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THE USE OF PLATELET-RICH FIBRIN COMBINED WITH FREE GINGIVAL GRAFT IN THE MANAGEMENT OF INSUFFICIENT ATTACHED GINGIVA AT TEETH WITH GINGIVAL RECESSION

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ABSTRACT

Aim: This study was carried to evaluate the effect of the placement of PRF membrane beneath the free gingival graft in gingival augmentation surgeries regarding the changes by time in graft horizontal dimension, vertical dimension and total surface area.

Subjects and Methods: Sixteen patients were selected. All the patients showed gingival recession with insufficient attached gingiva. The gingival augmentation surgery was decided to be performed as a primary surgery to increase the attached gingiva with no attempt to cover the recession during this study. Group 1 (test group) included eight patients where the gingival augmentation was performed using a free gingival graft in combination with a platelet rich fibrin membrane placed beneath the graft at the recipient site. Group 2 (control group) included eight patients where the gingival augmentation was performed using a free gingival graft only. The graft vertical dimension (GVD) and the graft horizontal dimension (GHD) were measured using William's graduated periodontal probe. Plaque index, gingival bleeding index and probing depth were used to monitor the oral hygiene status and gingival health condition throughout the study. All the measures were obtained at 2 occasions; the day of the surgery and 1.5 months after the surgery.

Results: Regarding the changes in GVD, a statistically significant greater mean and median percent decrease was noted in group 2 after 1.5 month. The same was obtained with the changes in GHD and graft surface area.

Conclusion: Using platelet-rich fibrin beneath free gingival graft in gingival augmentation surgery resulted in successful increase in the attached gingiva with less dimensional changes by time in the transplanted free gingival graft compared to using free gingival graft alone.

INTRODUCTION

The attached gingiva represents only part of the keratinized gingiva extending from the free gingival groove till the mucogingival junction while the keratinized gingiva extends from the gingival margin till the mucogingival junction ***(Joshi et al., 2016)***

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Although many studies showed that at least 1 mm of attached gingiva is necessary to maintain healthy periodontium, other studies believed that the exact amount of attached gingiva necessary for healthy periodontium is controversial. ***(Gargiulo et al.,1961; Dorfamn et al., 1980; Dorfman et al., 1982; Ericsson&Lindhe, 1984; Kennedy et al., 1985; Nevins et al., 1986; Wennstrom, 1987; Freedman et al., 1999).***

Some authors showed that the presence of moveable alveolar mucosa only without attached gingiva may be kept healthy for a long period when the patient oral hygiene and plaque control measures were well performed ***(Kisch et al., 1986; Salkin et al., 1987).*** Other authors proved that teeth with narrow zone of attached gingiva suffers higher inflammatory and recession indices than teeth with enough amount of attached gingiva believing that the wide attached gingiva better withstands trauma from mastication and tooth brushing and better resists gingival inflammation and pulling forces of the muscles.***(Wennstrom et al., 1983; Ericsson &Lindhe, 1984; Stetler & Bissada, 1987)***

Long tapered teeth are more susceptible to gingival recession when compared to square shaped teeth since wider zone of attached gingiva is usually associated with the square shaped teeth ***(Weisgold, 1977; Olsson et al., 1993)***. The gingival biotype commonly known as being thick or thin ***(Weisgold, 1977)***, has been recently classified as thin scalloped, thick flat or thick scalloped.***(De Rouck et al., 2009; Eghbali et al., 2009)***. It can be measured via direct visual assessment,ultrasonic devices and Cone beam computed tomography while the most applicable commonly used method is the probe transparency through the gingiva ***(Greenberg et al., 1976; Muller et al., 2000; Barriviera et al., 2009;; Stein et al., 2013)***. Studies concluded that thin scalloped biotype is associated with narrow zone of attached gingiva and is subjected to higher risk of gingival recession compared to thick flat biotype ***(Olsson et al., 1993; Anderegg et al., 1995; Baldi et al., 1999; Pontoriero & Carnevale, 2001; Hwang and Wang, 2006; Gobbato et al., 2012)***

Wilson in 1983 monitored teeth with inadequate attached gingiva &showed that they were subjected to mucogingival defects and further recession ***(Wilson, 1983)***. Surgical augmentation of the attached gingiva improves patient comfort and facilitates the control of dental plaque ***(Lindhe et al., 1996)***. Long term studies showed that gingival augmentation procedures helped in avoiding gingival recession overtime ***(Agudio et al., 2008; Agudio et al., 2009)***

Free gingival autografts (FGG) is considered simple, easy, highly predictable gingival augmentation modality. The free gingival autograft first described by Bjorn in 1963 and Sullivan &Atkins in 1968 has been used to increase the zone of attached gingiva, deepen the vestibule and in treatment of gingival recession ***(Bjorn, 1963; Sullivan & Atkins, 1968; Holbrook & Ochsenbein, 1983; Miller, 1985).*** The most common site for harvesting the FGG is the palate in the zone between the canine and the first molar palatal root .The greater and lesser palatine nerves & blood vessels should be avoided during graft harvesting .They enter the palate through the greater and lesser palatine foramina close to the third molar and pass across the palate into the incisive foramen. Usually, they are located 7-17 mm from the CEJ of upper premolars and molars being closer to the CEJ in shallow palatal vaults ***(Reiser et al., 1996).*** The palatal rugae area should not be included in the graft since it may lead to poor esthetic results and will keep its morphology permanently ***(Cohen, 1994; Breault et al., 1999)***

The thickness of the palatal graft should range from 0.75 -1.5 mm to make sure that adequate amount of connective tissue has been included in the graft. This was based upon the fact that the mean thickness of the palatal epithelium is

0.34 mm. ***(Soehren, 1973; Carranza, 1976; Goldman et al., 1976; Camarago et al., 2001)***

It should be noted that thicker grafts may end up with unaesthetic results in addition to deep palatal donor site, while thin grafts are associated with ideal color matching and accepted esthetic outcomes. ***(Sullivan and Atkins, 1968; Brackett & Gargiulo, 1970; Lampert et al., 1976).*** Moreover thin grafts revascularize and heal faster than thicker grafts since thick connective tissue shows greater primary contraction, collapse of blood vessels and delayed revascularization ***(Sullivan & Atkins, 1968; Camargo et al., 2001)***

During the first day after grafting and before the revascularization of the graft, the graft is totally dependent on plasmic circulation from the recipient bed ***(Forman, 1960; Reese & Stark, 1961).*** Capillaries proliferation into the graft starts by day 2 after the surgery and sufficient blood supply appear by day 8. The connective tissue union between the graft and bed start by day 4 and is completed at day 10 and may be responsible for some of the contraction of the graft.***(Davis & Traut, 1925; Egli et al., 1975)***

Many studies proved that the transplanted tissue showed changes in the horizontal and vertical dimensions over time after grafting and especially during the first postoperative month. (***Ericsson 1984; Hatipoglu et al 2007)***. The shrinkage of the free gingival graft is about 32%-45% and most of the graft shrinkage has been shown to occur during the first year after surgery then the dimensions stabilized ***(Dreeskamp & de Jacoby, 1973; Soehren et al., 1973; Ward, 1974; Egli et al., 1975; James and Mc Fall, 1978; Rateitschak, 1979; Orsini et al., 2004; Barbosa et al., 2009)***. Many authors proved that the graft shrinks vertically more than horizontally. ***(Orsini et al., 2004; Hatipoglu et al., 2007)***

Platelet –rich fibrin (PRF) is a platelet concentrate with leukocytes in a dense fibrin matrix. It is an autogenous material prepared by centrifuging the patient's own non-anticoagulated blood ***(Choukroun and Diss, 2006).*** PRF is considered second generation of platelets concentrates and an improved formulation of the traditional platelet-rich plasma (PRP***). (Choukroun et al., 2001; Dohan Ehrenfest et al., 2010)***. PRF was introduced in France by choukroun et al., in 2001. The patient's whole non-anticoagulated blood is immediately centrifuged after withdrawal from the patient resulting in PRF in short period of time (***Choukroun et al., 2001; Simonpieri et al., 2009)***. Being prepared without adding anticoagulant to the blood samples, the PRF can be considered purely autologous. The fibrin matrix obtained is rich in platelets , leukocytes and many growth factors including transforming growth factor beta 1 (TGF-β1), vascular endothelial growth factor (VEGF), interleukin beta (IL-1β), platelet derived growth factor PDGF, IL-4, IL-6. ***(Dohan et al., 2006)***. The L-PRF membrane is modified PRF to contain most of the platelets and leukocytes present in the blood plus the platelet growth factors and stem cells that are also trapped within the fibrin network with enhanced strength (***Dohan Ehrenfest 2018).***

The original idea behind platelet concentrates is that concentrated platelets and autologous growth factors are collected in a structure that can be used to enhance cellular migration, adhesion & proliferation at the surgical sites thus promoting rapid organization of the tissues and early healing. ***(Anfossi et al., 1989; Fijnheer et al., 1990; Whitman et al., 1997; Marx et al., 1998; Marx, 2004; Dohan & Choukroun, 2006 ; Jameson, 2007; Kutkut & Andreana, 2012)***

Since the recipient site healing following the free gingival grafts is a complicated mechanism as discussed above and depends initially on the plasmic circulation which requires excellent free gingival graft adaptation and stabilization at the recipient site. Moreover, since postoperative graft shrinkage is an inevitable process according to

previous literature. Thus, FGG combined with PRF in augmentation of attached gingiva by placing the PRF membrane beneath the FGG may have a beneficial effect on healing since PRF is rich in many critical factors that may aid healing and may improve the final grafting outcomes.

AIM OF THE STUDY:

This study was carried to evaluate the effect of the placement of PRF membrane beneath the free gingival graft in gingival augmentation surgeries regarding the changes over time in graft's horizontal dimension, vertical dimension and total surface area.

SUBJECTS AND METHODS:

Sixteen patients were selected from the outpatients' clinic of the department of Oral medicine and periodontology, faculty of dentistry, Ain Shams University.

All the patients showed gingival recession (class 1 or 2, Miller 1985) at two lower anterior neighboring teeth with insufficient attached gingiva <1mm and thin gingival biotype ≤1mm. The gingival augmentation surgery was decided to be performed as a primary surgery to increase the attached gingiva with no attempt to cover the recession during this study.

All the patients included in the study were free from any systemic diseases as evidenced by Burket's oral medicine health history questionnaire ***(Glick et al., 2008),*** age range 30-45, non-smokers. Any patients with mobility, crowding or subgingival restorations in the lower anteriors were excluded from the study. Any patients with probing depth ≥ 2mm were excluded from the study. Patients with palatal abnormalities or anatomic variations and patients who did not follow the oral hygiene instructions for proper plaque control after the conventional periodontal therapy were also excluded from the study. Roll technique was applied to determine the keratinized tissue width 2 weeks after initial periodontal treatment.

Patients were randomly divided in 2 groups. Group 1 (test group) included eight patients where the gingival augmentation was performed using a free gingival graft (FGG) in combination with a platelet rich fibrin (PRF) membrane. The PRF membrane was placed beneath the FGG at the recipient site. Group 2 (control group) included eight patients where the gingival augmentation was performed using a free gingival graft only. All the patients were informed about the full details of the surgery and an informed consent was taken.

Treatment protocol

Conventional periodontal treatment was performed to all patients two weeks before the surgical procedure and oral hygiene instructions were given. Preoperative photographs and periodontal measures (plaque index, gingival bleeding index, probing depth) were performed the day of the surgery just before starting the procedure.

Surgical steps: (Figure 1)

Preparation of the Leukocyte Platelet-Rich Fibrin (L-PRF) (for group 1 only):

Blood was collected in 9ml glass-coated plastic tube and then rapidly (before 1 minute) centrifuged at room temperature (2700 rpm for 12 minutes) producing L-PRF clot. The clot was placed in the sterile adapted surgical box and compressed into a membrane to be used later beneath the free gingival graft (Corso et al., 2010, Dohan Ehrenfest et al., 2018)

Preparation of the recipient site (for both groups)

After the administration of local anaesthesia to the recipient site *(*Articaine hydrochloride 4% with 1/100000 epinephrine, septanest SP, Septodont), Horizontal incision was performed below the gingival recession at the mucogingival junction using the scalpel with no 15-c blade. The horizontal

incision should extend one tooth mesial and distal to the defect. Two vertical incisions were performed at the end of the horizontal incisions extending into the alveolar mucosa beyond the mucogingival junction (MGJ).

Partial thickness flap was dissected using the scalpel (blade 15c) starting at the apical portion of the vertical incision in apicocoronal fashion leaving the periosteum on the alveolar bone. The partial-thickness flap was displaced apically enough. Special care was taken during reflection in order to dissect as much as possible close to the periosteum to be sure that the epithelium, connective tissue and muscles were elevated thus recipient bed without moveable soft tissues was present which ensures that the free gingival graft later on will be stable during healing. The partial thickness flap was then sutured with interrupted sutures to the periosteum apically (polypropylene 4/0 interrupted sutures- blue monofilament, Assut sutures). Measurement of the denuded recipient area vertically and horizontally was performed using the periodontal probe to prepare the suitable size of free gingival graft.

Graft harvesting from the palate (donor site) (for both groups):

The free gingival graft (FGG) was harvested with a scalpel (blade 15c) from the hard palate opposite to the premolars region according to the measurement of the recipient site. The FGG rectangular outline was performed by the scalpel not reaching the palatal bone then the FGG was dissected with the scalpel used parallel to the outer surface of the graft . The graft obtained was thinned using sharp curved scissors to obtain a uniform graft thickness around 1.5mm thickness. Graft suturing at the recipient site (polypropylene 4/0 interrupted sutures- blue monofilament, Assut sutures) was then performed starting with interrupted sutures to the neighbouring teeth attached gingiva mesially and distally securing the graft at the recipient bed. Single intra- periosteal cross over suture was then performed aimed to immobilize the graft and achieve better adaptation. For group 1only (test group), the formerly prepared PRF membrane was placed beneath the sutured FGG after the interrupted sutures and before performing the cross over suture. For both groups, after performing the cross over suture, gentle firm pressure with gauze was applied for 5 minutes over the graft to displace any blood below the graft ensuring graft adaptation, stabilization and minimal blood clot thickness and rapid revascularization . Finally, the palatal wound in both groups is covered by a small piece of PRF

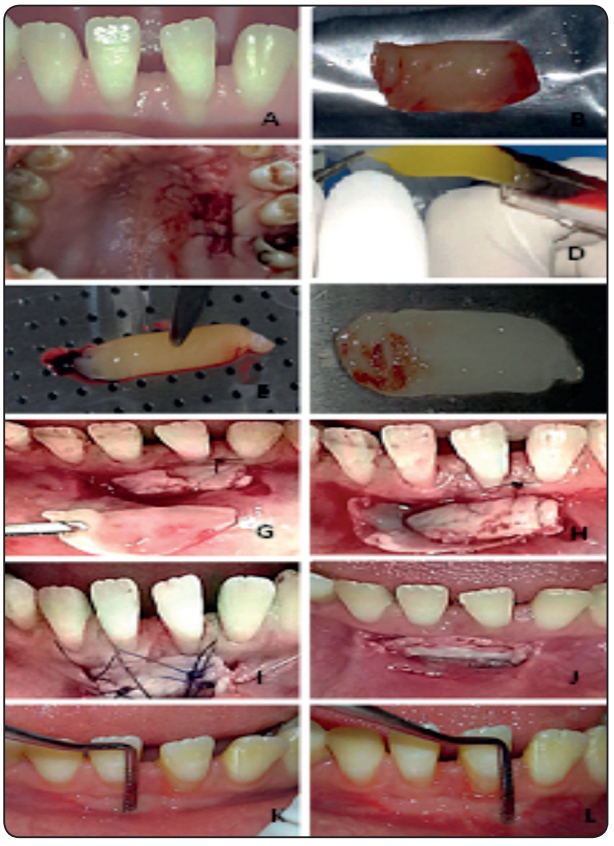

Fig. (1) A-L Free gingival graft combined with PRF surgical procedure. A: Lower incisors lack attached gingiva. B,C: Free gingival graft harvested from the hard palate. D, E, F: preparation of the PRF membrane. G, H: PRF membrane placed beneath the free gingival graft at the recipient site. I: suturing of the free gingival graft. J: recipient site 10 days after surgery. K, L: recipient site 1.5 month after surgery.

membrane stabilized by horizontal matress suture.

For both groups, postoperative medications were prescribed [amoxicillin 875mg /clavulanic acid 125 mg - orally 1 tablet every 12 hours for 7 days (Hibiotic 1gm tablets/ Amoun Pharmaceuticals) and dexamethasone phosphate 8 mg I.M. single injection (Epidron ampoule 2ml; Eipico)] and the patients were advised to avoid brushing and flossing the surgical site and to use chlorhexidine HCL mouth wash three times daily until suture removal 10 days after the surgery.

Evaluation and measures:

The graft vertical graft dimension (GVD, apicocoronal measure) and the graft horizontal dimension (GHD, mesiodistal measure) were measured throughout the study to the nearest mm using William's graduated periodontal probe. Graft surface area (GSA) was obtained by multiplying the graft horizontal and vertical dimensions. Plaque index, gingival bleeding index and probing depth were used to monitor the oral hygiene status and gingival health condition throughout the study.

Plaque Index (PI) (Löe 1967) was scored as follows: 0: No plaque in gingival area, 1: No plaque visible by the unaided eye, but plaque is made visible on the point of the probe after it has been moved across surface at entrance of gingival crevice, 2: Gingival area is covered with a thin to moderately thick layer of plaque; deposit is visible to the naked eye, 3: Heavy accumulation of plaque within the gingival pocket and/or on the gingival margin and adjacent tooth surface. Sulcus Bleeding Index (BI) (Mühlemann & Son 1971) scored as follows: 0: No inflammation, no bleeding on probing. 1: Bleeding from gingival sulcus on gentle probing; tissues otherwise appears normal, 2: Bleeding on probing plus change in color due to inflammation, 3: Bleeding on probing plus change in color and slight edema, 4: Bleeding on probing, color change and obvious edema, 5: Spontaneous bleeding, color change and marked edema. Probing Depth (PD) (Glavind & Löe 1967) was measured from the gingival margin to the base of the pocket to the nearest mm.

All the previous measures were obtained in this study at 2 occasions; baseline and follow up. The baseline measures were obtained the day of the surgery. The bleeding index, plaque index and probing depth were performed before the surgery while measurement of the graft dimensions was performed after graft suturing. The follow up measures were obtained 1.5 months after the surgery. Regarding the measurements of the graft dimensions, for every patient at each occasion the vertical measure was performed at three points along the length of the graft; one mesial, one distal and one in the middle of the graft then the mean graft vertical dimension was calculated. The same was performed with the horizontal graft dimension, the horizontal measure was performed at three points; one coronal, one apical and one in the middle of the graft then the mean graft horizontal dimension was calculated.

Statistical analysis

Numerical data were explored for normality using Kolmogorov-Smirnov test of normality. Normally distributed (parametric data), were presented as mean, standard deviation (SD), minimum and maximum and were compared between groups using independent t test (intergroup). Paired t test was used for intergroup comparison in different observation times. For non- parametric data, percent change groups were compared using Mann Whitney U test.

RESULTS

Probing depth, bleeding index and plaque index: In group I, Wilcoxon signed Rank test revealed no significant difference between baseline and 1.5 month values of PD, BI, PI (p=0.317,

p=0.317, p=0.157 respectively). In group II, Wilcoxon signed Rank test revealed no significant difference between baseline and 1.5 month values of PD, BI, PI (p=1, p=0.157, p=0.157 respectively). The same median baseline PD, BI, PI values were recorded in both groups, with no significant difference as indicated by Mann Whitney test (p=0.317, p=1, p=1 respectively) .The same median 1.5 month PD, BI, PI values were recorded in both groups, with no significant difference as indicated by Mann Whitney test (p=1, p=0.591, p=1 respectively).

Graft vertical dimensions: In group 1, Mean value decreased from (5.75±0.46) at baseline, to (5.25±0.46) at 1.5month. Paired t test indicated that the difference between baseline and 1.5 months was statistically significant (p=0.033), (Table 1, Fig.2). In group 2, Mean value decreased from (5.75±0.46) at baseline, to (3.75±0.71) at 1.5month. Paired t test indicated that the difference between baseline and 1.5 month was statistically significant (p=0.00), (Table 1, Fig.2). At baseline, there was no statistically significant difference between groups (p=1). At 1.5 month, a higher mean value was recorded in group 1, with extremely significant difference (p=0.00), (Table1, Fig.2)

Graft horizontal dimensions: In group 1, Mean value decreased from (12.88±0.64) at baseline, to (11.50±1.2) at 1.5month. Paired t test indicated that the difference between baseline and 1.5 month was statistically significant (p=0.004), (Table 2, Fig.3). In group 2, Mean value decreased from (13.38±0.52) at baseline, to (9.63±0.52) at 1.5month. Paired t test indicated that the difference between baseline and 1.5 month was statistically significant (p=0.00), (Table 2, Fig.3). At baseline, there was no statistically significant difference between groups (p=0.11). At 1.5 month, a higher mean value was recorded in group 1, with a statistically significant difference (p=0.00), (Table 2, Fig.3)

Graft Surface area: In group 1, Mean value

TABLE (1) Descriptive statistics of graft vertical dimension (mm), intergroup comparison (independent t test) and intragroup comparison (paired t test)

Groups		Baseline	1.5 month	Mean difference	P
Group 1	Mean	5.75	5.25	.50	.033*
	Std. Dev	.46	.46		
	Std. Error	.16	.16		
	Median	6.00	5.00		
	Range	1.00	1.00		
	Min	5.00	5.00		
	Max	6.00	6.00		
Group 2	Mean	5.75	3.75	2.00	0.00*
	Std. Dev	.46	.71		
	Std. Error	.16	.25		
	Median	6.00	4.00		
	Range	1.00	2.00		
	Min	5.00	3.00		
	Max	6.00	5.00		
Intergroup comparison (P)		1.00 NS	0.00*	------	-------
Mean difference		0.00	1.50		
Confidence interval of difference		-.50 to .50	.85 to 2.15		

Significance level p<0.05, *significant, ns=non significant

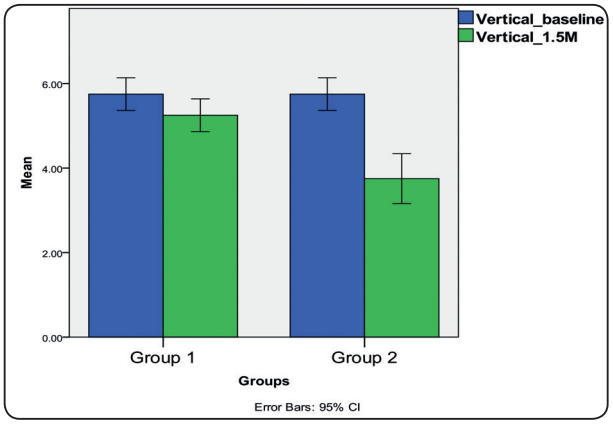

Fig. (2) Bar chart showing mean vertical graft dimension (mm) in both groups

TABLE (2) Descriptive statistics of graft horizontal dimension (mm), intergroup comparison (independent t test) and intragroup comparisons (paired t test)

Groups		Baseline	1.5 month	Mean difference	P
Group 1	Mean	12.88	11.50	1.38	.004*
	Std. Dev	.64	1.20		
	Std. Error	.23	.42		
	Range	2.00	3.00		
	Min	12.00	10.00		
	Max	14.00	13.00		
Group 2	Mean	13.38	9.63	3.75	0.00*
	Std. Dev	.52	.52		
	Std. Error	.18	.18		
	Median	13.00	10.00		
	Range	1.00	1.00		
	Min	13.00	9.00		
	Max	14.00	10.00		
Intergroup comparison (P)		.11 NS	.00*	----------	-------
Mean difference		-.50	1.88		
Confidence interval of difference		-1.12 to .12	.84 to 2.91		

Significance level p<0.05, *significant, ns=non significant

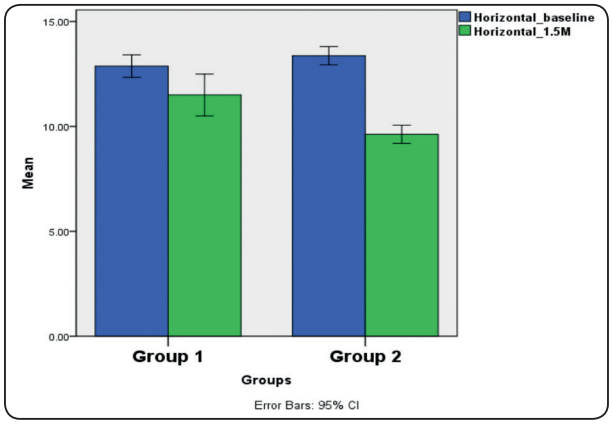

Fig. (3) Bar chart showing mean horizontal graft dimension (mm) in both groups

decreased from (74.13±7.97) at baseline, to (60.75±11.3) at 1.5month. Paired t test revealed that the difference between baseline and 1.5 month was statistically significant (p=0.01), (Table 3, Fig.4). In group 2, Mean value decreased from (76.88±6.49) at baseline, to (35.88±5.62) at 1.5month. Paired t test revealed that the difference between baseline and 1.5 month was statistically significant (p=0.00), (Table 3, Fig.4). At baseline, there was no statistically significant difference between groups (p=0.46). At 1.5 month, a higher mean value was recorded in group 1, with a statistically significant difference (p=0.00), (Table 3, Fig.4)

TABLE (3) Descriptive statistics of surface area (mm^2), intergroup comparison (independent t test) and intragroup comparisons (paired t test)

Groups		Baseline	1.5 month	Mean difference	P (paired t test)
Group 1	Mean	74.13	60.75	13.38	.010*
	Std. Dev	7.97	11.30		
	Std. Error	2.82	3.99		
	Median	78.00	57.50		
	Range	24.00	28.00		
	Min	60.00	50.00		
	Max	84.00	78.00		
Group 2	Mean	76.88	35.88	41.00	0.00*
	Std. Dev	6.49	5.62		
	Std. Error	2.29	1.99		
	Median	78.00	36.00		
	Range	19.00	15.00		
	Min	65.00	30.00		
	Max	84.00	45.00		
Intergroup comparison (P)		.46 NS	0.00*	----------	----
Mean difference		-2.75	24.88		
Confidence interval of difference		-10.58 to 5.08	14.97 to 34.78		

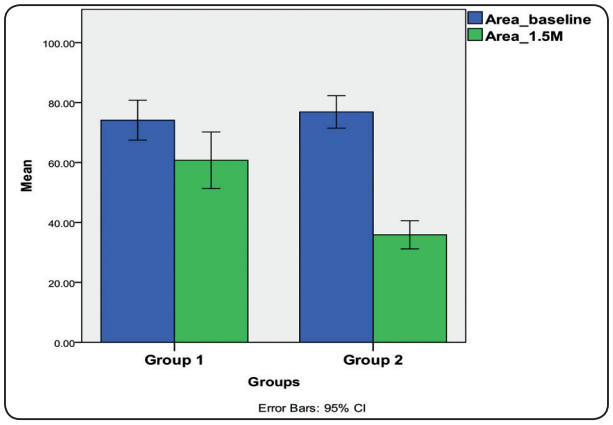

Fig. (4) Bar chart showing mean surface area (mm2) in both groups

Significance level p<0.05, *significant, ns=non significant

TABLE (4) Descriptive statistics of percent change (%) and intergroup comparisons (Mann Whitney U test)

Groups		Vertical dimension	Horizontal dimension	Surface area
Group 1	Median	-8.33	-14.84	-22.62
	Mean	-8.33	-10.76	-17.81
	Std. Dev	8.91	7.23	13.57
	Std. Error	3.15	2.56	4.80
	Range	16.67	16.67	30.56
	Min	-16.67	-16.67	-30.56
	Max	.00	.00	.00
Group 2	Median	-33.33	-28.57	-53.85
	Mean	-35.00	-28.02	-53.30
	Std. Dev	9.43	3.22	6.31
	Std. Error	3.33	1.14	2.23
	Range	33.33	7.69	21.98
	Min	-50.00	-30.77	-64.29
	Max	-16.67	-23.08	-42.31
P (Mann Whitney U test)		.001*	.001*	.001*
Mean difference		26.67	17.26	35.49
Confidence interval of difference		16.83 to36.51	11.00 to 23.53	23.68 to 47.30

Significance level p<0.05, *significant

Percent change: Regarding the graft vertical dimension; a greater mean and median percent decrease was noted in group 2. Mann Whitney U test revealed that this difference was statistically significant (p=0.001). Regarding the graft horizontal dimension; a greater mean and median percent decrease was noted in group 2. Mann Whitney U test revealed that this difference was statistically significant (p=0.001). Regarding the graft Surface area; a greater mean and median percent decrease was noted in group 2. Mann Whitney U test revealed that this difference was statistically significant (p=0.001). (Table 4)

DISCUSSION

Many studies showed that the presence of attached gingiva is critical for the prevention of gingival recession and maintenance of healthy periodontium (Lang & Loe, 1972). The free gingival graft (FGG) is a gingival augmentation technique introduced many years ago to increase the keratinized gingiva (H.B. 1963, Sullivan &Atkins, 1968, Agudio et al., 2008), but many studies proved that the FGG showed changes in the graft horizontal dimension (GHD) , graft vertical dimension (GVD) and graft surface area (GSA) overtime after grafting and specially during the first postoperative month (Ericsson &Lindhe, 1984, Hatipoglu et al., 2007). This study was carried to evaluate the effect of the placement of PRF membrane beneath the free gingival graft in gingival augmentation surgeries regarding the changes over time in graft's horizontal dimension, vertical dimension and surface area.

All the patients selected were of thin gingival biotype since studies showed that thin biotype is more commonly associated with narrow attached gingiva (Olsson et al.,1993, Anderegg et al., 1995,

Baldi et al.,1999, Gobbato et al., 2012) . Patients with crowding, subgingival restorations or who didn't follow the plaque control instructions after conventional periodontal treatment were excluded from the study to avoid the effect of any local predisposing factors on the final results.

The FGG was harvested from the palate between the palatal root of the first molar and the distal line angle of the canine where the thickest tissue is usually available (Reiser et al., 1996). The submucosa of the anterior palate is rich in fat (Orban 1996), thus after graft harvesting curved scissors was used to remove any fatty tissue from the connective tissue surface of the graft to obtain a uniform graft thickness around 1.5mm thickness before suturing at the recipient site to allow quick graft revascularization.

A small piece of the PRF membrane was used to cover the wound at the donor site stabilized by horizontal mattress suture as a recent study reported that PRF significantly accelerated the palatal wound healing after graft harvesting and reduced donor site morbidity. (Femminella et al., 2016) .

A follow up of 1.5 month was selected in our study since previous studies showed that the main change in transplanted tissue dimensions occurred during the first month. (Ericsson &Lindhe, 1984, Hatipoglu et al., 2007). Also literature showed that the FGG shrinkage between baseline and first month is statistically more significant than shrinkage between first and third month. Also the gain in keratinized gingiva after grafting is significant from zero to one month and not significant from one to three months. (Cifsibasi et al., 2015)

The same median baseline PD, BI, PI values were recorded in both groups, with no significant difference. In both groups, no significant difference between baseline and 1.5 month values of PD, BI, PI. Thus, the patient oral hygiene and the plaque control can be excluded to have any effects on the final study results.

Regarding the baseline GVD; there was no statistically significant difference between the groups. Also no statistically significant difference was found between the groups regarding the baseline GHD. This was since all the cases in this study were of two mandibular incisors with insufficient attached gingiva thus nearly similar grafts dimensions were required in all cases.

In both groups, the mean value of GVD decreased significantly 1.5month after the surgery and the same results were obtained with the GHD and the GSA. This was in accordance with the previous literature that proved that transplanted FGG showed significant changes in the horizontal and vertical dimensions overtime after grafting and specially in the first postoperative month ((Ericsson &Lindhe, 1984, James & Mc Fall, 1978, Orsini et al., 2004 Hatipoglu et al., 2007, Hatipoglu et al., 2007, Barbosa et al., 2009)

In Group 2 (FGG alone) the mean percent decrease in GVD was 35% which was in accordance with previous clinical studies that showed vertical shrinkage of FGG percentage from 31% to 45% (Ward, 1974, Silva et al., 2010, Barbosa et al., 2009). Other studies reported less shrinkage of 16.6% -22.4% (Guncu et al., 2012)

Regarding the GHD shrinkage, the mean percent decrease in group 2 was 28.02% at 1.5 month while previous study by Silva et al 2010 reported 17% and 22% mean percent decrease in GHD at 30 and 90 days respectively (Silva et al., 2010). Another study by Hatipoglu et al reported 5.8% and 10.2% percent decrease in GHD at day 21 and day 180 respectively while Guncu et al showed 9.8% and 14.25% decrease at day 21and 180 respectively (Hatipoglu et al., 2007, Guncu et al., 2012).

Regarding the graft surface area (GSA) obtained by multiplying the horizontal and vertical dimensions, the mean decrease in GSA in group 2 was 53.3%.Other studies evaluated the change in GSA , one showed 37% mean percent decrease in

GSA in 1 month period (Silva et al 2010) and the other showed 23.8% and 32.1% decrease in 21 days and 180 days recpectively (Hatipoglu et al., 2007).

Regarding Group 1 (Test group; FGG combined with PRF) the mean percent decrease in GVD was 8.33%, GHD was 10.76% & surface area was 17.81%. To the best of our knowledge no previous studies tested the graft dimensional changes when FGG is combined with PRF.

Regarding the percent of change in GVD, greater mean and median percent decrease was noticed in group 2 and on comparing both groups statistically significant difference was found. The same was obtained with GHD and GSA. This means that group 1 (FGG +PRF) showed significant less shrinkage of the graft which may be related to the PRF used beneath the graft. To the best of our knowledge, no previous studies compared FGG alone to FGG combined with PRF.

Literature studying the healing of FGG showed that in the first days after surgery, the grafted tissues survive with the plasmatic circulation from the recipient bed, before anastomosis between the blood vessels of the recipient bed and the grafted tissue which occurs 4-5 days after surgery. This is followed by capillaries proliferation and fibrous union between the graft and underlying connective tissue.(Oliver et al 1968, Nobuta et al 1988)

Taking in consideration that PRF is rich in platelets, leukocytes and growth factors, this directly promote the proliferation of endothelial cells and fibroblasts (He et al., 2009, Dohan Ehrenfest et al., 2009, Carlson & Roach, 2002, Roy et al., 2011, Chen et al., 2011, Toffler et al., 2009). This may help in accelerating the anastomosis and proliferation of new capillaries thus promoting rapid tissue repair and descrease the shrinkage of the transplanted tissue. Also the sticky nature of PRF helps in stabilization of the graft which is essential to achieve therapeutic success. (Whitman et al.,1997, Vikov et al., 2005). Moreover, the leukocytes within the PRF helps to eliminates any pathogens at the wound after surgery thus decrease the bacterial contamination so promoting direct healing and may minimizes the graft shrinkage (Clark, 2001).

CONCLUSION

Using platelet-rich fibrin beneath free gingival graft in gingival augmentation surgery resulted in successful increase in the attached gingiva with less dimensional changes in the transplanted free gingival graft compared to using free gingival graft alone. Further studies should be carried to additionaly explain the results obtained and to study the histological structure of the obtained grafted tissues by the two modalities.

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