Necessity of Keratinized Tissues for Dental Implants: A Clinical, Immunological, and Radiographic Study

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ABSTRACT

Background: Necessity of keratinized tissues (KTs) for maintaining health around dental implants (DIs) remains as a controversial issue.

Purpose: The aim of this study was to investigate the effects of KT width (KTW) on peri-implant tissues by evaluating peri-implant clinical and inflammatory parameters.

Materials and Methods: Sixty DIs were included in this 6-month longitudinal study. After classifying DI based on the presence of KTs at the buccal aspect as with adequate/inadequate KTW, DIs were randomly assigned into three study groups. In the first group, while free gingival graft (FGG) was performed, DIs in maintenance (M) group were followed up by standardized maintenance procedures at baseline, first, third, and sixth months as with DI with adequate KTW (Control). Clinical parameters, peri-implant sulcular fluid (PISF) volume, PISF Interleukin 1β concentration, and bone loss were analyzed.

Results: Significant improvements in clinical and immunological parameters were noted only for FGG for the whole study period. Statistical differences detected between the treatment groups (FGG vs M) were for gingival index at all time points and for PISF volume at sixth month. For the other parameters evaluated, while lower values were observed for FGG, statistically no differences were noted between the groups.

Conclusions: Based on the results of this study, it can be suggested that FGG performed around DIs lacking KT is a reliable method, leading to significant improvements in clinical and inflammatory parameters. Further long-term studies including more DIs are needed to clarify the role of KT on maintenance of DIs.

KEY WORDS: clinical study, implant, inflammation, radiographs, soft tissue grafting

INTRODUCTION

Dental implants (DIs) have been used in modern clinical practice for over the past 50 years, presenting an indispensable role in rehabilitation of fully[1–4] and partially edentulous patients[1,5–8] and single-tooth restorations as well.[9–11] Despite being used with high success rates and superior patient satisfaction, esthetic, technical, and biological complications may occur, which may jeopardize the success of dental rehabilitation.[4,12] In this aspect, several factors influencing the success and survival of DIs have been reported in the literature. Keratinized tissue (KT) width, determined as the distance between the free gingival margin and the mucogingival junction,[13] is considered as one of the local risk factors influencing

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success of DI therapy.[14,15] Based on the preliminary studies of Lang and Loe[16] focusing on the importance of KTs on maintenance of periodontal health, similar studies have been performed to determine the role of KT on peri-implant health. In an early animal study, Warrer and colleagues[17] reported more pronounced attachment loss (AL) and gingival recession (GR) for DIs with inadequate KT width (KTW) in a ligature-induced peri-implantitis model in monkeys. In a similar study design,[18] shallower peri-implant pocket depth (PPD) was recorded in ligature-induced DIs with adequate KTW, pronouncing the importance of KTW especially in patients lacking oral hygiene. In more recent clinical studies, while increased mucosal/gingival inflammation,[19–22] plaque formation,[19–22] alveolar bone loss,[20,23] and GR[19,23,24] were reported for DIs lacking KT, data presenting similar plaque index (PI),[23,24] gingival index (GI),[23,24] and PPD[19–23] exist in the literature, suggesting that the less pronounced necessity of KTs in case of good oral hygiene and maintenance can be achieved.

Differing from the other study designs by means of the investigated parameters, Zigdon and Machtei[24] evaluated peri-implant sulcular fluid (PISF) prostaglandin E_2 (PGE_2) levels in addition to the clinical parameters in DIs with adequate/inadequate KTW. Besides the increased GR and AL in sites with inadequate KTW, the results of the study demonstrated significantly increased PGE_2 levels. Similarly in a recent longitudinal study, Boynuegri and colleagues[21] investigated the role of KTW on peri-implant clinical and immunological parameters in a 12-month study period and reported similar PISF Interleukin 1β (IL-1β) levels, increased Tumor necrosis factor α (TNF-α) levels, and increased GI and PI between the sites with adequate/inadequate KTW.

Although controversies exist in the literature based on the results of the studies performed, it has been reported that wide KT band takes role in formation of a resistant barrier against mechanical trauma during oral hygiene procedures especially in patients with severe bone and soft tissue atrophies and helps in formation of peri-implant tissues in which nonkeratinized epithelium may become ineffective. In addition, it has been reported that adequate KTW prevents tissue prolapses during the intervals between prosthetic procedures and by preserving the junctional epithelium during functional movements of the mucosa, precludes mucogingival stress, which aids in maintenance of peri-implant tissue health.[25,26]

Based on these knowledge, the purposes of this longitudinal study were the following: (1) to evaluate the effects of KTW on peri-implant tissue health by clinical, immunological, and radiological parameters; (2) to compare the results of two treatment strategies [maintenance/free gingival grafts (FGGs) + maintenance] for peri-implant sites with inadequate KTW; and (3) to determine the optimal treatment and maintenance procedures for peri-implant sites with inadequate KTW.

MATERIAL AND METHOD

Sixty DIs performed in 18 patients (10 female, 8 male, mean age: 47.5 ± 11.26) with at least one DI-supported prosthetic restoration functioning for minimally 1 year in their dentition were included in this longitudinal study. None of the individuals had any known systemic disorders or used antibiotics and anti-inflammatory medications within 3 months of the experiment. Participants with active infectious diseases such as hepatitis, Human Immunodeficiency Virus , and tuberculosis or who were chronically treated with medications (phenytoin, cyclosporin-A, or calcium channel blockers) and smokers, as well as females who were lactating or pregnant, were excluded.

This study was approved by The Ethical Committee of Hacettepe University Faculty of Medicine (FON 10/11). Prior to the initiation of the study, patients were informed about the study design and informed consent forms were obtained. Patients able to achieve appropriate oral hygiene standards (full mouth plaque and GI scores <15%) following phase I treatment were included in the study group. DIs participated in the study (DI) were classified into two major groups based on the width of KT at the midbuccal aspect of each DI-supported fixed prosthesis as followed: DI with adequate KTW (>2 mm) and DI with inadequate KTW (≤2 mm).[16] DIs in the adequate KTW group were then randomly assigned to one of the treatment groups: FGG group or maintenance (M) group. Study design and distribution of the characteristics of the study group are presented in Figure 1 and Tables 1 and 2, respectively.

FGG Procedure

After recording the clinical parameters and obtaining PISF samples in FGG, FGG procedure was performed according to the technique described by Newman and colleagues.[27] Briefly, local infiltration anesthesia

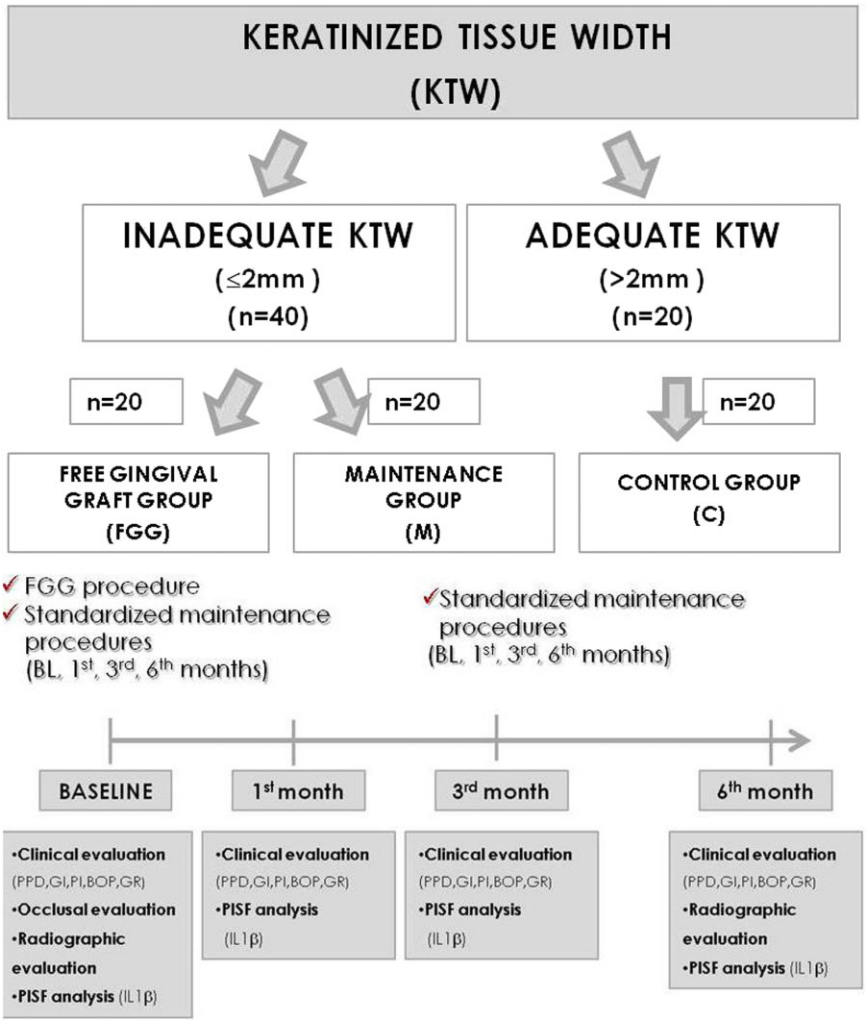

Figure 1 Study design.

(Ultracaine D-S Forte, Hoechst Marion Roussel, Frankfurt, Germany) was performed to the recipient and palatal donor site. Following an incision at mucogingival junction in the recipient site, a FGG was obtained from the donor site and secured to the neighboring area using 6-0 sutures (Ethicon, St Stevens-Wolume, Belgium). The site was then covered with periodontal dressing (Voco Pac, Cuxhaven, Germany) to avoid any damage to the site during the early postoperative period. Sutures and periodontal pack were removed 10 days following the

TABLE 1 Location of Dental Implants among the Study Groups

	(*n*/%)			
Location	FGG	M	C	Total
Maxillary anterior	4 (20.00)	2 (10.00)	1 (5.00)	7 (11.66)
Maxillary premolar	3 (15.00)	4 (20.00)	6 (30.00)	13 (21.66)
Maxillary molar	2 (10.00)	2 (10.00)	8 (40.00)	12 (20.00)
Mandibular anterior	2 (10.00)	0 (00.00)	0 (00.00)	2 (3.33)
Mandibular premolar	4 (20.00)	5 (25.00)	4 (20.00)	13 (21.66)
Mandibular molar	5 (25.00)	7 (35.00)	1 (5.00)	13 (21.66)
Total	20 (100)	20 (100)	20 (100)	60 (100)

C = control; FGG = free gingival graft; M = maintenance.

TABLE 2 Opposing Dentition Profile of the Study Groups

	(*n*/%)			
Opposing Dentition	FGG	M	C	Total
ND	6 (30)	10 (50)	7 (35)	23 (38.3)
FPR	1 (5)	2 (10)	2 (10)	5 (8.3)
DFPR	13 (65)	6 (30)	10 (50)	29 (48.3)
RPR	0 (0)	2 (10)	1 (5)	3 (5)
Total	20 (100)	20 (100)	20 (100)	60 (100)

C = control; DFPR = dental implant-supported fixed prosthetic restoration; FGG = free gingival graft; FPR = fixed prosthetic restoration; M = maintenance; ND = natural dentition; RPR = removable prosthetic restoration.

surgical procedure and the patient was allowed to perform oral hygiene procedures.

Maintenance Procedure

After recording clinical parameters and obtaining PISF samples at first, third, and sixth months for FGG and at baseline (BL), first, third, and sixth months in M and C, standardized professional oral hygiene procedures were performed including supra and subgingival scaling and polishing. Patients were advised to brush their teeth three times a day and proximal brushes were prescribed twice a day. The use of mouthwashes or other hygiene appliances were avoided in both study groups in order to avoid any effect on clinical/immunological parameters and to ensure the standardization of the study.

Clinical Peri-Implant Parameters

Clinical peri-implant parameters were recorded for each DI by the same calibrated periodontist (S.B.A.). *PPD* was recorded at six sites (mesiobuccal, midbuccal, distobuccal, mesiolingual/palatal, distolingual/palatal, and midlingual/palatal) using a periodontal probe (Michigan O Color-Coded Probe, Hu-Friedy, Chicago, IL, USA). *GI*[28] *and PI*[29] were measured at four sites for each DI (midmesial, midbuccal, mid-distal, and midlingual/palatal). *The presence of bleeding on probing* (*BOP*) was recorded as positive/negative using the Ainamo and Bay index[30] and calculated as the percentage of BOP (+) sites per each study group. GR was calculated by measuring the distance from the abutment-DI interface to the gingival margin at six sites per each DI by using a periodontal probe. To measure the *width of keratinized gingiva*, mucogingival junction was assessed by the "rolling technique" and the distance between the free gingival margin to the mucogingival junction was measured at the midbuccal aspect.[31] In addition to other clinical parameters, *type of the opposing dentition* was recorded based on the clinical status (no occlusion, fixed prosthetic restoration [FPR], removable prosthetic restoration [RPR], DI-supported fixed prosthetic restoration [DFPR], DI-supported RPR, and natural dentition [ND]).

PISF Analysis

PISF sampling was performed according to the method described by Rudin and colleagues[32] prior to the recording of clinical parameters. Briefly; supragingival plaque was removed, the sampling area was isolated using sterile cotton rolls, and the site was gently air dried in order to avoid any contamination during the sampling process. Standardized paper strips (Periopaper, no.593525; Ora Flow, Amityville, NY, USA) were placed into the peri-implant sulcus taking care to minimize mechanical irritation[33] and regardless of the probing depth, one paper strip was inserted approximately 1 mm at each site. For each DI, PISF samples were obtained at four sites (midmesial, midbuccal, mid-distal, and midpalatal/lingual). Strips contaminated by blood were excluded from the sampling group. Following 30 seconds of sampling time, paper strips were immediately transferred to a previously calibrated and warmed up Periotron 8000 (Ora Flow) device. Before measuring, the Periotron device was adjusted to reading zero by placing a standardized paper strip. Care was taken to minimize the period between sampling and the transfer of the paper strips to the device in order to eliminate the risk for evaporation.[34] The PISF volume was then electronically measured in "Periotron units" that then were converted to microliters by using MLCONVERT.EXE software program (Ora Flow). The PISF samples were placed in sterile Eppendorf tubes and carefully wrapped to be stored in −20°C until the

laboratory analysis. All the sampling processes were performed by the same calibrated periodontist (S.B.A.).

IL-1β Analysis

PISF samples obtained were analyzed for IL-1β using a commercially available enzyme-linked immunoabsorbent assay (eBioscience, Bendermed Human IL-1β Instant ELISA, San Diego, CA, USA). Analysis were performed in duplicate, according to the manufacturers' instructions. Results were calculated using the standard curves for the assay. The total amount of IL-1β was expressed in picograms and total concentration of IL-1β was expressed in picogram per microliter by proportioning the total amount of IL-1β to PISF volume.

Radiographic Analysis

Standardized periapical radiographs were obtained for each DI at BL and sixth months using a paralleling device (Dentsply Rinn, Rinn Cooperation, Elgin, IL, USA) and paralleling technique (Kavo In Exam, dental X-ray unit, 70 kVp, 7 Ma, 0.115 seconds). Radiographs obtained were then digitized at 2400 dpi using a flatbed scanner (Epson Expression 10000 XL, Seiko Epson Co., Nagano, Japan). To determine marginal bone loss (MBL), the distance from the first bone-implant contact to the implant shoulder was measured based on the actual distances between the two threads of DIs provided by the manufacturers. An image analysis program (ImageJ 1.43n, NIH, Bethesda, MD, USA) was used at ×400 magnification for the measurements. Bone loss was calculated for mesial and distal aspects of each DI and the values were averaged to calculate the mean proximal MBL. To evaluate observer's consistency, all the measurements were performed one more time after 2 weeks and intraclass correlation coefficient test revealed 93% compatibility between the two measurements. All the exposures and measurements were performed by the same calibrated radiologist (S.U.) blinded to the study groups.

Statistical Analysis

Friedman and Kruskal-Wallis tests were used for analyzing the intragroup differences and the differences between the study groups, respectively. For determination of the intragroup and differences between the groups for BOP (+), Cochran Q, Mc Nemar, and Qi Square tests were performed.

RESULTS

Clinical Parameters

Data recorded for clinical parameters are presented in Table 3.

PI. At BL, significant differences between the study groups were noted among all groups, presenting higher values for FGG followed by M and C, respectively. At first, third, and sixth month evaluations, only significant difference detected was between C versus M ($p < .05$). When *intragroup comparisons* were performed, M showed increased PI values at sixth month evaluation compared with the BL values ($p < .05$). In FGG, after increasing KTW following BL evaluations, PI values decreased significantly during the whole follow-up period when compared with BL values.

GI. At BL, statistically significant differences noted for GI were between M versus C and FGG versus C, presenting higher values for both test groups ($p < .05$). For the other follow-up periods evaluated, only significant differences were between M versus C and M versus FGG, showing higher values for M ($p < .05$). For *intragroup comparisons*, while GI showed significant increase at first month when compared with BL values in C, similar changes in FGG were detected, showing significant decreases following the increase in KTW by first month ($p < .05$).

GR. Statistically no differences were detected among the study groups for the follow-up periods examined ($p > .05$). Similarly, when *intragroup differences* were taken into account, the differences presented statistically no differences ($p > .05$).

PPD. Significantly no differences were noted between the groups at all follow-up periods for PPD ($p > .05$). When *intragroup differences* were evaluated, significant increases were noted in C at third and sixth months when compared with BL and prior follow-up periods ($p < .05$). For M, similar to the pattern observed for C, significant increases were recorded between first, third, and sixth months ($p < .05$). In FGG, a significant increase was observed at third month when compared with BL, first, and sixth months, respectively ($p < .05$).

GR. When intragroup and intergroup differences were taken into account, significantly no differences were

TABLE 3 Clinical Parameters among the Study Groups

	Baseline (BL)			First Month			Third Month			Sixth Month		
	FGG	M	C	FGG	M	C	FGG	M	C	FGG	M	C
PPD	1.97 ± 0.47	1.76 ± 0.73	2.05 ± 0.74	1.94 ± 0.53	2.04 ± 0.66§	2.16 ± 0.74	2.51 ± 0.63§,††,§§	2.24 ± 0.61§,††	2.34 ± 0.58§,††	2.29 ± 0.50**,‡‡	2.29 ± 0.65**,‡‡,§§	2.43 ± 0.81**,‡‡,§§
PI	0.58 ± 0.29†*,§,§,**	0.38 ± 0.30‡	0.16 ± 0.28	0.20 ± 0.26	0.36 ± 0.39‡	0.05 ± 0.15	0.23 ± 0.34	0.38 ± 0.30‡	0.13 ± 0.26	0.21 ± 0.32	0.45 ± 0.44‡,**	0.06 ± 0.27
GI	1.33 ± 0.51†,§,§,**	1.17 ± 0.59‡	0.35 ± 0.39	0.60 ± 0.42	1.38 ± 0.43‡,*	0.72 ± 0.63§	0.66 ± 0.43	1.42 ± 0.52‡,*	0.68 ± 0.47	0.65 ± 0.42	1.32 ± 0.33‡,*	0.56 ± 0.44
GR	0.20 ± 0.38	0.06 ± 0.18	0.02 ± 0.08	0.22 ± 0.39	0.06 ± 0.18	0.03 ± 0.08	0.24 ± 0.42	0.07 ± 0.19	0.04 ± 0.15	0.29 ± 0.48	0.09 ± 0.19	0.04 ± 0.15
KTW	0.35 ± 0.48§,§,**	0.60 ± 0.50	3.80 ± 1.23†,‡	4.80 ± 1.82*	0.60 ± 0.50	3.80 ± 1.23‡	4.60 ± 1.45*	0.60 ± 0.50	3.80 ± 1.23‡	4.40 ± 1.50*	0.60 ± 0.50	3.90 ± 1.29‡
BOP (*n* %)	17†,§,§,** (85%)	17§ (85%)	8 (40%)	6 (30%)	19*,‡ (95%)	9 (45%)	4 (20%)	18*,‡ (90%)	7 (35%)	6 (30%)	19*,‡ (95%)	5 (25%)

$p < .05$, statistically significant difference between *FGG versus M, †FGG versus C, ‡M versus C, §BL versus first month, §BL versus third month, **BL versus sixth month, ††first month versus third month, ‡‡first month versus sixth month, and §§third versus sixth month.

BOP = bleeding on probing; C = control; FGG = free gingival graft; GI = gingival index; GR = gingival recession; KTW = keratinized tissue width; M = maintenance; PI = plaque index; PPD = peri-implant pocket depth.

noted for any of the groups for the whole study period observed ($p > .05$).

KTW. At BL, C showed statistically higher values for KTW when compared with M and FGG ($p < .05$). For the other follow-up periods observed, significant differences were noted between C versus M and M versus FGG, presenting lower values for M ($p < .05$). When *intragroup comparisons* were considered, only significant difference noted was detected for FGG, presenting increased values for all follow-up periods evaluated when compared with BL ($p < .05$).

BOP. When the percentage of sites showing BOP was compared between the groups, statistically significant differences were noted between M versus C and FGG versus C, presenting higher values for FGG and M at BL ($p < .05$). For the first, third, and sixth month evaluations, significant differences were recorded between M versus C and M versus FGG ($p < .05$). *Intragroup comparisons* revealed significantly decreased values for only FGG when compared with BL values ($p < .05$).

Immunological Parameters

The mean values for PISF volume, IL-1β levels, and concentrations are presented in Table 4.

PISF Volume. At BL, the only significant difference noted was recorded between M versus C ($p < .05$). While the only difference noted was between M versus C at first month evaluation, at sixth months significant differences were noted between M versus C and M versus FGG ($p < .05$). While *intragroup differences* were considered, PISF volume significantly decreased at first and sixth months when compared with third month in C ($p < .05$). For M, only significant difference noted was between BL versus third month, presenting higher values for BL ($p < .05$). In FGG, significant decreases were recorded in PISF volume during the follow-up period observed when compared with BL values ($p < .05$).

Total IL-1β Amount. While study groups were compared, statistically no differences were detected between the groups for any of the follow-up periods evaluated ($p > .05$). For *intragroup evaluations*, IL-1β levels significantly increased at third month when compared with first month values in C ($p < .05$). For FGG, PISF IL-1β

TABLE 4 Immunological Parameters among the Study Groups

	BL			First Month			Third Month			Sixth Month		
	FGG	M	C	FGG	M	C	FGG	M	C	FGG	M	C
PISF volume (μL)	0.29 ± 0.13[§,¶,**]	0.34 ± 0.17[†,‡,¶]	0.22 ± 0.12	0.23 ± 0.12[†,‡,‡‡]	0.30 ± 0.12[‡]	0.19 ± 0.10[‡‡,††]	0.17 ± 0.10	0.27 ± 0.15	0.23 ± 0.16[§§]	0.13 ± 0.07	0.34 ± 0.22[‡,*]	0.18 ± 0.10
IL-1β (pg)	47.95 ± 39.98[§,¶,**]	34.74 ± 23.78	39.34 ± 49.43[**]	40.47 ± 36.29[‡‡]	32.93 ± 26.47	31.68 ± 32.98	29.32 ± 24.95[§§]	35.61 ± 30.84	37.14 ± 33.57[§§]	23.53 ± 28.33	30.03 ± 23.13	34.04 ± 30.18
IL-1β concentration (pg/μL)	165.30 ± 120.17	140.51 ± 151.81	172.08 ± 146.41	164.49 ± 100.78	112.85 ± 79.88	157.82 ± 129.73	193.37 ± 127.35[¶,§§]	142.48 ± 118.04[§§]	178.10 ± 179.51	179.38 ± 155.31	91.18 ± 55.47	180.68 ± 92.1[‡]

$p < .05$, statistically significant difference between *FGG versus M, †FGG versus C, ‡M versus C, §BL versus first month, ¶BL versus third month, **BL versus sixth month, ††first month versus third month, ‡‡first month versus sixth month, §§third versus sixth month.
BL = baseline; C = control; FGG = free gingival graft; IL-1β, Interleukin-1β; M = maintenance; PISF = peri-implant sulcular fluid.

levels significantly decreased by time when compared with the BL values ($p < .05$).

IL-1β Concentration. At sixth-month evaluation, significant differences were noted between C versus M ($p < .05$). Only *intragroup difference* noted for IL-1β concentration was for M at sixth-month evaluation, showing a significant decrease when compared with third month values ($p < .05$).

Radiographic Parameters

The mean values for MBL are presented in Table 5.

MBL. Mean proximal MBL determined at the end of the 6-month follow-up period showed similar values between the study groups, presenting statistically no differences ($p > .05$).

DISCUSSION

Necessity of KTs at peri-implant sites for achieving and maintaining health still remains as a controversial issue in the literature. Arising from the preliminary studies of Lang and Loe,[16] suggesting the fundamental role of KTs for maintaining periodontal health for natural teeth, several studies have focused on the importance of KTW on DIs. In preliminary animal studies in which the effects of KTW on ligature-induced plaque accumulation were investigated, increased loss of attachment, GR, and deeper peri-implant pocket formation were demonstrated for DIs lacking adequate KTW.[17,18] On the other hand, in clinical studies, while increased gingival inflammation, plaque accumulation, GR, bone loss, and pocket formation have been reported for DI lacking KT,[19–22] studies exist in the literature showing no significant differences for gingival inflammation and plaque accumulation for DI with/without adequate KTW in

TABLE 5 Marginal Bone Loss among the Study Groups

Marginal Bone Loss	BL	Sixth Month	MBL (Sixth Month–BL)
FGG	−0.39 ± 0.26	−0.55 ± 0.39	0.16 ± 0.15
M	−0.60 ± 0.52	−0.81 ± 0.61	0.21 ± 0.22
C	−0.56 ± 0.47	−0.72 ± 0.49	0.15 ± 0.22

BL = baseline; C = control; FGG = free gingival graft; M = maintenance; MBL = marginal bone loss.

case good oral hygiene can be maintained.[23,24] Although the differences in study designs (cross sectional/retrospective/longitudinal), characteristics of the study populations (overdentures/FPRs), and other factors that may have affected the study results (smoking/surface roughness, etc.) preclude conducting definitive conclusions regarding the need of KT for DIs, when the differences between the periodontal and peri-implant tissues are taken into account, in which immunological and physiological differences result in diminished defense mechanisms for DIs,[35–37] the importance of KTs should be more pronounced for prevention of the progression of a possible peri-implant inflammation.

In the present study, the effects of KTW on peri-implant clinical and immunological parameters were evaluated. The results demonstrated lack of adequate KTW resulted in increased inflammation indicated by increase in GI and presence of BOP. Furthermore, GI and BOP values significantly decreased in FGG following the increase in KTW. Contrarily, no correlations have been reported in the literature between KTW and presence of BOP.[14,22–24] The diversity between the results of the studies might be attributed to the smoking status of the study groups in which while only nonsmokers were included in the present study, smoking status has not been reported in the inclusion criteria of mentioned studies. It has been well documented in the literature that smoking suppresses clinical signs of inflammation in the gingival by changing the microvascular responses against plaque accumulation.[38,39] In the present study design, in which only nonsmokers are included, PISF total IL-1β amount showed positive correlation with presence of BOP, presenting the role of BOP in reflecting the inflammatory status of DIs. Based on these results, careful evaluation of BOP can be advised for DIs lacking KT for prevention of progression of inflammation. Similar to the presence of BOP, increased GI values were recorded for DIs lacking KTW in this study. While Bouri and colleagues,[20] Adibrad and colleagues,[19] Chung and colleagues,[22] and Boynuegri and colleagues[21] demonstrated similar results to our study indicating the increase in gingival inflammation in sites lacking KTW, Zigdon and Machtei[24] and Kim and colleagues[23] have reported no differences for GI in sites with adequate/inadequate KTW. In addition to the nature of peri-implant tissues that depend on the soft tissue color and texture prior to implantation, it has been reported that peri-implant tissues might be affected by the surface texture of DIs,[40] which can explain the differences in the results of the studies in the literature showing different GI values among sites with or lacking KTW.

The relationship between bacterial plaque accumulation, composition, and peri-implant inflammation has been well documented in the literature, which emphasizes the role of plaque elimination in preventing peri-implant inflammation.[41] Although, one of the most pronounced advantages of adequate KTs have been reported as the facilitation of plaque elimination, contradictory results regarding plaque accumulation present in the literature. While Bouri and colleagues,[20] Adibrad and colleagues,[19] Chung and colleagues,[22] and Boynuegri and colleagues[21] demonstrated higher plaque accumulation for DIs lacking KTW, other studies reported similar PI values for DIs with adequate/inadequate KTW.[23,24] In order to determine the effects of KTW on plaque elimination efficiency, PI has been recorded in the present study. While the results demonstrated increased values for M and FGG groups, in which inadequate KTW was diagnosed at BL, significant differences were only observed between C and M for the 6-month study period. Although lower values were detected for FGG, the reason why the difference could not reach statistical significance may be explained with the sensitivity and discomfort occurring at the surgical site that might have precluded efficient oral hygiene applications. While increased values were detected for M in the 6-month study period, decreases were recorded for FGG when compared with BL values. Contrary to our results, in a 12-month longitudinal study, Boynuegri and colleagues[21] reported a significant decrease in PI values for 36 DIs supporting overdentures for the study period observed. Despite the similarities in study design, the reason of the diversities in the results can be explained with the location of the DIs included for both studies, in which only DIs in the interforaminal region were included in the study of Boynuegri and colleagues and 90% of DIs in the present study were located in the posterior region, which may have had a negative influence on plaque elimination efficiency.

Although several diagnostic techniques are being used to determine the inflammatory status in the peri-implant area, detecting the inflammatory status prior to the occurrence of clinical signs has been approved as a prerequisite for conservative treatment approaches.[42–44] PISF, the osmotically mediated exuda originating from

the vascular plexus of the gingiva, is considered as the analogue of gingival crevicular fluid (GCF) and believed to have the potential of detecting early peri-implant inflammatory changes by means of its inflammatory mediator content.[43,45] Cytokines, soluble cell secreted proteins that are capable of affecting the properties of other cells, are the most common mediators that have gained clinical attention to determine the inflammatory status of DIs based on their potential in reflecting the inflammatory status in GCF.[46] Among the investigated cytokines, IL-1β has a considerable place in most studies based on its proinflammatory characteristics, showing increased levels in periodontal and peri-implant inflammatory conditions depending on the severity of the lesion and significant decreases in response to treatment.[47–62] In this study, in addition to the clinical peri-implant parameters, PISF IL-1β was investigated to detect early inflammatory changes prior to the occurrence of clinical signs and to support the findings with an inflammatory parameter based on the potential of IL-1β in reflecting inflammatory status. Our results revealed that, although increasing KTW in FGG decreased all clinical and immunological parameters when compared with BL values, on the other hand, similar values were recorded in the M group for the follow-up period. In addition, while FGG presented lower values for all clinical and immunological parameters evaluated, significant differences were observed only for GI for all follow-up periods and for PISF volume at sixth month. Similar to our results, Boynuegri and colleagues[21] reported lack of adequate KTW resulted in increased GI and plaque accumulation and while PISF TNF-α levels were consistent with clinical parameters, no significant differences were detected between the groups (adequate/inadequate KTW) regarding IL-1β levels. When the differences in GI values indicating the differences in peri-implant inflammatory status between M and FGG are considered in our study, differences between IL-1β levels and PISF volume could not reach statistical significance, which aroused the question whether other factors than the peri-implant inflammatory status may have influenced PISF volume and IL-1β levels. Although factors that have been shown to affect GCF IL-1β levels like sampling time, smoking, and systemically health status were standardized in this study design,[63–70] effects of the occlusal forces on PISF cytokine profile remain still unknown. Based on the knowledge that orthodontic forces may affect cytokine profiles of GCF,[67] factors effecting occlusal forces – like the opposing dentition and the localization of DIs (anterior/posterior) – might have an influence on the cytokine profile as well. When the study groups are considered from this point of view, despite showing statistically no differences, while the dominant opposing dentition was DFRP in C and FGG, ND was dominant in M. In addition, while 95% of DI in C remained in the posterior region, it was recorded as 90 and 70% for M and FGG, respectively. It has been well reported that the highest chewing forces are generated in ND, followed by FPR, RPR, and total prosthesis. In addition, the highest occlusal forces are documented to occur in the posterior area.[71,72] When the differences in the opposing dentition and the location of DIs performed are taken into account, it can be suggested that PISF profile might have been influenced by these factors, which may explain the similar IL-1β levels observed for the study groups that differ in their peri-implant inflammatory status.

CONCLUSIONS

Based on the results of this study presenting significant decreases in clinical and inflammatory parameters in peri-implant tissues lacking KTs following FGG procedure, it can be concluded the following: (1) in peri-implant sites lacking KTs, increasing the KTW by FGGs can be considered as a reliable and effective method for achieving and maintaining health status; (2) in cases where increasing KTW is not feasible, supporting the maintenance phase with accessory hygiene procedures (mouthwash, proximal brush, and irrigator) should be advised to eliminate and control peri-implant inflammation and frequent follow-up evaluations are needed to detect early inflammatory changes prior to the progression of inflammation; and (3) in addition to clinical parameters, PISF IL-1β can be used as an early diagnostic method for detection of inflammatory changes in peri-implant tissues based on its potential in defining active periodontal tissue breakdown. Further studies including more DIs standardized in DI-related factors (diameter and location of the DIs performed and type of the opposing dentition) and "in-patient" designed clinical studies in which control and treatment groups can be evaluated at the same patient level are needed to clarify the exact role of KTW on peri-implant clinical and inflammatory parameters.

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