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# Bone Grafting History Affects Soft Tissue Healing Following Implant Placement

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# Abstract

## Background

This study aimed to determine and compare soft tissue healing outcomes following implant placement in grafted (GG) and non-grafted bone (NGG).

## Methods

Patients receiving single implant in a tooth-bound maxillary non-molar site were recruited. Clinical healing was documented. Volume and content of wound fluid (WF; at 3, 6, and 9 days) were compared with adjacent gingival crevicular fluid (GCF; at baseline, 1, and 4 months). Buccal flap blood perfusion recovery and changes in bone thickness were recorded. Linear mixed model regression analysis and generalized estimating equations with Bonferroni adjustments were conducted for repeated measures.

## Results

Twenty-five patients ( $49 \pm 4$  years; 13 males; nine NGG) completed the study. Soft tissue closure was slower in GG ( $P < 0.01$ ). Differential response in WF/GCF protein concentrations was detected for ACTH (increased in GG only) and insulin, leptin, osteocalcin (decreased in NGG only) at day 6 ( $P \leq 0.04$ ), with no inter-group differences at any time ( $P > 0.05$ ). Blood perfusion rate decreased immediately postoperatively ( $P < 0.01$ , GG) followed by 3-day hyperemia ( $P > 0.05$  both groups). The recovery to baseline values was almost complete for NGG whereas GG stayed ischemic even at 4 months ( $P = 0.05$ ). Buccal bone thickness changes were significant in GG sites ( $P \leq 0.05$ ).

## Conclusion

History of bone grafting alters the clinical, physiological, and molecular healing response of overlying soft tissues after implant placement surgery.

## 1 INTRODUCTION

Dental implant fixture placement often requires pre-implant surgeries to adequately develop the recipient site. This is especially true for the esthetic zone, where buccal bone plate is usually thin or lost after tooth extraction.<sup>1</sup> Bone grafting procedures, such as socket preservation, guided bone regeneration and/or simultaneous bone graft application at time of implant placement, are well-documented approaches to prevent bone loss or regenerate lost bone.<sup>2, 3</sup> In general, successful long-term implant outcomes have been reported following these procedures.<sup>4, 5</sup> It is also well-established that bone graft materials have variable resorption rates<sup>6</sup> and may remain at the implant/bone interface and at the hard/soft tissue border for prolonged periods of time.<sup>6, 7</sup> When an implant is placed 4 to 6 months after a bone grafting procedure, graft-induced soft and hard tissue changes may be already established and could affect recovery from surgical trauma, as expressed by blood supply and buccal bone thickness changes.<sup>8-10</sup> Such graft-induced tissue changes are not expected to occur in "pristine" sites, that is., ones that have not undergone a bone grafting procedure. Soft tissue wound healing after the inevitable surgical trauma inflicted during implant fixture placement may be differentially affected in grafted and pristine sites; however, there is no information on the comparison of early soft tissue healing outcomes after implant placement in grafted and non-grafted sites.

Blood supply, which is compromised by surgical trauma, is critical for wound healing following surgery.<sup>11, 12</sup> Fast blood perfusion recovery of operated tissues is essential for favorable, non-

complicated, functional and aesthetic short-term and long-term outcomes.<sup>13, 14</sup> Laser Doppler flowmetry (LDF) allows non-invasive determination of tissue blood perfusion<sup>15</sup> and has been used to ascertain gingival flap perfusion following various periodontal surgeries.<sup>16, 17</sup> Alsum et al.<sup>18</sup> used LDF to study buccal flap blood perfusion following bone grafting procedures performed before implant placement. Molecular evidence indicates that peri-implant soft tissues heal differently than periodontal tissues after surgery.<sup>19</sup> Despite these reported early wound healing differences between periodontal and peri-implant tissues, there are no clinical reports on gingival flap perfusion following implant placement.

Cone beam computed tomography (CBCT) allows three-dimensional evaluation of alveolar ridge changes at various treatment phases.<sup>20</sup> Evaluation of peri-implant buccal bone thickness in the esthetic zone has been challenging because of anatomical and technical limitations.<sup>21, 22</sup> In most studies, buccal bone measurements have been expressed as bone thickness at specific distances from the implant platform<sup>23</sup> or as area volume in the coronal 2 mm of the implant.<sup>24, 25</sup> However, information on buccal bone changes along the entire implant length is lacking.

This study aims to investigate soft tissue healing, including gingival flap perfusion recovery, following implant placement surgery and to identify possible differential healing responses at grafted and pristine alveolar ridge sites.

## 2 MATERIALS AND METHODS

### 2.1 Study design and study population

This study was designed as a prospective clinical observation with a four-month follow-up period. Patients referred to the Ohio State University (OSU) Graduate Periodontics Clinic for implant placement at single maxillary non-molar site with intact adjacent teeth were recruited. Inclusion criteria were non-smokers, aged 18 to 75 years, no systemic diseases/conditions affecting periodontal health or healing, no untreated periodontal disease, non-pregnant and non-lactating. Patient recruitment, treatment, and follow-up visits were conducted between December 2015 and April 2018. Clinical and wound healing parameters were documented before, during, immediately after the surgery and at 3, 6, 9 days, and 1 and 4 months, postoperatively. The study protocol and informed consent forms were approved by the OSU Institutional Review Board (protocol 2015H0125); the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. All participants provided written informed consent before any study procedures.

### 2.2 Surgical procedures

Implant placement surgeries were performed by periodontal residents under direct periodontal faculty supervision. Depending on previous bone grafting history with/without additional bone deficiency at future implant site participants were categorized into grafted (GG) or non-grafted (NGG) group. Standardized surgical protocol included local anesthesia administration, mid-crestal incision followed by full thickness flap elevation just past the mucogingival junction, osteotomy preparation followed by implant fixture placement. No vertical incisions were made. Any unintentional soft tissue tear was recorded. Freeze-dried bone allograft (FDBA) and collagen membrane used for bone regeneration were identical for all GG cases in the present study.<sup>18</sup> All implant fixtures used were bone level implants with roughened surface, placed under one or two stage surgical protocol. Patients were prescribed

perioperative prophylactic antibiotics and postoperative analgesics (7 days) and antimicrobial rinse (0.12% chlorhexidine, 2 to 3 times/day for 2 weeks). Patient diaries were used to document postoperative medication consumption.

### 2.3 Clinical measurements

Gingival tissue phenotype (GTP) was recorded on the midbuccal aspect of the surgical site 2 to 3 mm apical to bone crest level using a non-tension caliper during surgery and at 4 months. GTP was classified as thick (thickness >1 mm) or thin (thickness ≤1 mm; modified by Muller et al.<sup>26</sup>). Keratinized Tissue Width (KTW) measurements were taken preoperatively on the mid buccal portion of the surgical site using UNC-15 probe.<sup>18</sup> Clinical wound healing was scored for each of the following parameters as previously described<sup>18</sup>: erythema (increased redness compared to adjacent non-operated sites); bleeding (spontaneous bleeding at wound site); necrosis (visual soft and/or hard tissue necrosis at wound site). In addition, clinical wound exposure (farthest distance between flap margins or between flap margin and healing abutment for non-submerged implant cases, measured with UNC-15 probe) and hydrogen peroxide test result<sup>18</sup> were recorded. All wound healing measurements (clinical wound healing parameters, wound exposure/closure and hydrogen peroxide test) were recorded at the same time.

All clinical measurements were recorded by two trained examiners (V.K. or B.L.). A training session was conducted before study initiation to control intra- and inter-examiner differences.

### 2.4 Laser Doppler flowmetry

A calibrated LDF instrument\* equipped with a standard probe was used as previously reported.<sup>18</sup> Briefly, a stent was used for stabilization and standardization of LDF probe positioning during recordings at mid-portion of buccal surface approximately 5 mm apical to gingival margin.<sup>18</sup> Recorded signals were translated into Perfusion Units (PU) and were displayed with a software program.† LDF measurements were recorded before and immediately after surgery, at 3, 6, 9 days, 1 and 4 months. Blood flow at all time points after baseline was calculated for each patient as percentage of the corresponding baseline value. LDF instrument was calibrated following manufacturer's protocol.

### 2.5 Resonance frequency analysis

A metal insert‡ was screwed in the implant fixture. Implant Stability Quotient (ISQ) was recorded through RFA by using a related probe and device specifically designed for metal insert.§ RFA measurements were taken immediately following implant placement and at 4 months. Four measurements (from buccal, palatal, mesial and distal aspect) were recorded and averaged to obtain the implant value used for data analysis.

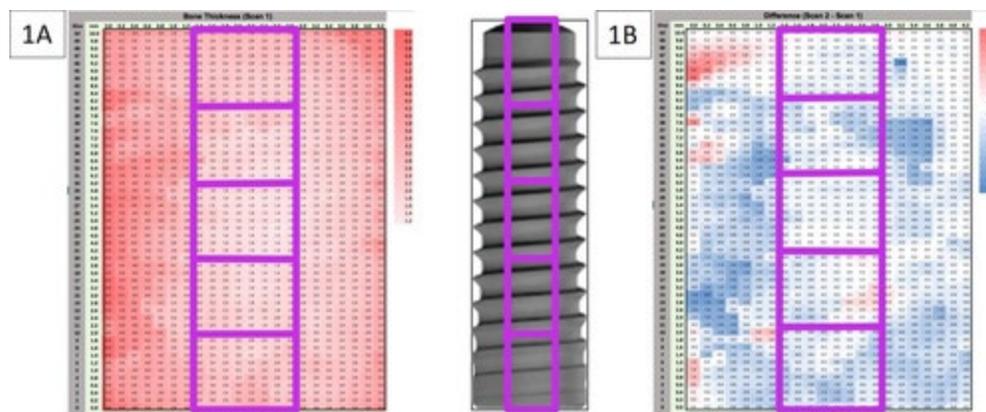
### 2.6 Wound and gingival crevicular fluid collection, sample preparation, and multiplex assays

GCF was collected from the two adjacent teeth before surgery, at 1 and 4 months. Wound fluid (WF) was collected from wound edges at 3, 6, and 9 days, as per published protocol.<sup>18, 19</sup> Briefly, sterile paper strips were gently inserted in the crevice or wound edges for 30 seconds (6 strips/tooth or implant). A calibrated electronic volume quantification unit¶ was used to determine the fluid volume

on each strip. Strips were placed in sterile vials and stored at  $-20^{\circ}\text{C}$ . The day of analysis, elution fluid was prepared.<sup>19</sup> A commercially available panel for multiplex assays# was used to determine molecular markers, including adrenocorticotrophic hormone (ACTH), Dickkopf-related protein 1 (DKK1), interleukin-1 beta (IL-1 $\beta$ ), interleukin-6 (IL-6), osteocalcin (OC), osteoprotegerin (OPN), insulin, and leptin.

## 2.7 CBCT analysis of buccal bone thickness

CBCT images were obtained under the following settings:  $8 \times 8$  cm Field of View, 14.7 seconds exposure time and 0.2 mm voxel size. CBCTs were obtained within first postoperative week and at 4 months. Data were converted to DICOM (Digital Imaging and Communications in Medicine) format and imported to specific software.\*\* CBCT images were superimposed using maxillary bone and teeth as landmarks. The buccal aspect of the implant was isolated and automatically selected by the software based on upper/lower grey value thresholds obtained from control areas (the lower control grey value threshold was the highest grey value of the palatal gingiva of the tooth contralateral to the implant site plus one grey value; the upper control grey value threshold was the highest grey value of the cortical bone at the interforaminal area of the lower border of the mandible). Briefly, peri-implant buccal bone thickness in the first CBCT was calculated using the segmented buccal bone region pixels. Starting from the first pixel coordinate in horizontal axis (left to right), maximum difference between pixel coordinates in vertical axis covered by the segmented area was calculated. Then, the pixel difference was multiplied by the distance between pixels (0.2 mm) and thickness was calculated for the first column of the ROI (region of interest). This calculation was done for each column in the implant ROI. Difference in bone thickness between first and second CBCT was calculated using the difference between the coordinates of the pixels farthest from the implant and covered by segmented areas for the two CBCTs. Following completion of these heat map-like tables of initial buccal bone thickness in the 1st CBCT (Figure 1A) and buccal bone thickness differences between 1st and 2nd CBCT (Figure 1B) along the width (diameter) and length (0 to 10 mm) of the implant, the measurements corresponding to the middle third of implant width were selected for statistical analysis (Figure 1). Registration of images, software development, selection of study areas and calculation of initial bone thickness and difference in bone thickness between the two scans were conducted by one medical imaging specialist (M.D.). Selection of control areas was performed by a clinician (V.K.).



**FIGURE 1** Heat map analysis to automatically calculate buccal bone thickness changes along implant fixture length. (A) An example of a heat map created based on initial buccal bone thickness along the buccal length of the newly placed dental implant fixture. Each cell represents the initial buccal bone thickness in each  $0.2 \times 0.2$

mm<sup>2</sup> area that it corresponds to (darker red means thicker bone, up to 6.2 mm; lighter red means thinner bone, down to 1.2 mm). Each purple box represents 2 × 2 mm<sup>2</sup> area on the mid-buccal aspect of the implant fixture, from coronal to apical. The mean of these boxes is reported as the initial buccal bone thickness of the selected area. **(B)** Peri-implant buccal bone thickness difference between first and second cone beam computed tomography (CBCT) heat map obtained from the same subject presented in Figure 1A. Each cell represents the buccal bone thickness difference in each 0.2 × 0.2 mm<sup>2</sup> area that it corresponds to (darker blue means bone loss, up to 2.2 mm; darker red means bone gain, up to 2 mm). Each purple box represents 2 × 2 mm<sup>2</sup> area on the mid-buccal aspect of the implant fixture, from coronal to apical. The mean of these boxes is reported as the buccal bone thickness difference of the selected area

## 2.8 Statistical analysis

Sample size was determined using a priori calculation method based on previously published data.<sup>18</sup> The patient (site) was the unit of analysis. When CBCT data were paired to clinical data, only cases with both data sets were included into analysis. Descriptive statistics are reported as mean ± SE and percentage. Data were analyzed in GraphPad Prism 5\* and SAS statistical Analysis Software, version 9.4† by a statistician (V.O.Y). Linear mixed model regression analysis with Bonferroni adjustments was used for repeated continuous measures with fixed and random effects within and between groups. Random effect (intercept and slope) regression analysis was conducted to estimate the slopes of the outcome over continuous time for non-grafted and grafted groups. For repeated measure binary outcomes, generalized estimating equations (GEEs) was used. *T*-test, chi-square test or Wilcoxon-Mann-Whitney test, as appropriate, were used to analyze all the other non-repeated data. *P* ≤ 0.05 following Bonferroni adjustments was chosen as statistically significant value.

## 3 RESULTS

### 3.1 Study population

Twenty-seven patients were recruited and 25 (49 ± 4 years; 13 males) completed all study procedures. Two patients were excluded at time of surgery because of surgical protocol changes. Study population and site demographics are detailed in Table 1. Each participant contributed a single site resulting in 25 total surgical sites (NGG = 9; GG = 16). NGG sites never received bone graft, whether before or during implant placement. Among GG sites, nine received an implant after extraction and socket preservation, four after guided bone regeneration and three were immediate implant sites with simultaneous bone graft placement, based on standard protocol that relies on primary stability. For the sites grafted before implant placement, average post-bone graft surgery healing time was 4 months (range: 3 to 5 months).<sup>18</sup> There were no statistically significant differences between NGG and GG for any of the baseline demographic parameters (Table 1).

**TABLE 1.** Demographics

	<b>Non-grafted (NGG; N = 9)</b>	<b>Grafted (GG; N = 16)</b>	<b>P</b>
Age (years)	47 ± 7	50 ± 4	0.684
Sex (females:males)	2:7	10:6	0.226
Anatomical location (maxillary tooth site)	1 incisor	6 incisors	0.357
	2 canines	2 canines	
	6 premolars	8 premolars	

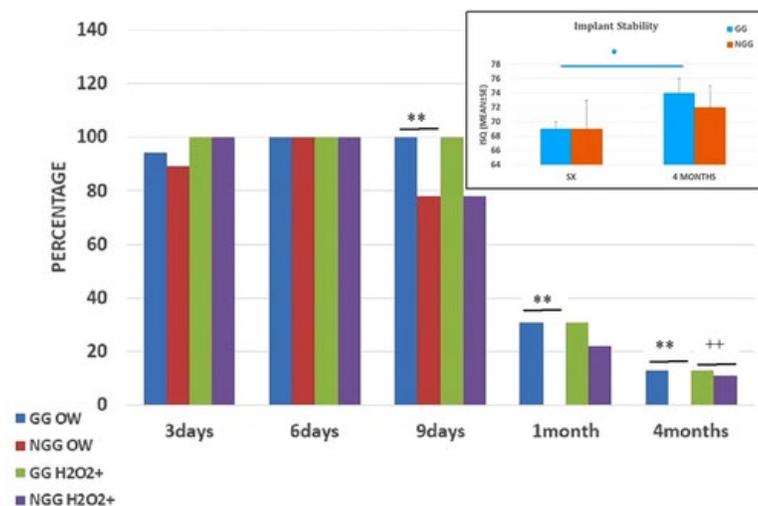
Gingival tissue phenotype	7 thin 2 thick	7 thin 9 thick	0.207
Implant diameter (mm)	3.9 ± 0.1	3.9 ± 0.1	0.575
Implant length (mm)	11.2 ± 0.3	11.2 ± 0.3	0.981
Healing time (days)	124 ± 4	143 ± 8	0.093

All implants were bone level; three implants (NGG = 1, GG = 2) were placed as one-stage based on initial stability.

### 3.2 Clinical parameters

Fifty-six percent of the cases (14 out of 25) were initially diagnosed with thin GTP (Table 1). Thin GTP was more common among NGG (78%) than GG sites (44%;  $P < 0.05$ ). Mean initial KTW was  $4.61 \pm 0.67$  mm in NGG and  $4.21 \pm 0.54$  mm in GG before the surgery ( $P = 0.66$  between groups; data not shown).

Healing was uneventful in all patients. At 3 days, wound closure was achieved in 11% of NGG and 6% of GG sites (Figure 2). All NGG site wounds were clinically closed by 1 month whereas 13% of the GG wounds remained open at 4 months ( $P < 0.01$ ). Statistically significant ( $P < 0.001$ ) intergroup differences were observed at 9 days, 1 month and 4 months (Figure 2). Hydrogen peroxide test was consistent with clinical evaluation of wound closure and detected statistically significant time-dependent changes only in GG ( $P < 0.01$ ; Figure 2).



**FIGURE 2** Clinical wound healing parameters and implant stability (insert). GG, grafted sites ( $n = 16$ ); NGG, Non-grafted sites ( $n = 9$ ); OW, Open Wound; H2O2+, Hydrogen Peroxide Positive; ISQ, Implant Stability Quotient.  $P < 0.01$ ; Time-dependent changes for open wounds for both groups; \*\* $P < 0.001$  between GG and NGG at 9 days, 1 month, 4 months.  $P < 0.01$ ; Time-dependent changes for H2O2+ sites; in GG only; ++  $P < 0.001$  between GG and NGG at 4 months. •  $P < 0.01$ ; Time-dependent changes for ISQ; in GG only. Data presented as mean + se

Time-dependent changes in erythema were statistically significant for both groups ( $P = 0.01$ ) with wound area appearing more erythematous in GG for a longer period of time (see Figure S1 in online *Journal of Periodontology*;  $P < 0.01$  intergroup differences at 9 days, 1 month, and 4 months). Necrosis was evident up to 9 days postoperative and time-dependent differences in tissue necrosis

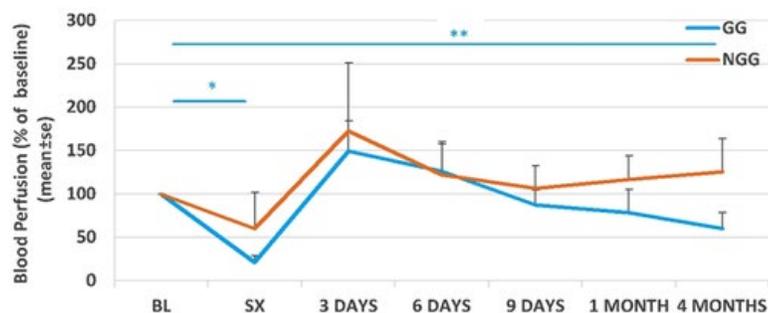
were statistically significant only for GG ( $P = 0.04$ ; see Figure S1 in online *Journal of Periodontology*) with no significant intergroup differences at any time point ( $P > 0.05$ ).

### 3.3 Resonance frequency analysis

Baseline mean ISQ was 69 for both groups with greater variability observed in NGG and increased for both groups at 4 months (NGG =  $72 \pm 3$ , GG =  $74 \pm 2$ ; time-dependent change statistically significant only for GG ( $P < 0.01$ ; Figure 2 insert) with no significant intergroup differences ( $P > 0.05$ ).

### 3.4 LDF responses

As expected, surgical manipulation caused a statistically significant decrease in gingival (flap) blood perfusion representing trauma-related ischemia (Figure 3;  $P = 0.01$  compared to baseline, GG only; no statistically significant intergroup differences). At 3 days postoperatively, blood perfusion in NGG was 173% of baseline and 149% of baseline in GG ( $P > 0.05$ ). Both groups exhibited an expected decrease to baseline values up to 9 days (Figure 3). Time-dependent changes in blood perfusion were statistically significant only in GG group ( $P = 0.05$ ). At 4 months, NGG blood perfusion was 125% of baseline blood perfusion (Figure 3;  $P > 0.05$  baseline to 4 months) and GG remained ischemic (60% of baseline blood perfusion;  $P = 0.05$ ). However, there were no statistically significant differences between groups at any time point ( $P > 0.05$ ).



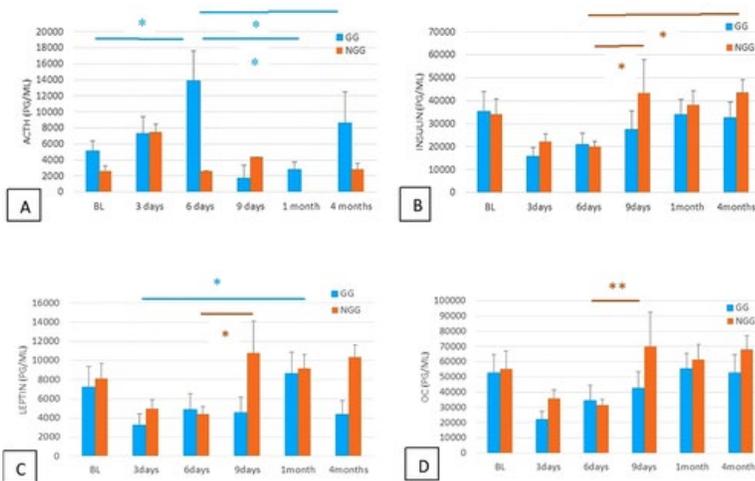
**FIGURE 3** Buccal flap blood perfusion. GG, grafted sites ( $n = 16$ ); NGG, Non-grafted sites ( $n = 9$ ). No significant differences between groups at any time point; Time-dependent differences statistically significant only for GG ( $P < 0.006$ ); \* $P = 0.01$ ; decrease between BL and SX; GG only. \*\* $P = 0.05$ ; Difference between BL and 4 months only in GG; no difference in NGG; Data presented as mean + se

### 3.5 Changes in WF volume and content

At baseline, GCF volume was similar in both groups ( $P = 0.13$  between groups; Figure S2 in online *Journal of Periodontology*), with intergroup differences not statistically significant at any time point ( $P > 0.05$ ). WF volume increases peaked at 3 days for GG ( $P = 0.007$ ) and at 6 days for NGG ( $P = 0.05$ ), with both groups returning to baseline levels at 9 days and remaining at baseline levels subsequently (see Figure S2 in online *Journal of Periodontology*).

When specific GCF/WF content was analyzed for whole study population, IL-6 and IL-1 $\beta$  concentrations increased from baseline to 3 and 6 days ( $P \leq 0.01$ ; IL-6 only), and OC, OPN, insulin and leptin concentrations decreased (3 and 6 days [ $P < 0.01$ ]) (data not shown). However, when content data were analyzed by group (NGG or GG), ACTH concentration was differentially increased at day 6 for GG only ( $P \leq 0.05$ ; Figure 4A). Insulin (Figure 4B), leptin (Figure 4C), and OC (Figure 4D) concentrations were non-significantly decreased from baseline at days 3 and 6, for each of the two groups, and

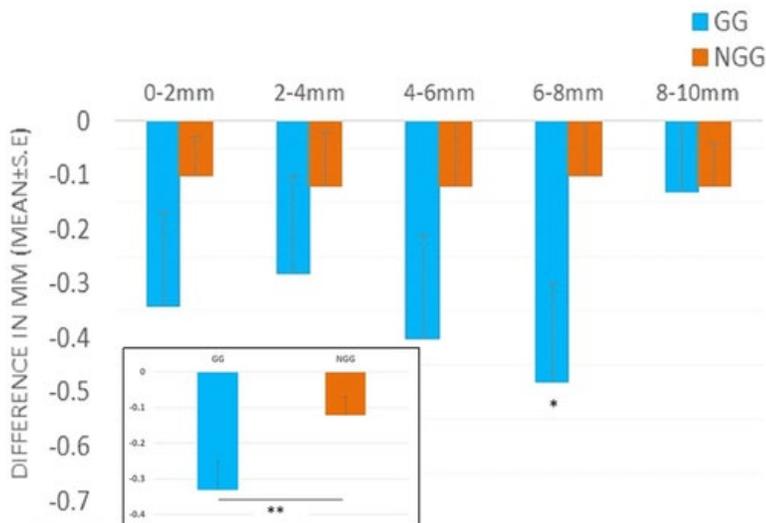
recovered (significantly increased) to levels matching or slightly higher than baseline by day 9 for the NGG group. Only leptin concentration recovered (significantly increased) in the GG group, by 1 month (Figure 4C). Non-significant time-dependent changes in concentrations of other mediators are presented in Figure S3 (see Figure S3 in online *Journal of Periodontology*).



**FIGURE 4** Wound Fluid Content. GG, grafted sites ( $n = 16$ ); NGG, Non-grafted sites ( $n = 9$ ); ACTH, corticotropin; OC, osteocalcin. Data presented as mean + se. **(A)**  $*P \leq 0.05$ ; ACTH concentration between BL and 6 days, 6 days and 1 month, and 6 days and 4 months GG. **(B)**  $*P \leq 0.05$ ; Insulin concentration between 6 days and 9 days and, 6 days and 4 months; NGG. **(C)**  $*P \leq 0.05$ ; Leptin concentrations between 3 days and 1 month; GG and, between 6 days and 9 days; NGG. **(D)**  $**P = 0.04$ ; OC concentration between 6 days and 9 days; NGG

### 3.6 Radiographic buccal bone thickness changes

CBCT data analysis were based on 12 GG and nine NGG implants (four GG cases had incomplete radiographic records). Initial peri-implant buccal bone thickness for NGG and GG was  $1.5 \pm 0.1$  mm (1.3 to 1.6 mm) and  $1.8 \pm 0.1$  mm (1.5 to 2.1 mm), respectively ( $P > 0.05$  at any coronal-apical vertical level; data not shown). Following 4 months of healing, CBCT analysis through superimposition and heat map analysis (Figure 1) revealed a mean loss of  $0.1 \pm 0.2$  mm (range: -1 to + 0.5 mm) in NGG and a mean loss of  $0.3 \pm 0.2$  mm (range: -1.5 to + 1.3 mm) in GG (Figure 5 insert;  $P < 0.01$  between groups). The most pronounced bone thickness loss was recorded at 6 to 8 mm length along the buccal aspect of implant fixture (Figure 5;  $P = 0.02$ , GG only). Among sites with thin GTP at baseline, GG lost more bone thickness compared to NGG at 0 to 6 mm length along the buccal aspect of implant fixture ( $P < 0.05$  between groups; data not shown).



**FIGURE 5** Peri-implant buccal bone thickness changes along implant length segments. GG, grafted sites ( $n = 12$ ); NGG, Non-grafted sites ( $n = 9$ ). Data presented as mean + se;  $*P = 0.02$  between groups; mean peri-implant mid-buccal bone loss at 4 months, at 6 to 8 mm coronal-apical level;  $**P < 0.01$  between groups; mean overall peri-implant mid-buccal bone loss at 4 months

## 4 DISCUSSION

Bone grafting for implant site development is a well-established treatment that makes it possible to place implant fixtures in areas that have experienced the inevitable post-extraction and/or post-trauma bone loss. Perhaps because of the predictability of bone grafting<sup>2-6</sup> and the success of dental implants placed in grafted bone,<sup>8-10</sup> the potential effect of incorporated graft materials on overlying soft tissue wound healing after implant fixture placement has not been investigated. The present study analyzed and compared soft tissue healing after implant placement surgery in pristine (NGG) and grafted (GG) sites; the results indicate that history of bone grafting alters the clinical (wound closure), physiological (blood perfusion), and molecular healing response of overlying soft tissues. The present study reports the first clinical data on gingival flap perfusion recovery following implant placement surgery and a novel approach for the analysis of postoperative peri-implant buccal bone thickness changes.

This study investigated single maxillary non-molar sites. This anatomical location was selected because previous studies have found that buccal bone in the esthetic area undergoes significant resorption post-extraction and following implant placement.<sup>20</sup> Limiting anatomical location to maxillary anterior sextant as well as limiting wound size to a single edentulous area with intact mesial and distal teeth allowed to minimize variability of hard and soft tissue phenotypes. Furthermore, and in conjunction with the controlled anatomical location and wound size, the standardized flap design (full thickness flap extending just past the mucogingival junction) and graft material used (FDBA), minimized the potential variability of the surgical trauma to which soft tissues were exposed. The clinical healing results indicate that peri-implant soft tissue healing in GG appeared to have a higher inflammatory clinical profile as expressed by prolonged erythema and delayed wound closure compared to NGG; these intergroup differences were evident despite the fact that NGG sites were much more likely to exhibit a thin gingival phenotype. Wound closure was previously reported as non-significant

determinant of GBR outcomes: volumetric and dimensional ridge changes post-GBR are not affected by wound opening during healing.<sup>27</sup> However, lack of wound closure can increase infection risk, wound mechanical instability, and may negatively affect biomaterial resorption rate, thus affecting quality more than quantity of regenerated bone.<sup>28, 29</sup> We previously reported a strong correlation between wound exposure and hyperemic flap response following bone regeneration procedures.<sup>18</sup> The current study reports increased erythema and wound exposure at grafted peri-implant sites. All implants, regardless of group, were osseointegrated at study end with no early healing complications and no need for additional bone grafting at time of uncovering/loading. Thus, despite the detected differences in clinical and physiological soft tissue characteristics, implant clinical data reported in the present study agree with previous publications on success/survival rate of dental implants placed at grafted compared to nongrafted sites.<sup>2, 3, 9</sup>

Buccal flap perfusion was monitored as soft tissue healing measure. Immediate post-surgical decrease in gingival blood perfusion, because of surgical trauma (flap elevation and use of local anesthetic with vasoconstrictive agent), has been previously reported.<sup>15-17</sup> In contrast to the present results, previous studies reported a hyperemic post-flap elevation response persisting up to day 7 post-operatively with blood perfusion returning to baseline 15 days after simple flap surgery.<sup>15-17</sup> The current results on post-implant surgery NGG perfusion response at 4 months are similar to our previously reported blood perfusion results following bone regeneration procedures before implant placement.<sup>18</sup> In contrast, the post-implant surgery GG response indicated continuous relative ischemic conditions up to 4 months. The current findings of more significantly decreased blood perfusion at clinically healed peri-implant GG but not at NGG sites agree with the study of Nakamoto et al.<sup>30</sup> who consistently found, using laser speckle imaging, significantly lower blood perfusion in all aspects of healthy gingiva (free, attached, and papillary) around maxillary anterior implants in previously bone grafted sites, compared to implants placed without any bone grafting.<sup>30</sup> This post-implant surgery ischemia response at GG sites may be related to soft tissue scarring response, differential response of regenerated bone to surgical trauma, and/or presence of residual graft material.

WF molecular content release over time also indicated differential responses within each group even though there was no difference between the two groups at any time point. Increase (up to 6 days) and decrease (up to 1 month) in ACTH was significant only in GG. ACTH (corticotropin) is locally released by immune cells and acts as autacoid during early and late phases of immune/inflammatory response.<sup>31</sup> It is generally accepted to play a role as pro-inflammatory mediator; however, the present study is the first to report data suggesting ACTH role in periodontal/peri-implant wound healing. The possible local involvement of ACTH in periodontal/peri-implant soft tissue healing merits further investigation. Bone mediators such as insulin, leptin, and OC, presented dynamic changes, with significant decreases at day 6 and recovery to baseline levels at day 9 in NGG sites. These bone mediators are generally reported as having protective role for periodontal tissues, thus expressed at high baseline concentrations within GCF.<sup>32, 33</sup> The temporarily decreased WF levels of these mediators shortly after surgery suggest a possible transient disruption of this protective role during early postoperative healing.

This study is reporting minimal changes in buccal bone thickness during early phases of healing independent of additional grafting procedures, which confirms previously reported outcomes<sup>34</sup> and is not surprising given the short-term observation period and non-loading conditions. The present study

is the first to report most pronounced bone thickness loss at 6 to 8 mm along the implant length. This novel finding may be related to the spatial distribution of mechanical stress during osteotomy preparation and angulation correction<sup>35, 36</sup> and/or the prolonged overlying soft-tissue ischemia. However, the magnitude of the observed bone changes may also reflect the technical limitations of CBCT in evaluating very thin bone tissue (<0.5 mm).<sup>21, 22</sup>

In order to evaluate buccal bone thickness throughout the entire implant length, an automatic registration of the two CBCTs (baseline and 4 months) was developed and the implant itself was used as reference to allow isolation of its buccal aspect. The creation of heat map outlines by using a color-coding scheme for buccal bone thickness at every  $2 \times 2 \text{ mm}^2$  area allowed detailed and precise spatial analysis of bone thickness changes. Previous work on CBCT measurements of buccal bone changes used linear, surface area, and volumetric calculations using either the implant or other landmarks.<sup>21, 22</sup> Superimposition of more than one CBCT with automatic measurements has been reported in more than ten publications,<sup>22</sup> with analyses based on manually selected peri-implant anatomical locations.<sup>22</sup> To our knowledge, this is the first study to use heat map generation to automatically calculate changes in buccal bone thickness along the length of the buccal surface of the newly placed fixture. This novel approach allowed greater standardization of study area isolation and automatic selection of bone grey threshold values. Nevertheless, beam hardening artefacts because of implant metal structure<sup>22-25</sup> and the aforementioned CBCT constraints in detecting thin bone in proximity to the implant<sup>36,37,39</sup> remain as limitations, even for this technique.

## 5 CONCLUSIONS

Within the limitations of this study, the early post-implant placement healing of soft tissues overlying grafted bone sites is characterized by greater wound closure delay, more persistent ischemia, and altered molecular content compared to soft tissues overlying non-grafted sites; these soft tissue differences are accompanied by greater buccal bone thickness changes at grafted sites. These novel findings suggest that sites regenerated after bone grafting may have differential responses to acute or chronic oral environment challenges; these altered responses could necessitate modified approaches to any intervention, including maintenance procedures, on such sites. Future research should examine other possible determinants of the soft tissue healing response following implant placement surgery.

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## AUTHOR CONTRIBUTIONS

Vrisiis Kofina contributed to patient recruitment, data collection, and analysis, data interpretation, preparation of figures, drafting, and editing the article. Mutlu Demirer contributed to data collection and analysis, data interpretation, preparation of figures, drafting and editing the article. Barbaros S. Erdal contributed to study design, data analysis, data interpretation, drafting and editing the article. Timothy D. Eubank contributed to data collection in relation to protein assays and editing the article. Vedat O. Yildiz contributed to data analysis, data interpretation, drafting, and editing the article.

Dimitris N. Tatakis contributed to data interpretation, drafting, editing, and revising the article. Binnaz Leblebicioglu conceived the study, wrote grant proposal, and established support, obtained IRB approval, contributed to patient recruitment, data collection, and analysis, data interpretation, preparation of figures, drafting, editing, and revising the article. All authors approved the final version of article.

## Supporting Information

Filename	Description
jper10610-sup-0001-FigureS1.jpg73.5 KB	Supporting Figure S1
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