The Effectiveness of the Influence of the Second Generation Platelet-rich Fibrin in the Treatment of Localized Individual Miller I and II Gingival Recessions (Case Report)

Aneta Terzievska¹, Daniela Veleska-Stevkovska², Gordana Apostolova²*, Zaklina Mencheva¹, Stavre Trajculeski¹

¹Department of Oral Surgery and Implantology, University Dental Clinical Centre “St. Panteleimon” Skopje, Republic of North Macedonia; ²Department of Oral Surgery and Implantology, Faculty of Dentistry, Ss. Cyril and Methodius University of Skopje, Skopje, Republic of North Macedonia

Abstract

BACKGROUND: The mucogingival surgical modality subepithelial connective tissue graft (SCTG) and the coronally positioned flap (CAF) in the treatment of Miller I and II gingival recessions are considered “gold standard,” that is, a technique for predictable and complete gingival root coverage defects, with long-term clinical stability of the obtained results. The counterpart of this type of technique is the relatively young platelet-rich fibrin (PRF) + CAF operational mode, due to the biological characteristics of this autologous platelet concentrate-PRF (angiogenesis, mitogenesis, osteopromotion, immunomodulation, and the capture of stem cells). The aim of this paper is to evaluate the clinical efficacy of the CAF + PRF combined technique, in the treatment of localized individual gingival recessions Miller I and II, through the comparison of the values of periodontal clinical parameters, measured preoperatively and 1 month postoperatively, as well as through the obtained immunohistochemical and histomorphometric analyzes of the taken biopsy material from the recipient site (the area of the grafted gingival defect), 1 month postoperatively.

CASE REPORT: A 37-year-old man was admitted to the Clinic for Oral Surgery at JZU USKC “St. Panteleimon”-Skopje for surgical treatment of localized maxillary gingival recession Miller II at tooth 31. Periodontal clinical parameters were measured preoperatively: Vertical dimension of gingival recession (RD/VGR), periodontal pocket depth (PPD), width of keratinized gingiva (KTW), and thickness of gingiva (GT). All measured in mm, as well as gingival biotype. A CAF + PRF therapy modality was used for the treatment of the gingival defect. One month postoperatively, repeated measurements of the values of the clinical periodontal indices were performed and they were compared with the measurements obtained preoperatively.

CONCLUSION: A significant decrease in the values of RD, PPD, and CAL was determined at the 1-month postoperative. A non-significant gain in KTW and GT values was noted 1 month post-operatively. There were no changes in the gingival biotype before and after surgery. PRF as second-generation autologous concentrates is not only an adjuvant and/or replacement of SCTG – the “gold standard” in the treatment of Miller I and II recessions but it is also a superior alternative in the surgical treatment of this type of shallow mucogingival defects.

Introduction

An estimated 90% of the global population under the age of 60 is affected by tooth recession to some extent, typically within the range of up to 1 mm. Furthermore, it is observed that approximately 40% of the overall population experiences recessions exceeding 3 mm in size [1]. Recession implies apical dislocation of the marginal gingiva toward the cementoenamel junction-CEJ [2], [3], associated with subsequent exposure of root surfaces, root caries, hypersensitivity, plaque accumulation, and esthetic compromise [4]. As a multifactorial change, it is a result of a complex etiology; traumatic occlusion, deficient or improper oral hygiene (rough tooth brushing), older prosthetic structures or dental fillings (inadequate marginal closure), as well as low inserted upper or lower labial frenulum, or shallow vestibulum, caused by muscle attachments – strong muscle pull [5]. Malocclusions and malpositioned teeth, as orthodontic anomalies, and the post-period of fixed appliances, are states of the anatomical challenge of the mucogingival complex as part of the periodontal complex, and those combined with the thin gingival biotype, are also one of the etiological causes of the recession [6]. The most common clinical manifestation of recession is a loss of interproximal attachment, but the conditions of vestibular or palatal “attachment loss” are not rare [7]. Assessment of periodontal status and etiopathogenesis that lead to changes in the periodontal complex as a whole is express through periodontal indices, which were published by Loe in 1967 [8]. In modern regenerative mucogingival surgery, the actual focus is more on the application of the second generation of autologous platelet concentrates (APCs). These autologous blood products are divided into four categories/classes (according to the content of leukocytes and fibrin): Pure platelet-rich plasma (P-PRP); leukocyte platelet pure plasma (L-PRP); pure platelet-rich fibrin (P-PRF), and leukocyte
platelet-rich fibrin (L-PRF) [9]. On the other hand, these four classes are divided into three generations of platelet concentrates [10].

PRF represents the second generation of APCs embedded in a fibrin matrix. The founder of the II generation ATCs-PRF is Prof. Choukroun (2001) and for the 1st time, they are described by Dohan et al. [11]. The accepted protocol of Dr. Choukroun includes the centrifugation process results in the separation of different layers in PRF. The uppermost layer, known as platelet-poor plasma, is acellular and often referred to as the serum layer. In the middle layer, called the PRF-Buffy coat or fibrin clot layer, large and dense platelet clusters indicate active aggregation and coagulation. These platelets are most concentrated within the first millimeter after the red part, gradually decreasing in concentration toward the upper portion of the yellowish fibrin body until they completely disappear over the second half. Finally, the lowest layer consists of red blood cells, making it rich in RBCs [12], [13].

The variety of centrifugation protocols, in terms of time and number of revolutions, as the type of centrifuge, the quality of the test tubes; glass, plastic coated with glass, and silicon (acute cytotoxic effect of silicate microparticles) [14], [15] or with silicone, the slant of their placement in the centrifuge, the volume of the collected blood as well as the speed of the venipuncture, and the manipulation of the already taken blood, are the reasons for the different concentration of cells and growth factors in PRF membranes [16]. In addition to the conventional protocol of Choukroun, in the past two decades, modifications of it have been developed, conditioned by the before mentioned protocols variations. Those new modalities are the following:

A-PRF (Choukroun, 2004) – the protocol is characterized by a lower speed of centrifugation, but a longer time –1300 rpm/8 min in sterile, ordinary glass tubes; plastic, coated with glass, and vacuum centrifugation, 700 rpm/3 min in plastic tubes without no lining [17], [18]. Those tubes have a hydrophobic surface and do not activate it in addition to the coagulation process. The total release of GFs at the end of the 24th day is significantly higher in A-PRF compared to PRP.

I-PRF (Choukroun, 2004) Liquid-injectable PRF this protocol is characterized by a lower speed of centrifugation, 700 rpm/3 min in plastic tubes without no lining [17], [18]. Those tubes have a hydrophobic surface and do not activate it in addition to the coagulation process. The total release of GFs at the end of the 24th day is significantly higher in I-PRF compared to PRP.

Microscopically, PRF consists of a 3D fibrin network that is constructed of thin, elastic, mature, and densely polymerized fibrin fibers in which they are trapped multitude of cells, predominantly platelets, as well as concentrates of growth factors and cytokines. It is this physiological/natural polymerization of PRF that makes it possible to enhance the trapping of circulating cytokines, which are released only in the 3D fibrin matrix remodeling, which is due to the slow, prolonged, and in equal dose release of GFs over a period of up to 28 days [12]. That 3D fibrin network in addition to stimulating the activity of the captured regulatory cytokines, it also enables the adhesion of new blood vessels. From there, the PRF can be interpreted as an ideal biomaterial in terms of tissue engineering: It contains a network that behaves as a three-dimensional lattice, viable cells for a tissue population, and molecules that stimulate reparation [19]. In addition to platelet, fibrin concentrate-PRF contains platelets, leukocytes, and circulating stem cells, it is also a reservoir of a large number of slow releasing (7, 10, 21–28 days) growth factors, cytokines, and matrix glycoproteins (thrombospondin-1) 60 that affect the regeneration process of soft and hard tissues, stimulating neoangiogenesis, fibroblast/osteoblast migration, proliferation, and differentiation as an integral part of wound healing, which is the central concept of surgical regenerative science [11]. The biological activity of PRFe results from the presence of biological modifiers, primarily Tr, due to alpha, the dense, and glycoprotein granules found in them. Alpha granules are the largest significance for the improvement of “wound healing,” due to the multitude of growth factors and cytokines, which contain: Platelet-derived growth factor (PDGF), transforming growth factor-beta 1, vascular endothelial growth factor (VEGF), insulin-like growth factor-1, basic-fibroblast growth factor, endothelial growth factor, interleukin 1 (IL)-beta, IL-4, and IL-6. All these growth factors together with the unique and slow remodeling of the 3D structure of the fibrin lattice (scaffold network) are responsible for accelerated wound healing. The whole biological activity of PRF is essentially presented through the following processes which are occurring in the wound: Angiogenesis (VEGF, PDGF, angiopoietin, and FGF-b), mitogenesis (TGF-β, thin, and elastic trimolecular bonds), immunomodulation (fibrin, fibronectin, leukocytes, and IL-4), wound recolonization (fibronectin, fibrinogen, vitronectin, tenascin, and fibrin), osteogenesis, and entrapment of circulating stem cells. The aim of the study is to evaluate the clinical and histological effects of surgical treatment of localized gingival recessions Miller I and II, during the application of PRF and simultaneously prove that PRF as a promoter of neoangiogenesis leads to accelerated and better revascularization of the operative field and thereby directly participates in the “wound healing” process.

From there, to prove that the adequate blood supply – nutrition of the newly created periodontal attachment is of exceptional importance in periodontal healing. Furthermore, one of the goals is to prove that PRF as a therapeutic modality, through stimulation of fibroblast/osteoblast proliferation, migration, and differentiation, gives stable and long-term results compared to keratinized (soft) tissue healing, as an integral part of gingival management recessions.
Materials and Methods

A 37-year-old man was admitted to the Clinic of Oral Surgery at the University Dental Clinical Center “St. Panteleimon” – Skopje, for surgical treatment of localized maxillary gingival recession Miller II on tooth 31. A few weeks preoperatively initial therapy on several occasions at the clinic for oral and periodontal diseases was provided (mechanical decontamination/debridement, scaling, and root planning). The tooth with the gingival recession and the adjacent agonists were mechanically brushed with an abrasive paste, and then polished. The patient was given guidance on proper oral hygiene maintenance and advised on the next steps to follow.

To address traumatic occlusion, a procedure called selective scraping was performed to remove existing premature contacts. Before the procedure, the patient underwent a thorough pre-operative preparation process. This involved the patient signing a survey questionnaire and an informed consent form in accordance with the Helsinki Declaration (1998/2008-revised). The patient was also provided with a detailed study protocol that clearly explained the procedure in an easily understandable manner. As part of the pre-operative preparation, the patient has prescribed a course of antibiotics, specifically Tbl/Metronidazole 400 mg, to be taken 3 times daily. The antibiotic regimen was initiated 3 days before the intervention and continued for a duration of 7 days.

Pre-operative values of periodontal clinical parameters: Vertical dimension of gingival recession (RD/VGR), periodontal pocket depth (PPD/PD), level of keratinized/attached gingiva (CAL), width of keratinized gingiva (KTW/KMW), and gingival thickness (GT) all noted in mm as well as biotype of gingiva (thin ≤1.5 or thick ≥2.5) were measured 1 day before the planned mucogingival surgical treatment. Before the intervention, the patient rinsed his mouth with 10 mL/0.05% cetylpyridinium chloride. Local plexus anesthesia (2% Scandonest) was administered using Carpule syringe, in the immediate vicinity of the recession zone. CAF+PRF therapeutic modality was applied to the patient for gingival treatment defect. Coronally advanced flap (CAF) was performed (Figure 1), starting with a sulcus incision on the recipient tooth, which was joined with horizontal incisions in the region of the adjacent interdental papillae, discretely oriented coronally near CEJ. The medial and the distal horizontal incisions started from the base of the anatomical papillae adjacent teeth to the CEJ of the tooth with recession. Their positioning from the tip of adjacent papillae was equal to the sum of RD+1 mm (de Sanctis and Zucchelli). These horizontal incisions were joined by two beveled vertical incisions that start at the corners of adjacent teeth and continue slightly divergently toward the vestibular fundus crossing the mucogingival border. Surgical papillae were raised in a split technique, which allows the preservation of the periosteum which plays a major role in the future flap and graft revascularization. Above the exposed root part, as well as 3–4 mm apically from the bony dehiscence projection, the raised flap was in full thickness. The exposed root surface was mechanically debrided with Gracey curettes and diamond bur. Afterward, the root was conditioned with 24% of ethylenediaminetetraacetic acid for 2 min and then with a concentrated solution of Doxycycline-2 min, (Figure 2) followed by abundant irrigation with 0.9% NaCl (Figure 3).
After the centrifugation process for obtaining PRF, the tubes were placed in the laboratory rack. From the A-PRF tubes, we removed the fibrin plugs. The plugs were placed on the metal grid of the PRF box (Figure 4) and pushed with another flat metal plate to squeeze out the PRF exudate. The membranes were positioned in the recipient bed, 1 mm above the CEJ, without sutures, gently fixing them under the mesiodistal borders of the recipient site (Figure 5).

We positioned the flap coronally above the enamel-cement border. The suturing was done with a non-resorbable polypropylene suture (5–0) (Figure 6). At the very end of the intervention, we aspirated the upper yellowish part-supernatant from both I-PRF tubes, which were injected into the surrounding tissue adjacent to the flap (Figure 7). In this way, we reintroduced concentrated amounts of growth factors that will accelerate the "wound healing." At the very end, with the rest of the I-PRF and PRF exudate, we gently rinsed the entire operative field (flap and sutures).

**Clinical periodontal parameters**

We performed the notification of clinical periodontal parameters 1 day before the intervention and 1 month postoperatively. We measured the values of:

- Depth of recession (GR/GD/VGR-the distance from the enamel-cementum border-CEJ to marginal gingiva)
- PPD/PD – the distance from the marginal gingiva to the bottom of the periodontal pocket
- Clinical level of attached gingiva (CAL=GR+PPD-the distance from CEJ to bottom of periodontal pocket)
- Width of keratinized gingiva (KA/WKM-WKG - the distance from the marginal gingiva to the mucogingival border-MGJ) Gingival biotype (thin ≤1.5 or thick ≥2.5)
- Gingival thickness (GT-the distance from the gingival surface to bone surface area measured in mm).

We performed the measurement of GR, CAL, KTW, and PPD using William’s graduated periodontal probe (Hu Freddy, Chicago, IL, USA) with a rounded tip, with subsequent graduations of millimeters starting from 1, 3, 5, 7 to 9 mm. The measurements again on the GT, we performed-transgingival probing (Vandana and Savitha) on the middle of the buccal surface of the affected tooth, with an endodontic #15 expander with a rubber stopper. After the application of surface anesthesia, in the middle region of the attached gingiva, in the middle distance between the mucogingival border and the base of the interdental papilla, we introduced the expander into the tissue.
in depth, from 1 to 2 mm below the marginal gingiva, perpendicular to the axis of the tooth (90° angle) until they touch the bone. We pulled the rubber stopper through the needle all the way to the surface of the gingiva, and the distance from the tip of the expander to the inner limit of the stopper, we measured to the nearest 0.1 mm, using a digital Vernier caliper or caliper/microscrew.

Paraclinical trials

Histomorphometry

We performed the histomorphometric analysis after 1 month of the intervention, with micro-gum punch biopsy of 2 mm, taken from the middle part of the grafted site, at a distance of 5 mm from the marginal gingiva. The material was analyzed at the Institute of Pathological Anatomy (Faculty of Medicine, University Sts. Cyril and Methodius in Skopje, North Macedonia. After fixing with a neutral solution formalin/formaldehyde, samples were embedded in paraffin. Microtome sections with a thickness of 5 µ were obtained. All sections then were further stained with hematoxylin/eosin (HE-staining) and Masson's trichrome (M&T) staining, through which the length of epithelial proliferations, the percentage of newly created blood vessels in relation to the surrounding field of view, as well as the quantification of new zones/areas of vascularization were determined using Image J software.

Immunohistochemical investigations

For immunohistochemical investigations, animal monoclonal and polyclonal antibodies were used for the detection of VEGF (polyclonal antibody Anti-rabbit Alexa Fluor 568 VEGF); CD31-(monoclonal antibody Alexa Fluor-mouse 635 and CD34-(Alexa Fluor-mouse monoclonal antibody), which are incubated in a dark place for 1 h, with the exception of the CD34 monoclonal antibody. The degree of vascularization 1 month postoperatively was visualized by fluorescence microscopy through the Dako EnVision Flex system for detection.

Results

One day before the patient’s surgical intervention, the value of the following clinical periodontal parameters was measured in mm:

From the above said, it can be concluded that this is a patient with a thin biotype gingiva, with a thickness of 0.8 mm and a depth of recession of 3 mm, Miller I class. One month postoperatively, the patient underwent the same clinical tests again; measurements and the following results were obtained: Reduction of the depth of the vertical gingival recession with a value of 2 mm, decrease in PPD value by 0.5 mm, and subsequently decreasing the CAL value. The width value of the keratinized gingiva has slightly increased by 0.2 mm (Table 2).

Discussion

The small width of the attached keratinized gingiva (KTW) <2 mm is one of the main reasons for the occurrence of recessions [22]. The principles of contemporary esthetic mucogingival surgery using autologous grafts (subepithelial connective tissue graft [SCTG] or PRF) are based on complete and predictable coverage of exposed roots surfaces, the establishment of homeostasis of the mucogingival complex, regeneration of the lost attachment, including the formation of new cementum through the insertion of connective tissue fibers, as well as alveolar bone regeneration [24]. The goal of periodontal plastic surgery is to generate a minimally invasive operative technique, which will favor and stimulate rapid regeneration, will cause minimal post-operative discomfort, and will allow maximum esthetic and functional patient satisfaction. The most commonly used grafts are free gingival graft and SCTG as before recognized gold standard in the treatment of this problem, while the most absolute and the most frequently used surgical technique is CAF (coronally advanced/displaced/positioned flap). The technique of taking SCTG is longer, it increases the risk of morbidity because it also involves a second operative field (donor site), thereby emphasizing

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<th>Table 2: Value of post-operative periodontal parameters</th>
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PPD: Periodontal pocket depth, RD: Recession depth, CAL: Clinical attachment level.
the patient’s discomfort, post-surgical pain, and the possibility of bleeding, dehiscence or necrosis of the flap [25]. Limiting the quantity and quality of donor tissue is also one of the disadvantages of CTG [3]. SCTG and PRF (platelet-enriched fibrin) are characterized by a common autologous background, representing different therapeutic concepts and strategies, but the analyzed studies suggest a similar clinical outcome. The development of platelet concentrates (biological agents) revolutionized the concept of tissue repair and regeneration [26]. Wound healing is a central concept of regenerative medicine surgical science. Healing is the result of complex relationships between different types of cells (fibroblasts, epithelial cells, and osteoblasts) and signaling molecules, cytokines, and growth factors GF [35]. Because there is no need to use an anticoagulant in the obtaining procedure of A-PRF, as well as due to the amount of platelets 97% and leukocytes 50% of the original blood volume [27], [28]. PRF is also defined as autologous platelet and leukocyte-enriched fibrin biomaterial [9], [27]. Advantages of PRF over PRP include shorter preparation time, absence of the use of biochemical modification/exogenous material, or anticoagulants such as CaCl$_2$ or bovine thrombin (economically more profitable, without the risk of bloodborne diseases or immunological Ag/At reactions), whereby the polymerization occurs naturally in a physiological style [29]. One of the more commercial properties is simplicity of application and manipulation.

The PRF membrane possesses mechanical adhesive properties and biological features similar to fibrin glue; thus, it keeps the flap in a stable state and strengthens angiogenesis [30]. These biological features correspond with the findings of Al Jasser et al. [31] as well as with the findings of the studies of Eren et al. [32] Madi and Elakel [33] and Shah et al. [12]. Regarding the decrease in RD value, our findings are similar to the findings by Eren and Atilla [25] and Mijiritsky et al. [10] where there is a significant reduction of value, also coincides with the significant decrease in the RD value in the study of Panda et al. [34], as well as with the results of the studies of Madi and Elakel [33]. A decrease in the RD value, but without significance, is described in the study by Al Jasser et al. [31]. While the discrepancy of our results with values in their studies was found in Moraschini and Edos [35] and the opposite result with increasing RD value was obtained in Kumar et al. [21]. A decrease in the value of PPD was found in the papers of Madi and Elakel [33], Mijiritsky et al. [10], and Miron et al. [36], while again a significant reduction of the same parameters is found in the paper by Kumar et al. [21]. Unchanged PPD values are reported by the authors; Eren and Atilla [25], Eren and Atilla [37] how and Miron et al. [14]. Except in our paper, the value of CAL shows a downward tendency in the studies of Eren and Atilla [25], Madi and Elakel. [33], and Mijiritsky et al. [10], but a significant decline in it was found in Panda et al. [34] and Kumar et al. [21]. CAL values remained unchanged in the studies of Moraschini and Edos [35], while the opposite effect with a significant increase in value exists in the papers of Öncü [38] and in Miron [14], Miron et al. [36]. Matching our results with respect to the value of KTW are present at Eren and Atilla [25] and Panda et al. [34], with a positive trend in the value of KTW but without significance. A similar relationship exists in the study of Eren and Atilla [37], whose results are stable up to 3 months postoperatively. An increase in the value of KTW with statistical significance was reported by the authors Mancini L et al. [39], Mijiritsky et al. [10], Miron et al. [14], and Öncü [38]. The marginal significance of an increased value of KTW exists in the paper of Al Jasser et al. [31]. We encountered a discrepancy in findings, in terms of the absence of any change in the value of KAW. The results of Eren and Atilla [24], Eren and Atilla [37], and Kumar et al. [21] in relative to the moderate increase in the value of GT showed correspondence with our findings in this paper, while a significant increase in GT values reported Öncü [38], Mancini et al. [39], Mijiritsky et al. [10], Panda et al. [34], and Shivakumar et al. [40].

Conclusion

With the use of the second generation of platelet-enriched fibrin-PRF (A-PRF and I-PRF), as an adjuvant to the widely accepted surgical method CAF in the treatment of localized Miller I and II gingival recessions, proved the thesis that this autologous biological additive is not only a substitute for the so far proven “gold standard” in the treatment of this type of gingival defects-SCTG, but it is an equally good alternative to the same, taking into account the wide spectrum of advantages, which unequivocally indicate the influence of this 3D matrix as a wound promoter, neoangiogenesis, initiator of rapid soft-tissue regeneration and repair and accelerator of the overall “wound healing,” which is the central concept of mucogingival surgery. Hence, the PRF has an immediate/direct role in the predictability of long-term success and stability of the resulting clinical “outcome” and, thus, becomes a potential grafting material in the treatment of shallow gingival recessions. The commercial sublimate from the above is the facts that modern autologous biological membranes (PRF) are economical, available in optimal amounts, unlike SCTG, easily manipulable from a surgical point of view (simpler for application and preparation), and superior in the elimination of post-operative discomfort-absence of a donor site morbidity.

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