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The effect of Injectable Platelet-rich Fibrin (I-PRF) on Peri-implant Soft Tissue Healing Following a Tuberosity Connective Tissue Graft Procedure: A Randomized Clinical Trail

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Connective Tissue Graft, I-PRF, tuberosity, implant.

ABSTRACT

The positive effect of blood concentrates on the healing of soft and hard tissues in periodontal and peri-implant surgeries has been proven. The growth factors contained in blood concentrates are the source of these positive properties. Injectable platelet-rich fibrin (I-PRF) is one of the recent generations of these extracts and its clinical form helps to be added to soft tissue grafts. I-PRF releases growth factors during the healing of these grafts. Therefore, our study aimed to evaluate the healing of the recipient site following the application the connective tissue graft to augment the peri-implant soft tissues. The research sample included 20 sites that needed peri-implant soft tissue management. In the test group, I-PRF was added to the Tuberosity connective tissue graft before it was sutured at the recipient site, while in the control group, the Tuberosity connective tissue graft was used at the recipient site without additions. Wound healing was assessed at weeks 1, 2, 3, and 4 to evaluate the ability of I-PRF to enhance the healing. Statistical tests were performed using SPSS v.22. The results showed that the soft tissue healing was similar in both groups with a minimum preference for the test group. The values of the significance level were greater than 0.05 when comparing the two groups in all observation periods, which means that the differences were not statistically significant. This study found that I-PRF enhances the healing of soft tissue wounds around implants when added to the connective tissue grafts. However, this result still needs more studies to be proven.

1. Introduction

In the past decade, it has been noted that studies investigating the peri -implant soft tissue augmentation procedures have increased significantly due to the increased knowledge of the crucial role of these tissues in maintaining the health status of dental implants and enhancing the functional and aesthetic results [1]. Autogenous soft tissue grafts could be considered the most effective in increasing the soft tissue volume around implants [2]. The periodontal flap healing is a more complex process than other sites in the oral

cavity because the connective tissue of the flap is in contact with a solid, avascular surface rather than in contact with another vascular wound bed. Furthermore, the healing process depends on the stability of the blood clot and the formation of an attachment resistant to mechanical tear. In periodontal and peri-implant surgeries, the mucoperiosteal flap is often displaced in order to be sutured in a more coronal position. As a result, functional forces on the flap increase and are transmitted to the fragile fibrin clot, leading to an increased risk of wound rupture [3]. One of the most important challenges is to find a biomaterial that could be used to promote tissue healing with maximum predictability [4]. Although knowledge of the tissue healing process is still insufficient, the role of platelets in tissue regenerative procedures is clear and scientifically proven [5]. Various studies have shown the effectiveness of blood concentrates in promoting the healing of hard and soft tissues around dental implants [6].

Platelet rich fibrin (PRF) is one of the latest generations of blood concentrates and is obtained without using of any anticoagulant. It contains a fibrin network and all components of the blood that promote healing and immune reaction. One of the most desirable features of PRF is the ability to deliver concentrated growth factors to the surgical site to stimulate the healing process. PRF is a therapeutic biomaterial that has a strong stimulatory effect on various aspects of soft and hard tissue healing; including angiogenesis, immune control, and recruitment of circulating stem cells [7]. More research is still needed to confirm the effectiveness of PRF in improving and maintaining bone and mucosa tissue around dental implants.

In 2014, by modifying spin centrifugation forces, injectable platelet-rich fibrin (I-PRF) was developed. Centrifuging blood in non-glass tubes at low speeds resulted in the formation of liquid PRF called I-PRF [8]. To date, numerous in vitro and human studies have been conducted regarding the role of I-PRF in promoting wound healing, accelerating orthodontic movement, and regeneration of bone, periodontal, and pulp tissues. I-PRF has been shown to enhance the regenerative potential of tissues by stimulating the proliferation and migration of mesenchymal stem cells (MSCs) and by inducing osteogenic differentiation of stem cells [9], [10]. In mucogingival surgeries, I-PRF has a positive effect on achieving root coverage with free gingival graft surgery [11]. In 2020, one study showed that combining I-PRF with micro-needling helped increase the thickness of the gingival tissue, so that the combination of these two techniques is considered the first step in non-surgical methods to enhance and improve the gingival thickness [12]. Ucak Turer reported that gingival recession decreased more in the group in which I-PRF was applied simultaneously with the coronally advanced flap and connective graft procedure, and the results also showed that the addition of I-PRF contributed to achieving an increase in keratinized tissue height compared to using the coronally advanced flap and connective graft procedure alone [13]. Previous studies have not evaluated the effect of I-PRF on connective tissue graft recipient site wound healing in the context of peri-implant soft tissue augmentation.

This randomized controlled clinical study was conducted to evaluate the effect of injectable platelet-rich fibrin (I-PRF) on peri-implant soft tissue healing after adding it to a connective tissue graft in a peri-implant soft tissue augmentation procedure.

2. Materials and Methods

This randomized controlled clinical trial (RCT) was conducted in the Department of Periodontology, the Faculty of Dentistry, Damascus University. The study included installing dental implants in 20 sites that needed peri-implant soft tissue augmentation (1 site for male compared to 19 sites for females) in the period between 2021 and 2023.

The inclusion criteria were:

- Placement of a single dental implant at least in a site that requires increased soft tissue volume due

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to the presence of a horizontal vestibular defect (2-3 mm concavity) or a thin mucosa phenotype.

- A sufficient thickness of mucosa in Maxillary tuberosity to allow connective tissue graft harvesting.
- Ability to maintain oral hygiene.

The exclusion criteria were:

- Any contraindication to collect the blood from the patient.
- Smokers.
- Pregnancy and breastfeeding.
- Systemic conditions or treatments that affect the general health or condition of the periodontal tissues (such as: radiation and/or chemical treatments during the previous 12 months, uncontrolled diabetes, etc.).
- Any previous surgery to increase the volume of soft tissues in the same site.
- The need for bone grafting procedure in the implant site.

All Patients who were selected agreed to participate in the research after being informed of the conditions, steps, and possible complications, and they signed the informed consent before being included and randomly distributed into one of two groups (10 sites in each group), which was as follows:

1. The test group (Tuberosity and I-PRF group): The connective graft was harvested from the Tuberosity and I-PRF was added to it before being placed in the recipient site, which is the same site of the dental implant procedure.
2. The control group (Tuberosity group): a connective graft was harvested from the Tuberosity and placed in the recipient site, which is the same site of the dental implant procedure.

The surgical procedure:

On the day of the surgery, the sealed randomization envelopes were opened and the patient was assigned to the study or control group.

The test group (Tuberosity connective tissue graft and I-PRF):

Preparing the recipient site:

The beginning was by preparing the recipient site which is the dental implant site that also requires peri-implant soft tissue augmentation procedure. After administering the local anesthesia, a horizontal incision was performed followed by elevating a partial-thickness flap, drilling the implant site according to the implant system's instructions, installing the implant, and placing the cover screw.

I-PRF preparation:

Before starting blood collection, all devices were prepared and the appropriate settings were set. In each patient in the study group, 10 ml of blood was drawn from a vein and transferred to a test tube without anticoagulants. Since anticoagulants were not used, blood was drawn and directly transferred into tubes, and centrifuged in order to enhance the regenerative capacity of I-PRF. To obtain the liquid form, centrifugation was performed as follows: 700 rpm for 3 minutes. After completing the centrifugation, a syringe was used to withdraw the I-PRF in order to be mixed with the connective tissue graft harvested from Maxillary Tuberosity [14].

Harvesting the connective tissue graft:

The Maxillary Tuberosity was anesthetized with local infiltration anesthesia and then the dimensions of the graft were determined according to the needs of the recipient site. A free gingival graft was harvested from tuberosity (Figure 1) and then the epithelium was removed outside the mouth with a 15-c blade (Figure 2). The donor site was managed by placing an absorbable collagen sponge and securing it with surgical sutures (5-0 nylon).

Suturing the connective tissue graft into the recipient site:
The connective tissue graft (CTG) harvested from the tuberosity was placed in a sterile container and mixed with I-PRF for a minimum of 5 minutes (Figure 3), and then the I-PRF-covered CTG was sutured at the recipient site covering the occlusal area and extending toward the vestibular area of the implant site. A horizontal mattress suturing technique was used to fix the CTG over the periosteum and ensuring its stability in the recipient site. The surgical flap was then closed with nylon sutures (5-0) so that the recipient site healed by the first intention.

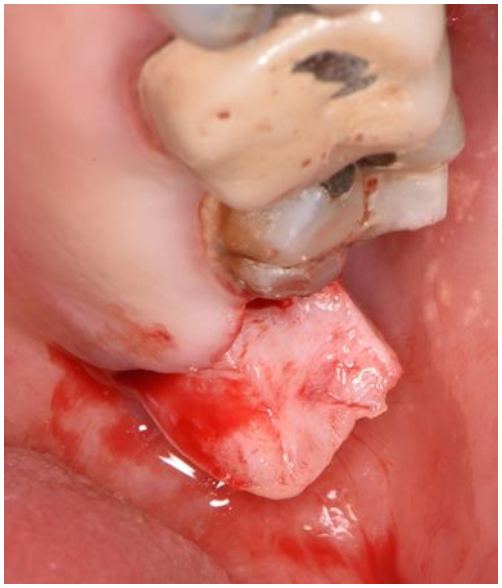

Figure 1: Harvesting CTG from the Tuberosity

Figure 2: De-epithelizing of the CTG

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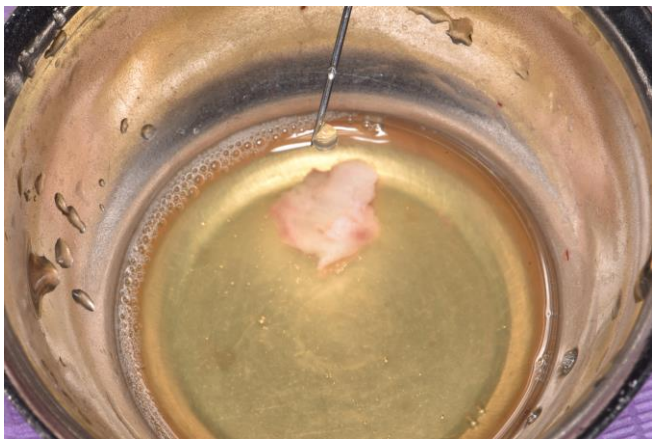

Figure 3: Mixing the I-PRF with the CTG

The control group (the Maxillary CTG):

In this group, the surgical procedure was accomplished through the same steps as before but without using I-PRF as the connective tissue graft was used alone in this group (Figure 4). The recipient site was prepared and the dental implant was installed. The CTG was harvested using the same previous method, and sutured to the recipient site. The surgical flap was closed with nylon sutures (5-0) and the recipient site was healed by the first intention.

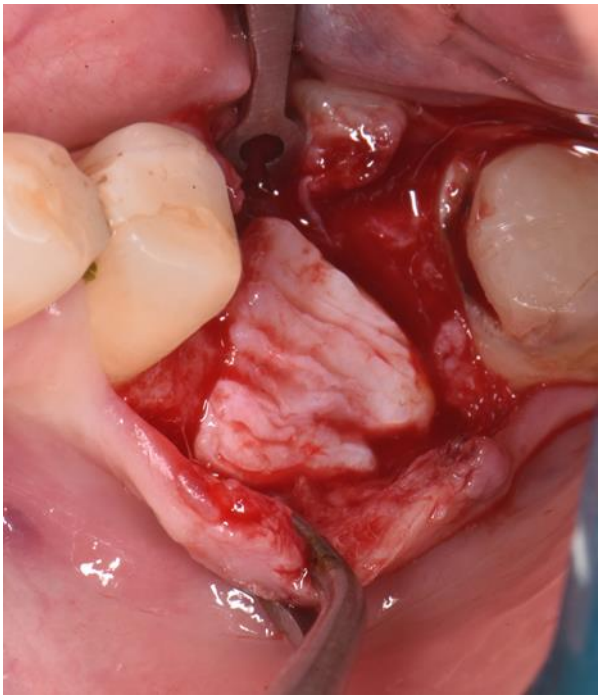

Figure 4: Suturing the CTG at the recipient site

Postoperative recommendations:

Each patient received post-operative instructions, which included prescribing an antibiotic (amoxicillin 500 mg every 8 hours for 7 days), and non-steroidal anti-inflammatory drugs (ibuprofen 600 mg when needed). Oral care instructions also included not brushing the recipient site and using an oral rinse (chlorhexidine 0.12%) twice daily for two weeks. Patients were also asked to avoid hard and hot foods on the day of surgery and to avoid foods and drinks that may contain irritating substances that cause irritation of the donor site. The sutures were removed after 2 weeks.

Primary outcome measure:

Wound Healing Index (WHI):

Wound healing at the recipient site (implantation site) was assessed at day 7 and weeks 2, 3, and 4 with the following grades [15]:

- Grade 1 = uneventful healing with no gingival edema, redness, suppuration, patient discomfort, or flap tearing.
- Grade 2 = uneventful healing with slight gingival edema, slight redness, slight patient discomfort, or slight flap tearing but no suppuration.
- Grade 3 = poor wound healing with significant gingival edema, redness, patient discomfort, flap rupture, or any suppuration.

Data were recorded on each patient's research card and collected for later study

Statistical analysis:

The research data was transferred to SPSS V.22 for statistical analysis. The data were analyzed using descriptive analysis (the mean, standard deviation, percentages), and inferential analysis was also used to study the existence of a significant difference between the values of the wound healing index between the two study groups, where the Kruskal-Wallis test was used to study the significant difference between the two groups.

3. Results

The study included 20 sites (1 site in male and 19 sites in females), where the number of sites in the Tuberosity CTG with I-PRF was 10 sites (50%) and in the Tuberosity CTG group was 10 sites (50%). All sample members completed the study without withdrawals.

Table .1 shows the descriptive analysis of wound healing index according to the study groups, where the Tuberosity CTG with I-PRF achieved complete healing at the third week. The Tuberosity CTG group also achieved complete healing by the third week.

Table .2 shows the results of applying the Kruskal-Wallis test to study the presence of a significant difference between the two groups in terms of wound healing. The test results showed that there was no significant difference between the two groups at all measurement times, as the value of the significance level was greater than (0.05), which indicates that the healing was similar between the two groups.

Table .1: Descriptive analysis of wound healing index at recipeint site by group

		Grade 1		Grade 2		Grade 3	
		Number	percentage	number	percentage	number	percentage
Control group (CTG)	7 days	4	40.0%	5	50.0%	1	10.0%
	2nd week	6	60.0%	4	40.0%	0	0.0%
	3rd week	10	100.0%	0	0.0%	0	0.0%
	4th week	10	100.0%	0	0.0%	0	0.0%
Test group (CTG+ I-PRF)	7 days	7	70.0%	2	20.0%	1	10.0%
	2nd week	8	80.0%	2	20.0%	0	0.0%
	3rd week	10	100.0%	0	0.0%	0	0.0%
	4th week	10	100.0%	0	0.0%	0	0.0%

Table .2: significant difference in WHI index

	Group	number	mean rank	P-value	sig.
7TH day	**CTG**	10	22.9	0.658	no significant difference
	CTG+I-PRF	10	17.65		

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	Total	20			
2nd week	**CTG**	10	23.1	0.743	no significant difference
	CTG+I-PRF	10	19.3		
	Total	20			
3rd week	**CTG**	10	19.5	0.104	no significant difference
	CTG+I-PRF	10	19.5		
	Total	20			
4th week	**CTG**	10	20.5	1.000	no significant difference
	CTG+I-PRF	10	20.5		
	Total	20			

4. Discussion

The health of peri-implant soft tissue is one of the most important factors in ensuring the success of implants. In the process of healing and regeneration of these tissues, the quality and quantity of the surrounding soft and hard tissues could be improved by increasing the regenerative capacity using appropriate stimuli. Different growth factors are expressed during different stages of tissue healing and could act as factors that promote and accelerate tissue repair. Blood concentrates are one of the various growth factors and PRF has proven its effectiveness in many fields of dentistry and periodontal surgery [6]. This study aimed to evaluate the ability of I-PRF to stimulate wound healing when added to a CTG in the context of peri-implant soft tissue augmentation. The results showed that the healing of the recipient site in the test group (CTG+ I-PRF) was better than the healing in the control group in which the CTG was sutured over the dental implant alone without adding I-PRF, but the difference was not statistically significant in favor of the test group, which means the need for further research on the application of I-PRF in this context is needed.

I-PRF is a new form of platelet concentrate enriched with leukocytes that could enhance soft and hard tissue regeneration phenomena [8], [16]. I-PRF remains liquid for approximately 15 minutes, so it provides dental practitioners with an additional practical form of PRF [14]. Studies have also shown that I-PRF has anti-inflammatory and antibacterial activity, which may contribute to faster tissue regeneration [17], [18]. I-PRF, on the other hand, is commonly used as an injectable biomaterial to help carry various biomolecules, or in combination with other biomaterials in a variety of clinical applications. Clinicians have recently used this method to facilitate agglomeration or encapsulation of biomaterials and grafts in order to improve the healing process of both soft and hard tissues [13], [19].

Platelets represent an endogenous source of more than 1,500 biological factors (including growth factors, immune system messages, and enzymes) that are vital for tissue repair and wound healing [20], [21]. The ability of these concentrated platelets to deliver 6-8 times higher than physiological doses of growth factors [22] represents the primary mechanism that stimulates wound healing. Growth factors released from platelets also stimulate further recruitment and proliferation of stem cells and other cells involved in the healing process [21]. Platelets are the dominant component of PRF and are responsible for the biological activity of PRF, have a crucial role in blood clot formation and also contain various protein molecules involved in wound healing signaling cascades [23].

Platelet-rich fibrin contains high levels of growth factors including platelet derived growth factors (PDGF), transforming growth factors β1 (TGF β1), and transforming growth factors β2 (TGF β2). Vascular endothelial growth factors (VEGF), platelet derived endothelial growth factors, interleukin 1 and 2, basic fibroblast growth factor (β-FGF), and platelet-activating factor-4. activating factor 4 (PAF-4) [24]. The cascade of interaction with these factors involves the immediate binding of secreted growth factors to membrane receptors located on the outer surface of cell membranes in the gingival graft, flap, or wound.

This results in the activation of an endogenous internal signal protein, which also initiates the expression of normal cell gene sequences such as extracellular matrix formation, cellular proliferation, bone production, and collagen synthesis [25], [6]. This adjuvant role of platelet-derived growth factors in bone and soft tissue healing has been shown in the medical literature [26]. All of the previous concepts reflect the ability of I-PRF to stimulate and accelerate healing when applied topically to soft and hard tissues, which was demonstrated by the results of this study.

In the medical literature, I-PRF has been applied several times with dental implants. A previous study noted an additional effect and clinical efficacy of PRF on bone tissue formation around single implants with immediate loading in the anterior region of the Maxillary. The available data showed that the application of PRF during implant placement has a stimulatory effect on bone formation [27]. Several previous studies have documented that PRF contains several growth factors that promote and stimulate the healing of soft and hard tissues around implants [24], [28]. There have been no studies examining healing when adding I-PRF to soft tissue grafts in the context of dental implants.

Healing in the control group was also good, although the I-PRF wasn't added to the recipient site. According to the medical literature, it could be said that suturing CTG tightly to the periosteum or adjacent soft tissue flap may positively affect the mechanical properties of the wound and increase the stability of the mucosal flap by improving the adhesion of the blood clot and its maturation. Without a CTG, the tension of the flap may directly affect the stability of the blood clot, which may lead to an increased risk of tearing between the surface of the lining tissue and the flap. Therefore, the prevailing hypothesis is that the CTG may actually improve the stability of the wound, as the tensile strength of the wound increases significantly with increasing time. The presence of a CTG layer helps increase the forces required to tear the flap from the wound bed compared to suturing the flap without the presence of a CTG layer [29]. The previous information is consistent with the results shown by the current study, which found that healing was similar in the two groups, with a non-statistically significant advantage for the test group, which benefited from all the features of I-PRF in improving healing.

To our knowledge, there is no study that has shown the effect of I-PRF on the healing of peri-implant soft tissue. Monitoring wound healing after surgery is an interesting topic in dentistry and has been extensively studied. Several studies have been conducted recently to review all the information about the clinical and histological features of the uncomplicated oral wound healing process, however no consistent parameters on how to monitor wound healing after specific surgical procedures have been reported [30]. Our current study monitored wound healing in one of the most common procedures in dentistry today, which is peri-implant soft tissue augmentation using connective tissue graft that was harvested from the Tuberosity, In the current study, dental implantation and soft tissue augmentation were performed simultaneously, and I-PRF was applied by mixing it with the CTG before suturing it to the recipient site. This study has several limitations as the initial monitoring point for wound healing was 7 days after the procedure and we were unable to perform daily monitoring to investigate the benefit of I-PRF in improving healing during the early stages of this procedure. More research needs to be done to evaluate the ability of I-PRF to improve healing and apply it in different ways, perhaps by injecting it into the recipient site, taking advantage of its liquid form, and investigating the benefit of applying it multiple times on the healing process.

5. Conclusions

Application of I-PRF with the CTG improved wound healing at the recipient site in the context of peri-implant soft tissue augmentation, but there was no statistically significant difference compared to application of the CTG alone.

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