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Original

Stem cell-based therapy in periodontal regeneration: a systematic review and meta-analysis of randomized clinical studies / Campagna, Alessandro; Baima, Giacomo; Romano, Federica; Amoroso, Federico; Mussano, Federico; Oteri, Giacomo; Aimetti, Mario; Peditto, Matteo. - In: DENTISTRY JOURNAL. - ISSN 2304-6767. - (2024). [10.3390/dj12050145]

Availability:

This version is available at: 11583/2988112 since: 2024-05-16T12:49:06Z

Publisher:

Mdpi

Published

DOI:10.3390/dj12050145

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Orally-derived stem cell-based therapy in periodontal regeneration: a systematic review and meta-analysis of randomized clinical studies

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Abstract: The present systematic review was performed study the application in the periodontal regenerative therapy of the orally-derived stem cells, because of this, the following PICO question was proposed: “In patients with periodontitis, can the adjunctive use of orally-derived stem cells provide additional clinical and radiographic benefits for periodontal regeneration?” Randomized clinical studies were electronically and manually searched up to December 2023. Quantitative analyses were performed with the aim to evaluate mean differences (MD) between the treatment and control groups in terms of clinical attachment level (CAL) gain, probing pocket depth (PPD) reduction, gingival recession (GR), and radiographic bone gain (RBG) using random effect models. A total of 7 studies were selected for the systematic review. Meta-analyses excluding studies with high risk of bias highlighted a non-statistically significant result for the use of stem cells compared to the control groups in terms of CAL gain [MD = 1.05; 95% CI (-0.88, 2.97) p = 0.29] and PPD reduction [MD = 1.32; 95% CI (-0.25, 2.88) p = 0.10]. The same also applied to GR [MD = -0.08; 95% CI (-0.79, 0.63) p = 0.83] and RBG [MD = 0.50; 95% CI (-0.88, 1.88) p = 0.48]. Based on high heterogeneity, there is not enough evidence to consider the adjunctive application of orally-derived mesenchymal stem cells as a preferential approach for periodontal regenerative treatment compared to standard procedures.

Keywords: periodontitis; periodontal regeneration; stem cells; biomaterials.

1. Introduction

Periodontitis is a biofilm-mediated disease with an important inflammatory component which causes the progressive breakdown of the supporting periodontal tissues [1–3]. The objectives of the first steps of periodontal therapy are the control of the microbial infection and the resolution of the inflammation, which clinically refers to the absence of bleeding on probing (BoP) and presence of shallow probing pocket depths (PPD ≤ 4 mm) [4–6]. However, residual pockets often persist after non-surgical treatment, especially in sites with important furcation involvement (FI) and/or deep intrabony defects [7,8]. After the XVI European Workshop in Periodontology, there is a strong recommendation to treat dental elements with deep residual PPD associated with intrabony defects of ≥ 3 mm with periodontal regenerative surgery [9–11]. Similarly, the recommendation was expressed for the treatment of class II maxillary and mandibular molars [9]. As reported in the literature, the various surgical techniques and biomaterials developed in the last 30–40 years with the aim of predictable periodontal regeneration have achieved variable success [12–

14]. The benefits reported are often limited to deep intrabony defects and class II mandibular FI, while supracrestal defects, non-containing intrabony defects and maxillary class II or III FI still have a less predictable outcome [15–18]. For this reason, new tissue engineering strategies are being sought, and **the implementation of innovative techniques using orally-derived stem cells** is growing in terms of scientific research in periodontology [19,20].

Compared to biomaterials, which are scaffolds characterized by unique chemical, mechanical and biological properties, mainly osteoinductivity and osteoconductivity [21], cell therapy relies on replenishing and/or empowering the inner healing body potential [22,23]. In recent years, cell regeneration therapy has been introduced in many areas of medicine, such as cardiology, neurology or traumatology [24,25], as well as for the treatment of orofacial dystrophies, diabetic problems and autoimmune diseases [26,27]. Regenerative medicine commonly employs stem cells, particularly mesenchymal stem cells (MSCs), which possess unique **faculties** like self-renewal, clonality, and potency. These adult stem cells exhibit anti-inflammatory properties and contribute to tissue repair processes, secreting mediators with various beneficial effects [28,29]. The expression of specific surface antigens, including CD44, CD73, CD29, CD90, and CD105, helps characterize MSCs, while lacking certain hematopoietic and endothelial markers [30]. Indeed, MSCs are defined by their plastic adherence, capacity of self-renovation, and the potential for differentiation *in vitro* into **different types of cells, like** osteoblasts, adipocytes, and chondroblasts under specific stimuli [31,32]. Many intraoral and dental sources of MSCs are available, for example **dental pulp, periodontal ligament, bone marrow from alveolar bone, dental follicle, gingival connective tissue or apical papilla** [33–36]. In virtue of their self-renewal, multipotentiality, immunomodulation, and tissue regeneration capacities, MSCs can promote the growth of various periodontal tissues, like **alveolar bone, root cementum and periodontal ligament, even in situation with low intrinsic potential** [23,37–41]. A recent study assessed periodontal regenerative approaches in animal models, observing that **mesenchymal stem cells used** alone or mixed with other biomaterials, such as **bovine bone, beta-tricalcium phosphate (β -TCP), or platelet-rich plasma (PRP)**, offered better regenerative outcomes than those of the group with biomaterials alone [42]. Most preclinical studies have indeed supported the biological rationale of employing MSCs to promote osteoinduction and tenogenesis, while decreasing inflammation [26,43–45].

In humans, recent systematic reviews evaluated the clinical results of periodontal regeneration by MSCs derived from different sources [46,47], reporting a significant advantage of using cell therapy in terms of final outcomes. However, due to the presence of highly heterogeneous results and the detection of methodological inconsistencies in data handling, the **purpose of the present systematic review** was to elucidate through a meta-analytic approach the adjunctive clinical and radiographic effect of using orally-derived stem cells for periodontal regeneration.

2. Materials and Methods

A systematic review protocol was written in the planning stages and registered on the **International prospective register of systematic reviews (PROSPERO; CRD42024525702)**. The PRISMA statement was followed in both the planning and reporting of the review [48].

2.1. Focused question

This systematic review aimed to answer the following **PICO** question: “In patients with periodontitis, can the adjunctive use of orally-derived stem cells provide additional clinical benefits measured as clinical attachment level (CAL) gain, probing pocket depth (PPD) reduction, recession (GR) and radiographic bone gain (RBG) for periodontal regeneration procedures?”

2.2. Eligibility criteria

In the present systematic review, the criteria used to select the clinical studies were based on the PICOS method and were the following:

- (P) Population: Adult patients with stage III-IV periodontitis presenting with residual pockets and intra-bony defects with at least 3 mm of intra-bony component after the completion of steps I-II of periodontal therapy (causal-related therapy; supra- and sub-gingival instrumentation) [9];
- (I) Intervention: Periodontal regeneration with the use of orally-derived stem cells;
- (C) Comparison: All other strategies for periodontal regeneration;
- (O) Outcome measures:

Primary outcomes: CAL gain, and PPD reduction.
Secondary outcomes: GR and RBG.

- (S) Types of studies: Only randomized controlled clinical trials (RCTs) were considered.

The following additional inclusion criteria were applied:

- English language;
- At least 6 months of follow-up;

These exclusion criteria were also applied to the selection process:

- Lack of pretreatment and post-treatment outcome measures
- Case reports, case series, retrospective studies, animal studies, *in vitro* studies.

2.3. Search strategy

The search was conducted through various sources, both electronically and manually. The electronic research included Medline (PubMed), Scopus, and CENTRAL (Cochrane Central Register of Controlled Trials) databases. All articles published until December 2023 were searched adopting the strategy reported in Table 1. A screening of the reference lists of the included studies and related reviews was also carried out to identify any additional article of relevance. Hand search was also implemented of the following journals by the authors: *Journal of Clinical Periodontology*, *Journal of Dental Research*, *Journal of Periodontology*, and *Journal of Periodontal Research*.

Table 1. – Search strategy.

(Periodontal defect OR periodontal lesion OR periodontal osseous defect OR intraosseous defect OR intra-osseous defect OR intrabony defect OR infra-bony defect OR angular defect OR bony defect OR osseous defect OR crater)
AND
(stem cells OR stem OR stem cell therapy OR cell therapy OR MSC OR mesenchymal stem cells OR human cord stem cells OR BMMSC OR bone marrow mesenchymal stem cell OR pluripotent stem cells OR embryonic stem cells OR ESC OR cell technology OR oral stem cells OR stem cell-delivery therapeutics OR induced pluripotent stem cells OR iPSC OR adipose-derived stem cells OR dental stem cells OR pulp stem cells OR periodontal ligament stem cells OR PDLSC OR progenitor cells OR apical papilla stem cells OR dental follicle stem cells OR human exfoliated deciduous tooth cells)
AND
(clinical trial OR case series OR prospective study OR longitudinal study OR cohort study OR RCT OR randomized clinical trial)
AND
(GTR OR guided tissue regeneration OR periodontal regeneration)

2.4. Study selection

The results obtained from the manual search and from the various electronic database were downloaded and imported jointly into a reference management software, and duplicates or non-English language articles were automatically removed. The identified

articles were checked based on the pre-defined eligibility criteria. During the initial phase the screening of potentially suitable titles and abstracts was performed: abstracts that at this time met the inclusion criteria or did not provide sufficient information were admitted for the subsequent review phase. Once the eligible articles were defined, they were re-evaluated after reading the full-text by applying the selection criteria again. The studies that satisfied all the inclusion criteria were included in the systematic review. Two reviewers (A.C. and M.P.) evaluated the abstracts, titles and full text for selection, and when differences occurred, they were solved by discussion with a third party (G.O.).

2.5. Data extraction for analysis

The relevant data identified in the included studies were reported in a standardized extraction form, including the following:

- Author(s) and year of publication;
- Number of patients included in the study;
- Number of defects treated in both the test and control groups;
- Type of stem cells used in the test group;
- Type of bone defect treated;
- Type of treatment of the test group;
- Type of treatment of the control group;
- CAL gain;
- PPD reduction;
- GR;
- RBG;
- Study duration.

2.6. Risk of bias of individual studies

The quality evaluation of the selected studies was independently performed by two review authors (A.C. and M.P.) through risk of bias analysis as it could impact on the overall results and conclusions. The Cochrane Collaboration's tool was used for assessing risk of bias [49,50]. We considered seven domains (sequence generation, allocation concealment, blinding of the outcome assessor, blinding of participants and personnel, incomplete outcome data, selective outcome reporting and other bias) and included in a specific table the results of the assessment. Then, the compressive risk of bias in the included studies was categorized as below:

A: Low risk of bias: little chance that bias would significantly affect the outcomes if all criteria were fulfilled;

B: Unclear risk of bias: possibility of bias that casts some doubt on the outcomes if one or more criteria were only partially met;

C: High risk of bias: likelihood of bias that substantially undermines confidence in the outcomes if one or more criteria were not met.

2.7. Statistical analysis

Studies were firstly summarized in a narrative form by key characteristics and according to type of regenerative surgery. A meta-analysis was carried out in the presence of at least two studies of similar design. The variables were registered at patient level. In each patient, only one tooth per technique was assessed. Weighted mean differences (MD) and 95% confidence intervals (95% CI) were calculated for CAL gain, PPD reduction, GR reduction [51], and RBG using the generic inverse variance method. Forest plots were graphically depicted to summarize the difference in outcomes between the groups using the patient as the analysis unit.

We used the χ^2 test to assess the statistical heterogeneity among the different studies and the percentage of variation in the global estimate due to heterogeneity was calculated using I² index (25%: low; 50%: moderate; 75% high). [49] In case of values higher than 50%, the random effect method was applied. Results were considered statistically significant

for p values < 0.05 . Statistical analyses were carried out using the RevMan software version 5.4 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

3. Results

3.1. Study selection

The selection process was conducted according to the PRISMA guideline (**Figure 1**). The search on the MEDLINE/PubMed, Scopus and Cochrane databases provided a total of 4086 studies; there were 65 duplicates, while articles discarded for non-English language were 334. A number of 3687 studies were screened and, of these, 3678 were excluded after first-stage reading of titles and abstracts due to the type of publication (chapter of book or thesis), objective and/or design of the study. Two articles were removed after full-text reading. Finally, 7 articles met all the inclusion criteria and were included into the qualitative analysis.

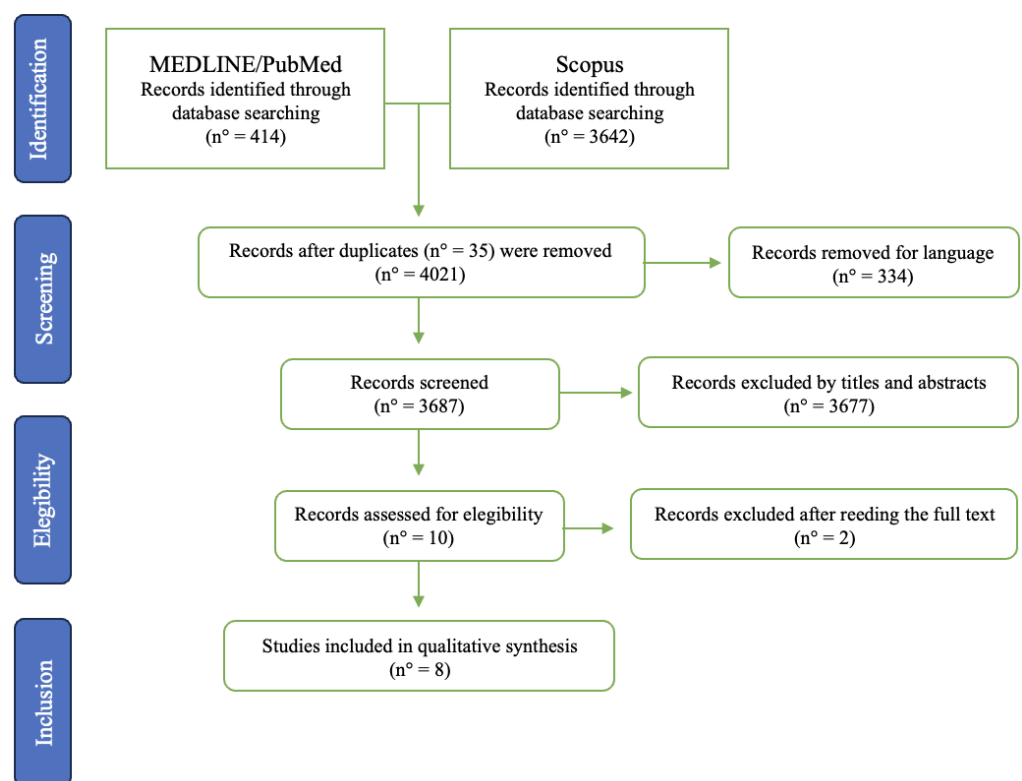


Figure 1. – PRISMA flowchart illustrating the experimental study search and selection process.

3.2. Risk of bias

Out of the 7 included RCTs, 2 were not included in the meta-analysis because were rated at high risk of bias [52,53]. Of the remaining 5 studies, 3 were considered as unclear risk of bias and only 2 as low risk of bias (**Figure 2**) [19,54–57]. The lack of blinding of the outcome assessor, among the seven domains, was the most frequent source of bias.

Study	Random sequence generation	Allocation concealment	Selective reporting	Other bias	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Compressive evaluation
Abdal-Wahab et al. (2020)	+	+	+	+	?	X	+	Unclear Included
Apatzidou et al. (2021)	+	?	+	+	X	?	+	Unclear Included
Chen et al. (2016)	+	+	+	+	+	+	+	Low Included
Ferrarotti et al. (2018)	+	?	+	+	+	+	+	Low Included
Hernández-Monjaraz et al. (2020)	+	?	+	+	?	?	X	High Excluded
Sánchez et al. (2020)	+	+	+	+	?	X	+	Unclear Included
Shalimi & Vandana (2018)	+	?	X	X	X	X	X	High Excluded

Figure 2. – Assessment of the risk of bias in the included studies.

3.3. Study characteristics

Data extracted from the RCTs included in the review are presented in Table 2. There was a certain heterogeneity about the specific type of stem cells used in the control groups between the 5 studies included in the meta-analysis. Indeed, 2 studies [19,57] used periodontal ligament stem cells (PDLSCs), 1 study [56] applied dental pulp stem cells (DPSCs), 1 study [55] used bone marrow mesenchymal stem cells extracted from alveolar bone (AB-MMSCs) and 1 study [54] gingival mesenchymal stem cells (GMSCs). The follow-up lasted 6 months in 1 study [54] and 12 months in 4 studies [19,55–57].

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Abdal-Wahab and colleagues [54] included a total of 20 patients, excluding current smokers. A full-mouth non-surgical periodontal therapy was performed in all selected patients and, then, they were randomly assigned in the following test or control groups:

- Test: 10 intrabony periodontal defects were treated with GMSCs associated with a β -TCP scaffold and a collagen membrane.
- Control: 10 intrabony periodontal defects treated with β -TCP and collagen membrane alone.

Apatzidou and colleagues [55] included 27 patients which were allocated in the following 3 groups:

- Test: 9 intrabony defects were treated with ABMMSCs embedded on a collagen scaffold enriched with a fibrin lysate and autologous platelets, using the minimally invasive surgical technique (MIST) [9].
- Control B: 10 intrabony defects were treated using MIST with only the collagen scaffold enriched with fibrin lysate and autologous platelets.
- Control C: 8 intrabony defects were treated with the MIST technique alone.

Chen and colleagues [19] selected 30 patients, randomly assigned to one of the two groups:

- Test: 20 intrabony defects treated with heterologous bone graft and the adjunctive use of PDLSCs
- Control: 21 intrabony defects treated with heterologous bone graft only.

Ferrarotti and colleagues [56] enrolled 29 patients with severe periodontitis, randomly assigning them to one of two groups:

- Test: 15 intrabony defects accessed with the MIST technique and treated with DPSCs soaked on a collagen sponge.
- Control: 14 intrabony defects treated with only insertion of collagen sponge using MIST technique.

Sánchez and colleagues [57] included a total of 20 patients. After initial periodontal therapy, the subjects were placed in one of two groups with a quasi-randomized approach, i.e. the patients assigned to the treatment group have previously obtained successful in vitro stem cell expansion process:

- Test: 10 intrabony defects treated with PDLSCs together with a heterologous bone substitute.
- Control: 10 intrabony defects treated with heterologous bone substitute alone.

Table 2. Summary of studies included in the systematic review.

Study	MSC type	Defect inclusion criteria	Group characteristics		Number of patients		Number of defects		Primary outcomes
			Test	Control	Test	Control	Test	Control	
Apatzidou et al. 2021	Autologous alveolar bone marrow mesenchymal stem cells (ABMMSCs)	Infrabony defect (5 1-wall defects in control groups; 3 2-wall defects in test group and 3 in control groups; 6 3-wall defects in test group	ABMMSCs + autologous fibrin/platelet lysate (aFPL) + collagen scaffold + MIST	Group B: autologous fibrin/platelet lysate (aFPL) + collagen scaffold + MIST	Group C: MIST	9	10 + 8	9	Group B: 10 Group C: 8 CAL; PPD; GR; BDD; BC-BD

		and 10 in control groups)							
Sanchez et al. 2020	Autologous periodontal ligament-derived mesenchymal stem cells (PDLSCs)	Infrabony defect (3 1-wall defects in test group and 10 in control group)	PDLSCs + bone xenograft	Bone xenograft	10	10	10	10	CAL; PPD; GR
Abdal-Wahab et al. 2020	Autologous gingival associated mesenchymal stem cells (GMSCs)	Infrabony defect (7 2-wall defects in test group and 6 in control group; 3 3-wall defects in test group and 4 in control group)	GMSC + (beta-tricalcium phosphate (β -TCP) + collagen membrane	Beta-tricalcium phosphate (β -TCP) + collagen membrane	10	10	10	10	CAL; PPD
Hernández-Monjaraz et al. 2020	Autologous dental pulp stem cells (DPSCs)	Infrabony defects	DPSCs + collagen scaffold	Collagen scaffold	11	10	11	10	PPD
Ferrarotti et al. 2018	Autologous dental pulp stem cells (DPSCs)	Infrabony defect (7 1-wall defects in test group and 5 in control group; 4 2-wall defects in test group and 5 in control group; 4 3-wall defects in test group and 4 in control group)	DPSCs + MIST + collagen sponge	MIST + collagen sponge	15	14	15	14	CAL; PPD; GR; BC-BD
Shalini & Vandana 2018	Autologous periodontal	Infrabony defects	OFD + PDLSCs	OFD	14	14	14	14	CAL, PPD

	ligament-derived mesenchymal stem cells (PDLSCs)						
Chen et al. 2016	Autologous periodontal ligament-derived mesenchymal stem cells (PDLSCs)	Infrabony defect (Defects characteristic not mentioned)	PDLSCs + GTR + Bio-oss	GTR + Bio-oss	20	21	CAL; PPD; GR; BDD

Legend: CAL: clinical attachment levels; PPD: probing pocket depth; GR: gingival recession; BDD: linear distance from cemento-enamel junction to bottom of defect; BC-BD: linear distance from bone crest to bottom of defect; MIST: minimally invasive surgical technique; GTR: guided tissue regeneration; OFD: open flap debridement.

3.4. Results of the analyses

The results of individual studies as they relate with the main outcomes are reported in Table S1. The mean improvements reported in the meta-analyses for the different study outcomes can be summarized as follows:

- CAL gain: A total of 4 studies [54–57] compared post-operative CAL gain with a minimum of 6-month follow up between the test and control groups. Very high heterogeneity was encountered between the groups ($P < 0.001$; $I^2 = 90\%$). The meta-analysis conducted using a random-effect model revealed a non-statistically significant improvement in the test group [$MD = 1.05$; 95% CI (-0.88, 2.97) $p = 0.29$] (Figure 3).
- PPD reduction: A total of 4 studies [54–57] compared post-operative PPD reduction with a minimum of 6-month follow up between the experimental group and the control group. There was high heterogeneity between the groups ($P < 0.001$; $I^2 = 83\%$). A non-statistically significant adjunctive improvement in PPD reduction in the experimental group [$MD = 1.32$; 95% CI (-0.25, 2.88) $p = 0.10$] was shown by the meta-analysis (Figure 4).
- GR: 3 studies [55–57] compared GR between the test and the control arm with a 12-month follow-up. The meta-analysis results displayed low heterogeneity between the groups ($P = 0.37$; $I^2 = 0\%$), so using a random effect model they revealed a non-statistically significant difference between the test and control groups [$MD = -0.08$; 95% CI (-0.79, 0.63) $p = 0.83$] (Figure 5).
- RBG: A total of 3 studies [19,55,56] compared RBG between the test group and the control group. The heterogeneity was high between the groups ($p < 0.01$; $I^2 = 84\%$). There was no statistically significant difference in RBG between test and control groups [$MD = 0.50$; 95% CI (-0.88, 1.88) $p = 0.48$] (Figure 6).

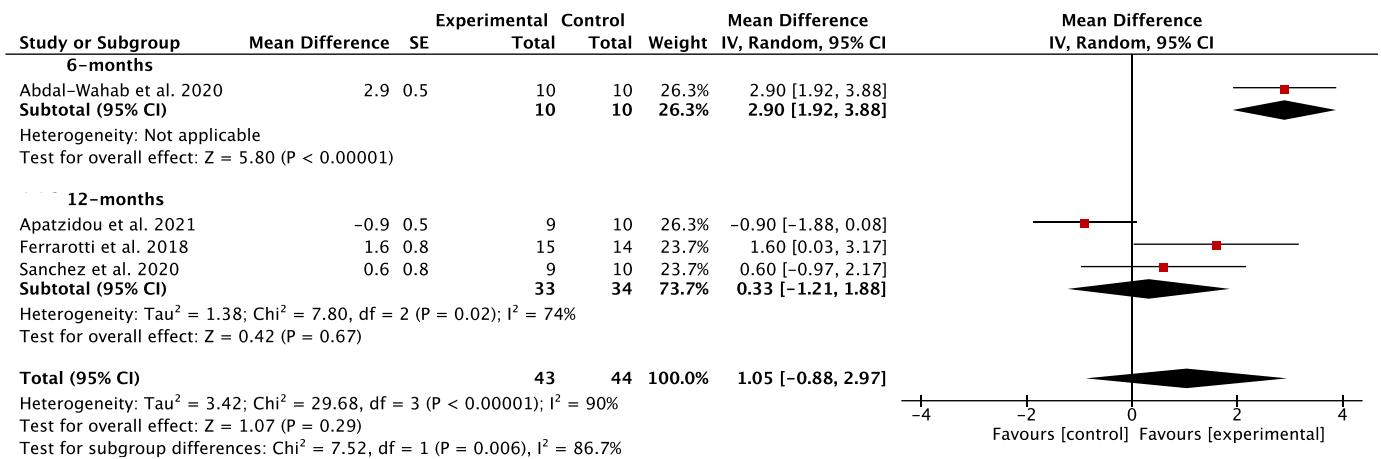


Figure 3. – Comparison between the results of studies comparing periodontal regeneration with or without the adjunctive use of orally-derived stem cells in terms of clinical attachment level (CAL) gain.

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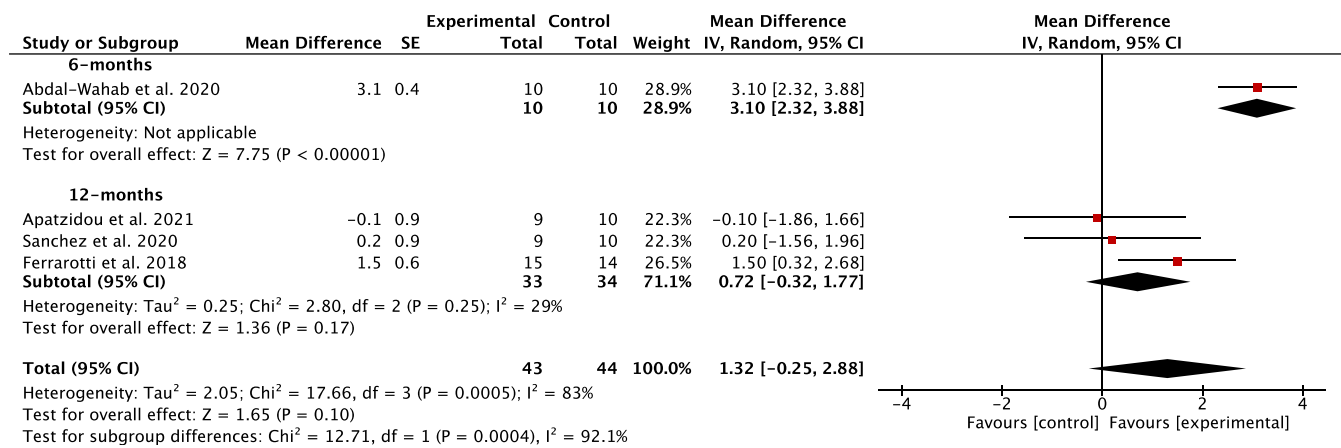


Figure 4. – Comparison between the results of studies comparing periodontal regeneration with or without the adjunctive use of orally-derived stem cells in terms of probing pocket depth (PPD) reduction.

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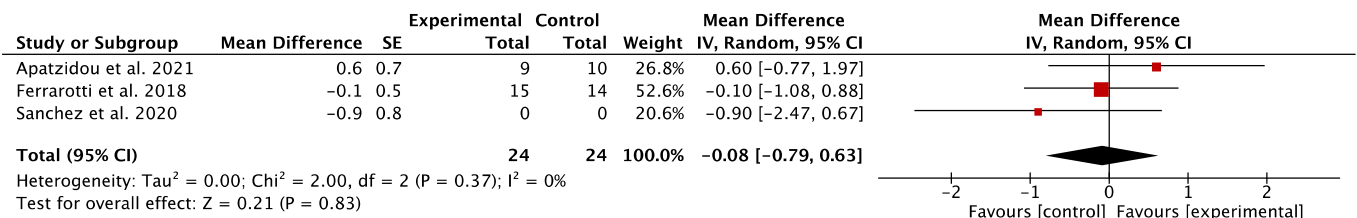


Figure 5. – Comparison between the results of studies comparing periodontal regeneration with or without the adjunctive use of orally-derived stem cells in terms of gingival recession (GR).

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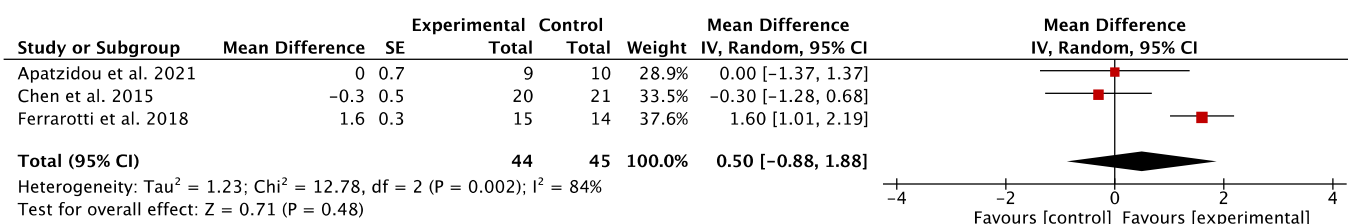


Figure 6. – Comparison between the results of studies comparing periodontal regeneration with or without the adjunctive use of orally-derived stem cells in terms of radiographical bone gain (RBG).

4. Discussion

The objective of this systematic literature review was to assess the clinical and therapeutic effectiveness of regenerative periodontal treatment when orally-derived stem cells are used as adjunctive therapy by selecting RCTs. To date, MSCs can be isolated from diverse sources in the oral cavity, with dento-periodontal derived stem cells seeming the best candidates for periodontal tissue regeneration [37,39,58]. Therefore, the focus question of this systematic review was: “In patients with periodontitis, can the adjunctive use of orally-derived MSCs provide additional clinical benefits measured as CAL gain, PPD reduction, GR, and RBG for periodontal regeneration?”. A total of 7 RCTs including a total of 186 patients were included after screening. Risk of bias test led to the exclusion of 2 RCTs for the meta-analyses. The overall findings showed a lack of significant benefits in the adjunctive use of orally-derived MSCs at 12 months during periodontal regeneration procedures. Heterogeneity in methodology, study design, and outcomes was high.

When considering the primary outcome CAL gain, 4 studies were selected. The confidence interval of the data relating to the study by Apatzidou et al. and the study by Sánchez et al. exceeded the vertical line of the reference value, so there was no statistically significant difference between the test and control groups. Conversely, Ferrarotti et al. and Abdal-Wahab et al. provided a statistically significant advantage for the adjunctive application of MSCs both at 6- and 12-month follow-up. When the studies were combined, the final meta-analysis revealed a non-statistically significant improvement in CAL gain for the test group of 1.05 mm [95% CI (-0.88, 2.97) $p = 0.29$]. For PPD reduction, the same 4 studies were selected [54–57], with the overall result of the meta-analysis being not statistically significant at 12 months, but with a significant advantage in the study at 6 months [54]. This finding may suggest a greater rapidity of the periodontal regeneration process following the use of MSCs, although in the long term the results of regenerative surgical treatment appear to be comparable to those of other regenerative methods. When delving deeper into study characteristics, Ferrarotti et al. [56] showed the highest difference in outcome measures between test and control group with respect to other included studies with low risk of bias. This discrepancy can be both ascribed to (i) the use of DPSCs and (ii) the nature of the regenerative procedure in the control group. Indeed, DPSCs hold significant promise due to their accessibility, shared origin, and similar antigenic pattern with PDLSCs, making them particularly attractive for therapeutic applications [33]. DPSCs exhibit an extended lifespan, display compatibility with biomaterials, and can be safely preserved through cryopreservation methods [59]. Building on this foundation, experimental findings from studies conducted in vivo and in animal models suggest that DPSCs have the capability to produce lamellar bone with proper vascularization. Moreover, DPSCs demonstrate the potential for differentiation into various periodontal tissues, emphasizing their versatility and potential therapeutic efficacy in the field of periodontal regeneration [59,60]. Regarding PDLSCs, they can be found both on the root and alveolar bone surfaces after tooth extraction, although those on the root demonstrate superior differentiation capabilities [61]. Recognized for their safety and efficacy, they became the pioneering treatment in periodontal regeneration therapy [35,41,62]. Indeed, PDLSCs

exhibited the ability to differentiate into mesenchymal cell lineages, generating cells capable of forming collagen, adipocytes, cementum tissue, Sharpey's fibers, and osteoblast-like cells *in vivo* [63]. However, translatability of PDLSCs to the clinics has been hindered by several limitations, including the necessity for tooth extraction and the possibility that the chronic exposure to a chronic inflammatory environment could lead to the depletion of their potential through senescence [36]. Finally, bone marrow-derived mesenchymal stem cells are a specific type of multipotent MSCs that can be obtained from the alveolar bone during surgery, proving comparable biologic features to iliac BMMSCs [64]. They have also shown the potential of inducing not only reconstruction of bone, but also periodontal and dental tissue regeneration in preclinical models. Indeed, they have the ability to increase the expression of genes related to tooth development, and they can transform into cells resembling ameloblasts and periodontal tissue cells [65]. Lastly, although presenting a biological rationale to hypothesize their use [66], no study was found testing the application of adipose-derived stem cells in periodontal tissue regeneration.

Three recent systematic reviews are present in literature focusing on this topic [46,47,67], with their results and conclusion disagreeing substantially from the present study. Indeed, their meta-analyses revealed significant differences between the experimental and control groups in terms of PPD, CAL, radiographic intrabony defect depth, and GR, emphasizing how the use of MSCs can be beneficial in periodontal regeneration. In contrast to these optimistic trends, the present systematic review revealed an overall lack of significant benefits at 12 months. Notably, the observed heterogeneity in methodology, study design, and measured outcomes was consistently pronounced. Indeed, in a plausible attempt to broaden the focus, previous systematic review combined studies using MSCs derived from diverse body sources (such as umbilical stem cells) for different oral surgical interventions (i.e., alveolar bone reconstruction) at different time-points (3, 6, and 12 months). Indeed, the inclusion of multiple follow-up groups from the same RCT may lead to excessive weight in the meta-analyses. Overall conclusions cannot overlook these important heterogeneities, in order to provide a clear snapshot of the state-of-the-art and guide future research endeavors. This raises important considerations about the standardization of protocols and the need for more homogeneity in future research endeavors to elucidate the specific conditions under which orally-derived MSCs may or may not be effective in enhancing periodontal regeneration.

The attention towards the use of MSCs in periodontal therapy derives from the need to implement treatment options for lesions resulting from periodontitis, due to its prevalence globally [68]. In recent years, MSCs have achieved increasing success in the treatment of many pathologies studied by various branches of medicine, based on their regenerative and immunoregulatory properties. Our knowledge is still limited, and this means that the prospects regarding their clinical use are very broad. It should be considered that there are critical steps to increase the frequency of stem cell-based therapeutic approaches. In fact, it must be said that the safety of cell therapies, in general, has not yet been fully evaluated. Notably, no RCT in the present review showed adverse events for the use of dento-periodontal derived stem cells. Furthermore, questions such as cell delivery, immunogenicity, use of autologous or allogeneic cells, culture quality control, and cost-effectiveness are critical to address. The next phase of research should aim to identify the tissues that can optimally serve as the source of stem cells and, in this sense, future attention should be even more marked on dental and periodontal tissues. It should be emphasized that until recently, medical stem cell research has not prioritized periodontal tissues due to the non-life-threatening nature of periodontitis.

Although efforts were made to enhance the quality of data regarding the topic, this study has certain limitations primarily stemming from the nature of the existing literature. Indeed, the RCTs provided periodontal regeneration with very diverse flap design, scaffolds, MSCs vectors and cell handling technologies. Despite the promising outcomes highlighted in the broader literature, our synthesis points to a nuanced perspective, suggesting that the use of orally-derived MSCs may not consistently confer additional clinical

benefits in the specified timeframe. While recognizing the potential of stem cell therapies, including MSCs, our findings underscore the complexity of translating these approaches into consistently successful clinical outcomes in the context of periodontitis.

5. Conclusions

In conclusion, it was not possible to demonstrate that the additional use of dento/periodontal stem cells in periodontal regenerative surgical procedures determines an improvement in clinical and radiographic parameters compared to other biomaterials or techniques more studied in the literature. The regenerative approach supported by tissue engineering and cell therapy should be explored in depth with a significantly higher number of randomized controlled clinical trials, with larger samples and **at least 12 months follow-up** to allow the detection of even long-term outcomes. In consideration of the results expressed, the low number of RCTs, the inherent costs of using MSCs and the possibility of adverse events still too little addressed in the literature, regenerative periodontal surgery with the use of stem cells for bone defects could not be currently considered a preferential approach for clinical treatment compared to other periodontal regeneration procedures.

6. Indications for future research

- **RCTs evaluating the clinical efficacy, as well as patient related outcomes and cost-benefit analyses of periodontal regeneration using dento-periodontal stem cells.**
- This RCTs should be designed with an increased number of patients enrolled and long-term follow-up.
- Studies focused on clinical protocols to obtain an efficient number of MSCs from the oral cavity.
- Studies focused on side effects in short-term and long-term with the use of MSCs.

List of abbreviations: MD (mean difference); CAL (clinical attachment level); PPD (probing pocket depth); GR (gingival recession); RGB (radiographic bone gain); CI (confidence interval); BoP (bleeding on probing); FI (furcation involvement); MSCs (mesenchymal stem cells); β -TCP (beta-tricalcium phosphate); PRP (platelet-rich plasma); RCTs (randomized clinical trials); BMMSC (bone marrow mesenchymal stem cells); ESC (embryonic stem cells); iPSC (induced pluripotent stem cells); PDLSCs (periodontal ligament stem cells); GTR (guided tissue regeneration); DPSCs (dental pulp stem cells); ABMMSCs (alveolar bone marrow mesenchymal stem cells); GMSCs (gingival mesenchymal stem cells); MIST (minimally invasive surgical technique); BDD (linear distance from cemento-enamel junction to bottom of defect); BC-BD (linear distance from bone crest to bottom of defect); OFD (open flap debridement).

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Included studies: summary of the results for the main outcomes of interest.

Author Contributions: Conceptualization, A.C., M.P. and G.O.; X.X. and Y.Y.; methodology, A.C., G.B., M.P., F.R. and G.O.; validation, A.C. and M.P.; formal analysis, A.C., G.B. and F.R.; investigation, A.C., M.P. and F.A.; resources, X.X.; data curation, F.M.; writing—original draft preparation, A.C., M.P., F.A.; writing—review and editing, G.B., F.R., F.M., M.A. and G.O.; supervision, M.A. and G.O.; project administration, F.M. and G.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data used in this study are available in the manuscript or its supporting material.

Conflicts of Interest: The authors declare no conflicts of interest.

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