Evaluation of Advanced Platelet-Rich Fibrin Associated with Zucchelli Technique in the Treatment of Cairo Type 1 Gingival Recession: Randomized Clinical Trial

ABSTRACT

Aims: To evaluate the effectiveness of the Coronally Advanced Flap Modified by Zucchelli with A-PRF in root coverage in patients with Cairo type I gingival recession.
Study design: Mention the design of the study here.
Place and Duration of Study: Sample: Department of Periodontics, between January and April 2022.
Methodology: The CONSORT 2010 protocol was followed for sample selection and distribution. It was a prospective study, split-mouth parallel randomized, including 54 recessions within Group MCAF+SCTG: Zucchelli Technique + SCTG; and Group 2: Zucchelli Technique + A-PRF; 6 blood samples were collected; centrifugation (1300 rpm for 14 minutes, with G force of 210); and preparation of A-PRF membranes in the Stainless Steel Kit (Intra-Luck®). For the analysis of the results, the periodontal indices of height and width of the GR, probing depth, clinical insertion level, gingival level, keratinized tissue width, keratinized gingival tissue thickness, and sensitivity level, were used in the initial period, with 90 and 180 days of follow-up. GraphPad Prism 8.0 program® was used for statistical analysis. ANOVA and TUKEY tests were performed. Kruskall-WALLIS and DUNN tests were performed to analyze the pain parameter, with significance level of 5%. **Results:** Parameters probing depth and bleeding index remained without statistically significant changes in both groups. There was improvement in parameters height and width of the recession, height of the inserted gum, thickness of the gum and level of sensitivity in both groups. There was no statistically significant difference between groups.
Conclusion: There was clinical improvement in all criteria evaluated for both groups. Thus, the Coronally Advanced Flap modified by Zucchelli associated with A-PRF is as effective as the Coronally Advanced Flap modified by Zucchelli associated with SCTG.

Keywords: Gingival Recession, Surgical Flap, Platelet-Rich Fibrin, Connective Tissue.

1. INTRODUCTION

Gingival recession (GR) is defined as the apical positioning of the gingival margin in relation to the cemento-enamel junction (CEJ), which results in root exposure[1–14]. Around 50% of the world's population is affected [9]. It is often diagnosed in clinical routine and has varied etiology, such as anatomical, pathological and physiological factors[1, 15]. As the effects of GR include dentinal hypersensitivity, aesthetic changes, and predisposition to carious/non-carious cervical lesions [2, 5, 8, 9, 12, 14], treatments should be indicated to reduce sensitivity, create/increase the keratinized tissue width [1]. Thus, the result is evaluated according to the percentage of root coverage or as total root coverage [1]. Treatment and prognosis depend on the GR classification [15]. Miller's classification was widely used until

2018 and included classes I, II, III and IV[1, 14, 15], Periodontal Consensus by the American Academy of Periodontics and European Periodontic Federation, in 2018. Of all, Cairo classification is the most recommended[16].

This classification takes into account the height of the recession, compared to the loss of interproximal insertion tissue, and comprises Type 1 Recession (RT1), Type 2 Recession (RT2), or Type 3 Recession (RT3).[14, 16] RT1 group includes those recessions that maintain the interproximal insertion, while the papilla is intact, and the JCE in the mesial or distal can't be clinically visualized. In the RT2 group there is partial loss of insertion, and the distance from the JCE to the bottom of the groove/pouch is less than or equal to the loss of vestibular insertion. The RT3 group, on the other hand, presents an extensive loss of insertion, with the loss of papilla greater than the height of the recession [14, 16].

Although the management is varied, surgical indication seems to be the best choice for definitive resolution[2, 6, 17–19], with complete coverage and integration between the coating and adjacent tissues[18, 20]. The patient's needs and local anatomy close to the RG influence the selection of the surgical technique[21, 22]. Among the options, the most predictable management, which can reach up to 100% root cover, is the association of the coronally positioned flap (CPF) and the connective tissue graft (CT)[2, 5, 13, 23, 24] . The CAF technique was initially modified by Zucchelli for multiple recessions[22] and then for isolated recession[21]. The advantages of the PRC technique modified by Zucchelli include greater thickness of keratinized tissue in the flap region over root exposures, better coronal displacement of the flap, and anchorage and blood supply to the surgical papilla in the interproximal areas between root exposures[21, 22].

The use of connective tissue requires a second surgical procedure to remove the palate graft, which is associated with increased morbidity of the patient, thus being one of the main reasons for the use of alternative materials[4–6, 9, 18, 25, 26]. Autologous platelet concentrates (APC) have emerged as potential regenerative material, used in isolation or with other techniques[2, 27]. APC is critical in soft tissue healing due to the release of growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), insulin growth factor (IGF), platelet-derived growth factor-AB (PDGF-AB) and interleukin-1β(IL-1β)[2] . These growth factors stimulate the repair and regeneration of soft and hard tissues and reduce inflammation and consequently pain and discomfort[28]. They are divided into generations; platelet-rich plasma (PRP), platelet-rich fibrin (PRF) and concentrated growth factors (CGF).[2, 29] PRP is the first generation and contains high concentrations of platelets obtained by centrifugation and using chemical additives[2, 3, 18, 27, 29, 30]. And iIt didn't have significant acceptance by clinicians[4]. PRF is the second generation and was proposed by Choukroun *et al*. (2006). It is a fibrin concentrate rich in platelets obtained after centrifugation and without the addition of chemical additives[2–4, 18, 27, 29–31] . Based on the speed and duration of processing, PRF is classified as L-PRF (platelet-rich fibrin – rich in leukocytes), A-PRF (platelet-rich fibrin – advanced) and PRF prepared with titanium[3] . CGF is the third generation of APCs and is a concentrate with variation in centrifuge speed without the addition of any products[29, 32]. Both PRP and PRF have been used in soft tissue reconstruction in the treatment of GR[29]. Moreover, there is distribution of neutrophilic granulocytes within the A-PRF clot that favors the functionality of monocytes/macrophages and lymphocytes, and their implantation to support tissue[19, 33].

Although APC is being studied and used for the treatment of gingival recessions, its results are still controversial[2, 10, 17, 27]. Different methods are employed for the preparation of membranes, with consequent obtention of membranes with different characteristics[17, 27]. The A-PRF may influence soft tissue regeneration, especially for the presence of monocytes/macrophages and their growth factors[19, 33]. Thus, the need for further studies

in the area is justified[10, 17, 27]. Therefore, the objective of this study was to assess the effectiveness of the use of A-PRF associated with Zucchelli technique in root coverage in patients with Cairo type 1 gingival recession.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

This was a paralell randomized split-mouth prospective study. The project was approved by the Human Research Ethics Committee of Unioeste, under number 48868921.1.0000.0107 and by the National Ethics and Research Commission, under number 5210320. The study was registered in the Brazilian Registry of Clinical Trials (ReBEC) under number RBR-8kbd5xn. It was performed in the University Clinics. All patients received the informed consent form to sign.

For the sample size, the number was based by the T Test for dependent samples, through a test power of 80% and alpha level of 0.05, with a minimum of 20 recessions. These data were also based on previous studies by the group of researchers [34, 35]. After the initial periodontal clinical examination and the preparation of the patients, each one received treatment of the split mouth, randomly according to the proposed treatments Group MCAF + SCTG (Control): Zucchelli Technique + SCTG; MCAF + A-PRF Group (Test): Zucchelli + A-PRF technique.

The sample consisted of consecutive patients, recruited between January and April 2022. For the sample, the CONSORT 2010 protocol [36] (figure 1) was followed, and 13 patients, both genders, between 20 to 50 years, who presented bilateral Cairo type 1 gingival recession (figures 2A and 3A) in at least one of the arches were selected. So, 54 recessions were included. The other teeth of the dental arch should be in periodontal health, with all sites presenting probing depth smaller than or equal to 3 mm, with probing bleeding index less than or equal to 5%, without gingival inflammation and caries-free. As exclusion criteria, patients with positive history of antibiotic therapy in the six months before the study, anti-inflammatory drugs, steroids or non-steroids, in the three months before the study, or bisphosphonates; positive history of pregnancy; positive history of smoking or definitive cessation of the habit for five years; positive history of any systemic problem that contraindicates surgical procedure (hypertension, diabetes, coagulopathies, cancer); endodontic treatment or pulp pathology in the tooth involved; pathogenic occlusal interferences and previous surgery on site. The medical history of each patient was obtained through anamnesis and all participants underwent physical examination.

All patients received basic periodontal treatment and oral hygiene instruction. Periodontal treatment was performed by a single operator and manual and ultrasonic instrumentation was performed under the effect of local anesthesia. For manual instrumentation, 5/6, 7/8, 11/12 Gracey curettes and 13/14 periodontal curettes were used, as well as a piezoelectric apparatus for ultrasonic instrumentation.

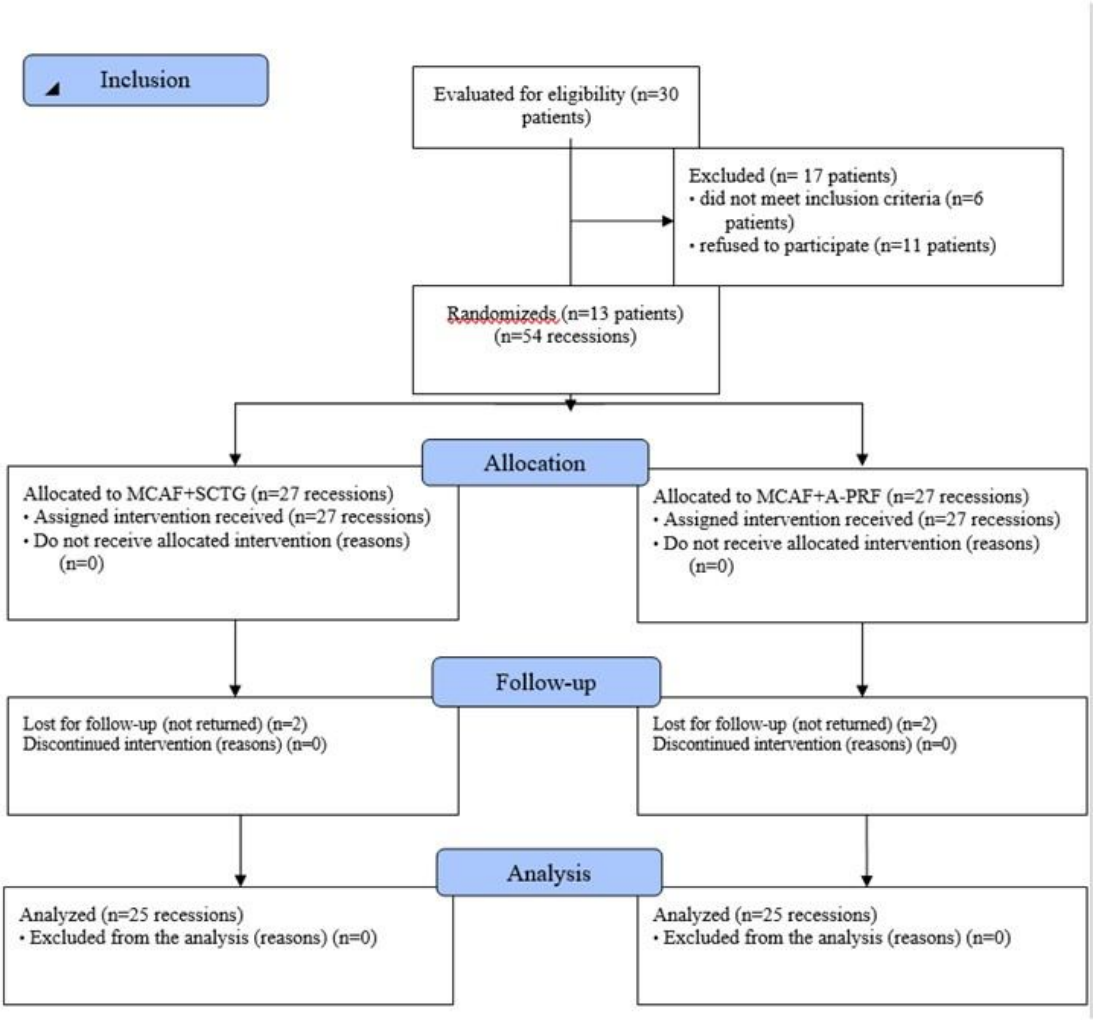

Figure 1: CONSORT flow chart.

Clinical/periodontal examination was performed initially (T0) and then at 90 days (T1) and 180 days (T2) after the procedure. The evaluations were performed by a previously trained researcher who, by means of Type 23 Periodontal Probe, which determined:

1. Probing depth (PD) and bleeding on probing (BOP);
2. Level of clinical insertion;
3. Height and width of the recession;
4. Height of the clinical crown;
5. Height of inserted gingiva.
6. Thickness of keratinized gingival tissue in three points;

Patients used the visual analogue scale (VAS) score to assess their discomfort after air jet stimulus; the extremes of the scale range from no sensitivity to extreme sensitivity (from 0 to 10) [37]. They respond to two scales, each referring to one side, in T0, T1 and T2.

The MCAF + SCTG group was submitted to subepithelial connective tissue graft with flap positioned coronally, modified by Zucchelli[21, 22], according to the technique. The recipient area was the one with nerve block, according to the region, with anesthetic Mepivacaine 2% and Epinephrine 1:100,000 (DFL, Rio de Janeiro, RJ, Brazil). An incision was made with a 15c blade (Swann-Morton®, Swann-Morton Ltd., Sheffield S6 2BJ England). The flap of the envelope consisted of oblique submarginal incisions in interdental areas, incisions that continued with intrassulcular incision in the recessions (figure 2B). Each surgical papilla was displaced in relation to the anatomical papilla by the oblique submarginal interdental incisions; the mesial surgical papilla to the midline of the flap was displaced more apically and distally, while the papilla distal to the midline was displaced in a more apical and mesial position. The flap of the envelope was elevated with a split-total-split approach in the coronal-apical direction: the oblique interdental incisions were performed keeping the blade parallel to the long axis of the teeth, in order to dissect the tissue in partial thickness in the direction of the papillae. Apical gingival tissue at root recessions was elevated to total thickness to provide that critical flap part to the thickest root cover. Finally, the apical part of the flap was increased in partial thickness to facilitate coronal displacement of the flap (figure 2C). The root surfaces received mechanical treatment with the use of curettes. The subepithelial connective tissue graft was obtained from the palate, by linear technique (figures 2D, 2E and 2F), then positioned on the recession and stabilized in the proximal ones with Polypropylene 5.0 (TECHSUTURE®, Bauru, São Paulo, Brazil) at the CEJ level (figure 2G). Subsequently, the flap was sutured on the graft with the same thread (figure 2H).
For the MCAF + A-PRF Group, the protocol for obtaining the A-PRF was according to Choukron *et al* (2018)[38] .

The sequence of the process of obtaining the A-PRF was divided into three stages:

Stage 1: Venous puncture and blood collection.

Step 2: Cell separation (centrifugation).

Step 3: Preparation of A-PRF membranes.

The 1st Stage was performed before the beginning of the surgical procedure for root covering. A qualified professional (nurse) performed the venopuncture of 60 ml of blood. The venopuncture protocol followed the recommendations of the Ministry of Health, with vacuum collection device, following the steps: Threading the needle in the adapter (cannon); Adjusting the garrote and choosing the vein; Antisepsis of the collection site with cotton and 70% alcohol; Performing the puncture; Introduction of the tube into the support by pressing it to the limit; Release of the garrote as soon as the blood flows into the tube; Guidance to the patient to press the punctured part with cotton, keeping the arm outstretched, without bending it.

In the 2nd Stage, the 6 tubes, 10 mL each, of blood were immediately taken to the centrifuge (Montserrat FibrinFUGE25, Montserrat, China) (figure 3B). Fibrin membranes were obtained by centrifugation at approximately 1300 rpm for 14 minutes. At the end of the centrifuge, the tubes were not removed immediately. They were left for 30 minutes, until the time of their use in the surgical bed.

In the 3rd Stage, the kit was used to make PRF membranes in stainless steel (Intra-Luck®). The centrifuged intermediate part, the fibrin clot, was separated from the red cell and platelet-poor plasma, and deposited in the stainless-steel box, the compressive cap was positioned using its own weight. The weight itself (130g) was sufficient to compress the clot and obtain the membranes (figures 3C and 3D), without offering damage to the cellular structures present in the fibrin mesh.

Figure 2: A. Cairo type 1 gingival recessions on the upper left side. B. Incisions; C. Divided-total-divided flap in coronal-apical direction. D. D. Removal of subepithelial connective tissue; E and F. Dimensions of the harvested subepithelial connective tissue; G. Subepithelial connective tissue in position and sutured; H. Coronally Advanced flap and sutured; Post-operative: I. Fifteen days of the Zucchelli Technique + SCTG; J. J. Ninety days of MCAF + SCTG Technique; K. One hundred and eighty days of MCAF + SCTG Technique.

The recipient bed was made, according to the same technique as that of the MCAF+SCTG group (figures 3E and 3F), and the membranes were inserted in the site (figure 3G) [39]. Four A-PRF membranes were overlapped one over the other, positioned and sutured with

Prolipropylene 5.0 thread (TECHSUTURE®, Bauru, São Paulo, Brazil), below the flap. Subsequently, the flap was sutured on the graft with the same thread (figure 3H).

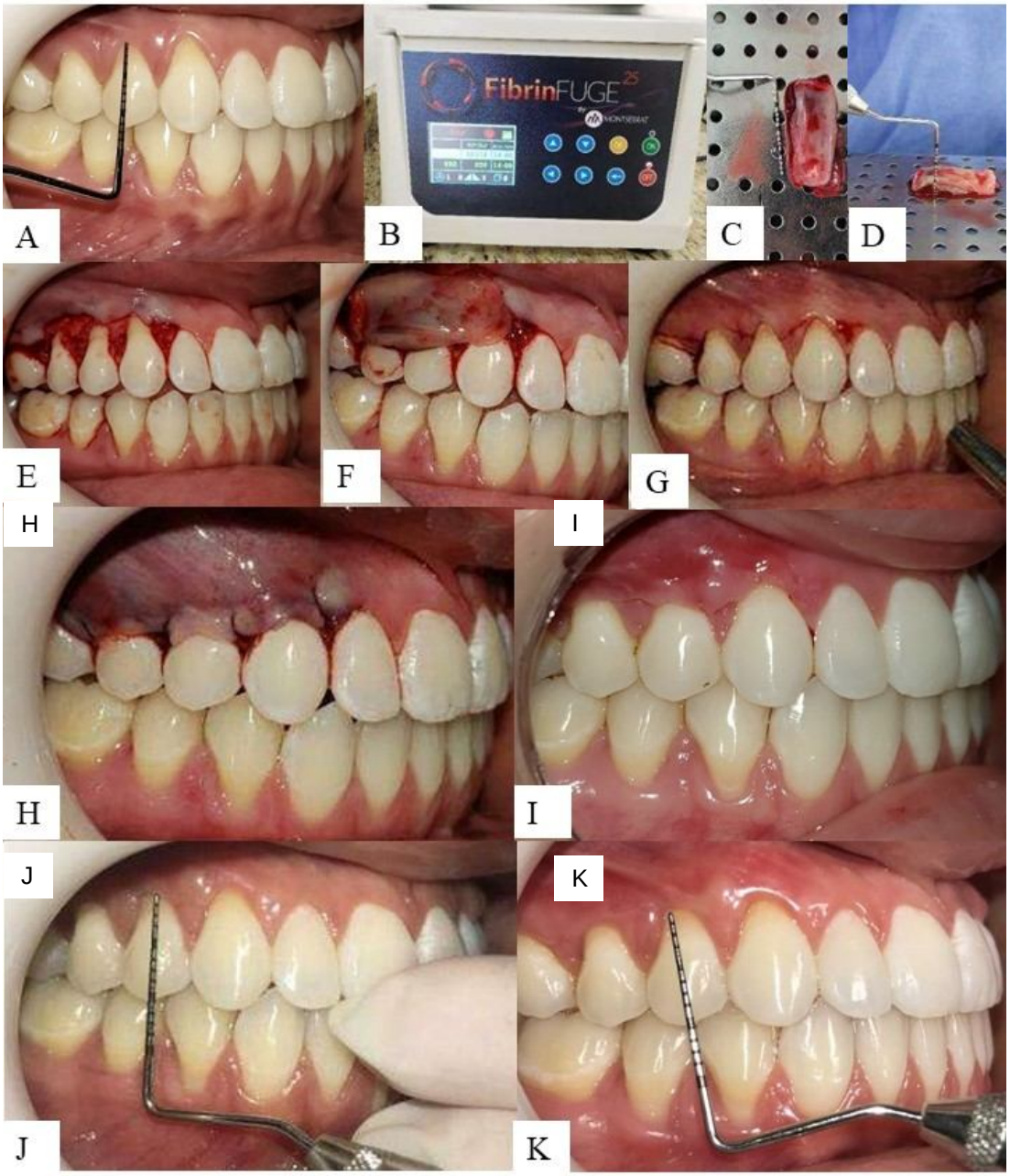

Figure 3: A. Cairo type 1 gingival recessionson the upper right side. B. Centrifuge configured according to the protocol; C and D. A-PRF membrane (4 overlapping membranes). E. Incisions; F. Split-total-split flap in coronal-apical direction; G. A-PRF mebranes positioned in the reception bed; H. Sutured flap. Post-operative: I. Fifteen days of Zucchelli + A-PRF Technique; J. Ninety days of Zucchelli + A-PRF Technique; K. One hundred and eighty days of Zucchelli + A-PRF Technique.

Both periodontal surgical techniques were performed by a single professional who was experienced and trained to perform them. Patients received the following medication: Amoxicilline 500mg every 8 hours for seven days, 1g dipyrone every 6 hours for three days, and chlorhexidine digluconate 0.12% every 12 hours for 15 days. The sutures of the donor region were removed at seven days and the sutures of the recipient bed at 15 days (figures 2I and 3I). Patients were instructed not to perform mechanical hygiene (brushing or flossing) on the teeth involved in the procedure, but should perform only chemical hygiene, for fifteen days after surgery.

Patients were evaluated in interval T0, T1 and T2 (figures 2A, 2J, 2K, 3A, 3J, 3K) in all criteria of periodontal examination. After the 180 days of the experiment, the patients were included in a periodontal maintenance program.

Regarding the statistical analysis, performed using GraphPad Prism 8.0® program, all clinical data obtained were initially analyzed and evaluated using SHAPIRO-WILK tests to verify normality of the distribution; then, ANOVA and TUKEY tests were used. Kruskall-WALLIS and DUNN tests were used to analyze pain parameter. A significance level of 5% was considered for all analyses.

3. RESULTS

Fifty-four Cairo type 1 gingival recessions (in 13 patients), 27 in the MCAF + A-PRF group and 27 in the MCAF + SCTG group were evaluated. There were 12 female and one male patients. Their age ranged from 20 to 50 years, with mean age of 32±11 years. We had 24 GR in anterior teeth (44.44%) and 30 GR in posterior teeth, premolars (55.55%). Regarding dental arches, 30 of the GR were located in the upper arch (55.55%) and 24 in the lower arch (44.44%). We observed the total cover-up of the recession (100% of covering) in 17 teeth (62%) of the group that received the SCTG, 12 in the maxilla and five in the mandible, of which eight were in anterior teeth and nine in posterior teeth; and 11 teeth (40%) in the group with A-PRF, five in the maxilla and six in the mandible, five anterior teeth and six posterior teeth. The mean root coverage in the MCAF+SCTG group was 82.70% and in the MCAF+A-PRF group it was 70%.

Correlations between T0, T1 and T2 were made intragroup (Tables 1). The MCAF + SCTG group presented results without statistical alteration for the three periods evaluated for probing depth in the three sites and probing bleeding index (Table 1). When T0 and T1 are compared, improvement in all other parameters evaluated is observed (Table 1). When comparing T1 and T2, it was observed that there was no statistically significant difference, with the maintenance of the improvement in parameters (Table 1). The comparison between T0 and T2 showed that there was a statistically significant improvement for all these parameters (Table 1).

Table 1: Comparative analysis of periodontal parameters of MCAF+ SCTG and MCAF + A-PRF groups in the initial, 90-day and final periods

	MCAF + SCTG			MCAF + A-PRF		
	Preoperative	90 days Postoperative	180 days Postoperative	Preoperative	90 days Postoperative	180 days Postoperative
MPD (mm)	1.92±0.54A	1.59±0.57A	1.60±0.58A	1.96±0.63A	1.67±0.54A	1.70±0.54A
VPD (mm)	1.29±0.47A	1.37±0.49A	1.37±0.49A	1.42±0.57A	1.42±0.50A	1.37±0.49A
DPD (mm)	1.63±0.68A	1.48±0.57A	1.52±0.51A	1.74±0.59A	1.57±0.50A	1.48±0.51A
PBI (%)	0.00%±0.00A	0.00%±0.00A	4.00%±0.43A	0.00%±0.00A	3.44%±0.40A	3.70%±0.42A
LCI (mm)	3.85±1.16A	2.11±0.89B	1.84±0.80B	3.64±1.02A	2.53±1.26B	2.18±0.88B
HR (mm)	2.667±0.94A	0.74±0.81B	0.44±0.71B	2.42±0.93A	1.17±1.03B	0.74±0.71B
WR (mm)	3.44±0.80A	1.25±1.34B	0.76±1.16B	3.46±1.01A	1.64±1.24B	1.22±1.31B
HC (mm)	11.63±1.54A	9.74±1.25B	9.40±1.08B	11.18±1.76A	9.92±1.65B	9.74±1.37B
HKT (mm)	1.85±0.86A	3.18±1.24B	3.16±0.85B	1.78±0.99A	2.46±1.10AB	2.78±1.33B
TKT (mm)	1.037±0.19A	2.11±0.80B	2.08±0.70B	1.03±0.18A	1.89±0.62B	1.63±0.63B
SI	3.88±3.26A	1.03±1.93B	0.76±1.45B	3.96±3.47A	1.89±2.26AB	0.81±1.33B

MPD: Mesial probing depth; VPD: Vestibular probing depth; DPD: Distal probing depth; LCI: Level of Clinical Insertion; HR: Height of recession; WR: Recession width; HC: Height of the clinical crown; HKT: Height of the keratinized tissue; TKT: Gingival thickness; PBI: Bleeding index. SI: Sensitivity index. Different letters mean that they are statistically significant difference within each treatment group ($p<0.05$).

The probing depth in the three sites and the bleeding index in the MCAF + A-PRF group also showed no statistically significant difference for the three periods evaluated (Table 1). Except for sensitivity index, all other parameters showed statistically significant improvement when comparing T0 and T1 (Table 1). Between T1 and T2, there was maintenance of the improvement achieved in T1, because there was no statistically significant difference (Table 1). The alteration occurred between T0 and T2 demonstrates that all parameters showed statistically significant improvement (Table 1).

According to Table 1, the intragroup comparison, MCAF + SCTG and MCAF + A-PRF, showed that there was no statistically significant difference for any of the parameters evaluated. The height of the recession was slightly higher in MCAF + A-PRF. Moreover, in

the MCAF + SCTG group, the thickness of keratinized tissue and height of the inserted gingiva were higher.

Although not statistically analyzed, it was observed that no episodes of trans or postoperative hemorrhage occurred. In addition, some patients reported discomfort with the suture thread, but none complained of uncontrolled pain.

Table 2: Comparative analysis of variation (difference from 0 – 180 days) of periodontal parameters' values of groups MCAF + SCTG and MCAF + A-PRF

	MCAF+SCTG	MCAF+A-PRF
MPD (mm)	0.43±0.96A	0.32±0.82A
VPD (mm)	0.04±0.77A	0.11±0.62A
DPD (mm)	0.28±0.76A	0.22±0.97A
LCI (mm)	2.15±1.56A	1.53±1.13A
HR (mm)	1.71±0.71A	2.26±1.01A
WR (mm)	2.74±1.48A	2.28±1.53A
HC (mm)	2.92±3.35A	1.78±2.63A
HKT (mm)	1.07±1.30A	0.96±1.58A
TKT (mm)	0.89±0.89A	0.53±0.74A

MPD: Mesial probing depth; VPD: Vestibular probing depth; DPD: Distal probing depth; LCI: Level of Clinical Insertion; HR: Height of recession; WR: Recession width; HC: Height of the clinical crown; HKT: Height of the keratinized tissue; TKT: Gingival thickness. Different letters mean that there are statistically significant differences between treatment groups ($p<0.05$).

4. DISCUSSION

GRs, when left untreated, tend to progress [11]. Although there is evidence that SCTG is the gold standard, due to predictability, stability and height and thickness gain of the covered region, treatment alternatives have been studied [6, 9, 24, 40]. The choice of SCTG substitute material should be based on scientific evidence [8]. However, the wide variety of publications on the subject may even hinder clinical decision making [27]. According to Bhatia *et al.* (2021), there are not enough studies to evaluate the CAF modified by Zucchelli, which justifies the present study. Therefore, this study evaluated periodontal clinical parameters in root coverage of RT1 with a coronally advanced flap modified by Zucchelli associated with the A-PRF membrane and compared it with the same flap associated with the subepithelial connective tissue graft.

The horizontal incisions in the papilla, performed with CAF, may compromise the tissues and the adaptation of the grafted tissue [6]. Vertical incisions that can hinder the blood supply to the inserted material[5, 6, 41], and increase surgical trauma and healing period are also necessary [5]. This influences the aesthetic result, with chances of formation of keloid[6] . Alternatives for flap design are still controversial [9], moreover, Zucchelli and De Sanctis (2000) suggested the elimination of vertical incisions [6, 22]. Thus, the incisions in the papillae are oblique which allows for a better adaptation on the deepithelialized papilla, in the coronary advanced of the new papilla [6, 22]. Research also shows that, since the design of the flap is split-total-split, there is improvement in the passivity of displacement and preservation of the thickness of the marginal tissue to the recession [6, 22].

Researchers state that although the adoption of CAF associated with biomaterials is better than using CAF alone, there are still controversies regarding the choice of the best

biomaterial option, and this choice should be made with care [9]. The literature shows that SCTG is the gold standard for offering aesthetic results, complete closure and primary healing of the donor area, in addition to the results of the recipient area[25, 40]. The SCTG acts as a framework to stabilize the clot and increase gingival thickness [4], as well as offering stable root coverage in the long term [4, 8]. From two weeks on, the biomaterial can already receive enough blood supply, which is dependent on the revascularization conferred by the periosteum and the flap [40], and this revascularization can occur, as well as complete healing, between 28 and 60 days after the procedure [5]. It is also considered that SCTG has a morphogenetic stimulus for histodifferentiation of the tissue of the region [40]. Our results confirm the excellent results of SCTG, since the improvement was observed in all parameters evaluated (Table 1).

However, harvesting the SCTG from the palate may be associated with complications and difficulty to acquire enough volume [40]. Regardless of the harvesting technique, transoperative and postoperative bleeding due to vessel injury is the most common complication, in addition to postoperative pain [8, 25], which increases surgical morbidity [8]. These risks may discourage some patients from opting for this procedure [8]. Thus, the best way to prevent complications is to know the anatomy [25]. According to Tavelli *et al.* (2019), there is still no agreement in the definition of the safer region for the collection of the connective without lesion to the major palatine artery (MPA), although many researchers have studied the subject. The greater thickness of the connective tissue of the palate is in the region of the second premolar and second molar and the distance from the larger palatine artery to the cementoenamel junction is also greater in these regions [25]. APM emerges from the palatine forame major, which can be palpated in most cases and the most common location is in the middle palatal region of the third molar [25]. Thus, the limit of the collection would be the distal of the canine and the mesial of the second molar[25]. We can confirm that this location was respected in this research, because we had no episodes of hemorrhage.

The use of connective tissue graft substitutes does not cause postoperative pain and does not affect quality of life [9]; however, they may have limitations [8] such as financial and clinical restrictions[13]. Among the alternatives, we found the acellular dermal matrix, xenogenic collagen matrix and APCs [4, 6]. APC's are considered easy to collect and handle, with low cost [10]. PRF membranes provide a structure with viable cells for tissue repopulation, and molecules that stimulate tissue repair[13]. Although membrane quality and platelet concentrate quantity are key to results [5], differences in centrifugal, speed and G-force can alter membrane quality [9]. L-PRF has been widely defended for promoting release of growth factors[4]. These growth factors can be released within 21 days and accelerate soft tissue healing [8]. In *vitro studies* have shown that the L-PRF membrane favors the migration and proliferation of fibroblasts [8], but root coverage results are still controversial[4]. While comparing L-PRF to A-PRF, it is observed that A-PRF has more platelets and living progenitor cells, with significant protein release, an advantage in its use[19]. The best membrane quality is due to reduced speed and increased centrifugation time, resulting in an A-PRF structure that offers fibrin remodeling in more resistant connective tissue [26].

Although the literature suggests that six months would be enough for healing and tissue stability after mucogingival surgery [8, 9], we observed that the results found in both groups were statistically significant for LGI, HR, WR, HC, TKT and SI parameters, between T0 and T1, except for HKT in the MCAF+A-PRF group (Table 1). And that there was stability in these parameters, since when T1 (three months) and T2 (six months) were evaluated, the results remained the same, because they were not significant. Between T0 and T2, it was observed that all variables showed statistically significant differences (Table 1).

The recommended variables to evaluate the result of the root coverage were height of the keratinized tissue and percentage of coverage [9]. A clinical study evaluated RPMC+SCTG and an initial value of inserted gums was found from 1.15±0.59 to 2.05± 1.56 [24]. In both groups, increase in the height of the keratinized tissue was observed, showing statistically significant difference, with mean from 1.85±0.86 to 3.16±0.85 in the MCAF+SCTG group and 1.78±0.99 to 2.78±1.33 in the MCAF+A-PRF group, with no statistically significant differences between the groups (Table 1). Bhatia *et al.* (2021) conducted a systematic review evaluating the effectiveness of the MCAF and all the studies included showed increase in HKT. The justification is that after the coronary advance, there is tendency of the mucogingival junction to migrate to apical, due to the presence of granulation tissue derived from the periodontal ligament that stimulates the formation of keratinized tissue [6]. SCTG decreases the chances of apical flap contraction in the early stages of healing [6]. Despite the fact that there is no consensus on what the ideal amount of keratinized tissue would be[13], HKT is an important factor in the root coverage outcome [4, 9], with 2mm suggested as the minimal for stability, and considered essential for long-term maintenance, with lower risk of recurrence [6, 9]. We observed that in both groups HKT was found with at least this value in the six months after the procedure, and the MCAF+SCTG group showed even better outcomes. However, the difference between the two was not statistically significant (Table 2).

The literature showed decrease in the recession for MCAF+SCTG, in one year of follow-up, from 2.91±0.87 to 0.09±0.29 [24]. In our study, decrease from 2.66±0.94 to 0.44±0.71 was observed in this group, in six months of follow-up and, although we did not have studies to compare in the MCAF+A-PRF group, there was also reduction from 2.42±0.93 to 0.74±0.71. We observed total coverage in 62% of the teeth in MCAF+SCTG group and 40% in MCAF+A-PRF group, with mean coverage of 82.70% and 70%, respectively. Bhatia *et al.* (2021) showed in a systematic review that the total coverage rate for MCAF associated with SCTG in the literature is 55.6% to 93.10%, which may be greater than 97.14%. Once RPMC+SCTG was compared to MCAF+PRF, the SCTG group was better [6]. The MCAF exhibits moderate to high level of efficacy in root coverage of Cairo type 1 gingival recessions [6]. They also compared MCAF with CAF and stated that there was no difference between the techniques in relation to the rate of recession coverage, but that the MCAF presented better aesthetic results [6]. Partial dissection for passive coronally advanced and preservation of the total thickness of marginal tissue may explain the positive results [6].

Another parameter to be evaluated is gingival thickness[4], the structure of the SCTG stabilizes the clot in order to favor increase in thickness, on the other hand, the L-PRF membrane is less stable and thinner, consequently the gain may be lower [4]. To offer better quality, four membranes would be ideal for root coverage [5, 10], or two folded membranes [10]. In the present study, four A-PRF membranes were used, which were sufficient to maintain thickness gain in the evaluation made after six months of the procedure. In both groups statistically significant increase was observed; from 1.03±0.19 to 2.08±0.70 and 1.03±0.18 to 1.63±0.63, respectively for, MCAF+SCTG and MCAF+A-PRF. The ideal thickness to ensure the long-term result would be 1.2 mm [4], therefore, both groups presented results within this parameter (Tables 1 and 2). In addition to the no statistically significant difference between the two groups, with increase of 0.89±0.89 with the SCTG and 0.53±0.74 with A-PRF (Table 2). The increase in gum thickness favors root coverage and long-term stability of the results achieved[6], because keratinized gingiva is protective and creates resistance to muscle use, restorative interventions, traumatic brushing, and plaque [13].

The keratinized tissue stabilizes soft and hard tissues and contributes to oral hygiene and better appearance[13]. Maintaining thickness is associated with low plaque indexes [4]. It can be pointed out that PRF membrane improves the soft tissue, because there is a junction of the connective tissue with the root region, which occurs through repair with the junctional epithelium [41]. Bhatavadekar *et al.* (2019) studied the MCAF technique and observed that the probing depth did not present statistically significant difference. It was observed that the patients in the present study maintained gingival health, since no patient presented probing depth greater than 2 mm, with no statistically significant difference between the terms evaluated, as well as with the LCI, in both groups and between groups. LCI was also found normal with the use of APC in the literature [10]. For the maintenance of gingival health, it is recommended to follow up oral hygiene[9]. Thus, the patients in this study were included in a follow-up program.

The location of teeth is also a relevant parameter for the prognosis of treatment [4]. GR in lower teeth are often challenging, because anatomically, there is a shallow vestibule, possible presence of hypertrophic brakes, thin gingiva, as well as thin vestibular bone, making the coverage questionable [41]. In the present study, our sample consisted of 24 (44.44%) recessions in lower teeth. In the MCAF+SCTG group, five teeth reached total coverage and in the MCAF+A-PRF group there were six teeth, and it was a higher amount than that of the superior ones that resulted in total coverage in this group.

GR are often underestimated, so publications on the perception and acceptance of their treatments are limited [12]. One of the goals of treatment is to eliminate hypersensitivity, which affects the quality of life of patients, and is often reported by them [12]. This sensitivity can be understood as a response to the exposed dentin; therefore, root coverage can hide the dentin, and thus improve this parameter [12]. In the MCAF+SCTG group, it was 3.88±3.26, initially, and decreased statistically significantly to 0.76±1.45 at 180 days (Table 1). In the MCAF+A-PRF group, we also observed statistically significant reduction from 3.96±3.47 initial to 0.81±1.33 in the long postoperative term (Table 1). Treatment of GRs should be carried out to prevent them from progressing and further affecting the quality of life of the patients [11].

5. CONCLUSION

Thus, it can be concluded that root coverage of RT1 with coronally advanced flap modified by Zucchelli associated with the A-PRF membrane was as effective as the same flap associated with subepithelial connective tissue graft.

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