KEY WORDS
multiple gingival recessions, gingival fibroblasts, biotransplant, mucogingival surgery, tissue engineering

TREATMENT OF MULTIPLE GINGIVAL RECESSIONS IN ANTERIOR MAXILLARY TEETH USING PLATELET-RICH FIBRIN AND AUTOLOGOUS GINGIVAL FIBROBLASTS: A CASE SERIES

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PURPOSE. To evaluate the clinical outcome of biografts containing autologous fibroblast cell culture (AFCC) injected into platelet-rich fibrin (PRF) membrane and placed under coronally advanced flap (CAF) for the treatment of multiple gingival recessions in the anterior area of the upper jaw.

MATERIAL AND METHODS. Ten patients with 43 gingival recessions, Miller class I, II and III, located in the anterior maxillary teeth were studied. Patients' AFCCs were isolated from biopsied palatal mucosa and cultured for cell-suspension preparation. The PRF membrane was made of whole blood taken before surgery. During the mucogingival surgery, the AFCC was introduced into the PRF membrane, which was placed under the CAF. Root coverage, clinical attachment level (CAL), probing depth (PD), gingival recession depth (GRD), keratinized tissue height (KTH), full-mouth plaque score (FMPS), and full-mouth bleeding score (FMBS), were recorded prior to treatment and at 3, 6 and 12 months after surgery.

RESULTS. All patients completed the study. The mean root coverage was 83%. After 12 months, mean GRD was significantly reduced from 1.63 ± 0.28 mm to 0.26 ± 0.12 mm (P = 0.0002). Mean KTH significantly increased from 2.8 ± 0.37 mm to 3.63 ± 0.27 mm (P = 0.0003). PD showed no significant changes at any timepoint (1.52 ± 0.07 mm at baseline and 1.43 ± 0.06 mm 12 months after surgery). After 12 months, complete root coverage was 100% at Miller class I and II teeth, and 54% at Miller class III teeth.

CONCLUSIONS. Application of PRF membrane containing AFCC represents a promising new therapeutic concept, based on living cells, in the treatment of gingival recession. However, well-designed randomised controlled trials are needed to evaluate its effectiveness in comparison with conventional procedures.

CONFLICT OF INTERESTS STATEMENT. The authors declared no conflict of interest related to this article to report.

INTRODUCTION

Gingival recession is one of the most common problems seen in dentistry. It not only compromises the aesthetic appearance of the gingiva, but also increases tooth sensitivity, risk of caries and abrasive defects in the cervical region of the teeth[1,2].

The gold standard for treatment of multiple gingival recessions is the application of a connective tissue graft (CTG) in combination with a coronally-advanced flap or tunnelling technique[3,4]. However, CTG surgery exposes the donor site to the risk of several side effects, including pain, post-surgical inflammation, bleeding, infection, and additional surgical trauma[5]. Furthermore, in cases of multiple gingival recessions, due to the limited donor area, patients may have to undergo several interventions to achieve coverage of all exposed root surfaces,

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sometimes over the course of several years. In the search for new approaches for gingival recession correction with less biological cost and comparable clinical outcomes, several new techniques have been introduced, including alternative soft tissue substitutes[6]; regenerative therapies;[7] and tissue engineering approaches[8].

Tissue engineering is a rapidly growing field that aims to create bioartificial tissues and organs by replacing or regenerating cells, tissues or organs in order to restore normal biological function. The success of tissue engineering and the results of research into stem/differentiated cells provide grounds for the development of new approaches to restoring the volume of lost tissues through transplantation of cells obtained *in vitro*[9-13]. The increasing collaboration between different disciplines, such as biochemistry, biology, biomaterials, medicine and engineering, has led to promising outcomes in tissue regeneration. In the dental field, tissue engineering is a new promising therapeutic approach that has been applied for treatment different pathological conditions and diseases[14].

There are three key elements for tissue regeneration: natural or synthetic scaffolds (extracellular matrices), cells, and growth factors. Most studies that have been carried out in this field have chosen fibroblasts, cells of mesenchymal origin, as a source of cell material, because they form the basis of connective tissue, producing collagen and growth factors[6,15-22]. At the same time, different scaffolds have been applied to carry cell cultures, namely hyaluronic acid matrix[6], acellular dermal matrix[15], collagen gel[22], collagen membrane[8], chitosan-based scaffold[19] and polymer scaffolds (polyglactin mesh)[20,21].

In periodontology, tissue engineering techniques are being applied to the ongoing challenge of hard and soft tissue reconstruction. To reconstruct periodontal soft tissues, specifically the attached gingiva, several authors have proposed the use of autologous human fibroblasts delivered in different carriers, namely collagen membranes or hyaluronic acid membranes[8,23-25]. Fibroblasts are the main cellular component of the connective tissue plate of the gingiva. They provide local homeostasis and morphofunctional organization, and have demonstrated physiological and reparative potential[26]. In culture, fibroblasts actively produce such extracellular matrix components as procollagen, proelastin, glycosaminoglycans, growth factors, and others[27]. Fibroblasts have been shown to maintain these activities after transplantation into connective tissue. Furthermore, fibroblasts may accelerate angiogenesis, capillary endothelial cell proliferation and, accordingly, may increase regeneration of damaged periodontal tissues[6,27,28]. Autologous fibroblasts do not conflict with their host's immune system, and do not cause allergic or other adverse reactions[6,29]. However, information about the applications of autologous fibroblast cell cultures (AFCCs) in dentistry is thus far limited.

In a parallel line of research, in recent decades there has been growing interest in molecular mediators and other biological agents, along with various forms of platelet concentrates. Haemoderivatives in particular have yielded some inspiring results in terms of both soft tissue and bone regeneration,[30,31] and the growth factors and cytokines contained in platelet-rich plasma (PRP) appear to have an impact on regeneration of soft and hard periodontal tissues[30-34]. PRF is another product obtained from the patient's own blood plasma. It is a dense fibrin matrix enriched with platelets and white blood cells[35]. Due to its structure, PRF provides long-term release of growth factors (VEGF, IGF1, PDGF-AB) and cytokines, and stimulates cell migration, which is clinically manifested by faster healing of the periodontal tissues and reduced postoperative discomfort[36-39]. In addition, being obtained from the patient's own blood and therefore autogenic, PRF is highly biocompatible with the patient's tissues and anticoagulants are not required[38].

To the best of our knowledge, there have been no studies exploring the application of PRF combined with AFCC for gingival plastic surgery. Thus, the aim of this case series was to evaluate the effects of PRF+AFCC under CAF in the treatment of multiple gingival recessions.

MATERIALS AND METHODS

Study design

This study was designed as a prospective case series. Ten patients (2 males and 8 females, aged 28 to 57 years; mean age ± SD: 44.2 ± 8.4) with multiple gingival recessions in the anterior maxillary area were selected for this study. A total of 43 recessions, Miller classes I, II and III, were treated in non-smokers referred to a private dental practice in Saint Petersburg, Russia, from April 2018 to August 2019.

Inclusion criteria

At least two Miller class I, II or III gingival recessions on adjacent maxillary front teeth in patients of 18 to 70 years of age.

Exclusion criteria

Patients were not included in the study if any of following exclusion criteria applied:

- Systemic conditions that generally preclude surgery or could influence the outcome of therapy (e.g., diabetes with HbA1c > 7%, INR > 3, etc.);
- Pregnancy or lactation;
- Long-term use of phenytoin, cyclosporine, systemic corticosteroids, or calcium channel blockers;
- Any antibiotics, anti-inflammatory drugs, or corticosteroids taken during the previous 3 months;
- Poor oral hygiene with full-mouth plaque score (FMPS) and/or full-mouth bleeding score (FMBS) lower than 15%;
- Poorly delineated cementoenamel junction (CEJ) in teeth with gingival recessions;
- Smoking.

Informed written consent was obtained from each patient prior to any procedure or assessment. Patients were explained the nature of the research, as well as the procedures, objectives, potential discomfort, risks and benefits of participating in the study.

Primary and secondary outcome variables

The primary outcome variable was root coverage. Root coverage was calculated using the formula[40]: [(baseline gingival recession depth - gingival recession depth at 3/6/12 months) / baseline gingival recession depth] × 100 [%].

The secondary outcome variables were the following.

- Gingival recession depth (GRD): distance from the CEJ to the gingival margin, measured at the midline of the vestibular surface of each tooth.
- Keratinized tissue height (KTH): distance from the free gingival margin to the mucogingival junction, assessed mid-facially.
- Probing depth (PD): distance from the gingival margin to the bottom of the pocket or sulcus, evaluated at 6 points for each tooth (3 vestibular and 3 palatal/ lingual) using a graduated probe.
- Clinical attachment level (CAL): the sum of PD and GRD at the midline of the vestibular surface of the tooth.

For assessments of periodontal parameters, a UNC 15 periodontal probe (Hu-Friedy, Chicago, USA) was used, and all dimensions were rounded to the nearest mm.

In addition, the following scores were calculated:

- Full-mouth plaque score (FMPS);
- Full-mouth bleeding score (FMBS).

Clinical parameters were measured before surgery (baseline), and at 3, 6 and 12 months after surgery by a calibrated independent examiner (A.A.).

Gingival biopsy

For the purposes of obtaining autologous tissues, all participants underwent a biopsy of the palatal mucosa (epithelium + connective tissue) at the level of the molar teeth (**FIG. 1**). After local anaesthesia (Sol. Ultracain DC - 1.7 ml Aventis Pharma, Hamburg, Germany) biopsy samples of 2 to 3 mm diameter were harvested using a surgical blade. Biopsy samples were placed in a sterile medium containing a-MEM (Sigma Aldrich, St. Louis, USA), 2% FBS (HyClone, Logan, USA), penicillin (200 U/mL), streptomycin (200 mkg/mL), and amphotericin (200 U/mL) (Stem Cell Technologies, Vancouver, Canada), then labelled and delivered to the laboratory at +4 to 8˚C within 24 hours of biopsy sampling.

Cell preparation and testing

Patients' AFCCs were isolated and cultured from the samples using standard methods with minor modifications[41]. In particular, each sample was transferred into 15 mL tube in DMEM/F12 (Invitrogen, Thermo Fisher Scientific, Waltham, USA) supplemented with 10% FBS (HyClone), gentamicin (20 mg/ml) and a 0.05% solution of collagenase type II (Sigma), and incubated for 12 hours at 37 ˚C. Before enzymatic digestion, samples were minced, and then the cell suspension was centrifuged at 200 g for 10 min. The cell pellet was resuspended in DMEM/F12 culture medium (HyClone) supplemented with 15% FBS (HyClone) and gentamicin (20 mkg/mL), and plated in 25 cm^2 culture flasks (NUNC, Roskilde, Denmark) at a density of 3–5x104 cells/cm^2. Cells were cultured at 37 °C, 95% humidity and 5% CO^2, with a medium change every 3 days. Cell cultures were passaged at 70% confluence. The cell culture medium was replaced every 72 hours. When confluent, the primary cells were trypsinized with 0.05% trypsin/EDTA solution (Bioind, Kibbutz, Israel). Visual observation of culture growth and cell morphology was conducted by phase contrast microscopy (Carl Zeiss Axiovert-200, Jena, Germany). Cell cultures at passages 1 to 3 were used for research experiments.

The finished cell preparation comprised a suspension of the patient's AFCCs in saline solution at a concentration of $20x10^6$ cells/ml. For safety purposes, the cell material was tested to exclude contamination by viral, bacterial and fungal agents using polymerase chain reaction (PCR), along with cell karyotyping.

PCR and standard bacteriological analysis were performed at a certified diagnostic centre to exclude the following:

1. Antibodies to HIV 1.2, hepatitis B virus (HBs Ag), hepatitis C virus (anti-HCV), and antibodies to *Treponema pallidum*;
2. *Staphylococci*, *Streptococcus* spp, *Enterococcus* spp, *Candida* spp, *Aspergillus* spp, and *Escherichia coli* via standard bacteriological analysis (seeding of fibroblast suspension for sterility);
3. DNA of *Ureaplasma* species, HIV 1.2, hepatitis B, cytomegalovirus, *Toxoplasma gondii*, *Treponema pallidum*, human papillomavirus 16, human papillomavirus 18, *Mycoplasma genitalium* and *Chlamydia trachomatis*[26], and RNA of hepatitis C virus via PCR (biomaterial cellular suspension of fibroblasts).

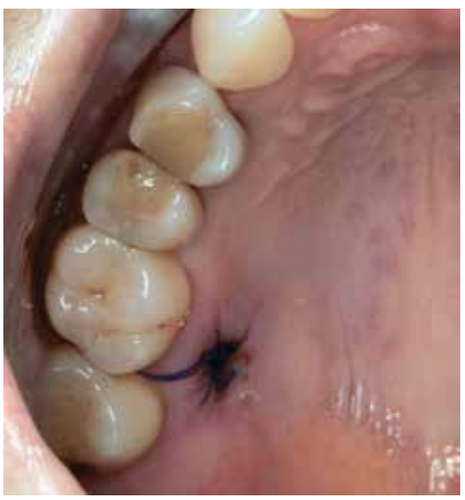


FIG. 1: *A biopsy is harvested from the palate*

The final cell products were thoroughly washed for 24 hours before harvesting to remove any traces of growth media supplements. Karyotyping was performed by standard techniques. Specifically, cells were fixed and treated with demecolcine (0.05 mkg/ml) for 1 hour followed by treatment with 0.56 KCL (37°C) for 1 hour. Air-dried chromosome slides were prepared by routine methods. G-banding was performed using the trypsin-Giemsa technique. Karyotypes were described according to the ISCN[42,43].

Additionally, expression of mesenchymal and epithelial markers was analysed using FACS flow cytometry (BD FACS Canto-II), as described elsewhere[44,45]. Cells were positive for mesenchymal (CD73+, CD90+, CD105+, collagen I/III+, elastin+, vimentin+, P4HB+) markers and negative for epithelial (CD324-, cytokeratin 14/15/16/19-) and endothelial/haematopoietic (CD31-, CD34-, CD45) markers.

All the above procedures were performed according to the new medical technology and registered with the Russian Federal service for supervision of healthcare as FS No. 2010/419, "Collection, transportation, isolation, cultivation, cryopreservation, storage and clinical use of oral mucosal fibroblasts for treatment of patients with mucosal recessions and gingival deficiency in the area of teeth and dental implants", on 9 December 2010.

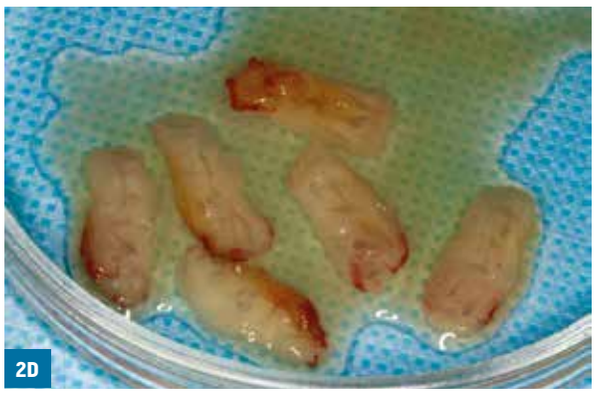
Biotransplant preparation

One PRF membrane required 9 ml of blood taken from the ulnar vein of the patient just before surgery. Using a centrifuge (Scilogex DM0412, RU No. RZN 2015/3442, Dragon Laboratory Instruments Limited, Tumwater, USA) according to the manufacturer's instructions (13 minutes at 1300 rpm) we obtained a fibrin clot, which was transformed into a PRF membrane by compression in a dedicated box (Bonart 4F-11. №3, New Taipei City, Taiwan, R.O.C.) for 5 minutes. On the day of surgery, an AFCC cell preparation was delivered to the dental office. One ml of AFCC suspension comprising $20x10^6$ cells/ml was injected into the newly formed PRF membrane using a syringe (Omnifix Syringe. B. Braun, Melsungen. Germany) with a 13-mm needle (30 G, BD Microlance, Becton, Dickinson, Dublin, Ireland). For cell culture seeding, the needle was fully inserted into the PRF membrane, and the AFCC suspension was administered, applying a linear-retrograde method releasing 50–100 μl of suspension. Then the needle was removed and a new injection was made parallel to the previous one at a distance of approximately 1 mm, until the entire cell suspension had been introduced into the PRF membrane (**FIGS. 2A-D**). The number of biotransplants required was calculated for each patient individually, depending on the number of gingival defects: every 3 recessions required 1 PRF membrane supplemented with 1 ml of AFCC containing $20x10^6$ fibroblasts.

Surgical procedure

In the two weeks before surgery, each patient was scheduled for professional prophylaxis including full-mouth ultrasonic scaling and polishing. Oral hygiene instructions were provided, and an electric toothbrush with soft bristles was recommended for home brushing[46].

All interventions were performed by the same experienced surgeon (G. E.) after administration of intraoral antisepsis (0.12% chlorhexidine rinse). After administration of local anaesthesia, root surfaces in the region between the bottom of the sulcus and the CEJ were carefully scaled and planed with hand instruments. A modified coronally-advanced flap (CAF) technique was performed together and PRF-membrane + AFCC was applied[47]. Specifically, an oblique paramarginal and intrasulcular incision was made in the recession area of the teeth where root coverage was scheduled. The incision was extended mesially and distally to the adjacent teeth on each side. A sufficiently mobile split mucosal-periosteal flap was raised, and the denuded root surface was treated chemically using Pref-gel (Straumann, Basel, Switzerland)



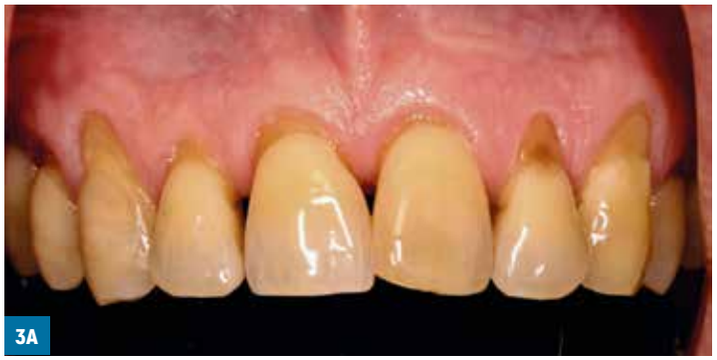
FIGS. 2A-D: *Biotransplant preparation: PRF membrane (A); AFCC suspension in a sterile container (B); syringe with AFCC, ready for injection into PRF membrane (C); PRF membrane seeded with AFCC (D)*

- a pH-neutral root surface conditioner containing 24% ethylenediaminetetraacetic acid (EDTA). Then the vestibular surfaces of the gingival papillae were de-epithelized. The AFCC-/PRF biotransplant was positioned and stabilized using horizontal or cross mattress sutures in the desired position, and fixed to the base of the de-epithelized papillae with vertical mattress sutures, starting from the palatal surface and using Pro-len 7.0 non-resorbable suture material (Ethicon, Somerville, USA). The mobilized mucosal-periosteal flap was placed coronally and fixed in the interdental spaces with sling sutures, completely covering the biotransplant (Pro-len 6.0 and 7.0). After suturing, the flap was located at least 1 mm coronally to the CEJ of the treated teeth (**FIGS. 3A-G**).

Postoperative period

To prevent postoperative pain and discomfort, patients were prescribed 400 mg of ibuprofen every 12 hours during the next day after the surgery. During the first 2 weeks after surgery, tooth brushing in the surgical area was suspended. For dental plaque control, patients were recommended to rinse with a 0.12 % chlorhexidine digluconate solution twice a day for two weeks. All subjects were advised not to brush their teeth in the surgical area until the sutures had been removed, not to exercise, and not to eat hard food during first 2 weeks after the intervention. Sutures were removed 14 days post-surgery (**FIGS. 3H-J**). After suture removal, patients were advised to brush their teeth with a soft toothbrush in the direction from the gingiva to the tooth margin.

All patients were followed up to 1-year after the grafting procedures (**FIGS. 3K-O**).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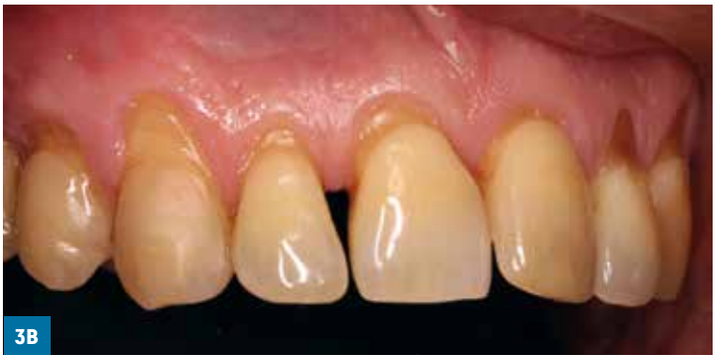
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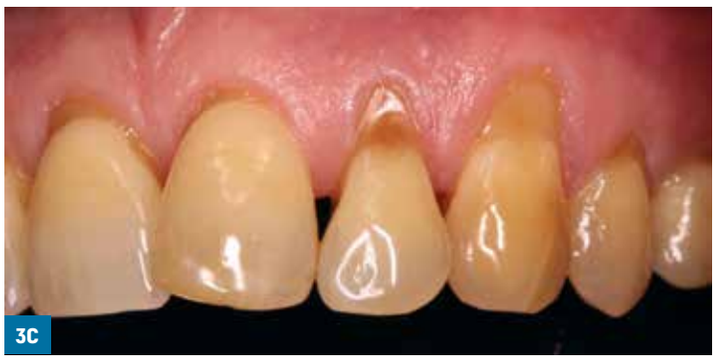
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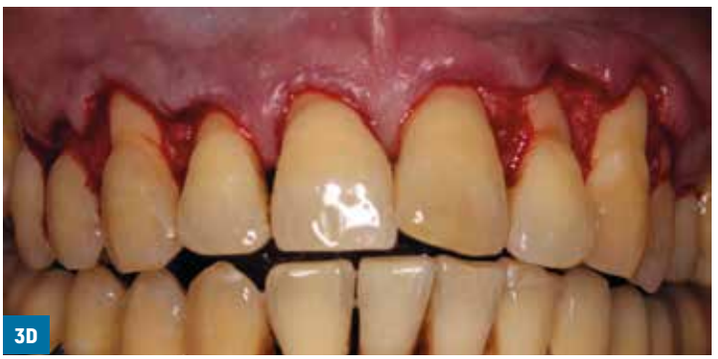
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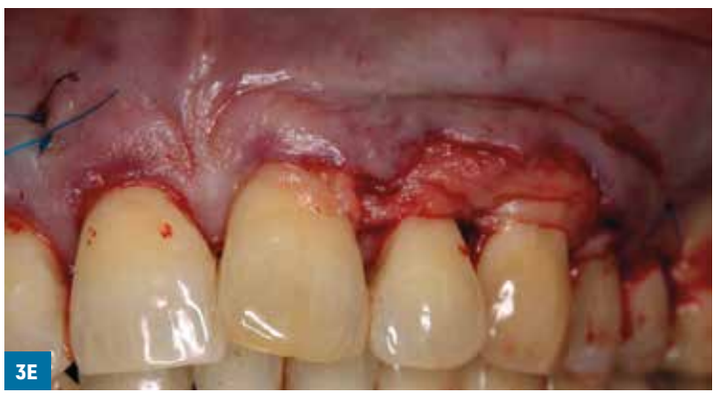
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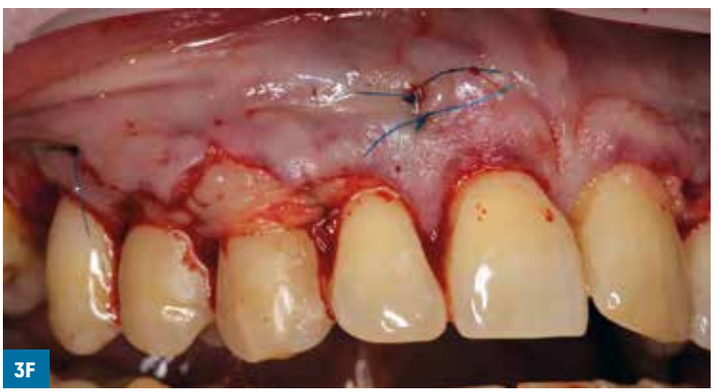
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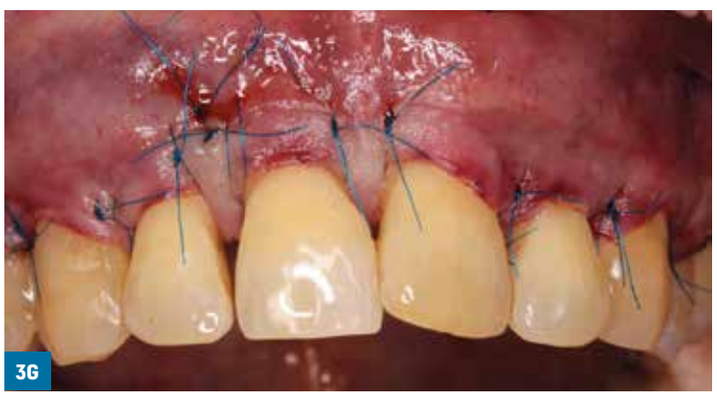
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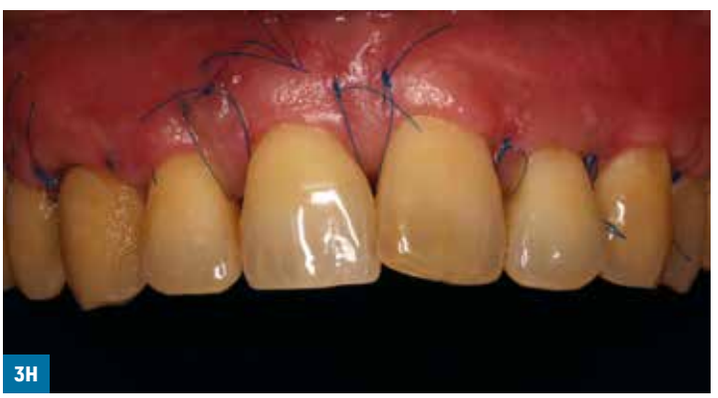
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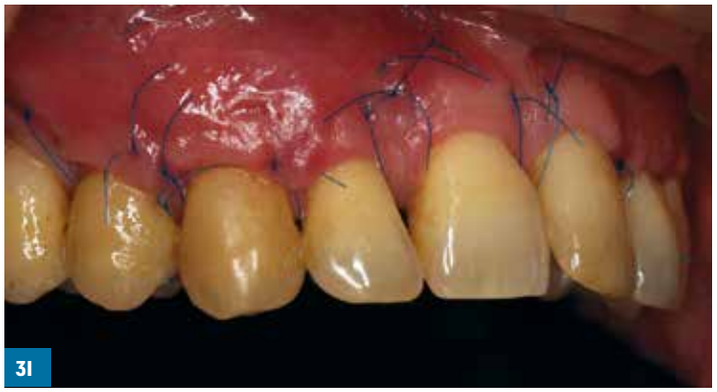

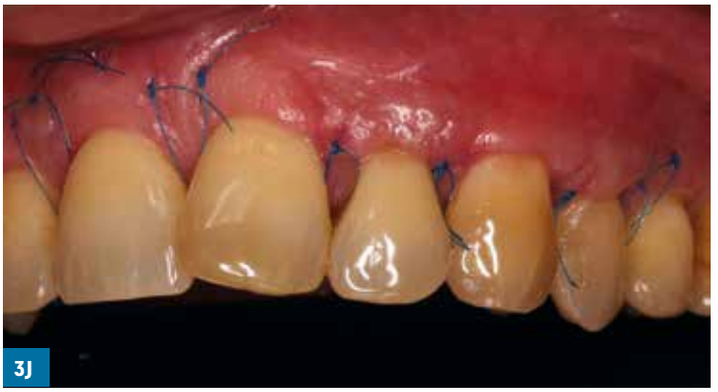

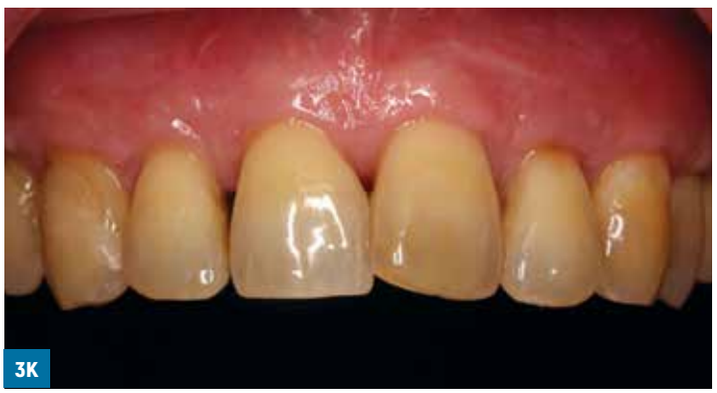

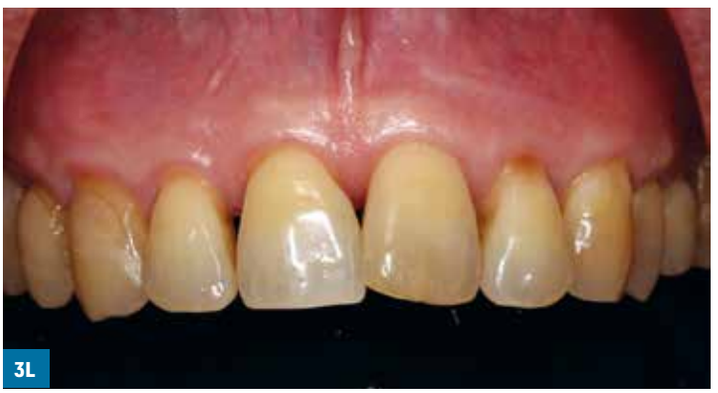

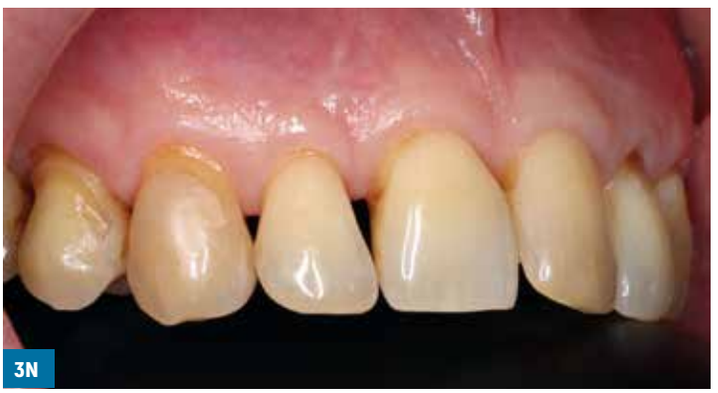

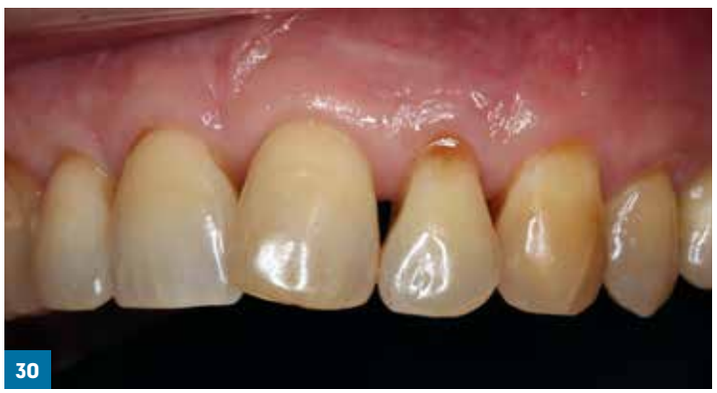

FIGS. 3A-O: *Preoperative views. Frontal (A), left lateral (B) and right lateral (C) views of multiple maxillary gingival recessions before treatment. Surgical procedure: prepared modified CAF (D); PRF membrane + AFCC inserted under the flap and sutured to the recipient site (E, F); tension-free closure of the CAF (G); PRF membrane is completely covered by the flap. Postoperative views. Intraoral frontal view 14 days after surgery (H); intraoral left and right lateral views 14 days after surgery (I, J); clinical outcome 3 months after surgery (K); clinical outcome 6 months after surgery (L). Twelve-month follow-up (M-O): intraoral frontal and lateral views one year after mucogingival surgery*

Statistical analyses

No sample size calculation was performed due to the exploratory nature of the study. Statistical analysis of the data obtained was performed using the statistical software package StatSoft Statistica using methods of variation statistics and Student's t test. Descriptive statistics were calculated via means ± standard deviation (SD) for the following variables: GRD, PD, KTH, FMBS, FMPS, CAL. Clinical parameters were evaluated at the baseline (T0), and at 3 (T3), 6 (T6) and 12 months (T12) after the surgery.

The main indicator of success was considered to be a high percentage of root coverage and an increase in KTH and CAL 12 months after the procedure relative to the initial value. Comparison of initial data and results at 12 months postoperatively was performed using the Wilcoxon paired test. A P value < 0.05 was considered statistically significant.

RESULTS

Twenty-two patients were screened for eligibility, but 12 patients were not included in the study for the following reasons: four patients were smokers, three had cervical abrasions, and five had restorations in cervical area of the teeth with multiple gingival recessions. Ten patients were considered eligible and were consecutively enrolled in the study. All 10 patients completed the study, and were seen at all control and follow-up appointments. The data from all ten patients were recorded and statistically analysed. The main baseline patient characteristics are presented in **TABLE 1**.

The postoperative healing period was uneventful in all patients. No evidence of serious adverse local or systemic side effects was observed in any patient throughout the study. Clinical parameters, and changes thereof are summarised in **TABLE 2**. Individual patient data is reported in **TABLES 3-7**.

Clinical attachment level significantly increased 3 months after surgery, from 3.15 ± 0.2 mm at T0, to 1.77 ± 0.08 at T3 (P = 0.0001). Further follow-up assessments did not reveal any significant changes in mean CAL values (**TABLE 2**). One year after mucogingival surgery, mean CAL displayed a statistically significant improvement, from 3.15 ± 0.2 mm at baseline to 1.67 ± 0.09 mm at T12 (P = 0.0002). The mean difference in CAL between T12 and T0 was 1.48 ± 0.14 mm (**FIG. 4**).

There was a statistically significant reduction in GRD three months after surgery, from 1.63 ± 0.28 mm to 0.24 ± 0.11 mm (P = 0.001). Then, from T3 to T12, no statistically significant changes in GRD were observed (P = 0.17). At T12, the mean GRD was significantly lower than at T0, being 0.26 ± 0.12 mm vs. 1.63 ± 0.28 mm, respectively (P = 0.0002) (**FIG. 5**). The mean difference in GRD between T12 and T0 was 1.37 ± 0.16 mm (**TABLE 2**).

Keratinized mucosa height significantly increased from 2.82 ± 0.37 mm at baseline to 3.33 ± 0.28 mm at T3 (P = 0.001). Mean KTH did not change significantly from T3 to T12 (P = 0.1), and one year after surgery, mean KTH was significantly higher than at baseline, at 3.63 ± 0.27 mm vs. 2.82 ± 0.37 mm (P = 0.0003) (**FIG. 6**). The mean difference in KTH between T12 and T0 was 0.81 ± 0.10 mm (**TABLE 2**).

Differences between baseline PD measurements and 12-month postoperative values were not statistically significant, being 1.52 ± 0.07 mm and 1.43 ± 0.06 mm, respectively (P = 0.12) (**TABLE 2**).

Three months after surgery 100% of Miller class I and II gingival recessions retained complete root coverage, while only 10% of Miller class III gingival recessions remained completely covered at T3. Comparison of the results obtained at the stage between 3 and 12 months showed no statistically significant difference (P = 0.17). The mean percentage of root coverage 12 months after surgery was 83%. After 12 months of follow-up, complete root coverage was maintained at 100% of Miller class I and II teeth and 54% of Miller class III teeth. Check-ups during the entire follow-up period showed good oral hygiene in all patients: FMPS and

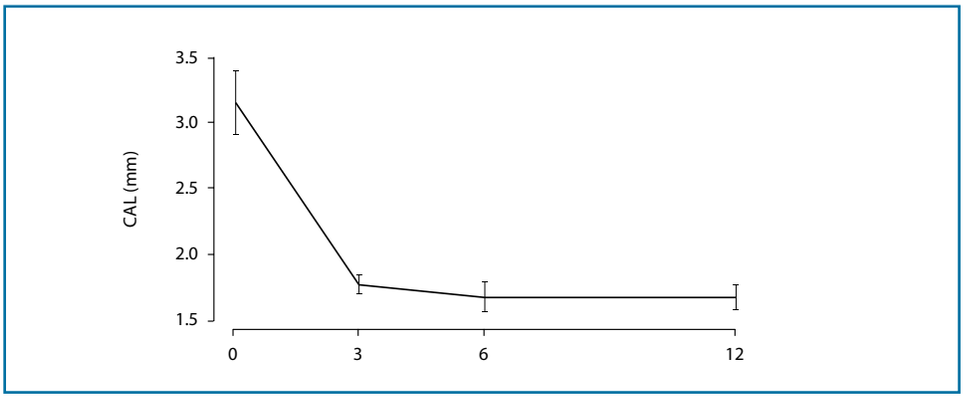

FIG. 4: *Pattern of clinical attachment level changes. Differences between 0 and 3 months are statistically significant (P = 0.0001). There was no statistically significant difference between 3 and 6 or 12 months (P = 0.1)*

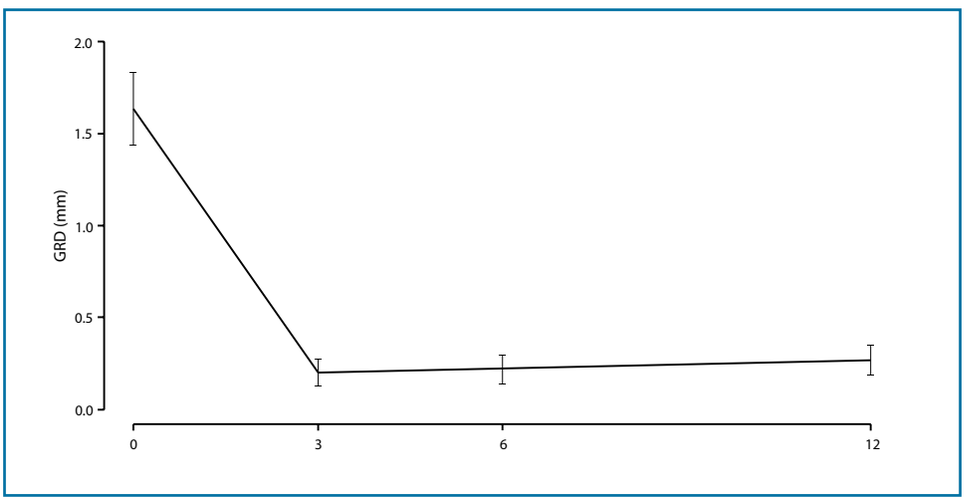

FIG. 5: *Pattern of changes in the gingival recession depth. The differences between 0 and 3 months are statistically significant (P = 0.001). There was no statistically significant difference between 3 to 6 or 12 months (P = 0.17)*

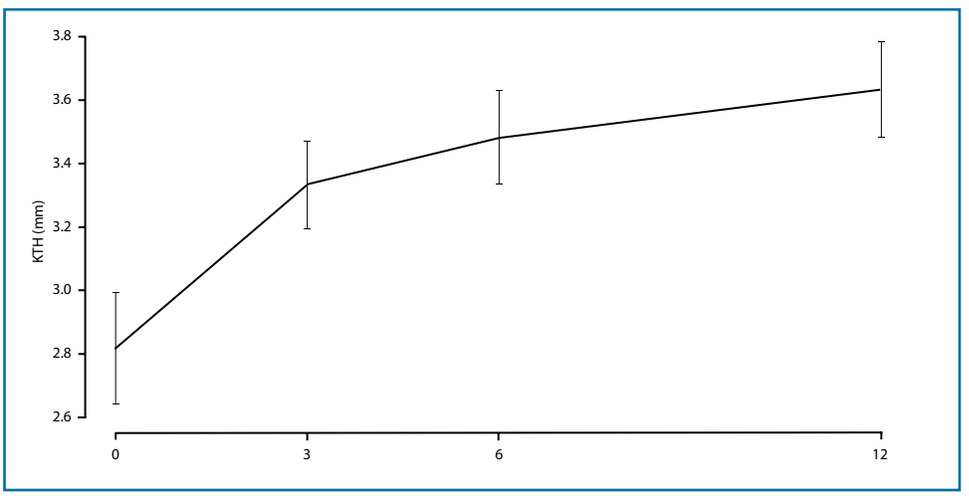

FIG. 6: *Pattern of keratinized tissue height changes. Differences between 0 and 3 months are statistically significant (P = 0.001). There was no statistically significant difference between 3 and 6 or 12 months (P = 0.1)*

TABLE 1 DEMOGRAPHIC DATA AND DISTRIBUTION OF GINGIVAL RECESSIONS AT BASELINE

Parameter	Value
Age (average ± SD)	44.2 ± 8.4
Sex: Male Female	 2 8
N. of recessions (n)	43
Miller class: Class I (n - %) Class II (n - %) Class III (n - %)	 24-61% 6-18% 13-21%

TABLE 2 MEAN CLINICAL PARAMETERS AT BASELINE AND AT DIFFERENT TIME POINTS DURING FOLLOW-UP

Parameter	T0	T3	T6	T12	P-value T0–T3	P-value T3–T12	P-value T0–T12	Mean T12–T0 difference
CAL (mm)	3.15 ± 0.23	1.77 ± 0.08	1.67 ± 0.11	1.67 ± 0.09	0.0001*	0.14	0.0002*	1.48 ± 0.14
GRD (mm)	1.63 ± 0.28	0.24 ± 0.11	0.22 ± 0.11	0.26 ± 0.12	0.001*	0.17	0.0002*	1.37 ± 0.16
KTH (mm)	2.82 ± 0.37	3.33 ± 0.28	3.48 ± 0.29	3.63 ± 0.27	0.001*	0.1	0.0003*	0.81 ± 0.10
PD (mm)	1.52 ± 0.07	1.57 ± 0.08	1.46 ± 0.09	1.43 ± 0.06	0.17	0.03	0.12	0.12 ± 0.01
FMPS (%)	7.03 ± 1.02	6.93 ± 0.77	6.96 ± 1.2	6.2 ± 0.42	0.38	0.32	0.36	
FMBS (%)	0.44 ± 0.3	0.37 ± 0.25	0.62 ± 0.3	0.66 ± 0.24	0.46	0.39	0.49	

Values represent mean ± SD; patients n = 10

TABLE 3 MEAN PROBING DEPTH (MM)

Patient	T0	T3	T6	T12
1	1.58 ± 0.62	1.72 ± 0.63	1.72 ± 0.65	1.16 ± 0.42
2	1.72 ± 0.63	1.69 ± 0.64	1.72 ± 0.62	1.42 ± 0.59
3	1.58 ± 0.69	1.66 ± 0.65	1.63 ± 0.70	1.44 ± 0.52
4	1.69 ± 0.67	1.63 ± 0.59	1.19 ± 0.38	1.33 ± 0.51
5	1.21 ± 0.39	1.0 ± 0.29	1.16 ± 0.36	1.0 ± 0.31
6	1.44 ± 0.56	1.77 ± 0.59	1.72 ± 0.61	1.63 ± 0.67
7	1.52 ± 0.63	1.36 ± 0.69	1.38 ± 0.70	1.38 ± 0.63
8	1.55 ± 0.65	1.5 ± 0.56	1.5 ± 0.53	1.55 ± 0.67
9	1.72 ± 0.62	1.77 ± 0.70	1.41 ± 0.38	1.42 ± 0.46
10	1.66 ± 0.59	1.83 ± 0.68	1.38 ± 0.42	1.41 ± 0.59

**Values represent mean ± SD for each study subject*

TABLE 4 MEAN GINGIVAL RECESSION DEPTH (MM)

Patient	T0	T3	T6	T12
1	2.0 ± 1.0	0.5 ± 0.5	1.0	1.0 ± 1.0
2	1.66 ± 0.5	0	0	0
3	1.66 ± 0.5	0	0	0
4	1.25 ± 0.6	0	0	0
5	4.0 ± 2.3	1.2 ± 1.3	1.2 ± 1.3	1.4 ± 1.3
6	2.5 ± 2.3	0.75 ± 0.5	0.75 ± 0.5	1.0 ± 0.5
7	1.8 ± 0.4	0	0	0.2 ± 0.5
8	2.0 ± 1.0	0	0	0
9	1.75 ± 1.0	0	0	0
10	3.16 ± 1.9	0.33 ± 0.6	0.16 ± 0.5	0.16 ± 0.5

Values represent mean ± SD for each study subject

TABLE 5 MEAN KERATINIZED TISSUE HEIGHT (MM)

Patient	T0	T3	T6	T12
1	1.83 ± 0.24	1.66 ± 0.18	1.66 ± 0.22	2.33 ± 0.32
2	3.51 ± 0.22	3.66 ± 0.31	3.83 ± 0.29	4.33 ± 0.36
3	4.16 ± 0.51	4.5 ± 0.43	4.5 ± 0.39	4.5 ± 0.42
4	4.0 ± 0.49	4.0 ± 0.41	4.66 ± 0.56	4.5 ± 0.49
5	1.2 ± 0.23	2.8 ± 0.42	3.2 ± 0.48	3.2 ± 0.46
6	2.33 ± 0.33	3.83 ± 0.29	3.83 ± 0.31	3.16 ± 0.29
7	1.5 ± 0.18	3.0 ± 0.39	3.0 ± 0.42	2.66 ± 0.36
8	2.5 ± 0.45	3.0 ± 0.42	3.33 ± 0.56	3.5 ± 0.48
9	4.33 ± 0.56	4.33 ± 0.49	4.16 ± 0.38	4.5 ± 0.55
10	3.0 ± 0.48	3.0 ± 0.51	3.16 ± 0.49	4.16 ± 0.37

Values represent mean ± SD for each study subject

TABLE 6 MEAN CLINICAL ATTACHMENT LEVELS (MM)

Patient	T0	T3	T6	T12
1	2.92 ± 0.21	2.06 ± 0.09	2.39 ± 0.12	1.83 ± 0.07
2	2.56 ± 0.18	1.7 ± 0.07	1.72 ± 0.09	1.42 ± 0.06
3	2.42 ± 0.25	1.67 ± 0.04	1.64 ± 0.05	1.44 ± 0.04
4	2.56 ± 0.19	1.64 ± 0.06	1.19 ± 0.08	1.33 ± 0.1
5	4.33 ± 0.28	2.0 ± 0.08	1.86 ± 0.12	2.17 ± 0.07
6	3.11 ± 0.23	2.11 ± 0.06	2.06 ± 0.07	2.14 ± 0.09
7	3.03 ± 0.22	1.36 ± 0.09	1.39 ± 0.1	1.56 ± 0.08
8	3.22 ± 0.25	1.53 ± 0.05	1.5 ± 0.12	1.56 ± 0.04
9	2.81 ± 0.19	1.81 ± 0.07	1.42 ± 0.09	1.44 ± 0.08
10	4.58 ± 0.22	1.83 ± 0. 06	1.56 ± 0.07	1.78 ± 0.09

Values represent mean ± SD for each study subject

TABLE 7. MEAN FULL-MOUTH PLAQUE AND BLEEDING SCORES (%)

	FMBS (%)				FMPS (%)			
Patient	T0	T3	T6	T12	T0	T3	T6	T12
1	0	0	0	0	6.2	5.0	7.5	5.0
2	0	0	0	0	8.9	6.2	5.3	7.1
3	0	0	0	0	7.8	8.5	9.3	6.3
4	0	1.9	2.8	0.9	11.5	11.5	12.5	7.6
5	0	0	0	0	2.7	4.6	11.1	5.5
6	0	0	0.8	1.6	6.2	9.3	6.2	7.0
7	1.8	0	0.9	1.8	6.4	4.6	10.1	8.3
8	0	0	0.7	1.5	4.6	5.4	1.5	4.6
9	0	0	0	0	3.5	5.3	2.6	4.4
10	2.6	1.8	1.8	0.8	12.5	8.9	3.5	6.2
Mean (%)	0.44	0.37	0.7	0.66	7.03	6.93	6.96	6.2
SD (±)	0.90	0.74	0.90	0.72	3.05	2.30	3.57	1.25

FBMS were always lower than 15%, and did not change significantly from T0 to T12 (P = 0.36 and P = 0.49, respectively; **TABLE 2**).

DISCUSSION

In this case series we introduce a novel approach for the treatment of multiple gingival recessions using PRF membrane seeded with autologous palatal fibroblasts placed under CAF. PRF displays excellent cell cytocompatibility, and it was chosen as a scaffold material because it provides good cell adhesion, migration and differentiation[48,49]. In a systematic review[50] dedicated to the effects of PRF on soft tissue wound healing, authors suggested that PRF may enhance soft tissue regeneration. However, in the current study postoperative healing and patient discomfort were not assessed, and there were no controls, consequently no conclusions can be drawn on this aspect.

However, the clinical outcomes we did record are comparable with those reported in the recent literature on the application of tissue engineering. Specifically, in our study, significant improvements in the clinical parameters CAL, GRD and KTH were observed 3 months after the surgery and were successfully maintained for 12 months.

In gingival recession management, the percentage of root coverage represents one of the most significant clinical parameters. Milincovic et. al.[9] studied AFCCs seeded on a collagen scaffold (test) placed under a CAF, as compared with CTG + CAF (control) in patients with single or multiple Miller class I or II maxillary gingival recessions. Twelve months after surgery, they observed statistically significant changes in coverage between baseline measurements and postoperative measurements in both groups (roughly 90%), but no significant difference in coverage between test subjects and control. Similarly, Jhaveri et. al.[15], studying the efficacy of acellular dermal matrix allograft (ADMA) seeded with autologous gingival fibroblasts (test group) under a CAF in comparison with the CTG + CAF for the treatment of Miller class I or II recessions, reported mean root coverage of 83.33% in both test and control groups 6 month after the surgery. In a case series reported by Murata et. al.[51] Miller class I and II gingival recessions were treated with cultured gingival dermal substitute (CGDS) composed of gingival fibroblast and matrix, fabricated using tissue-engineering techniques, in combination with CAF. Their reported average root coverage after surgery was 79.1% ± 25.7%. In our study, mean root coverage was higher 12 months after surgery, at 83% for the whole group and 100% when considering only Miller class I and II recessions.

An increase in keratinised tissue height has been reported by several authors after application of autologous gingival fibroblasts combined with different carriers[9,13,16,22,51]. Our results in line with reported results, and mean KTH was significantly higher than at baseline, at 0.81 ± 0.10 mm, one year after surgery. This, alongside the high percentage of root coverage obtained suggests that PRF seeded with autologous gingival fibroblasts is a promising graft material for mucogingival plastic surgeries.

The main potential advantages of the proposed method in comparison to the other currently used methods are the possibility of exploiting an unlimited number of autologous biotransplants for mucogingival surgeries, and a low biological cost due to limited damage to the donor site (palatal biopsy instead of connective tissue harvesting). Future studies are therefore warranted to validate these potential advantages.

Being a pilot study, the number of patients treated in this series was small. Other limitations of this study were a lack of patient randomization and postoperative morbidity assessment. Despite the growing interest in tissue engineering, data from clinical research is currently scarce. This is likely due to the need for a highly equipped laboratory, and, as a consequence, increased research costs. Even though cell technologies are of current interest, it will take a long time to apply this kind of technique in daily practice.

CONCLUSIONS

The results of this study suggests that PRF + AFCC may be a predictable and effective alternative treatment procedure for the treatment of multiple Miller class I and II gingival recessions using autogenous soft tissue grafts requiring a smaller donor site than the conventional procedure. Randomised controlled trials are needed to evaluate the effectiveness of this approach in comparison with conventional surgical procedures.

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