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A novel therapeutic strategy for periodontal regeneration of non-contained intrabony defects using autologous micrografts from the palatal mucosa

Abstract

Aims
Due to their accessibility and biological features, mesenchymal stem cells (MSCs) derived from the hard palate mucosa (PMSCs) hold significant promise for periodontal re-generation. This case series investigates the clinical and radiographic efficacy of autologous micrografts (AMGs) enriched in PMSCs for the treatment of non-contained defects in patients with severe periodontitis.

Materials and methods
Five patients presenting with at least one predominantly 1- or 2-wall intra-bony defect requiring periodontal regenerative surgery were consecutively enrolled. A small connective tissue sample was harvested from the palate, mechanically dissociated chair-side, and filtered to obtain AMGs enriched in PMSCs. The selected intrabony defects were filled with a resorbable scaffold seeded with a suspension containing AMGs.

Results
At the 6-month follow-up, a mean clinical attachment gain of 4.8 ± 1.8 mm was observed, along with a residual mean probing depth of 4.2 ± 0.8 mm and a radiographic bone fill of 3.6 ± 4.3 mm Characterization of AMGs was performed in two patients, demonstrating progenitor cells expressing MSC-specific surface markers.

Conclusions
These preliminary findings suggest that AMGs derived from the palatal mucosa may offer a promising approach for the regenerative treatment of intrabony defects with unfavorable architecture.

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Keywords

Mesenchymal stem cells, palate, guided tissue regeneration, periodontal, periodontitis, tissue engineering.

INTRODUCTION

Periodontitis is a biofilm-mediated inflammatory disease of tooth-supporting tissues, resulting in progressive periodontal attachment and alveolar bone loss (1). Intrabony defects, which are anatomic deformities surrounded by one, two, three bony walls or a combination thereof, are commonly detected in the advanced periodontitis stages and represent a site-specific risk factor for disease progression (2,3). For the treatment of such periodontal defects, regenerative treatment is recommended, with the goal of completely restoring the tooth's supporting apparatus over a previously diseased root surface (4,5). However, periodontal regeneration is challenging, especially in defects with uncontained configuration (predominantly 1 and/or 2 walls), as the current surgical procedures and biomaterials have shown variable success in achieving predictable regenerative outcomes (6). One of the major limitations may be due to an inadequate number of resident stem/progenitor cells to regenerate the lost periodontal tissues (7–9). It is also possible that the inflammatory environment in the deep periodontal pocket may lead to the exhaustion of the stem cells' regenerative potential, impairing the outcomes of clinical procedures (10–12).

In the last years, insights into the reparative capability of the periodontium in conjunction with advances in stem cell biology and tissue engineering have prompted extensive research into the application of cell-based treatments (11). In fact, the supply of adult mesenchymal stem cells (MSCs) has obtained positive results in the attempt to repair or to replace compromised tissues or cells. MSCs can be isolated from several tissues like, bone marrow, umbilical cord blood, adipose tissue and peripheral blood (12) as well as from intraoral and dental sources, such as the dental pulp, dental follicle, periodontal ligament and apical papilla (13–17). These multipotent cells can differentiate into adipocytes, chondrocytes and osteocytes if properly stimulated under specific conditions (18–20).

Recent studies have demonstrated that autologous micrografts (AMGs) obtained by mechanical disaggregation and filtering of fresh oral tissue contain a significant amount of progenitor cells, which may enhance tissue regeneration (21–23). In particular, AMGs made out of dental pulp connective tissue samples have achieved clinically successful periodontal regeneration when endorsed on a collagen scaffold (24–26). Since AMGs do not undergo immune rejection, have good cell compatibility and are free of ethical barriers, they are considered ideal candidate for tissue engineering and regenerative medicine applications (27).

More recently, given the need for tooth extraction to harvest MSCs from dental pulp and periodontal ligament, hard palate mucosa (HPM) has been identified as an alternative and easily accessible source of undifferentiated cells, which could be a feasible tool for periodontal regeneration. Jiang et al. isolated hard palate-derived mesenchymal stem cells (PMSCs) from the lamina propria of rat's HPM (28). They exhibited clonogenicity, self-renewal, migration, and multipotent differentiation capacities. Scaffold-free 3D PMSC aggregates constructed using light-controlled cell free technology and serum-free culture medium were able to mimic native cellular microenvironments, promoting bone formation and angiogenesis, while controlling inflammation in a tibial defect model (28). Even though systematic reviews of preclinical animal studies in dentistry have demonstrated that tissue engineering can enhance periodontal tissue regeneration, these studies serve primarily as groundwork for clinical trials, and there is an urgent need for evidence supporting its clinical application (29,30).

Thus, the aim of this case series was to assess the clinical and radiographic effectiveness of HPM-derived AMGs for periodontal regeneration of non-contained intrabony defects in patients with severe periodontitis. Secondary aim was to isolate and to characterize MSCs from AMGs.

MATERIALS AND METHODS

Study design and participants

This prospective case series study was conducted in accordance with the Helsinki Declaration, at the Section of Periodontology, C.I.R. Dental School, University of Turin (Italy) from January to September 2024. The research protocol was approved by the Institutional Ethical Committee (no. 0116829), and all patients gave written informed consent prior to the beginning of the study.

Adult patients with stage III/IV periodontitis who had completed step 1 and 2 of periodontal treatment (etiological therapy) were consecutively enrolled into the study, following a screening visit including full-mouth probing and radiographic examination (31). Inclusion criteria comprised the following: 1) completion of etiological therapy at least 2 months prior to screening; 2) percentages of plaque-harboring tooth surfaces [full-mouth plaque score (FMPS)] and bleeding gingival units [full-mouth bleeding score (FMBS)] < 15%; 3) at least one predominantly 1- or 2-wall intrabony defect on natural teeth with a residual probing depth (PPD) of ⩾ 6 mm and an intrabony radiographic component of ⩾ 3 mm, deemed suitable for regenerative treatment. The defect had to extend to the lingual/palatal side, as assessed by preoperative bone sounding, requiring flap elevation on both the buccal and oral side for its accessibility.

Exclusion criteria were: 1) current smoking; 2) contraindications for periodontal surgery; 3) compromised systemic health (i.e., diabetes, quantitative

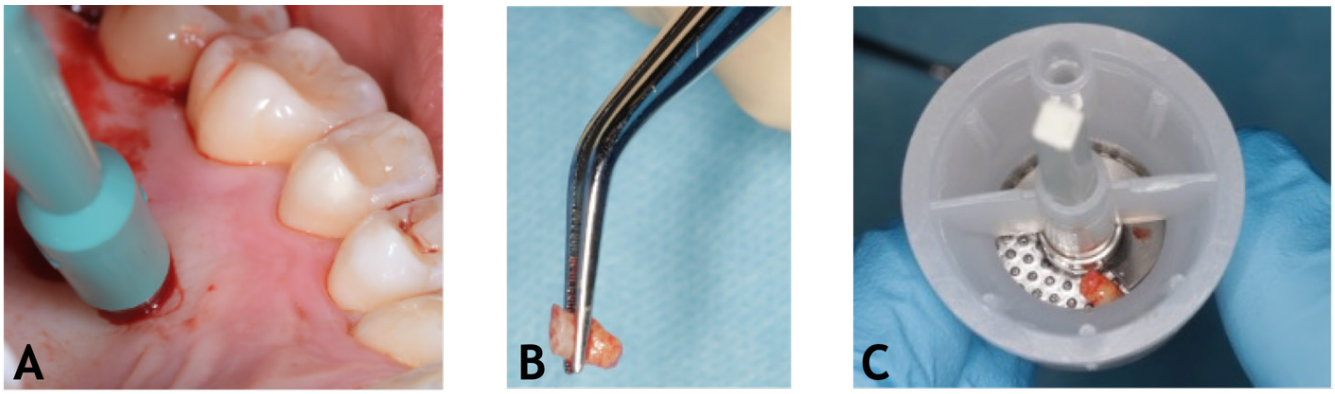

Fig. 1 Autologous micrograft preparation in accordance with the Rigenera® protocol: (A) A small punch of the palatal mucosa was collected directly from the premolar region and washed with sterile saline; (B) Autologous tissue sample; (C) The sample was inserted in the Rigenera® device with sterile saline to obtain the autologous micrografts.

and/or qualitative polymorphonuclear neutrophils defects, other immune system disorders); 4) ongoing drug therapy that could interfere with periodontal tissue health and healing (i.e., anti-inflammatory agents, diphenylhydantoin, calcium channel blockers, cyclosporin A, immunostimulants/ immunomodulators). 5) pregnancy and lactation; 6) previous periodontal surgery on the involved sites; 7) furcation involvement ⩾ II degree.

Surgical procedure and clinical measurements

Clinical and radiographic parameters were recorded at baseline (before surgery) and 6 months postoperatively by the same calibrated examiner. Clinical measurements were recorded to the nearest millimeter using a manual periodontal probe with 1 mm markings (UNC15, Hu-Friedy, Chicago, IL. USA) at the deepest location of the selected interproximal defect. They included plaque accumulation (PI), bleeding on probing (BoP), PPD, clinical attachment level (CAL) and recession of the gingival margin (REC).

Digital standardized long cone intra-oral radiographs were obtained at the same time points. The radiographic angle (RA) and the linear distance from the bone crest to the base of the defect (BC-BD) were recorded by means of an image analysis software (ImageJ, Bethesda, USA). The difference between BC-BD recorded at baseline and 6-month examination was identified as the amount of bone fill within the intrabony defect.

Mobile teeth were splinted to adjacent teeth before surgery or immediately after surgery if deemed necessary to avoid compromising access or visibility. For the access flap, modified or simplified papilla preservation technique was selected based on the width of the interdental papilla (32,33). The elevation of the flap was kept at a minimum to allow the exposure of the defect and the careful debridement of the root surface. Vertical releasing incisions were placed mesial or distal to the treated defect, if considered necessary to improve visibility and/or to achieve a tension free flap closure. Minimally invasive flap elevation and papilla reflection were done followed by the degranulation of the defect with micro-curettes. Then, the root surface was debrided using ultrasonic devices with periotips and mini-curettes followed by EDTA application on a dry surface for 120 seconds.

A small punch of tissue (3 mm of diameter and 4-5 mm of depth) was harvested from the palate in the premolar region, then the graft was mechanically disaggregated using the chair-side Rigenera® System (Human Brain Wave, LLC, Turin, Italy) that is a mechanical disruptor working at a rotating speed to 80 rpm, in 1.0 ml sterile physiologic solution (24,34). After dissociation, the micrografts suspension was passed through a disposable grid (Rigeneracons with about 100 hexagonal holes filtering cells and components of extracellular matrix with a cut-off of 80 µm in an average time of 3 minutes) (Fig. 1). The suspension containing AMGs was seeded on a resorbable polylactic-co-glycolic acid dextran and hydroxyapatite scaffold (New Shore, Ghimas Spa, Bologna Italy) and subsequently compacted to completely fill the defect.

Flaps were positioned at the pre-surgical level without any tension using non-resorbable 6-0 e-PTFE sutures. Selection of the suturing technique was based on the flap design, i.e., a horizontal internal mattress suture at the base of the papilla and a second internal mattress suture (vertical or horizontal) between the most coronal portion of the flap and the most coronal portion of the palatal/ lingual papilla.

Post-operative care

Antibiotics (875 mg amoxicillin + 125 mg clavulanic acid) were prescribed twice a day for 6 days and analgesics (600 mg ibuprofen) three times a day for 3 days post-surgery. Subsequent doses were taken only if necessary to control pain. Patients were instructed to discontinue toothbrushing in the surgical area for the first 4 weeks post-operatively and to maintain plaque control with 0.2% chlorhexidine digluconate mouthwash. Thereafter, they resumed mechanical tooth cleaning of the treated areas, and during weeks 4 to 6 they

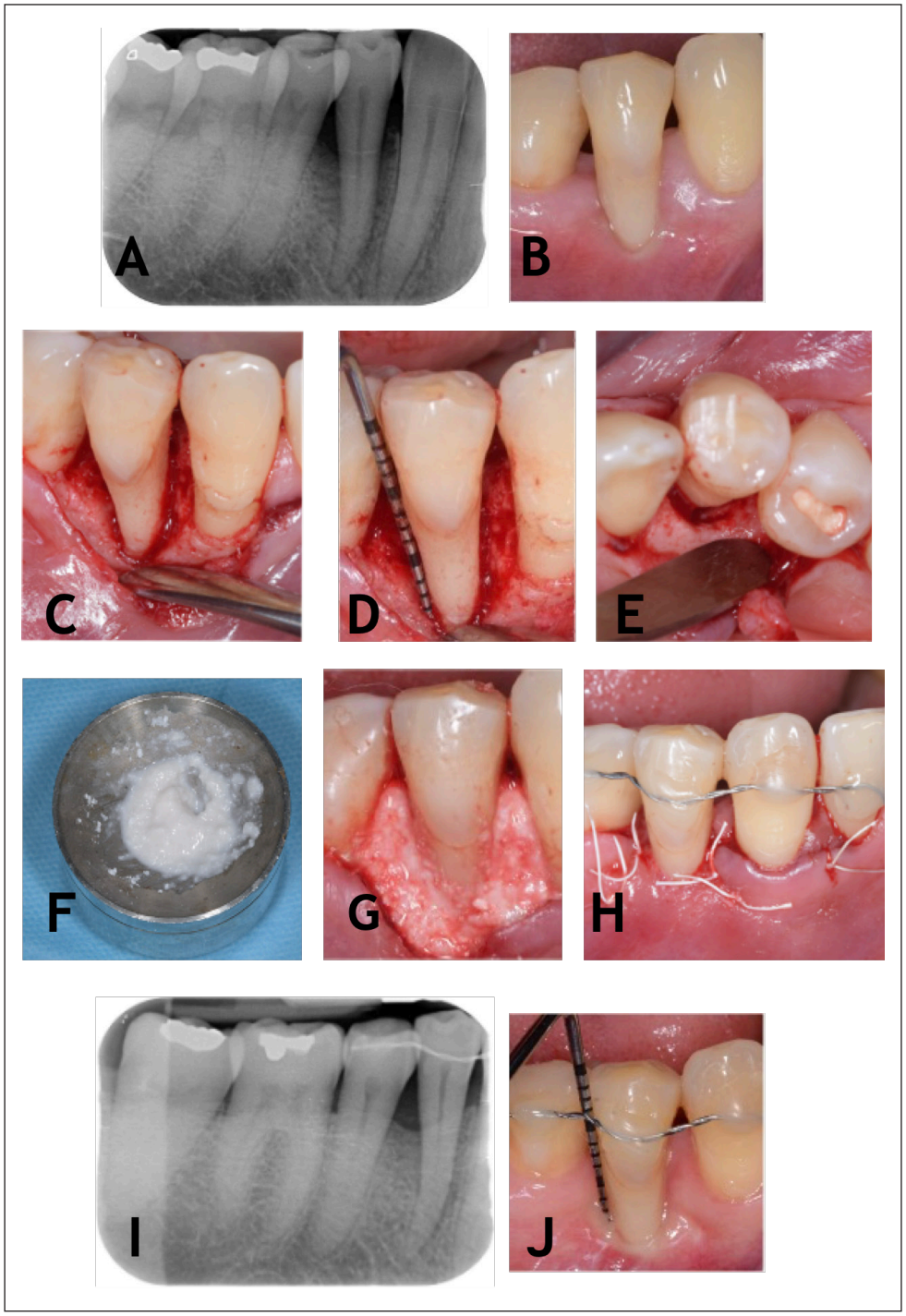

Fig. 2 Case 3, circumferential defect associated with mandibular right first premolar.
(A) Preoperative radiograph.
(B) Preoperative clinical image.
(C-E) Flap elevation and intra-surgical defect assessment.
(F) Autologous micrografts endorsed on the scaffold to form a biocomplex.
(G) Biocomplex entirely filling the intrabony component of the defect.
(H) Primary closure by means of horizontal mattress and simple interrupted sutures.
(I) Periapical radiograph at 6 months post-operatively showing the complete closure of the defect.
(J) Six-month post-operative periodontal probing depth.

were instructed to use a soft toothbrush. Sutures were removed after 2 weeks. During the first month after surgery patients were recalled weekly for professional tooth cleaning of the treated area, subsequently at 3 and 6 months for re-inforcement of oral hygiene procedures and supra-gingival debridement.

Laboratory procedures

Isolation of MSCs from the palatal mucosa

A second tissue punch with identical dimensions as the first one was harvested during the surgical session from the same palatal area. It was disaggregated using the Rigenera® medical device as previously described. The cell suspension obtained was cultured in α-MEM with 15% of FBS, 5% of penicillin/streptomycin (Life Technologies, Carlsbad, CA, USA) and gentamicin at 5 µg/ml (Merck, Darmstadt, Germany) to avoid culture contamination. After 20-30 days, in two of the five samples, small colonies of cells were detectable, and cells were allowed to grow until confluence.

Analysis of the MSCs' immunophenotype

After the cells reached the confluence in a cell culture dish, they were detached and analyzed for the expression

Case	Baseline					6 Months				
	PPD	REC	CAL	BC-BD	RA	PPD	REC	CAL	BC-BD	RA
1	6	0	6	4.4	22.1	3	0	3	2.7	26.2
2	11	2	13	4.4	46	5	2	7	3.5	51.2
3	10	0	10	11.2	7.7	4	1	5	0	0
4	12	0	12	7.6	24.6	5	0	5	6.3	27.9
5	7	0	7	4.6	38.1	4	0	4	2.9	48.7
MEAN	9.2	0.4	9.6	6.5	27.7	4.2	0.6	4.8	2.9	30.8
SD	2.6	0.9	3.1	3.0	14.9	0.8	0.9	1.5	1.9	20.7

Table 1. Clinical and radiographic outcomes

PPD = Probing Depth (mm), REC = distance between gingival margin and cement-enamel junction (mm), CAL = Clinical Attachment level (mm), BC-BD = Radiographic distance from the bone crest to the base of the defect (mm), RA = Radiographic Angle (°)

of the typical MSCs' markers. A standard labelling protocol for surface antigens was performed utilizing monoclonal antibodies fluorochrome-conjugated: human CD105 PE (Invitrogen, Camarillo, CA, USA), CD73 VioBright 515, CD45 PerCP (Miltenyi Biotech, Bergisch Gladbach, Germany), CD90 PerCP (Biolegend, San Diego, CA, USA). As control, unstained cells were examined. Data were acquired on a MACsQuant 10 and analyzed with MACsQuantify software (Miltenyi Biotech, Bergisch Gladbach, Germany).

Data analysis

Due to the small sample size, only descriptive statistics was performed and quantitative variables were summarized using mean ± standard deviation (SD).

RESULTS

Clinical Results

Five patients (2 males, 3 females, mean age: 58.8 ± 8.7 years) with Stage III Grade B periodontitis contributed one intrabony defect requiring periodontal regenerative surgery. All defects were predominantly 1- to 2-wall non-contained defects, and were localized at maxillary premolars (two defects) or mandibular premolar (one defect), maxillary first molar (one defect), and maxillary third molar (one defect). Defect

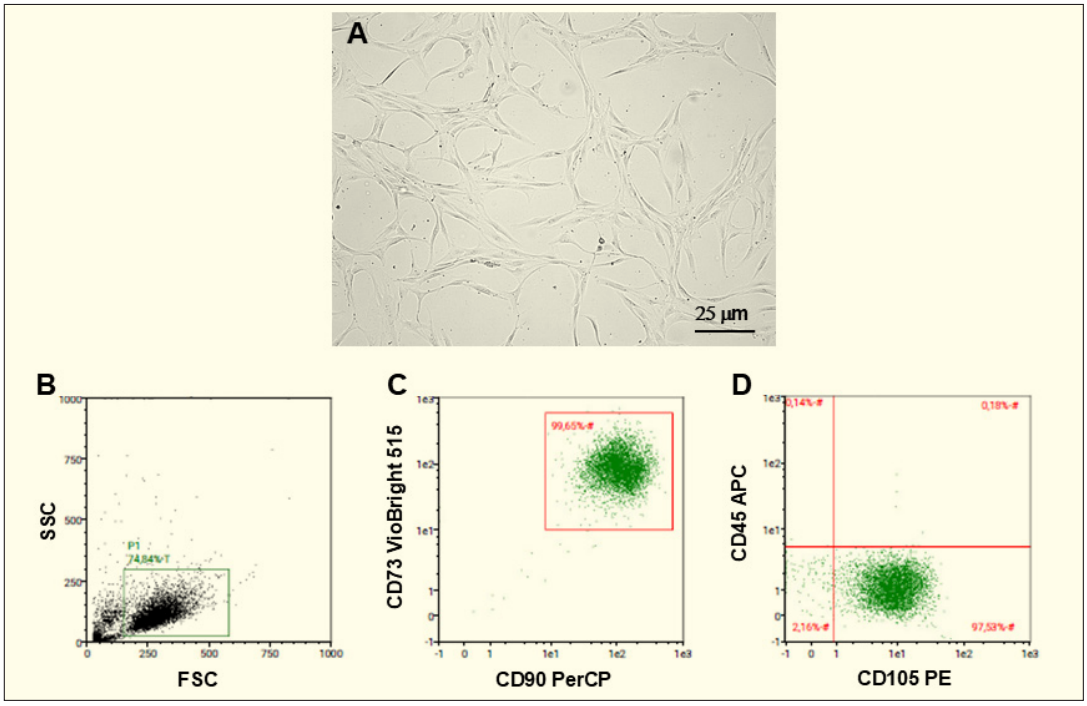

Fig. 3 Palatal MSCs. (A) In the image MSCs with the typical spindle-shape are evident. (B) The dot plot shows the physical parameters of cells: forward scatter (FSC) and Side Scatter (SSC), with the gate on the examined population (P1). (C) A population positive for CD73 and CD90 was gated and on these cells, we also show the expression of CD105 and the lack of CD45 (D).

morphology was further characterized intrasurgically. The mean distance from cemento-enamel junction to the bottom of the defects was 13.8 ± 2.6 mm, the intrabony component of the defects was 8.2 ± 2.8 mm and the width measured on average 6.6 ± 4.0 mm.
Healing was uneventful for all patients, and no materials exposure occurred. As reported in Table 1 the application of the AMGs resulted in an average CAL gain of 4.8 ± 1.8 mm associated with a residual mean PPD of 4.2 ± 0.8 mm and a remarkable stability of the gingival margin at 6 months. None of the sites displayed any plaque deposit, and only one site showed persisting BoP.
No sites lost attachment and all sites gained 3 mm or more. Three sites achieved successful regeneration based on the composite outcome measure (PPD ⩽ 4 mm and CAL) gain ⩾ 3 mm) [35]. Clinical outcomes were supported by the radiographic analysis with a 6-month mean bone fill of 3.6 ± 4.3 mm. In the periapical radiographs defects were filled by bone-like tissue. A clinical case is described in Fig. 2.

MSC isolation and phenotypic characterization

Disaggregated palatal mucosa samples were harvested from patients to characterize the cells present in the suspension derived from Rigeneracons.
Cells grew in culture, showing the classical spindle-shape (Fig. 3A) morphology of MSCs. The MSCs' immune-phenotype was assessed, through the flow cytometric analysis (Fig. 3B), on cells detached after reaching the confluence at the first passage that expressed CD73, CD90 and CD105, while they were negative for CD45 (Fig. 3C-D).

DISCUSSION

In this report for the first time, we describe preliminary data on the use of HPM-derived AMGs in periodontal regeneration. The clinical and radiographic findings seem to support the effectiveness of such cell-based therapy when applied to deep and non-containing intrabony defects in patients with advanced periodontitis. At 6-month evaluation considerable clinical improvement in terms of CAL gain (4.8 mm) and PPD reduction (5.0 mm) was obtained together with a minimal change in REC (0.2 mm). However, due to the lack of histologic evidence, no definitive statement could be made concerning the tissue properties achieved.
The observed clinical benefits are greater than those reported in previous systematic reviews evaluating reconstructive surgery in intrabony periodontal defects using bio-logical inductors or resorbable membrane application (36,37). They are also more favorable with respect to those achieved when a minimally invasive surgical approach, based on the papilla preservation technique, was used alone or in combination with enamel matrix derivatives or collagen resorbable membranes (38). These findings are even more relevant considering that the defects' regenerative potential is related to their anatomy, with non-containing 1-2-walled intrabony defects displaying the lowest chance of obtaining successful regeneration (39).
We used the chair-side Rigenera® system to form AMGs from a few millimeters sample of HPM, which were transplanted into the area of periodontal defects. PMSCs were promptly isolated through a mechanical process allowing for the filtration of cells under 80 µm of diameter, in contrast with the traditional enzymatic digestion method that requires the use of chemical reagents and long culture time (23,26). The cut-off of 80 µm promotes the discharging of old differentiated cells and the enrichment of young progenitor cells (23,26). Indeed, under this dimension the percentage of cells expressing stem antigens increases significantly, avoiding a magnetic or flow cytometric sorting (40). The main advantage of this protocol is to obtain an adequate number of micrografts containing viable cells, thus avoiding in-vitro cell expansion and manipulation. It also avoids the possible complications arising from the use of non-autologous micrografts, as donor and acceptor are the same individual.
Previous studies reported encouraging results for the application of AMGs containing progenitor cells in bone and periodontal regeneration (41). A mean CAL gain of 4.5 mm and PPD reduction of 4.9 mm was achieved using AMGs containing MSCs from dental pulp tissues of wisdom teeth (42). Nonetheless, results from meta-analyses comparing the efficacy of stem cell-based therapy with conventional regenerative therapy for the treatment of periodontitis are heterogeneous (29,43–45), suggesting the need for further high-quality clinical research. This inconsistency may be ascribed to the limited number of clinical studies and to their variability in terms of methods of cell collection and extraction.
When coming to decide the donor site for MSCs, the hard palatal mucosa stands out due to its excellent healing ability, convenience of access, and mechanical properties compared to other harvesting sites (35). Dental pulp-derived MSCs, although being promising for periodontal regeneration, require a vital tooth free of carious lesions and not periodontally involved (24,25). In addition to the ease of collecting a palate tissue graft, MSCs isolated from HPM exhibit various properties, including clonogenicity, self-renewal capability, and pluripotent differentiation potential (28,35,46,47). Moreover, they have a resistance to inflammatory stimuli enabling uninterrupted osteogenesis in the presence of inflammation, have a notable migratory capacity, facilitating prompt arrival at the site of injury (28).
Consistent with previous preclinical studies in humans

(48,49), the present study confirmed that HPM cells display the basic characteristics of MSCs such as plastic adherence and specific surface antigen composition (49). Indeed, cell characterization, performed by FACS, showed that they expressed some specific MSC markers such as CD105, CD73, and CD90, but they were negative for CD45. In the above-mentioned studies connective tissue grafts were obtained by de-epithelialization of a free gingival graft or by a split flap approach and cells were extracted by enzymatic digestion. In the present study AMGs were obtained by mechanical dissociation of a palatal mucosa tissue graft. Thus, it can be hypothesized that cells different from MSCs, exosomes and growth factors contained in the tissue particles may also affect the wound-healing process through the stimulation of host cells (50,51). Zanzottera et al. reported a high number of pericytes in AMGs obtained from periosteum samples, which may enhance tissue regeneration, promoting revascularization (21).

Periodontal tissue regeneration requires an adequate scaffold as well as cells that are capable to attach to the scaffold, proliferate, and differentiate (19). We used a synthetic copolymer, PLGA, combined with hydroxyapatite to form a resorbable alloplastic scaffold on which the AMG suspension was endorsed. PLGA is one of the most attractive biopolymers for tissue engineering due its high biocompatibility and customizable bio-degradability and, when combined with a hydroxyapatite filler, it displays improved mechanical and osteoconductive properties, which may enhance bone regeneration in periodontal defects (52). This copolymer has been successfully used for bone tissue re-generation during sinus lift procedures combined with AMGs from human periosteum using the Rigenera® protocol (53).

There are some limitations of this study. First, the sample size is small and data are preliminary. Secondly, the lack of a control group does not allow to discriminate the role of the scaffold material in the clinical outcomes achieved. Moreover, the study is short-term. However, the meta-analysis by Zhang et al. (40) comparing stem-based periodontal regenerative therapy with conventional procedures reported the greatest improvement for PPD at 6-month examination with respect to longer follow-up times. Finally, no histologic analysis was carried out to demonstrate the evidence of new root cementum, alveolar bone and periodontal ligament formation in the treated sites.

CONCLUSION

Within the limits of the study design and sample size of this case series, the application of AMGs from the palatal mucosa into deep non-containing intrabony defects coupled with minimally invasive surgical procedures would seem to enhance the intrinsic regenerative potential of the periodontal defects. Randomized controlled clinical trials should be performed to confirm these preliminary findings and to address the potential advantages of this approach compared to conventional regenerative procedures.

Author Contributions

Conceptualization, M.A., C.J. and F.M.; methodology, A.B., and F.R.; laboratory analysis, I.R. and F.M.; investigation, C.J., and A.B.; data curation, F.R.; writing—original draft preparation, A.B., F.R., G.B. and I.R.; writing—review and editing, M.A. and F.M.; project administration, M.A. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of AOU Città della Salute e della Scienza di Torino (n° 0116829, date of approval 09/10/2023).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

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