

Cite as:

Sarduy L, Véliz OL, Veitia F, Arce MA, Barreto EE. Efficacy of the membranous form of platelet-rich fibrin in root coverage. *Rev Estomatol Herediana*. 2024; 34(3): 209-217. DOI: 10.20453/reh.v34i3.5834

Received: August 24, 2023

Accepted: May 30, 2024

Online: September 30, 2024

Conflict of interest: The authors declare that they have no conflict of interest.

Funding: Self-funded.

Ethical approval: Approved by the Ethics Committee from the Faculty of Stomatology of Villa Clara on February 6, 2017.

Author contributions:

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OLVC: formal analysis, writing – original draft, writing – review & editing.

FVC: conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft, writing – review & editing.

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Efficacy of the membranous form of platelet-rich fibrin in root coverage

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ABSTRACT

Objective: To evaluate the efficacy of the membranous form of platelet-rich fibrin associated with the coronally advanced flap in patients attended by consultation in periodontics at the Faculty of Stomatology of Villa Clara. **Materials and methods:** A longitudinal study was conducted at the Faculty of Stomatology, Villa Clara, Santa Clara, Cuba, from January 2016 to March 2017. The sample comprised 17 individuals selected using a purposive, non-probabilistic sampling method. The traditional coronally advanced flap procedure was applied to teeth with periodontal recession in this cohort. Additionally, a platelet-rich fibrin membrane, derived from autologous blood and prepared in the Regenerative Medicine Service of the same institution, was placed beneath the flap. The study analyzed the following variables: age, sex, recession length, Miller classification, root coverage, width and gain of inserted gingiva, evaluation categories, and therapeutic efficacy. **Results:** Root coverage percentage was higher in class I and increased over time in all Miller classes. Inserted gingival width and gain increased from one month to one year. The percentage efficacy was 93.18%. **Conclusions:** Periodontal recession therapy, with the membranous form of platelet-rich fibrin associated with the coronally advanced flap, was considered effective in patients attended by consultation in periodontics at the Faculty of Stomatology of Villa Clara.

Keywords: platelet-rich fibrin; periodontal plastic surgery; gingival recession.

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INTRODUCTION

Gingival recession (GR) has been conceptualized as the apical shifting of the gingiva in connection with the cemento-enamel junction (CEJ), which may extend to the mucogingival junction or beyond it (1). The clinical importance of this condition lies in the fact that the exposed root surface makes the tooth susceptible to the development of root caries, dentin hypersensitivity and consequent impact on aesthetics, which is the main reason for patients to turn to periodontology services searching for solutions (2). The therapeutics of these mucogingival deformities has been directed towards their coverage through various surgical grafting techniques, both free and pedicled, included within the currently referred to as periodontal plastic surgery (3). According to studies conducted by the author, and scientific literature reports, a decrease in the results obtained with conventional grafting mucogingival techniques over time has been confirmed (4-9).

In recent years, the use of the potential of platelet derivatives has been exploited in various specialties of stomatology. The regenerative biomaterials obtained from blood components offer advantages that, according to its physical form, can be combined with the conventional procedures of root coverage. The platelet-rich fibrin (PRF), used as a membrane, is considered a bioproduct with physical and biological properties that allow it to be combined with techniques such as the coronally advanced flap (CAF), providing the bilaminar procedure with regeneration at the site of the GR (10). The tri-dimensional structure of the PRF membrane ensures the trapping of platelets, leukocytes, stem cells, cytokines, and other biological elements, which, together with growth factors resulting from platelet degranulation, promote the tissue repair process (11).

When a biomaterial is tested for the first time in the treatment of periodontal injuries of any kind, it is important to determine its efficacy through its application in ideal circumstances that demonstrate its usefulness and use feasibility (10, 11). Although, initially, these studies focus on smaller samples, subsequent research would arise to assess their effectiveness in actual conditions. Therefore, as a first approach to the subject of this study, the objective was established to assess the efficacy of the PRF used as a membrane in connection with the CAF in the treatment of GR in patients who attended the periodontology clinic at the Faculty of Stomatology in Villa Clara, Cuba.

MATERIALS AND METHODS

A before-and-after longitudinal study was carried out at the Faculty of Stomatology of the Universidad de Ciencias Médicas de Villa Clara, Santa Clara, Cuba, in partnership with the Biomedical Research Unit (UNIB, from the Spanish acronym) located in the same center, from January 2016 to March 2017. It had a population of 27 patients affected by GR, who were eligible for root coverage using the CAF technique. The cohort was selected through a non-probabilistic purposive sampling based on criteria. It consisted of 17 people, who met the inclusion criteria established for this study, and out of which a total of 44 teeth with gingival recession were studied.

A dental examination was carried out in the periodontology clinic. The data collected were recorded on a data collection form created for these purposes. To exclude the GR associated to periodontitis and determine the situation of the interdental bone to establish the Miller classification (12), each patient underwent the corresponding radiographic examination.

Regarding participation criteria, the study included patients between 18 and 60 years old with GR in the buccal surfaces of permanent teeth, patients carrying GR classified by Miller as stages I, II or III, presenting CEJ in teeth carrying GR, having pulp vitality, keratinized gingiva of over 2 mm on teeth with GR, patients who met the requirements for the preparation of the biomaterial (PRF), and motivated patients willing to solve aesthetic and functional impact caused by GR. Likewise, the study excluded smokers, people with systemic diseases impairing cicatrization such as diabetes mellitus, hematological, immunological and auto-immune diseases, and malignant neoplasms; pregnant patients; patients with poor oral hygiene, with GR associated with periodontitis, widespread GR, GR associated to frenulum and muscular insertions. Patients who had previous periodontal surgical procedures in the GR study of no longer than 24 months before the examination, and patients whose teeth carrying GR present cervical restorations, were also excluded. It is worth mentioning that outbound criteria were considered for the study, where patients hold no responsibility with the therapeutics applied, and for patients who present sloughing of the biomaterial placed (PRF).

To perform the root coverage, a PRF membrane was prepared and placed under the CAF during the surgical procedure. For the preparation of the bioproduct

(PRF) used as a membrane, the patient was referred to UNIB, where the protocol established was followed by the scientific and technical service of Regenerative Medicine. Within the specific exclusion criteria for the preparation of the PRF membrane are transplanted, or immuno-suppressed patients, or both; patients with blood, endocrine, and cardiovascular disorders; being pregnant or breastfeeding; and undergoing treatment with cytostatic agents.

For its preparation, a 10 mL blood collection was made from the cephalic vein at the elbow crease, after fasting for at least eight hours. It was deposited in test tubes without anticoagulants and centrifuged for 10 minutes at 3000 rpm. Subsequently, the middle portion of the tube, where the PRF is, is extracted (13). Once the biomaterial is prepared, it is set on a Petri dish, covered with a sterile drape, in a cool environment and immediately transferred to the surgical unit of the Faculty of Stomatology. The surgical procedure takes place at the faculty where the CAF is placed during a periodontal plastic procedure by the main author of the research, who has the necessary experience to perform it. Measurements to evaluate the clinical changes were taken at 1 month, 6 months, and 1 year after of the treatment.

Variables

The variables analyzed in this study were the length of gingival recession (measured in millimeters) and the root coverage (calculated by subtracting the length of the initial recession from that of the final recession; after that, the result is divided by the initial recession's length; finally, this quotient is multiplied by 100 to obtain the percentage). Additionally, the width of the attached gingiva (measured in millimeters), the gain in the attached gingiva (measured in millimeters), the Miller classification (12), the evaluation categories (favorable, moderately favorable, and not favorable), the therapy efficacy (efficacious/inefficacious), sex, and age were measured.

For the favorable evaluation category, the root coverage was thought to be above 70% and the attached gingiva width above 3 mm. For the moderately favorable category, the root coverage was thought to be equal or higher than 50% but less than 70%, whereas the width was thought to be equal or higher than 2 mm. Lastly, the not favorable category would have a root coverage of less than 50% and the width of the attached gingiva, less than 2 mm.

A treatment is considered effective if the sum of the favorable and moderately favorable categories for the treated teeth is 75% or higher. Conversely, it is deemed ineffective if this sum is less than 75%.

Ethical considerations

Ethical considerations were considered for research involving human participants, covered in the Declaration of Helsinki. The approval of all the people participating in the study was granted and confirmed by signing an informed consent template. Participant data were protected by the researcher, and they were only used for research purposes. This study was approved by the Research Ethics Committee of the Faculty of Stomatology of the Universidad de Ciencias Médicas de Villa Clara, on February 6th, 2017.

Statistical analysis

The data collected were recorded in a 2016 Microsoft Excel workbook, and were later processed in the SPSS 22.0 statistics software. The information obtained from the data summary and the application of statistical hypothesis tests was presented in tables and figures showing absolute frequencies, percentages, as well as values corresponding to the mean and standard deviation in variables with a quantitative measurement scale. As part of the inferential statistics, 95% confidence intervals, Friedman test, and chi-square goodness-of-fit test were applied. This enables the comparison of the frequencies observed with those expected under the assumption that a determined hypothesis holds. As a result of the hypothesis tests, the statistical value is shown, as well as the significance associated to it (p). According to the p -value, the difference or association was classified as highly significant ($p < 0.01$), significant ($0.01 \leq p < 0.05$), and not significant ($p \geq 0.05$).

RESULTS

During this study, we worked with a total of 17 patients (11 women, who represented 64.71%, and 6 men, who were 35.29%). One month after the surgical procedure, a reduction of the GR length was found for all Miller classes, which continued through time for classes I and II; class III showed the same value at 6 months and continued to decrease at one year. The percentage achieved for root coverage was higher in Miller's class I, followed by class II. Then, an increase was observed for said classes over time.

Class III remained stable at 6 months and showed an increase one year after the procedure. The Friedman test showed highly significant differences between the mean lengths of the GR at different periods of being measured for Miller classes I and II ($p < 0.01$).

The mean value of the GR's length for classes I and II, at the beginning, differ greatly from the rest. Among the means at one month, 6 months and one year, the

differences detected were not significant. In Miller's class III there were no significant differences between the mean of the corresponding lengths during the time of the study ($p > 0.05$). There were highly significant differences in classes I and II between the root coverages in different stages ($p < 0.01$). There were no significant differences between the coverage at one month and 6 months, neither between the latter and at one year for Miller classes I and II (table 1).

Table 1. Gingival recession length and root coverage in teeth grouped as per the Miller's classification in different periods of the study.

Periods	Variables	Miller Class						Total	
		Class I		Class II		Class III			
		Mean*	SD	Mean*	SD	Mean*	SD	Mean*	SD
At the start	Length (mm)	2.65 ^a	0.51	3.00 ^a	0.75	3.25 ^a	1.06	2.81 ^a	0.64
1 month	Length (mm)	0.67 ^b	0.69	1.16 ^b	0.72	2.25 ^a	0.35	0.92 ^b	0.78
	Coverage (%)	75.26 ¹	25.32	59.78 ¹	27.21	28.75 ¹	12.37	67.51 ¹	27.62
6 months	Length (mm)	0.35 ^b	0.54	0.84 ^b	0.68	2.25 ^a	0.35	0.61 ^{b, c}	0.72
	Coverage (%)	87.24 ^{1, 2}	20.41	70.66 ^{1, 2}	25.67	28.75 ¹	12.37	78.56 ^{1, 2}	25.69
1 year	Length (mm)	0.29 ^b	0.49	0.63 ^b	0.70	1.75 ^a	0.35	0.48 ^c	0.65
	Coverage (%)	89.81 ²	17.95	79.17 ²	23.26	45.00 ¹	7.07	83.90 ²	21.84
Length	x ² (p)**	69.29 (<0.001)		41.19 (<0.001)		6.00 (0.112)		114.69 (<0.001)	
Coverage	x ² (p)**	18.73 (<0.001)		15.55 (<0.001)		4.00 (0.135)		35.77 (<0.001)	

Mean values are presented: length expressed in millimeters and coverage expressed in percentage. *Friedman test: i) length: in columns, the non-significant differences ($p > 0.05$) between means are expressed with a common letter; ii) coverage: in columns, the non-significant differences ($p > 0.05$) are expressed with a common digit. **Chi-square goodness-of-fit test: χ^2 (p).

An increase in the width and gain of the keratinized tissue over time of the analysis was observed during the study. When applying the Friedman test, there were highly significant differences for the width and the gain over time ($p < 0.01$). The mean of the dimensions of the attached gingiva, at the start, showed significant differences with the rest of the

means; this was not the same between the mean width values at one month, 6 months and one year, where no significant differences were found. In regard to the gain of the attached gingiva, there was no evidence of significant differences between the first month and after 6 months, and between the latter and one year (table 2).

Table 2. Width and gain of attached gingiva as per the periods established in the research.

Periods	Variables			
	Width (mm)		Gain (mm)	
	Mean*	SD	Mean*	SD
At the start	2.82 ^a	0.56		
1 month	3.85 ^b	0.64	1.03 ^a	0.45
6 months	4.02 ^b	0.70	1.20 ^{a, b}	0.51
1 year	4.14 ^b	0.74	1.32 ^b	0.53
χ^2 (p)**	113.93 (<0.001)		26.12 (<0.001)	

*Friedman test: in columns, the non-significant differences ($p > 0.05$) between means are expressed with a common letter. ** Chi-square goodness-of-fit test: χ^2 (p).

In most teeth, it was found that the therapy was assessed as favorable, 30 equals to 68.18%, followed by the moderately favorable category, 11 equals to 25.00%. Only 3 were evaluated as not favorable equals to 6.82%. When applying the chi-square goodness-of-

fit test, it was found that there were highly significant differences between the percentages corresponding to the favorable, moderately favorable and not favorable categories, which resulted from the evaluation of the procedure performed ($\chi^2 = 26.227$; $p = 0.000$) (table 3).

Table 3. Evaluation categories for the findings from the treatment performed.

Categories	n	%
Favorable	30	68.18
Moderately favorable	11	25.00
Not favorable	3	6.82
Total	44	100.00

**Chi-square goodness-of-fit test: $\chi^2 = 26.227$; $p < 0.001$.

Considering the “95% confidence interval” indicator, it was found that the percentage of treatment efficacy was between 85.73-100.00% (figure 1).

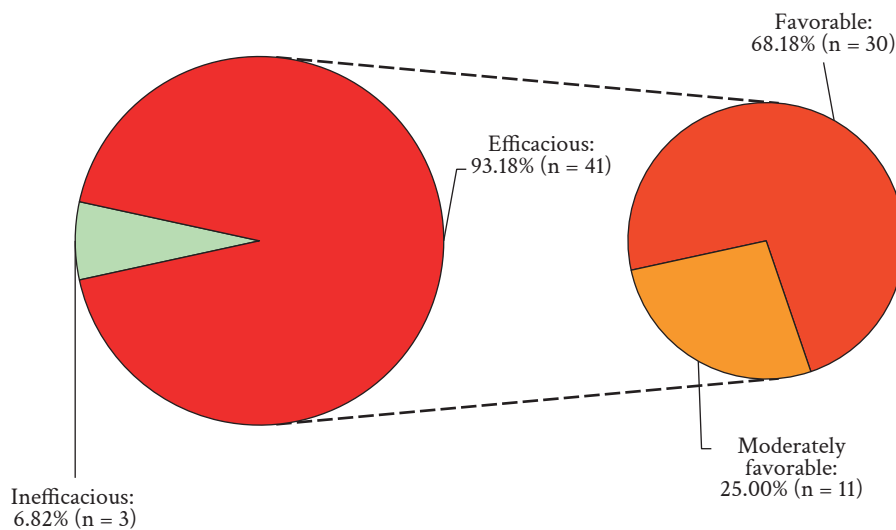


Figure 1. Treatment efficacy of gingival recession with platelet-rich fibrin used as a membrane. Confidence Interval (95%) for the efficacy percentage: 85.73-100.00%.

Figure 2 below is an example of one of those cases where GR is treated using the PRF membrane therapeutic procedure. The image shows the PRF membrane after being prepared and placed in the Petri dish to be taken to the recipient site during the surgical procedure (figure 2A), which was prepared using the CAF technique in the sites corresponding to the first and second right lower premolars (teeth

44 and 45) (figure 2B). Subsequently, the biomaterial was adjusted to the recipient site (figure 2C) and the flap was sutured in a coronal position relative to the recession (figure 2D). Once stitches are taken out, progress was presented at one month (figure 2E), at 6 months (figure 2F) and the complete cicatrization one year after the procedure was performed (figure 2G).

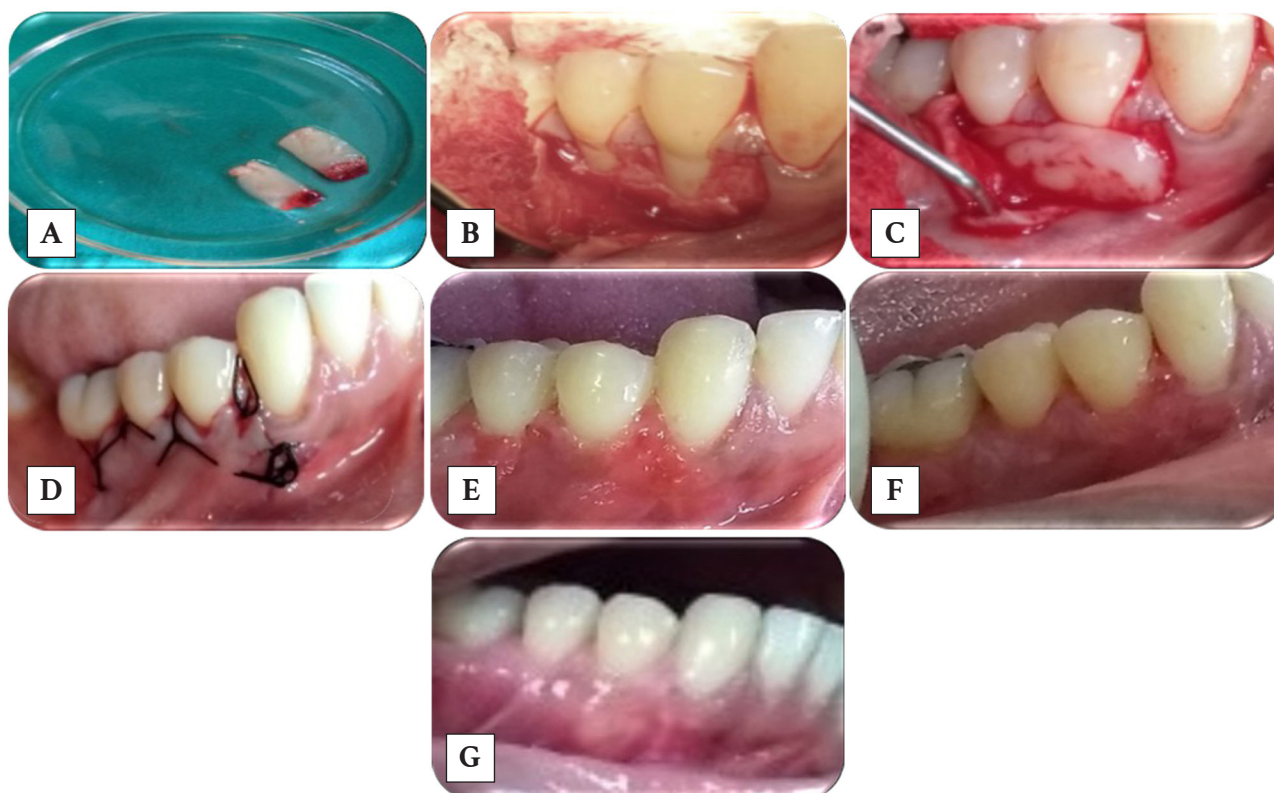


Figure 2. Repositioning flap surgical technique (CAF) associated to the platelet-rich fibrin (PRF). A) PRF membrane. B) CAF. C) PRF membrane placement associated to CAF. D) Sutured flap. E) Flap progress at one month. F) Flap progress at 6 months. G) Flap progress at one year.

DISCUSSION

PRF membrane associated with CAF offers great advantages in root coverage and the gain of attached gingiva, which constitutes the main evaluation parameters in the findings of periodontal surgery techniques. The contributions of the bioproduct associated with CAF can explain the percentage increase of coverage in areas treated with the new therapeutic procedure. The tri-dimensional structure of the solid fibrin matrix works as a step toward mesenchymal cells, whose differentiation contributes to tissue regeneration. All of this explains the graft growth over time (13, 14). There are a number of degrees of transformation and degeneration that occur in the biomaterial (PRF) once put in the treated sites; thus, it sometimes becomes practically invisible, but it remains present on the root surface, even though the surface may appear denuded in parts of the graft, with its regenerative potential (14, 15).

The aforementioned explains the bioproduct's extended action in the root's coronal portion of the apparently exposed graft. Multiple growth factors present in the membrane contribute to neoformation and favor stability to the relocated tissue. For instance,

the fibroblast growth factor stimulates those cellular elements for the production of collagen fibers and blocks bacterial protease activity, which may lead to a degree of tissue destruction. In the same manner, the insulin-like factor type I, together with the previous one, promotes cell division, proliferation and differentiation, which contributes to a continuous cellular growth while these are active (16). Similar findings are those outlined by Joshi et al. (17), who reported an increase that remained stable at 6 months of treatment using the PRF membrane.

The adequate width of the attached gingiva is one of the criteria to consider when performing the different periodontal plastic techniques. It is assumed that its dimensions will be adequate as long as health conditions are guaranteed for periodontal tissues. A wide band of the attached gingiva around the teeth, which had or have GR, halts GR progression as long as hygiene and the factors that caused the gingival margin recession are controlled. Gingival nutrition is vital to avoid dystrophy. With the procedure applied, there was an increase of the attached gingival band, which progressed along the measurements taken. This is determined by the effect of growth factors included in

the PRF membrane, such as insulin-like, transforming beta and fibroblastic growth factors, which foster cellular proliferation, division and differentiation, favor the deposition extracellular matrix, and increase collagen biosynthesis (18). The continuous action of the factors mentioned above explains the possibility of forming a band of attached gingiva that shows growth and maturation over time.

Similar findings to those presented above were obtained by Sarduy et al. (4), who used other associations to the CAF, which also showed an increase of the attached gingiva over time. However, with the proposed technique, lost tissue regeneration is guaranteed, enabling an increase of width as well as thickness. Coincidentally, Tadepalli et al. (19) reported an increase in the thickness of keratinized tissue with the PRF membrane in two dimensions as a result of the procedures performed in their study. This translates into stable clinical results and a lower probability of a new GR occurring when using a similar protocol to the one proposed in this research, and another one with less centrifugation force with the advanced PRF.

Authors such as Louis et al. (20) reported superior root coverage by using subepithelial connective tissue in connection with the PRF, which differs from this study where there were some differences in the bioproduct preparation protocol, primarily in the final outcome. This guarantees a higher stability of the results over time, based on the tri-dimensional structure of the fabricated membrane.

According to the Miller classification (12), the coverage prediction is superior in classes I and II, which is confirmed by the proposed course of action in this study. This confirms the prognosis established by the aforementioned author. Similar results are those presented by Abu-Ta'a (21), who proposes the course of action for the aforementioned classes. Tadepalli et al. (19) propose superior coverage in classes I and II in this same order, when it comes to advanced PRF, which presents a higher concentration of growth factors. However, in this study, results show this order with the proposed protocol. The estate of the interdental bone in these Miller classes enables a superior regeneration compared to the others, and the smaller longitudinal extension of the recession in class I enables better regenerative outcomes with the proposed bioproduct (22).

Root coverage and the width of the attached gingiva were the parameters established by the researcher in order to evaluate the results of the technique used. There were also important outcomes in them, which

led to a predominance of the favorable category. It is assumed that the use of the traditional CAF technique is a highly applicable surgical procedure for covering exposed roots. However, the combination of this procedure with certain biomaterials placed between the flap and the root transforms it into a bilaminar technique that leads to superior outcomes. When adding the regenerative benefits of the biomaterials used, the outcomes are superior and stable over time.

The fundamental purpose of periodontal plastic procedures is to obtain root coverage and its durability. But it also requires adequate dimensions of the inserted gingiva associated with the GR are required in order to maintain optimal results of the coverage obtained, or to prevent the progression of the dystrophic process, which highlights the clinical value of these techniques. When inserted gingival gain and root coverage are sustained over time, they allow for greater protection of the root surface. This avoids dentin hypersensitivity, offers better resistance to the passage of inflammation of deeper tissues of the periodontium and to functional and tensile forces of musculature, which could lead to margin inadaptation causing ischemia and a continuation of dystrophy (23-25).

These findings are superior to those published by Chekurthi et al. (26), who collected a 75.94% efficacy in root coverage, as well as Kuka et al. (27), who allude to a mean coverage of 86.36%. However, the values obtained by the author are similar to those expressed by Raja et al. (28) who allude to a root coverage of 94.17% in an 18-month measurement period. Stefanini et al. (29), and Pini-Prato and Gianfilippo (30) consider values above 70% to declare periodontal plastic procedures as effective. These numbers are surpassed by those obtained by the author.

The main methodological limitations of this study are due to the limited research on the efficacy of PRF in the treatment of GR, which would allow for comparisons, the sample size due to the specificities of root coverage techniques, as well as the lack of measurements over a longer period of time. Another limitation is the inability to histologically verify the regeneration achieved with this biomaterial, and only be able to report it based on clinical changes.

CONCLUSIONS

A higher efficacy of the PRF used as a membrane in gingival recessions, Miller class I followed by class II, was verified. The course of action proposed showed

outcomes of root coverage and gain of attached gingiva, which increased over time. The therapeutics of PRF used as a membrane for periodontal recession coverage was evaluated as efficacy.

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