older Adults. Stem Cells Int 2020; 2020: 8890873.

30) Sánchez N, Fierravanti L, Núñez J, Vignoletti F, González-Zamora M, Santamaría S, Suárez-Sancho S, Fernández-Santos ME, Figuero E, Herrera D, García-Sanz JA, Sanz M. Periodontal regeneration using a xenogeneic bone substitute seeded with autologous periodontal ligament-derived mesenchymal stem cells: A 12-month quasi-randomized controlled pilot clinical trial. J Clin Periodontol 2020; 47: 1391-1402.

31) Abdal-Wahab M, Abdel Ghaffar KA, Ezzatt OM, Hassan AAA, El Ansary MMS, Gamal AY. Regenerative potential of cultured gingival fibroblasts in

treatment of periodontal intrabony defects (randomized clinical and biochemical trial). J Periodontal Res 2020; 55: 441-452.

32) Chen FM, Gao LN, Tian BM, Zhang XY, Zhang YJ, Dong GY, Lu H, Chu Q, Xu J, Yu Y, Wu RX, Yin Y, Shi S, Jin Y. Treatment of periodontal intrabony defects using autologous periodontal ligament stem cells: a randomized clinical trial. Stem Cell Res Ther 2016; 7: 33.

33) Yamamiya K, Okuda K, Kawase T, Hata K, Wolff LF, Yoshie H. Tissue-engineered cultured periosteum used with platelet-rich plasma and hydroxyapatite in treating human osseous defects. J Periodontol 2008; 79: 811-818.

34) Ferrarotti F, Romano F, Gamba MN, Quirico A, Giraudi M, Audagna M, Aimetti M. Human intrabony defect regeneration with micrografts containing dental pulp stem cells: A randomized controlled clinical trial. J Clin Periodontol 2018; 45: 841-850.

35) Needleman IG, Worthington HV, Giedrys-Leeper E, Tucker RJ. Guided tissue regeneration for periodontal infra-bony defects. Cochrane Database Syst Rev 2006. doi: 10.1002/14651858.CD001724.pub2.

36) Esposito M, Grusovin MG, Papanikolaou N, Coulthard P, Worthington HV. Enamel matrix derivative (Emdogain) for periodontal tissue regeneration in intrabony defects. A Cochrane systematic review. Eur J Oral Implantol 2009; 2: 247-266.

37) Sculean A, Kiss A, Miliauskaite A, Schwarz F, Arweiler NB, Hannig M. Ten-year results following treatment of intra-bony defects with enamel matrix proteins and guided tissue regeneration. J Clin Periodontol 2008; 35: 817-824.

38) Wu YC, Lin LK, Song CJ, Su YX, Tu YK. Comparisons of periodontal regenerative therapies: A meta-analysis on the long-term efficacy. J Clin Periodontol 2017; 44: 511-519.

39) Cortellini P, Tonetti MS. Regenerative periodontal therapy. In: Lang NP, Lindhe J, editors. Clin. Periodontol. Implant Dent, 2015, p. 913.

40) Du M, Duan X, Yang P. Induced Pluripotent Stem Cells and Periodontal Regeneration. Curr Oral Health Rep 2015; 2: 257-265.

41) Kobolak J, Dinnyes A, Memic A, Khademhosseini A, Mobasheri A. Mesenchymal stem cells: Identification, phenotypic characterization, biological properties and potential for regenerative medicine through biomaterial micro-engineering of their niche. Methods 2016; 99: 62-68.

42) Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126: 663-676.

43) Novello S, Debouche A, Philippe M, Naudet F, Jeanne S. Clinical application of mesenchymal stem cells in periodontal regeneration: A systematic review and meta-analysis. J Periodontal Res 2020; 55: 1-12.