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Effects of Smoking on Palatal Thickness, Density, and Vascularization Assessed by High-Frequency Ultrasonography

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Summary: This study demonstrated that high-frequency ultrasonography is an effective, reliable, and non-invasive method for detecting smoking-induced structural and microvascular alterations in palatal donor tissue during the preoperative period; additionally, smoking was shown to increase epithelial thickness while reducing vascularization.

Author contributions: All authors have made substantial contributions to study conception and design. MA, SSA and HO performed data collection. MA, HO, SSA, EU, MAS, SK, and GK contributed to data analysis, data interpretation, drafting and critically revising the manuscript. All authors approved the final version submitted to be published.

ABSTRACT

Objective: This study aimed to evaluate tissue thickness and vascularization of graft donor sites in heavy/chronic smokers and non-smokers using high-frequency ultrasonography.

Materials and methods: This single-center case-control study included 42 systemically healthy individuals (21 smokers, 21 heavy/chronic smokers) requiring palatal graft harvesting. Smoking status was biochemically confirmed using salivary cotinine. Preoperative high-frequency ultrasonography evaluated tissue thickness, density, and vascularization.

Results: Heavy/chronic smokers showed significantly greater epithelial thickness in premolar and molar regions ($p<0.001$), with no differences in lamina propria or total tissue thickness ($p>0.05$). Echo intensity did not differ between groups ($p=0.288$), while Doppler/B-Flow values were lower in heavy/chronic smokers ($p=0.017$), indicating reduced vascularization. Salivary cotinine ($\rho=-0.493$, $p=0.001$) and daily cigarette use ($\rho=-0.359$, $p=0.020$) were negatively correlated with vascularization, and positively correlated with epithelial thickness (cotinine: $r=0.538$ [molar], $r=0.490$ [5–6 mid]; cigarette consumption: $r=0.666$ [molar], $r=0.652$ [5–6 mid], $r=0.561$ [premolar]; all $p<0.001$). Age correlated positively with lamina propria and total thickness, whereas sex showed no association.

Conclusion: High-frequency ultrasonography enables assessment of tissue thickness and vascularization in palatal graft donor sites. Smoking adversely affects vascularization without significantly altering overall tissue thickness. Ultrasonographic evaluation may aid in predicting graft healing and long-term tissue stability, particularly in heavy/chronic smokers.

Key words [MeSH terms]: Smoking, Palatal mucosa, Connective tissue graft, Ultrasonography, Vascularization, Periodontal plastic surgery

INTRODUCTION

Soft tissue autografts have long been widely used for the treatment of gingival recession and for augmenting the volume of peri-implant and periodontal tissues in implant-supported rehabilitations [1]. Among these, the autologous subepithelial connective tissue graft (CTG) is regarded as the gold standard in periodontal plastic surgery due to its high predictability, reliability, and well-documented clinical efficacy [2-5]. The lateral palate is the most frequently preferred donor site for soft tissue autograft procedures and provides a reliable source of donor tissue for various periodontal and implant-related interventions, including root coverage, alveolar ridge volume augmentation, and the establishment of adequate keratinized tissue width [6,7].

The clinical success of CTG procedure is closely associated with the thickness of the donor tissue and the collagen-to-adipose tissue ratio [8]. Therefore, accurate and reliable assessment of the structural characteristics of palatal fibro-mucosa is of critical importance for ensuring the predictability and success of grafting procedures [9]. The thickness of the palatal fibro-mucosa exhibits considerable variability both among individuals and across different palatal regions, with the premolar area generally reported to be thicker and the molar region thinner [6,8,10,11]. In addition, palatal tissue thickness has been shown to increase with greater distance from the gingival margin and with advancing age [12,13]. It has been suggested that women may present with thinner palatal tissue compared to men, the literature remains inconsistent regarding sex-related differences [13-16]. Smoking is a significant environmental factor that adversely affects the quality and healing capacity of periodontal and peri-implant tissues. In smokers, vascularization, collagen synthesis, and tissue remodeling processes have been shown to be impaired [17-19]. However, data specifically addressing the effects of smoking on the biological properties of palatal fibro-mucosa remain limited [20,21].

In recent years, high-frequency ultrasonography has emerged as a non-invasive method for assessing the thickness, density, and vascularization of soft tissues. Ultrasonography not only assists clinicians in graft planning but also enhances patient comfort by reducing the need for invasive measurement techniques [22,23]. According to the relevant literature, there is limited evidence regarding the detailed evaluation of palatal donor site by comparing smokers or non-smokers with ultrasonography. Addressing this gap may contribute to the refinement of graft selection strategies in clinical practice and improve the predictability of grafting outcomes.

Based on this background, our hypothesis was that the ultrasonographic thickness, density, and vascularization characteristics of the palatal fibro-mucosa represent the histopathological characteristics of the tissue and significantly differ between smokers and non-smokers. The aim

of the present study was to evaluate palatal donor tissues in smokers and non-smokers using high-frequency ultrasonography and to compare the thickness, density, and vascularization properties of the fibro-mucosa between the two groups.

MATERIALS AND METHODS

Study design and participants

This single-center, case-control clinical study included a total of 42 systemically healthy individuals who were scheduled to undergo mucogingival surgery involving palatal soft tissue graft harvesting at the Department of Periodontology, Faculty of Dentistry, Ankara University. The study population consisted of 21 smokers and 21 non-smokers. Participants were allocated into smoker (S, n=21) and non-smoker (NS, n=21) groups.

The study protocol was approved by the Human Research Ethics Committee of Ankara University Faculty of Medicine (Approval No: İ01-36-26). All participants were informed about the study procedures and provided written informed consent prior to enrollment, in accordance with the principles of the Declaration of Helsinki. No identifiable personal data were included.

Inclusion and exclusion criteria

Eligible participants were adults (≥ 18 years) who required CTG harvesting from the region of interest (ROI) and were systemically healthy with good oral hygiene, defined as a plaque index $< 10\%$. Individuals were classified as smokers if they reported smoking ≥ 20 cigarettes (≥ 1 pack/day) per day for ≥ 5 years and had salivary cotinine levels > 0.18 mg/l, while non-smokers were those who had never smoked in their lives. This threshold is consistent with the Centers for Disease Control and Prevention (CDC) definition of heavy smoking and aligns with recent prospective peri-implant evidence employing the identical criterion alongside cotinine-based verification of smoking status [24].

Individuals were excluded if they presented with untreated periodontitis or peri-implantitis, were pregnant or lactating, or had diabetes mellitus. Additional exclusion criteria included liver or kidney failure, severe hematologic disorders, any autoimmune or inflammatory disease, or the use of medications known to impair normal wound healing. Participants with a history of malignancy, radiotherapy, or chemotherapy within the previous 18 months were also excluded. The use of orthodontic appliances with palatal extensions, the presence of removable prostheses in the ROI, and refusal or inability to provide written informed consent were further grounds for exclusion.

Smoking status determination

Smoking status was objectively verified by biochemical assessment of salivary cotinine levels. Unstimulated whole saliva samples were obtained between 09:00 and 11:00 a.m. to minimize potential circadian variation. Prior to collection, participants were instructed to rinse their mouths thoroughly with distilled water. Subsequently, they were seated in an upright and relaxed position and asked to passively expectorate into a sterile plastic collection tube over a 5-minute period [18]. The collected saliva samples were centrifuged at $2800\times g$ for 10 minutes to remove cellular debris. The resulting supernatant was carefully transferred into sterile Eppendorf tubes and stored at -80°C until biochemical analysis. Quantification of salivary cotinine concentrations was carried out using liquid chromatography–tandem mass spectrometry (LC–MS/MS) with a TSQ Quantum Access MAX Triple Quadrupole Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Patients were classified as smokers if they reported smoking ≥ 20 cigarettes daily for ≥ 5 years and had salivary cotinine levels >0.18 mg/L, whereas nonsmokers were individuals who had never smoked in their lifetime. [18,24].

Ultrasonographic analysis

Ultrasonographic analyses were performed prior to surgery using a high-frequency ultrasound device (LOGIQ P10 XDClear R4.5, GE Healthcare, Republic of Korea). Examinations were conducted with an L8–18i-RS high-frequency linear “hockey stick” probe under the following settings: 8 MHz frequency, 25.2×4 mm physical footprint, 25.2 mm maximum field of view (FOV), and 80 mm maximum display depth. For each participant, three repeated measurements were obtained, and the mean values were used for analysis. All measurements were performed prior to graft harvesting, and the same protocol was applied to both smoker and non-smoker groups. Six-second ultrasonographic video recordings and static B-mode images were acquired from each patient and stored in DICOM format. Both the static B-mode images and the dynamic recordings were archived in DICOM format, and all analyses were conducted digitally using computer-based software. A 5-mm-thick standoff gel pad (Aquaflex Ultrasound Gel Pad; Parker Laboratories, Fairfield, NJ, USA) was placed over the transducer and covered with a rubber sleeve to minimize tissue compression during measurement.

Doppler / B-Flow assessment

Vascularization and blood flow parameters of the ROI were recorded using Doppler ultrasonography. For image acquisition, the probe was positioned longitudinally over the right maxillary palatal donor site, with its midpoint located between teeth 15 and 16. A 6-second color cine clip was digitally recorded in Color Doppler Ultrasound (CDUS) mode. The same L8–18i-RS linear probe operating at 8 MHz was used for both Color Doppler Ultrasound (CDUS) and B-Flow modes to evaluate palatal microvascular perfusion, thereby directly linking the Doppler/B-Flow measurements to vascularization assessment of the donor site. Color Doppler settings were standardized as follows: color velocity scale 15–30 cm/s, color gain adjusted to fill the vessel lumen without overflow into surrounding tissue, Doppler angle $\leq 60^\circ$, and a low wall filter to preserve slow-flow signals consistent with the palatal microcirculation [25].

Color Quantification (CQ) analysis was performed using dedicated software developed for the LOGIQ P10 XDClear R4.5 system (GE Healthcare, Republic of Korea). For each participant, the ROI was defined as the area between the palatal crestal bone and the outer palatal mucosal surface at the center of the image. Blood flow within each ROI was then quantified to assess vascularization in the corresponding region. In addition to CDUS, B-Flow mode a non-Doppler, grey-scale blood-flow imaging technique was employed to provide angle-independent visualization of slow palatal blood flow, complementing the color Doppler signal and improving sensitivity for the detection of low-velocity microvascular perfusion.

Linear measurements

The thickness of the lamina propria and submucosa was measured in millimeters using a high-frequency linear probe with the LOGIQ P10 XDClear R4.5 ultrasonography system (GE Healthcare, Republic of Korea). The probe was positioned longitudinally over the palatal mucosa, with its midpoint aligned between teeth 15 and 16.

Linear measurements were obtained at three anatomical locations: the midpoint between teeth 15 and 16, the premolar region, and the molar region (**Figure 1A**). At each site, epithelial thickness, lamina propria thickness, and total soft tissue thickness were measured in millimeters (**Figure 1B**). All linear measurements of the epithelium, lamina propria, and submucosa were performed three times, and the mean values were used for analysis.

Examiner blinding and calibration

The investigator performing the ultrasonographic measurements (SSA) was blinded to the participants' smoking status. Linear analyses were repeated three times for each patient at one-week intervals; each measurement set was performed independently and in a blinded manner relative to the previous results, and mean values were used for analysis. Prior to the study, the examiner responsible for measurements underwent calibration using ultrasonographic images obtained from individuals not included in the study, and training was considered complete once the intraclass correlation coefficient (ICC) exceeded 0.8.

Intra-rater reliability of ultrasound measurements was assessed in a subset of participants by the single operator (SSA), who repeated all measurements one week apart. Reliability was quantified using ICC (3, k) (two-way mixed model, absolute agreement, average measures), standard error of measurement ($SEM = SD \times \sqrt{1-ICC}$), and minimal detectable change ($MDC_{95} = SEM \times 1.96 \times \sqrt{2}$).

Echo intensity analysis

Tissue density and structural integrity were evaluated using echo intensity analysis. ROI was defined as the area between the palatal crestal bone and the palatal mucosal surface in the region between teeth 15 and 16. Within this ROI, grayscale pixel values were measured offline to quantify tissue echogenicity [26]. ImageJ software (version 2.0; National Institutes of Health, Bethesda, MD, USA) was used to calculate the mean grayscale value, standard deviation (SD), and pixel count within the selected ROI.

Statistical Analysis

Based on the periodontal ultrasonography study by Tavelli et al. (2021), which represented the closest methodological reference, a standard deviation for mucosal thickness of 0.26 mm and a clinically meaningful minimum difference of 0.13 mm were used for the calculation. Under these assumptions ($\alpha = 0.05$, power = 80%, two-tailed independent samples t-test), an estimated sample size of approximately 10 participants per group was found to be sufficient. The inclusion of 21 participants per group in the present study substantially exceeded this threshold, supporting the adequacy of the sample size for the primary outcome measure.

All statistical analyses were conducted using SPSS software (SPSS for Windows, version 26.0, IBM SPSS Inc., New York, NY, USA). Data normality was assessed using the Shapiro–Wilk test. Normally distributed data were analyzed using the student t-test, whereas non-normally

distributed data were evaluated using the Mann–Whitney U test. Categorical variables were analyzed using the chi-square test. Correlation analyses were performed using Pearson's r for pairs of variables in which both members satisfied the assumption of normality (Shapiro–Wilk $p \geq 0.05$), and Spearman's rank correlation coefficient (ρ) for pairs in which at least one variable was non-normally distributed. A significance level of $\alpha=0.05$ was set for all statistical tests.

RESULTS

Study Population

Demographic characteristics are presented in **Table 1**. A total of 21 smokers (8 females, 13 males; mean age: 33.3 ± 10.4 years) and 21 non-smokers (11 females, 10 males; mean age: 29.3 ± 9.4 years) completed all study procedures. No statistically significant difference was observed between the groups in terms of age or sex distribution ($p=0.198$ and $p=0.352$, respectively). Salivary cotinine levels were significantly higher in smokers compared to non-smokers ($p<0.001$).

Linear Measurements

Ultrasonographic measurement results are presented in **Table 2**. In the premolar region, epithelial thickness was significantly greater in the smoker group compared with non-smokers (0.45 ± 0.12 mm vs. 0.32 ± 0.08 mm, respectively; $p<0.001$). Although lamina propria thickness and total tissue thickness in the same region were higher in smokers, these differences did not reach statistical significance ($p=0.081$ and $p=0.090$, respectively).

Similarly, in the 5-6 mid and molar region, epithelial thickness was significantly higher in smokers than in non-smokers ($p<0.001$). However, no significant difference was observed within the groups in terms of lamina propria thickness or total tissue thickness in this region ($p=0.255$ and $p=0.310$ for 5-6 mid and $p=0.856$ and $p=0.893$ for molar region).

Intra-rater reliability statistics for all tissue thickness measurements are presented in **Table 2**. ICC values indicated excellent reliability across all parameters ($ICC > 0.95$). For epithelial thickness measurements, SEM ranged from 0.020 to 0.023 mm and MDC_{95} from 0.057 to 0.064 mm both less than half the smallest intergroup difference observed in the study (0.13 mm), confirming that the reported differences exceed measurement noise. Lamina propria and total thickness measurements similarly demonstrated excellent reliability ($ICC 0.987–0.997$); however, the correspondingly wider SEM (0.146–0.218 mm) and MDC_{95} (0.406–0.604 mm) values were consistent with the absence of statistically significant intergroup differences for these parameters ($p > 0.05$).

Doppler/B-Flow and echo intensity measurements

Table 3 presents the Doppler/B-Flow and echo intensity measurements of the palatal donor site in smokers and non-smokers. No significant inter-group difference was observed regarding echo intensity values ($p=0.288$). In contrast, Doppler/B-Flow measurements were significantly higher in the non-smoker group (smokers: 0.19 (0.14–0.35); non-smokers: 0.46 (0.25–0.56); $p=0.017$).

Correlation Analyses

Pearson correlation analysis revealed several significant associations between smoking-related variables, demographic factors, and ultrasonographic parameters (**Table 4**). Salivary cotinine levels showed a significant negative correlation with Doppler values ($p=-0.493$, $p=0.001$). Similarly, daily cigarette consumption was negatively correlated with Doppler measurements ($p=-0.359$, $p=0.020$). In contrast, both cotinine levels and daily cigarette consumption exhibited strong and significant positive correlations with epithelial thickness. The highest correlation coefficients were observed between daily cigarette consumption and epithelial thickness in the molar region ($r=0.666$, $p<0.001$) and the 5–6 mid-region ($r=0.652$, $p<0.001$). Cotinine levels were likewise positively correlated with epithelial thickness in the molar ($r=0.538$, $p<0.001$) and 5–6 mid-regions ($r=0.490$, $p<0.001$). Age was not significantly associated with Doppler or epithelial thickness parameters; however, it demonstrated consistent positive correlations with lamina propria thickness and total tissue thickness across regions. Notably, total tissue thickness in the 5–6 mid-region ($r=0.541$, $p<0.001$) and molar region ($r=0.509$, $p<0.001$) showed the strongest positive associations with age. Overall, these findings indicate that smoking exposure is mainly associated with alterations in the epithelial layer and vascular parameters, whereas aging appears to be associated with increased connective tissue and overall palatal soft tissue thickness.

DISCUSSION

In this case-control study, the effects of smoking on the structural and microvascular characteristics of the palatal fibromucosa were evaluated using high-frequency ultrasonography. Our findings demonstrated that palatal epithelial thickness was significantly higher in heavy/chronic smokers across all assessed anatomical regions, whereas lamina propria thickness remained comparable between heavy/chronic smokers and non-smokers. Furthermore, lower Doppler values were observed in the smoking group, and these values

exhibited a significant negative correlation with both daily cigarette consumption and salivary cotinine levels.

While the adverse effects of smoking on various oral mucosal sites have been extensively documented in the literature, data specifically concerning the palatal donor region remains scarce [21, 27-30]. One of the prominent findings of this study is the significant increase in palatal epithelial thickness observed across all assessed regions in heavy/chronic smokers. This histomorphological alteration is considered to represent a response to chronic thermal and chemical irritation from tobacco smoke, manifesting as epithelial hyperplasia and hyperkeratinization [31-33]. From a clinical perspective, this epithelial thickening may complicate the determination of the ideal incision depth during free gingival or connective tissue graft harvesting. Furthermore, it may increase the risk of epithelial remnants in superficial grafts, potentially predisposing to healing complications. The presence of residual epithelial components within the graft has been associated with undesirable outcomes such as cyst formation and edema [34,35]. Therefore, precise knowledge of the thickness of the different palatal tissue layers is essential to maximize lamina propria yield while minimizing epithelial remnants [36]. However, it has been reported in the literature that changes in epithelial thickness may not be solely dependent on smoking, as various biological and local factors can also influence this parameter. In this regard, a study conducted by Gültekin et al. (2008) stated that the increase in epithelial thickness was not directly related to smoking; instead, periodontal status and inflammatory processes were found to be more decisive factors [37]. Despite the marked increase in epithelial thickness observed among heavy/chronic smokers in our study, the fact that similar increases in thickness can occur in non-smokers suggests that epithelial tissue is a dynamic structure capable of adapting not only to chemical irritants but also to inflammation and functional or mechanical stimuli. This situation indicates that in clinical evaluations, epithelial thickness should be analyzed through a holistic perspective, considering the patient's overall periodontal health and the mechanical effects on the tissue, rather than focusing solely on smoking status.

Interestingly, despite the marked increase in epithelial thickness, the absence of a significant difference in lamina propria thickness suggests that the effects of smoking on connective tissue are manifested through cellular and functional alterations rather than quantitative volumetric changes [38,39]. This finding indicates that smoking-induced modifications in fibroblast activity, collagen synthesis, and extracellular matrix turnover may not always translate into measurable differences in tissue thickness. Consequently, graft quality in heavy/chronic

smokers should be evaluated not solely on the basis of tissue dimensions, but in conjunction with the biological and vascular potential of the tissue.

Although total palatal tissue thickness remained comparable between heavy/chronic smokers and non-smokers, the increased epithelial thickness observed in smokers may still have practical implications for connective tissue graft harvesting. From a surgical perspective, when total tissue dimensions are preserved, a proportionally thicker epithelial compartment may reduce the effective proportion of harvestable lamina propria in superficially harvested grafts, thereby potentially affecting the yield of high-quality connective tissue. Since successful connective tissue grafting relies on maximizing the lamina propria component while minimizing epithelial remnants, inaccurate incision depth in tissues with epithelial hyperplasia may increase the risk of epithelial inclusion and compromise graft quality [8,36]. Beyond the issue of epithelial remnants, smoking-associated biological impairments may negatively affect graft outcomes through multiple mechanisms: diminished vascularization and altered collagen metabolism may delay vascular integration between the graft and the recipient site, thereby increasing the risk of necrosis and graft failure, while impaired fibroblast function may predispose the graft to greater shrinkage over time and dimensional instability; furthermore, inadequate revascularization may impart a pale and patch-like appearance to the healing graft, resulting in pronounced color discrepancy with the surrounding gingival tissues and compromised aesthetic outcomes [38]. Nevertheless, because no statistically significant reduction in lamina propria thickness was observed in the present study, these considerations should be interpreted cautiously. Rather than a quantitative reduction in connective tissue volume, smoking-related alterations may primarily reflect qualitative biological impairments, including reduced vascularization, altered collagen metabolism, and impaired fibroblast function, which together may adversely influence graft healing potential despite preserved tissue dimensions [39,41].

While oral tissue perfusion during wound healing has previously been investigated using methods such as fluorescent angiography, laser Doppler flowmetry, and laser speckle contrast imaging [30,42], the present study evaluated palatal tissue perfusion non-invasively through Doppler and B-Flow modes of high-frequency ultrasonography. The findings demonstrated a significant adverse effect of smoking on the palatal microvascular network. Specifically, heavy/chronic smokers exhibited lower Doppler and B-Flow signals, and these parameters showed an inverse correlation with salivary cotinine levels. The observed negative association between cotinine concentration and palatal vascularization ($p = -0.493$) may indicate a

biologically meaningful dose–response relationship between nicotine exposure and perfusion impairment. Since cotinine is considered a reliable biomarker of cumulative nicotine exposure, the progressive reduction in vascularization with increasing cotinine levels further supports the mechanistic role of nicotine in microvascular compromise. These findings are consistent with the well-documented vasoconstrictive and endothelial effects of nicotine and reinforce the biological plausibility linking tobacco exposure to altered periodontal vascular homeostasis [43,44]. Nicotine-induced vasoconstriction, endothelial dysfunction, and suppression of angiogenic activity are therefore likely to represent the principal mechanisms underlying the observed reduction in local perfusion. The adverse effects of smoking on periodontal tissues extend beyond transient vascular alterations and include long-term impairments in tissue repair, such as reduced gingival blood flow, inhibition of cytokine and growth factor production, and impaired fibroblast attachment to root surfaces [39].

The biological significance of reduced Doppler/B-Flow signals may be particularly relevant when interpreted within the sequential stages of connective tissue graft healing. Following graft placement, the early survival phase depends initially on plasmatic diffusion from the recipient bed, after which inosculation and revascularization become essential to restore oxygenation and nutrient exchange. During subsequent healing stages, angiogenesis and extracellular matrix remodeling support graft maturation and long-term tissue stability [42,45]. In this context, the lower Doppler/B-Flow signals observed in heavy/chronic smokers may indicate a diminished microvascular reserve that could compromise early revascularization and subsequent tissue remodeling. Such vascular impairment may partially explain the well-documented reduction in wound-healing potential and clinical predictability of soft tissue grafting procedures in smokers [30,46]. Given that early and adequate tissue perfusion is critical for both donor site healing and graft integration [45], the microvascular restriction observed in heavy/chronic smokers is directly associated with diminished soft tissue healing potential and reduced clinical predictability. Indeed, systematic reviews in the literature consistently support the negative impact of smoking on the clinical outcomes of combined root coverage procedure [46,47].

It is well-established in the literature that palatal mucosal thickness exhibits variability both among individuals and across different intraoral regions within the same individuals [13,15]. This parameter is of critical importance regarding graft harvestability and long-term clinical stability [6,8]. In the current study, the differences in thickness observed between the premolar and molar regions are consistent with previous research investigating palatal morphology [10,14]. However, no significant difference was found in total palatal tissue thickness between

heavy/chronic smokers and non-smokers across all measured regions. This finding is in alignment with the results reported by Khalid N. Said (2020), suggesting that palatal tissue thickness may be a relatively preserved structural feature [48]. In contrast, the more pronounced impact of smoking on the level of vascularization supports the hypothesis that the effects of smoking primarily manifest at the level of the superficial epithelial layer and the microvascular network.

Interestingly, despite the significant differences observed in epithelial thickness and Doppler/B-Flow values, no intergroup difference was detected in echo intensity measurements. Since grayscale echo intensity derived from high-frequency ultrasonography primarily reflects tissue echogenicity and macroscopic structural organization, this finding may suggest that smoking-related alterations in palatal donor tissue are more pronounced at the epithelial and microvascular levels than at the level of gross connective tissue architecture detectable by HFUS. Nevertheless, this observation should be interpreted cautiously. Smoking is known to impair fibroblast function, collagen metabolism, and extracellular matrix turnover through oxidative and inflammatory pathways [39,40]. Thus, the absence of echogenic differences does not necessarily imply preserved connective tissue integrity but may instead indicate that smoking-related structural alterations occur at a microscopic level beyond the sensitivity of grayscale ultrasonographic analysis. Future histologic correlation studies are warranted to further clarify the biological significance of these findings.

The relationship between palatal mucosal thickness and age has been extensively investigated in the literature using diverse methodologies. Clinical studies based on transgingival probing have demonstrated a tendency for palatal mucosal thickness to increase with age [13,49,50]. Similarly, studies employing advanced imaging techniques, such as computed tomography and magnetic resonance imaging, have reported a positive correlation between age and mucosal thickness, particularly in the posterior palatal region [6,12]. Despite the methodological differences in measurement techniques, this consistent relationship indicates that the underlying biological process remains valid regardless of the measurement modality. In the current study, the age-related increase in tissue thickness detected via ultrasonography in the premolar and molar regions not only supports these established findings in the literature but also demonstrates that ultrasonography serves as a reliable, objective, and non-invasive alternative for evaluating the morphological and biological characteristics of the palatal donor site.

The high-frequency ultrasonography used in our study enabled a reliable and non-invasive evaluation of palatal tissue thickness, density, and vascularization. Consistent with the literature, these findings indicate that ultrasonography is an effective clinical tool that optimizes

graft planning in the palatal donor site and allows for the simultaneous analysis of tissue thickness and vascularization [22,23]. Unlike traditional methods, its ability to reveal both the morphological and hemodynamic characteristics of the tissue in real-time suggests that this technology could be utilized on a broader scale in clinical practice.

There are several limitations to this study that should be acknowledged. First, the single-center design and relatively small sample size may limit the generalizability of the findings. Additionally, the biological characteristics of the palatal donor site were evaluated solely through non-invasive high-frequency ultrasonography without histological confirmation; therefore, the direct cellular correlates of certain parameters could not be established. Individual metabolic variations and environmental exposure may influence the results. Moreover, this study exclusively enrolled heavy/chronic smokers (≥ 20 cigarettes/day for >5 years) confirmed by salivary cotinine assay, the findings should not be extrapolated to light, occasional, or former smokers, whose clinical outcomes may differ substantially. Finally, due to the case-control design and the exclusive focus on the preoperative period, the impact of the observed differences on long-term clinical outcomes could not be evaluated. Consequently, longitudinal prospective studies are required for a more comprehensive understanding of these relationships.

CONCLUSION

In conclusion, smoking adversely affects vascularization and increases epithelial thickness, whereas lamina propria and total tissue thickness remain unchanged. These findings suggest that ultrasonographic evaluation may offer clinical advantages in assessing graft healing potential and long-term stability in heavy/chronic smokers. Future studies involving larger sample sizes and advanced imaging modalities will help strengthen the generalizability of these results.

SOURCE OF FUNDING

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article. Specifically, the raw data can be obtained from the corresponding author, Merve Atak (merve.atak@gmail.com), upon reasonable request.

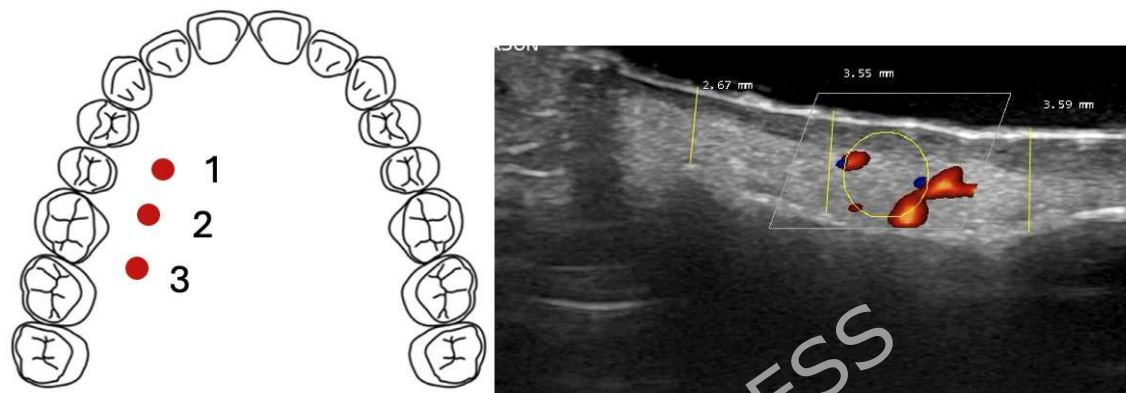


Figure 1: Palatal measurement sites and ultrasonographic assessment. Measurements were performed at three standardized points in the posterior palate, as illustrated schematically (left). Representative ultrasonographic image with Doppler mode demonstrates vascularization within the region of interest (right). No identifying patient information is presented in this figure.

Table 1: Demographic data and salivary cotinine levels of smoker and non-smoker groups.

Table 2: Comparison of ultrasound values between the groups and intra-rater reliability statistics for all tissue thickness measurements (Operator SSA).

Table 3: Comparison of echo intensity and microvascular parameters between smokers

Table 4: Correlation coefficients between demographic/smoking-related variables and ultrasonographic parameters

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Table 1. Demographic data and salivary cotinine levels of smoker and non-smoker groups.

Parameters	Smokers (n=21)	Non-Smokers (n=21)	p
Age (year)	33.3 ± 10.4	29.3 ± 9.4	0.198
Gender (female/male)	8 / 13	11 / 10	0.352
Smoking (n/day)	21 (20–31)	-	<0.001
Salivary cotinine (mg/L)	1.72 ± 0.65	0.06 ± 0.03	<0.001
Data were analyzed using Student's t-test for mean ± SD, Mann–Whitney U test for median (min–max), and Chi-square test for categorical variables.			

Table 2. Comparison of ultrasound values between the groups and intra-rater reliability statistics for all tissue thickness measurements (Operator SSA).

	Palatal Fibro-mucosal Thickness		Intra-rater reliability statistics
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		Smokers (n=21)	Non-Smokers (n=21)	p	ICC (95% CI)	SEM (mm)	MDC ₉₅ (mm)	Group difference S – NS (mm)
Premolar	Epithelium	0.45 ± 0.12	0.32 ± 0.08	<0.001	0.953 (0.920–0.973)	0.020	0.057	0.13
	Lamina propria	3.29 ± 0.85	2.79 ± 0.95	0.081	0.997 (0.995–0.999)	0.146	0.406	0.50
	Total	3.67 ± 0.85	3.19 ± 0.94	0.090	0.994 (0.990–0.997)	0.146	0.406	0.48
Molar	Epithelium	0.46 ± 0.14	0.29 ± 0.07	<0.001	0.966 (0.943–0.981)	0.023	0.064	0.17
	Lamina propria	2.47 ± 1.50	2.55 ± 1.27	0.856	0.997 (0.996–0.998)	0.218	0.604	–0.08
	Total	2.95 ± 1.49	2.89 ± 1.20	0.893	0.996 (0.993–0.998)	0.212	0.588	0.06
5-6 mid	Epithelium	0.47 ± 0.14	0.31 ± 0.07	<0.001	0.975 (0.959–0.986)	0.023	0.062	0.16
	Lamina propria	2.95 ± 0.97	2.58 ± 1.12	0.255	0.987 (0.979–0.993)	0.165	0.468	0.37
	Total	3.24 ± 0.95	2.91 ± 1.12	0.310	0.995 (0.992–0.997)	0.165	0.459	0.33

Data were analyzed using Student's t-test for mean (SD). Values are given in millimeters (mm). Data were analyzed using Student's t-test for mean values (SD). Measurements are presented in millimeters (mm). ICC = intraclass correlation coefficient (two-way mixed model, absolute agreement, average measures); CI = confidence interval; SEM = standard error of measurement ($SEM = SD \times \sqrt{1-ICC}$); MDC₉₅ = minimal detectable change at 95% confidence level ($MDC_{95} = SEM \times 1.96 \times \sqrt{2}$); S = smokers; NS = non-smokers. SEM and MDC₉₅ were calculated using the mean item SD.

Table 3. Comparison of echo intensity and microvascular parameters between smokers and non-smokers

Parameters	Smokers (n=21)	Non-Smokers (n=21)	p
Echo Intensity (dB)	-36.38 ± -6.38	-38.64 ± -7.21	0.288
DOPPLER/B-Flow (a.u.)	0.19 (0.14-0.35)	0.46 (0.25-0.56)	0.017

Data were evaluated using Student's t-test for mean (SD) values and the Mann–Whitney U test for median (IQR) values.
dB : decibel , a.u.: arbitrary units (relative vascularity measurement)

Table 4. Correlation coefficients between demographic/smoking-related variables and ultrasonographic parameters

Variables	Cotinine		Cigarette consumption		Age	
	r/p	p	r/p	p	r/p	p
Intensity ^r	0,179	0,257	0,111	0,485	0,212	0,177
Doppler ^p	-0,493	0.001	-0,359	0,020	0,011	0,946
5–6 Mid Epithelium ^r	0,490	0,001	0,652	< 0.001	0,120	0,449
5–6 Mid Lamina propria ^r	0,159	0,314	0,156	0,325	0,477	0,001
5–6 Mid Total ^r	0,128	0,417	0,144	0,364	0,541	< 0.001
Premolar Epithelium ^r	0,290	0,063	0,561	0,000	-0,146	0,358
Premolar Lamina propria ^r	0,220	0,161	0,230	0,143	0,484	0,001
Premolar Total ^r	0,210	0,182	0,228	0,146	0,488	0,001
Molar Epithelium ^r	0,538	< 0.001	0,666	< 0.001	0,101	0,525
Molar Lamina propria ^r	-0,070	0,660	-0,069	0,665	0,459	0,002
Molar Total ^r	-0,020	0,902	-0,011	0,946	0,509	0,001
Total thickness (epithelium + lamina propria). The correlation coefficient used for each pair is indicated by a superscript annotation (^r for Pearson, ^p for Spearman). Correlation analyses were performed using data obtained from all 42 patients.						