Human intrabony defect regeneration with micrografts containing dental pulp stem cells: A randomized controlled clinical trial

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Human intrabony defect regeneration with micro-grafts containing dental pulp stem cells: a randomized controlled clinical trial.

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Running title:
Periodontal regeneration with DPSCs.

Keywords: dental pulp; periodontal pocket; periodontal regeneration; randomized controlled trial; stem cells; tissue engineering.

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Abstract

**Aim:** The goal of this study was to evaluate if dental pulp stem cells (DPSCs) delivered into intrabony defects in a collagen scaffold would enhance the clinical and radiographic parameters of periodontal regeneration.

**Materials and Methods:** In this randomized controlled trial, 29 chronic periodontitis patients presenting one deep intrabony defect and requiring extraction of one vital tooth were consecutively enrolled. Defects were randomly assigned to test or control treatments which both consisted of the use of minimally invasive surgical technique. The dental pulp of the extracted tooth was mechanically dissociated to obtain micro-grafts rich in autologous DPSCs. Test sites (n=15) were filled with micro-grafts seeded onto collagen sponge, whereas control sites (n=14) with collagen sponge alone. Clinical and radiographic parameters were recorded at baseline, 6 and 12 months postoperatively.

**Results:** Test sites exhibited significantly more PD reduction (4.9 mm versus 3.4 mm), CAL gain (4.5 versus 2.9 mm) and bone defect fill (3.9 versus 1.6 mm) than controls. Moreover, residual PD < 5 mm (93% versus 50%) and CAL gain ≥ 4 mm (73% versus 29%) was significantly more frequent in the test group.

**Conclusions:** Application of DPSCs significantly improved clinical parameters of periodontal regeneration one year after treatment.
Clinical relevance

Scientific rationale for study: Research is seeking to identify ways to improve regenerative therapy predictability. This randomized and controlled study reports on the application of dental pulp stem cells (DPSCs) in periodontal regeneration in humans.

Principal findings: The application in intrabony defects of DPSCs seeded onto a collagen scaffold enhances clinical and radiographic parameters of periodontal regeneration at 1-year follow-up.

Practical implications: DPSCs may represent a promising regenerative procedure to treat intrabony defects in periodontitis patients. The need for a vital and intact tooth requiring extraction limits their clinical applicability.
1. Introduction

The goal of periodontal therapy is to arrest disease progression and ultimately to regenerate lost periodontal tissues (Karring, Nyman, Gottlow, & Laurell, 1993). Several studies over the past 30 years had demonstrated that blood clot stability plays a pivotal role in regeneration of tooth-supporting tissues (Wikesjo & Nilveus, 1990; Wikesjo et al., 2003), avoiding apical migration of epithelial cells during the first healing phase. In this respect, new surgical techniques designed to optimize flap and clot stability (Harrel, Nunn, & Belling, 1999; Cortellini & Tonetti, 2007; Cortellini & Tonetti, 2009; Trombelli, Farina, Franceschetti, & Calura, 2009) and new biological agents promoting periodontal ligament and root cementum formation (Hammarström, 1997; Trombelli & Farina, 2008) have been developed. However, the ability to predictably regenerate the periodontal damaged tissues still remains a major unmet objective of regenerative therapies (Aichelmann-Reidy, & Reynolds, 2008).

Tissue engineering (Palmer & Cortellini, Group B of the European Workshop on Periodontology, 2008) has recently been shown a promising approach for periodontal regeneration, and strategies using adult mesenchymal stem cells (MSCs) are promising (Hynes, Menicanin, Gronthos, & Bartold, 2012; Peng, Ye, & Zhou, 2009). Adult stem cells are undifferentiated cells found in specialized tissues and organs of adults that have the capacity of self-renewal and the potential to differentiate in a multiple, but limited, number of cells lineages (Lin, Gronthos, & Bartold, 2008). Teeth have been demonstrated to represent an important MSCs niche due to their embryogenic origin and post-natal development (Huang, Gronthos, & Shi, 2009). MSCs have been isolated from exfoliated deciduous teeth, apical papilla of immature permanent teeth, dental follicle, periodontal ligament and dental pulp. Human dental pulp stem cells (DPSCs) are especially attractive because they are easy accessible, share the same origin and similar antigenic pattern of periodontal stem cells and
can differentiate in the same lineages (Gronthos et al., 2002; Huang, Gronthos, & Shi, 2009; Tziafas & Kodonas, 2010). They have a long lifespan, interact with biomaterials, and can be safely cryopreserved (Papaccio et al., 2006; Graziano et al., 2008a).

Experimental evidence from in vivo studies and animal models suggests that DPSCs have the capacity of producing lamellar bone with appropriate vascularization and the potential to differentiate in periodontal tissues (Graziano et al., 2008b; Khorsand et al. 2013).

Despite these encouraging results, the need for culture expanding procedures to obtain a sufficient number of cells for delivery to the damaged site increases the regulatory difficulties for translating stem cells therapies to a clinical setting. Recently, autologous micro-grafts rich in progenitor cells were obtained by mechanical disaggregation of periosteum and dental pulp without the need for culture expanding procedures. Micro-grafts displayed in vitro high cell viability and optimal regenerative potential (Monti et al., 2017; Trovato et al., 2015).

Some initial reports in humans demonstrated the clinical and radiographic efficacy of dental pulp micro-grafts in post-extraction alveolar defects (d’Aquino et al. 2009; Monti et al., 2017) or in periodontal intrabony defects (Aimetti, Ferrarotti, Cricenti, Mariani, & Romano, 2014; Aimetti, Ferrarotti, Gamba, Giraudi, & Romano, 2018). These preliminary results suggest that DPSCs may represent a promising tool for bone and periodontal regeneration. Due to the lack of randomized controlled studies, the aim of the study was to assess if dental pulp micro-grafts delivered into deep intrabony defects in a collagen scaffold would enhance the clinical and radiographic parameters of periodontal regeneration.

2. Material and Methods

2.1 Study population and Experimental design

This article is reported according to the CONSORT statement. This study was designed as a parallel, double-blind, prospective randomized trial to evaluate the clinical and radiographic outcomes 12 months following two different surgical treatments of deep intrabony defects:
minimally invasive surgical technique (MIST) plus dental pulp micro-grafts in a collagen sponge bio-complex (test) versus MIST plus collagen sponge alone (control). The study was approved by the Institutional Ethical Committee (protocol n. 56/2016) and conducted in accordance of the principles of Declaration of Helsinki as revised in 2008. The trial was registered at clinicaltrials.gov as NCT03386877. Patients provided written consent for participation.

Consecutive periodontitis patients who had completed non-surgical periodontal treatment at the Section of Periodontology, C.I.R. Dental School, University of Turin were screened for inclusion. The following eligibility criteria were used: 1) diagnosis of advanced chronic periodontitis (Armitage, 1999); 2) full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS) < 15% at the time of enrolment; 3) aetiological periodontal therapy completed at least 3 months prior to screening; 4) presence of one natural tooth having a vertical defect with residual probing depth (PD) ≥ 6 mm and a radiographic intrabony component ≥ 3 mm; 5) presence of one vital and intact tooth requiring extraction due to impaction or malposition, as autologous source of DPSCs.

Exclusion criteria included: 1) smoking habits; 2) contraindications for periodontal surgery; 3) systemic diseases affecting periodontal healing; 4) pregnancy and lactation; 5) history of periodontal surgery or prosthetic restorations at the experimental teeth; 6) clinical evidence of furcation defects (Hamp, Nyman, & Lindhe, 1975).

2.2 Sample size and randomization

The difference in radiographic bone fill between test and control procedures was set as the primary outcome of the study. A difference of 1.5 mm was assumed to be clinically significant and the amount of variation was 1.1 mm (Cortellini & Tonetti, 2011). Therefore at 0.05 two-sided alpha error and 80% power, the calculated sample size was 24 patients (12 per group) that increased to 15 for compensation of possible dropouts. Post-hoc power calculation revealed that the study had 90% power to detect differences in the primary outcome measure.
After baseline visit, patients were randomized to the test or control treatment by using computer-generated random sequence allocation. To conceal assignment, forms with the treatment modality were put into opaque and sealed envelopes with the patient number on the outside. The envelopes were placed into the custody of the study coordinator. The surgeon, the examiner who performed the measurements and participants were blinded to treatment assignment. Code breaking was performed after statistical analysis.

2.3 Data collection

2.3.1 Clinical measurements

The following clinical parameters were assessed on the experimental teeth at baseline, 6 and 12 months after surgery using a periodontal probe (PCP 15/11.5, Hu-Friedy, Chicago, IL, USA): 1) presence/absence of plaque (PI 0/1); 2) presence/absence of BoP (0/1); 3) PD; 4) gingival recession (REC); and 4) CAL. FMPS and FMBS were also calculated as the percentage of gingival units (6 sites per tooth) around all teeth that revealed PI or BoP. During the surgical session defect morphology was characterized in terms of the distance in mm (INTRA) from the most coronal extension of the bone crest (BC) to the bottom of the defect (BD) and the extent in mm of one, two, and three wall subcomponents of the defect. One blinded and calibrated examiner (M.N.G.) carried out all clinical measurements. For the calibration exercise ten chronic periodontitis patients not enrolled in the study were evaluated on two separate occasions, 24 hours apart. Calibration was accepted if measurements of PD and CAL at baseline and at 24 hours were similar to the millimetre at ≥ 90%. The agreement was between 92% and 95%.

2.3.2 Radiographic measurements

Periapical standardized radiographs were taken by a clinician masked to the clinical measurements (M.G.) using the paralleling technique and individually customized bite-blocks (RINN XCP Film Holding Instruments, Dentsply, York, USA) at baseline, 6 and 12 months after surgery. The anatomical landmarks were identified as previously described (Schei,
Waerhaug, Lodval, & Arno, 1959). The radiographic angle (RA) and the linear distance from BC and BD (intrabony defect depth, IBD) were analysed using an image-analysis software (Image-J, NH). The difference between IBD values recorded at baseline and at 1-year examination was identified as the amount of radiographic bone fill.

### 2.4 Surgical procedures

Two clinicians (M.A. and F.F.) were involved in the surgical sessions. To ensure allocation concealment, one operator (M.A.) performed all the surgical procedures, while the other one (F.F.) carried out tooth extraction and filled the intrabony defects with the collagen sponge provided by the study coordinator (A.Q.). Defects were accessed with the MIST (Cortellini & Tonetti, 2007) using an operative microscope (Zeiss S7, Feldbach, Switzerland). The elevation of the flap was kept at minimum to allow the exposure of the defect and the careful debridement of the root surface using manual (mini five Gracey curettes, Hu-Friedy, Chicago, IL, USA) and ultrasonic (Cavitron® SelectTM, Dentsply, York, USA) devices.

Following intrasurgical data registration, the tooth scheduled for extraction was removed, washed in 0.2% chlorhexidine (CHX) for 60 seconds and given to the study coordinator, charged for DPSCs isolation. After opening the sealed envelope, he sectioned the tooth from the test group along the CEJ to expose the pulp chamber and collected the pulp tissue with a sterile Gracey curette. Then, the pulp was mechanically dissociated using the Rigenera Machine System (Rigenera® HBW, Turin, Italy), a biological tissue disaggregator working at a rotating speed of 80 rpm, in 1.0 ml sterile physiologic solution (Aimetti et al., 2014; d’Aquino et al., 2009). After dissociation, the cellular suspension was passed through a disposable grid (Rigeneracons) with 100 hexagonal holes filtering cells and component of extracellular matrix with a cut-off of 50 µm in an average time of 90 seconds. The obtained micro-grafts enriched in DPSCs were endorsed onto a collagen sponge scaffold (Condress®, Istituto Gentili, Milano, Italy) to form a bio-complex. In the control group the collagen sponge was only hydrated using physiologic sterile solution without any addition of pulp
The flaps were repositioned and tension-free primary flap closure was obtained using horizontal internal mattress and interrupted sutures.

2.5 Post-surgical care

Patients were prescribed antibiotics (875 mg amoxicillin + 125 mg clavulanic acid, 1 g b.i.d for 6 days) and analgesics (ibuprofen 600 mg, if needed). They were advised to avoid toothbrushing and flossing in the treated area for the first two weeks and to rinse with 0.12% CHX solution three times a day for 4 weeks. After two weeks sutures were removed and patients were instructed to use a soft toothbrush in the surgical area. After 4 weeks, they discontinued CHX mouthrinse and resumed conventional hygiene practices with medium toothbrush and interdental devices.

Recall appointments were scheduled weekly during the first month and every three months for the remainder of the observational period. They consisted of reinforcement of oral hygiene measures, polishing, full-mouth scaling and root planing and occlusal adjustment when needed. Subgingival instrumentation was not performed in the treated area over 12 months.

2.6 Statistical analysis

The primary outcome measurement was the radiographic bone fill. Secondary outcome measurements included changes in clinical parameters. Only one defect per subject was included in the study. Mean values and standard deviations (SD) were calculated for every variable and for every assessment time point.

To test whether the data were normally distributed the Kolmogorov-Smirnov test was done. The homogeneity of groups at baseline was tested using the unpaired $t$ test (PD, CAL, IBD, INTRA) and the Mann-Whitney $U$ test (FMPS, FMBS, REC). The repeated-measures ANOVA was used to assess the effect of time and treatment on continuous variables with a Gaussian distribution. The Friedman test was applied to variables without a normal distribution. Intragroup multiple comparisons were conducted with the post-hoc tests
(Newman-Keuls test or Dunn test). The intergroup differences were statistically explored using the unpaired student \( t \)-test or the Mann-Whitney \( U \) test. The Bonferroni correction was applied for multiple comparisons. The level of significance was set at 5%. Statistical analyses were conducted using commercially available software (SAS version 9.2).

3. RESULTS

The flow chart of the experimental design is presented in Fig 1. Twenty-nine patients (13 males and 14 females), 39 to 69-year old (mean age 50.7 ± 8.5 years), meeting the inclusion criteria were treated. Fifteen bony defects (6 maxillary and 9 mandibular) received MIST and dental pulp micro-grafts/collagen bio-complex, while 14 (8 maxillary and 6 mandibular) MIST and collagen sponge without cells. No subject discontinued participation in the study and no data points were missing for analysis. The first surgical procedure was carried out in January 2016. All 12-month follow-up visits were completed in April 2017.

Patient characteristics at baseline were not significantly different (\( p > .05 \)) between groups (Table 1). The distributions of intrabony defects according to teeth were: 20% incisive, 13.4% canine, 26.7% premolar, and 40% molar for the test group and 28.6% incisive, 21.4% canine, 14.3% premolar, and 35.7% molar, for the control group. As reported in Table 2, no statistically significant difference was detected for any of the baseline defect characteristics between test and control sites.

Mean ± SD of all clinical and radiographic parameters over the 12-month period are presented in Table 2. Primary closure was obtained and maintained in all the sites. Soft tissues achieved complete healing within 3 to 4 weeks. No infectious episodes related to both procedures were reported. FMPS and FMBS remained below 20% through the study period in both groups, indicating a good standard of plaque control.

Both surgical techniques resulted in statistically significant overall changes in PD and CAL between baseline and 12-month examination (\( p < .001 \)). As shown in Table 2, the greatest PD reduction and CAL gain occurred during the first 6 months post surgery (\( p < .001 \)).
In the DPSCs-treated group mean PD reduction and mean CAL gain amounted to $4.9 \pm 1.4$ mm and to $4.5 \pm 1.9$ mm during the 12-month period. A not statistically significant increase in REC values of $0.4 \pm 1.1$ mm was observed at the end of the experimental period.

In the control group mean PD reduction was $3.4 \pm 1.7$ mm and mean CAL gain was $2.9 \pm 2.2$ mm at 12-month follow-up when compared to baseline values. At this time point a not statistically significant REC increase of $0.5 \pm 0.9$ mm had taken place.

Changes in clinical parameters were significantly different between the two procedures ($p \leq .03$). The test group showed greater CAL gain and PD reduction at both re-evaluation assessments. The frequency distributions of residual PDs and CAL changes at 12 months are summarized in Table 3. At 12-month follow-up visit, complete pocket closure (PD ≤ 3 mm) was observed at a frequency of 66.7% in test sites and 14.3% at control sites. A CAL gain ≥ 4 mm was measured in 73.3% and 28.6% of test and control intrabony defects, respectively.

Radiographic data are summarized in Table 4. Statistically significant differences between test and control group in IBD values were observed at both 6- ($p < .001$) and 12-month follow-up ($p < .001$). At 12 months the mean radiographic bone fill was $3.9 \pm 1.5$ mm and $1.6 \pm 1.1$ mm in the test and control group, respectively. Two treated cases are illustrated in Figures 2 and 3.

4. DISCUSSION

In spite the number of current available regenerative procedures and biomaterials for the management of periodontal intrabony defects, clinicians continue to seek more predictable and less technique-sensitive regenerative therapies. Recent evidence from several in vitro studies and animal models as well as from small-scale clinical pilot studies indicate that DPSCs may be a powerful tool for bone and periodontal regeneration (d’Aquino et al., 2009; Khorsand et al., 2013; Monti et al., 2017). To the best of our knowledge this is the first randomized controlled study on the use of collagen sponge scaffold complexed with autologous dental pulp micro-grafts in the regenerative treatment of deep intrabony defects.
with a prevalent non-containing configuration.

The experimental therapy resulted in significantly more PD reduction, CAL gain and radiographic bone gain than the open flap instrumentation, demonstrating the bioactivity of the micro-grafts, and enhancement of the intrinsic healing potential in the human intrabony defect environment. The 93% of the test sites compared to 50% of control sites exhibited residual PD ≤ 4 mm at 12-month follow-up. Several studies reported the association between presence of residual pockets after treatment, especially with PD ≥ 5 mm and lack of periodontal stability (Lang & Tonetti, 2003; Matuliene et al., 2008; Matuliene et al., 2010).

The achieved CAL gain in the test group (4.5 ± 1.9 mm) was better than those reported in a systematic review by Graziani et al. (2012) concerning the papilla preservation flap methods (2.48 mm, CI: 1.44-3.52). The present findings are also more favourable with respect to those reported in controlled clinical trials evaluating the outcomes of regenerative surgery in intrabony defects using enamel matrix derivative and minimally invasive surgery (Cortellini & Tonetti, 2009; Jepsen et al., 2008; Ribeiro, Casarin, Júnior, Sallum, & Casati, 2011). At 12 months they reported CAL changes ranging from 1.8 mm to 4.9 mm and PD reduction from 2.6 mm to 5.2 mm. Notably, all DPCSs-treated sites were filled by radiographic bone-like tissue with a mean bone fill of 3.9 ± 1.2 mm.

Consistent with the results of previous studies, the magnitude of changes in CAL (2.9 ± 2.2 mm) and PD (3.4 ± 1.7 mm) were more modest in the control group and inferior to the regenerative technique evaluated in parallel (Graziani et al., 2012; Needleman, Worthington, Griedys-Leeper, & Tucker, 2006; Tu, Needleman, Chambrone, Lu, & Faggion, 2012).

In recent years several therapeutic protocols for autologous human stem cells transplantation have been conducted with different results (Hynes, Menicanin, Gronthos, & Bartold, 2012). The surgical access to these potential collection sites often represents a limiting point and the ratio between tissue collected and stem cells isolated is often disadvantageous. Dental pulp is a niche housing neural-crestal-derived stem cells. It is easily accessible and there is limited
morbidity after collection (Mitsiadis, Barrandon, Rochat, Barrandon, & De Bari, 2007). Recently, a novel method of cells extraction based on mechanical disaggregation of connective tissues has been introduced (d’Aquino et al., 2009). It produces in a few minutes millions of micro-grafts from a few millimetres sample of autologous dental pulp and filters them with a cut-off of 50 µm in order to promote the discharging of old differentiated cells and the enrichment of young progenitor cells (Trovato et al. 2015, Monti et al. 2017). Under this dimension the percentage of cells expressing stem antigens grows dramatically, avoiding a magnetic or flow cytometric sorting (Iohara et al., 2006).

DPSCs are fully comparable to cells obtained after enzymatic digestion but they are produced directly within the surgery and immediately used without any manipulation (Monti et al., 2017). They showed cell viability of 92% with 7AAD staining, tended to express surface markers of mesenchymal cells such as STRO-1, CD44 and CD90 and were able to differentiate in adipocytes, osteocyte and chondrocytes lines in vitro (Monti et al., 2017). Flow cytometry analysis demonstrated that human dental pulp micro-grafts are highly positive to Flk-1 and CD34, markers of stromal and vascular endothelial progenitors, and contain about 77% of progenitors cells, a sufficient quantity to perform in vivo experiments (d’Aquino et al., 2009). Pericytes are a heterogeneous population of mesenchymal cells associated with the microvasculature, which vary greatly in origin, morphology, surface markers and function in different tissues. Some pericyte markers are also expressed on other cell types, most notably endothelial and smooth muscle cells (Armulik, Genové, & Betsholtz, 2011). Similar to other types of stem cells, pericytes act as a repair system in response to injury by maintaining the structural integrity of blood vessels (Wong, Rowley, Redpath, Tilman, Fellous, & Johnson, 2015). The CD34 and Flk-1 cytotype seems to have a very interesting power to induce healing and regeneration process and to be the much suitable cell population for clinical approach (Graziano et al., 2008b).
Previous investigations in the canine model demonstrated that DPSCs have the ability to regenerate periodontal tissues in surgically created intrabony defects (Khorsand et al., 2013) and to form new cementum in furcation perforations (Bakhtiar et al., 2016).

The clinical efficacy of this method for DPSCs collection has been demonstrated in humans in sinus lift procedures and in bone regeneration of post-extraction alveolar defects (d’Aquino et al., 2009; Brunelli et al., 2013; Graziano, d’Aquino, Brunelli, Fanali, & Carinci, 2011). Preliminary data are available for periodontal regeneration of intrabony defects (Aimetti et al. 2014, 2018).

The same mechanical disaggregation protocol has been used in the present investigation to obtain a cellular suspension endorsed onto collagen III scaffold. Collagen sponge supports stem cells proliferation and differentiation within the first weeks, and stimulates the formation of calcified tissues (Sumita et al., 2006). Mechanical stability of the coagulum is a key factor for cell differentiation, and it is pivotal when the committed tissue is a mineralized tissue like the root cementum or the alveolar bone. Furthermore, collagen is radiolucent, inactive in terms of periodontal regeneration and with a rapid resorption rate.

An important aspect to take into account when interpreting the results is that this technique, requiring a vital and intact tooth as autologous donor site of DPSCs, has a limited applicability. There is preliminary evidence that periodontally involved teeth in aggressive periodontitis patients may contain putative stem cells with certain MSC properties (Sun et al., 2014). This may widen the clinical applicability of this procedure.

When extracting human teeth, another therapeutic option to reach periodontal regeneration is to use autologous periodontal ligament stem cells (PDLSCs). These cells exhibit multipotency, as demonstrated by their ability of differentiating into osteoblasts, fibroblasts and cementoblasts and forming cementum and periodontal ligament tissue (Seo et al., 2004). However, current PDLSCs-based periodontal cell therapies require ex vivo expansion with
animal serum in order to produce sufficient cell number before implantation in human intrabony defects (Feng et al., 2010; Cheng et al., 2016). This raises some concerns on immunological risks and limits PDLSCs applicability in periodontal clinical practice (Shahdadfar, Fronsdal, Haug, Reinholt, & Brinchmann, 2005). In contrast, dental pulp provides sufficient tissue for selection of progenitor cells without the need of prior culture expanding procedures, leading to easier application in cell-based periodontal therapy (Graziano et al., 2008b).

There are some limitations of this study. First, the sample size was small and data were from one institution. Second, it is not possible to confirm that periodontal regeneration had indeed occurred to the treated sites, because no histological analysis can be presented for ethical reasons. However, radiographic bony changes have been considered a valid parameter to clinically demonstrate the effectiveness of regenerative procedures (Cortellini, Pini Prato, & Tonetti, 1993). Moreover, the selected intrabony defects had limited self-regenerative potential and were filled with a biologically inert and radiolucent biomaterial.

5. Conclusions

Despite these limitations, the study findings add significantly to the existing literature on the clinical applicability of tissue engineering to regenerative therapies. In individuals with one or more vital teeth requiring extraction, the application of autologous dental pulp micro-grafts into deep non-containing intrabony defects coupled with minimally invasive surgical procedures, to optimize blood stability and to provide a stable space for regeneration, could enhance the intrinsic regenerative potential of the periodontal defect. In this way, using this protocol, the patient is, at the same time, the donor and the acceptor of DPSCs, and this system permits to increase the progenitor cell viability in the surgical site that needs to be regenerated. This could limit the need for grafting materials and barrier membranes. Independent clinical trials are needed to confirm the promising results of the present study.
REFERENCES


https://doi.org/10.1111/j.1600-051X.2009.01508.x


TABLE 1 Characteristics of study subjects at baseline

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test Group (n = 15)</th>
<th>Control Group (n = 14)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; mean ± SD)</td>
<td>51.9 ± 8.4</td>
<td>49.4 ± 9.3</td>
<td>0.404*</td>
</tr>
<tr>
<td>Females/males (n)</td>
<td>7/8</td>
<td>8/6</td>
<td>0.853**</td>
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<tr>
<td>FMPS (%; mean ± SD)</td>
<td>10.9 ± 3.9</td>
<td>10.1 ± 2.1</td>
<td>0.470***</td>
</tr>
<tr>
<td>FMBS (%; mean ± SD)</td>
<td>8.9 ± 3.7</td>
<td>9.6 ± 3.9</td>
<td>0.647***</td>
</tr>
</tbody>
</table>

FMPS, Full-Mouth Plaque Score; FMBS, Full-Mouth Bleeding Score; SD, standard deviation.
*Unpaired t-test.
**Chi-square test.
***Mann-Whitney U-test.

TABLE 2 Baseline characteristics of intrabony defect sites

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test Group (n = 15)</th>
<th>Control Group (n = 14)</th>
<th>p value</th>
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<tr>
<td>PD (mm; mean ± SD)</td>
<td>8.3 ± 1.2</td>
<td>7.9 ± 1.3</td>
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<td>CAL (mm; mean ± SD)</td>
<td>10.0 ± 1.6</td>
<td>9.4 ± 1.5</td>
<td>0.276 *</td>
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<td>INTRA (mm)</td>
<td>6.9 ± 1.5</td>
<td>6.3 ± 1.0</td>
<td>0.254*</td>
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<tr>
<td>1-wall (%)</td>
<td>44.2 ± 24.8</td>
<td>37.7 ± 19.4</td>
<td>0.440**</td>
</tr>
<tr>
<td>2-wall (%)</td>
<td>27.0 ± 13.5</td>
<td>36.0 ± 18.1</td>
<td>0.141**</td>
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<tr>
<td>3-wall (%)</td>
<td>28.8 ± 20.3</td>
<td>26.3 ± 16.4</td>
<td>0.726**</td>
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<tr>
<td>Radiographic angle (°; mean ± SD)</td>
<td>27.0 ± 4.8</td>
<td>25.9 ± 6.2</td>
<td>0.702*</td>
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<td>IBD (mm; mean ± SD)</td>
<td>6.4 ± 1.4</td>
<td>5.6 ± 1.0</td>
<td>0.115*</td>
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</table>

PD, probing depth; CAL, clinical attachment level; INTRA, intrasurgical intrabony defect depth; IBD, radiographic intrabony defect depth; SD, standard deviation.
*Unpaired t-test.
**Mann-Whitney U-test.
<table>
<thead>
<tr>
<th>Variable</th>
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<th>Δ0-6 months</th>
<th>12 months</th>
<th>Δ0-12 months</th>
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<tr>
<td>FMPS (%)</td>
<td>Test</td>
<td>10.9 ± 3.9*</td>
<td>10.0 ± 3.5</td>
<td>0.9 ± 6.1</td>
<td>9.7 ± 4.6</td>
<td>1.2 ± 6.7</td>
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<tr>
<td></td>
<td>Control</td>
<td>10.1 ± 2.1*</td>
<td>11.6 ± 3.7</td>
<td>-1.5 ± 3.5</td>
<td>12.3 ± 5.1</td>
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<td>Difference between groups</td>
<td>NS†</td>
<td>NS‡</td>
<td></td>
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<tr>
<td>FMBS (%)</td>
<td>Test</td>
<td>8.9 ± 3.7*</td>
<td>7.5 ± 4.7</td>
<td>1.4 ± 4.1</td>
<td>7.7 ± 2.9</td>
<td>1.2 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>9.6 ± 3.9*</td>
<td>8.1 ± 3.6</td>
<td>1.5 ± 3.6</td>
<td>7.4 ± 3.5</td>
<td>2.1 ± 5.4</td>
</tr>
<tr>
<td>Difference between groups</td>
<td>NS†</td>
<td>NS‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD (mm)</td>
<td>Test</td>
<td>8.3 ± 1.2**</td>
<td>3.5 ± 0.8</td>
<td>4.8 ± 0.9***</td>
<td>3.4 ± 0.9</td>
<td>4.9 ± 1.4***</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.9 ± 1.3**</td>
<td>4.6 ± 1.0</td>
<td>3.3 ± 1.6***</td>
<td>4.5 ± 1.0</td>
<td>3.4 ± 1.7***</td>
</tr>
<tr>
<td>Difference between groups</td>
<td>NS†</td>
<td>p = 0.002†</td>
<td></td>
<td></td>
<td>p = 0.001‡</td>
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</tr>
<tr>
<td>CAL (mm)</td>
<td>Test</td>
<td>10.0 ± 1.6**</td>
<td>5.4 ± 1.2</td>
<td>4.6 ± 1.4***</td>
<td>5.5 ± 1.1</td>
<td>4.5 ± 1.9***</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>9.4 ± 1.5**</td>
<td>6.6 ± 1.3</td>
<td>2.8 ± 1.7***</td>
<td>6.5 ± 1.2</td>
<td>2.9 ± 2.2***</td>
</tr>
<tr>
<td>Difference between groups</td>
<td>NS†</td>
<td>p = 0.03‡</td>
<td></td>
<td></td>
<td>p = 0.01‡</td>
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</tr>
<tr>
<td>REC (mm)</td>
<td>Test</td>
<td>1.7 ± 1.2*</td>
<td>1.9 ± 1.2</td>
<td>-0.2 ± 0.6</td>
<td>2.1 ± 1.3</td>
<td>-0.4 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.5 ± 0.8*</td>
<td>2.0 ± 1.1</td>
<td>-0.5 ± 0.9</td>
<td>2.0 ± 1.2</td>
<td>-0.5 ± 0.9</td>
</tr>
<tr>
<td>Difference between groups</td>
<td>NS†</td>
<td>NS‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBD (mm)</td>
<td>Test</td>
<td>6.4 ± 1.4**</td>
<td>2.7 ± 0.8</td>
<td>3.7 ± 1.1***</td>
<td>2.5 ± 0.7</td>
<td>3.9 ± 1.2***</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5.6 ± 1.0**</td>
<td>4.1 ± 0.9</td>
<td>1.5 ± 1.2***</td>
<td>4.0 ± 0.8</td>
<td>1.6 ± 1.1***</td>
</tr>
<tr>
<td>Difference between groups</td>
<td>NS†</td>
<td>p &lt; 0.001‡</td>
<td></td>
<td></td>
<td>p &lt; 0.001‡</td>
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</tbody>
</table>

FMPS, Full-Mouth Plaque Score; FMBS, Full-Mouth Bleeding Score; PD, probing depth; CAL, clinical attachment level; REC, gingival recession, IBD, radiographic intrabony defect depth.

NS, difference between groups is not statistically significant (p > 0.05)

* p > .05, p values represent changes among the three time points (ANOVA or Friedman’s test)

** p < .001, p values represent longitudinal changes from baseline (Tukey test or Dunn test)

TABLE 3 Changes in clinical and radiographic parameters (mean ± SD) over the 12-month experimental period.
Table 4 Inter-group comparison of frequency distribution of residual PD and CAL gain measured one year after treatment

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>CATEGORY</th>
<th>TEST (%)</th>
<th>CONTROL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual PD (mm)</td>
<td>0 to 1</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>2 to 3</td>
<td>10 (66.7)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td></td>
<td>4 to 5</td>
<td>5 (33.3)</td>
<td>10 (71.4)</td>
</tr>
<tr>
<td></td>
<td>≥ 6</td>
<td>0 (0)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>CAL gain (mm)</td>
<td>0 to 1</td>
<td>0 (0)</td>
<td>4 (28.4)</td>
</tr>
<tr>
<td></td>
<td>2 to 3</td>
<td>4 (26.7)</td>
<td>6 (42.9)</td>
</tr>
<tr>
<td></td>
<td>4 to 5</td>
<td>6 (40)</td>
<td>3 (21.4)</td>
</tr>
<tr>
<td></td>
<td>≥ 6</td>
<td>5 (33.3)</td>
<td>1 (7.1)</td>
</tr>
</tbody>
</table>

Figure legends

*Figure 1.* Consort diagram showing the study design.

*Figure 2.* Test site treated with minimally invasive surgical technique and autologous dental pulp micro-grafts/collagen sponge bio-complex (periodontal surgeon M.A.). Preoperative radiographic view of an intrabony defect on the distal aspect of the maxillary first molar (a), elevation of the flap and probing of the intrabony defect (b), intraoperative view of the defect before elevating the interdental papilla (c), mechanical dissociation of the dental pulp (d), placement of a collagen sponge with dental pulp micrografts (e), suture (f), clinical (g) and radiographic (h) aspects at 12 months after treatment.

*Figure 3.* Control site treated with minimally invasive surgical technique and collagen sponge alone. Preoperative radiographic (a) and clinical views (b) of an intrabony defect on the distal aspect of the mandibular first premolar, elevation of the flap and defect debridement (c), suture (d), radiographic (e) and clinical (f) aspects at 12 months after treatment.
Figure 1.
Figure 2.

Figure 3.