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# Periosteal Fenestration Procedure in Apically Positioned Flap Increases the Attached Mucosal Width: An In Vivo Experimental Study

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## ABSTRACT

**Aim:** To evaluate the efficacy of the apically positioned flap (APF) with periosteal fenestration in increasing the width of the masticatory mucosa (MM, including attached and keratinized mucosa) and to assess the histological properties of the MM gained through this technique.

**Materials and Methods:** Six mongrel dogs were treated using a split-mouth design, with APF alone (control) and APF with periosteal fenestration (test) applied from the first to the fourth maxillary premolars on both sides (total of 48 sites). Clinical MM width (cMW) and digitally scanned MM width (dMW) were measured at baseline, post-operatively, and after 8 weeks of healing. Histological observations included measuring the distance from the free gingival margin to the mucogingival junction (MGJ) and the width of non-keratinized attached mucosa (NKAM). A linear mixed model was employed to analyse changes in MM measurements.

**Results:** The mean gain of cMW was 3.17 mm (SD 1.63) in the control group and 4.27 mm (SD 1.91) in the fenestration group with a significant difference in favour of the fenestration group. The estimated mean difference was 1.45 mm (95% CI: 0.57–2.33; $p = 0.002$). Histologically, the increased mucosal width observed in the fenestration group, compared to the control group, was primarily characterized by non-keratinized epithelium.

**Conclusion:** APF with periosteal fenestration enhances the attached mucosal width; however, this increase is predominantly composed of NKAM rather than orthokeratinized mucosa.

## 1 | Introduction

Masticatory mucosa (MM) around teeth and dental implants has two key histological features: (i) dense fibrous tissue firmly attached to the periosteum, and (ii) orthokeratinization of the epithelium (Nanci 2013). These characteristics differ from those of the oral mucosa, which contains loose connective tissue (CT) and non-keratinized epithelium. Clinically, these tissues can be distinguished by the color of their keratinized or non-keratinized surfaces (Lee et al. 2020). The presence of keratinized mucosa (KM) is considered clinically beneficial, although its significance remains the subject of debate, particularly around implants (Mancini et al. 2024). A recent systematic review concluded that a KM width of <2 mm is associated with a higher prevalence of




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gingival recession, marginal bone loss, plaque accumulation, and peri-implantitis (Lin et al. 2013; Ramanauskaite et al. 2022).

It is believed that a stable mucosal seal by the MM, tightly attached to the underlying tissue, forms a physiological barrier against bacterial challenges (Strauss et al. 2024). The maintenance of the biological seal by MM appears to be essential for peri-implant health (Mancini et al. 2024; Ramanauskaite et al. 2022). Insufficient MM and reduced vestibular depth impair self-oral hygiene control, posing significant risks for peri-implantitis (Monje et al. 2023). A shallow vestibule complicates access to the mucosal margin of the implant prosthesis with a toothbrush, thereby increasing the risk of peri-implantitis (Halperin-Sternfeld et al. 2016; Monje et al. 2023). A recent cross-sectional study reported that a shallow vestibule (≤4 mm) and lack of attached mucosa are associated with peri-implantitis (Isler et al. 2024). Inadequate MM can also cause discomfort during brushing, thereby reducing the efficiency of self-plaque control (Perussolo et al. 2018). As a result, various surgical techniques have been advocated to increase MM and treat shallow vestibules.

The apically positioned flap (APF), combined with a free gingival graft (FGG), is widely regarded as the treatment of choice for shallow vestibules and insufficient MM width (Montero et al. 2022). A systematic review found that APF with FGG leads to an average gain of 3.54 mm in MM width, whereas APF alone results in a 2.39 mm gain (Tavelli et al. 2021). Additionally, APF with FGG shows greater resistance to post-operative shrinkage. A retrospective study indicated that the MM width achieved with either APF alone or APF combined with a collagen matrix decreased by approximately 50%–60% after 1 year. Conversely, the increase in MM width by APF with FGG shrank by only about 30% (Lim et al. 2018).

Despite these positive outcomes, the harvesting of free gingiva can lead to post-operative pain and may result in extensive bleeding, infection and sensory disturbances (Tavelli et al. 2020; Thoma et al. 2023). As a less invasive alternative, APF with periosteal fenestration has been proposed to achieve stable vestibular depth without an autologous graft (Yadav et al. 2014). The rationale for this technique is based on the assumption that denuding the alveolar bone induces the formation of granulation tissue, which eventually develops into new attached gingiva (Kon et al. 1978; Staffileno et al. 1966). In this technique, the fenestrated periosteum heals with scar-like tissue, preventing the coronal relapse of mucosal and muscular attachments. A retrospective study indicated that APF with periosteal fenestration results in an MM width gain comparable to that achieved by APF with FGG (Lee et al. 2021).

However, the histological characteristics of the MM obtained through APF with periosteal fenestration remain largely unexplored. This is particularly relevant in light of Karring's landmark study, which suggested that the differentiation between keratinized and non-keratinized mucosa is influenced by the underlying CT (Karring et al. 1975). Therefore, the aim of the present in vivo study was (i) to evaluate the width of MM obtained by APF and APF with periosteal fenestration, and (ii) to assess the histological properties of the MM area gained through APF with periosteal fenestration.

## 2 | Materials and Methods

### 2.1 | Animals and Materials

Six healthy mongrels weighing 25–30 kg and aged 18–24 months were fed in a facility accredited by the International Association for Assessment and Accreditation of Laboratory Animal Care, with a temperature of 15°C–20°C and humidity above 30%. The study was approved by the Institutional Animal Care and Use Committee of Yonsei Medical Center, Seoul, South Korea (Approval No. 2021-0100) and adhered to ARRIVE guidelines (Percie du Sert et al. 2020).

### 2.2 | Study Design

The study employed a split-mouth design, with control (APF alone) and fenestration (APF with periosteal fenestration) groups randomly assigned to each hemi-maxilla (Figure 1). The first, second, third and fourth premolars on each side were designated as the measuring sites. Twelve maxillary sites in six animals were included, and all parameters were measured at 48 sites across 12 unilateral maxillae. Clinical measurements were taken at baseline ($T_0$), post-operatively ($T_1$), and after 8 weeks of healing ($T_{8wk}$). Intraoral scans were taken at $T_0$ and $T_{8wk}$. At $T_{8wk}$, animals were euthanised for histological analysis.

### 2.3 | Surgical Protocols

General anaesthesia was induced using medetomidine (0.75 mg/kg, intramuscularly; Tomidin, Provet, Istanbul, Turkey), alfaxalone (2 mg/kg, intravenously; Jurox, Rutherford, NSW, Australia) and isoflurane inhalation (Forane, Choongwae Pharmaceutical, Seoul, South Korea). Local anaesthesia was administered using 2% lidocaine with 1:80,000 epinephrine (Kwangmyung Pharm, Seoul, South Korea).

All surgical procedures were conducted by one experienced periodontist (J.S.L.). Vertical grooves were made on the crown of the teeth with diamond burs for reference (Lee et al. 2020). An initial horizontal incision was made at the mucogingival junction (MGJ), extending from the distal surface of the canine to the mesial surface of the first molar. Then, a partial-thickness flap was raised, leaving the periosteum intact on the alveolar surface and ensuring that no movable mucosa was left on the exposed periosteal bed.

In the fenestration group, a periosteal fenestration was made at the intended apical positioning level using a blade and a periosteal elevator. Then, in both groups, the flap was apically positioned and sutured to the periosteal bed using horizontal mattress sutures (Monosyn 6–0, B.Braun, Bethlehem, USA).

Post-operative care included the daily administration of analgesics (0.2 mg/kg; meloxicam, Boehringer-Ingelheim, Ingelheim, Germany) and antibiotics (20 mg/kg; cefazolin, Yuhan, Seoul, South Korea) for 7 days. Weekly oral prophylaxis was performed under sedation as necessary. The animals were euthanised at $T_{8wk}$, and each maxilla was sectioned, dehydrated using ethanol solutions, trimmed and embedded in paraffin. Central bucco-lingual ground sections were taken from each measuring site along with the reference groove, produced at a thickness of 20–50 $\mu$m staining with haematoxylin and eosin (H&E) and Masson's trichrome.

## 2.4 | Clinical Analysis

An examiner (J.S.L.) measured the MM width using a UNC-15 periodontal probe (Hu-Friedy, Chicago, Illinois, USA). The periodontal probe was positioned along the reference groove, and the distance from the MGJ to the marginal gingiva was recorded (Figure 2A). The MGJ was identified by distinguishing the boundary between the movable and immobile mucosa while pulling the cheeks.

At $T_0$, $T_1$ and $T_{8wk}$, the following measurements were recorded:

- Clinical MM width (cMW): the distance from the MGJ to the gingival margin;
- Gained cMW: the difference between the cMW at $T_{8wk}$ and the cMW at $T_0$;
- Relapse of cMW: the difference between the cMW at $T_{8wk}$ and the cMW at $T_1$.

## 2.5 | Digital Analysis

An examiner (S.J.) measured the distance from the MGJ to the marginal gingiva on intraoral scans using a software (Medit, MEDIT, Seoul, South Korea). The measurements were taken parallel to the reference grooves (Figure 2B). The MGJ was identified by distinguishing the colour of the attached gingiva and the oral mucosa (Lee et al. 2020).

At $T_0$ and $T_{8wk}$, the following measurements were recorded:

- Digital MM width (dMW): the length from the MGJ to the gingival margin;
- Gained dMW: difference between the dMW at $T_{8wk}$ from the dMW at $T_0$.

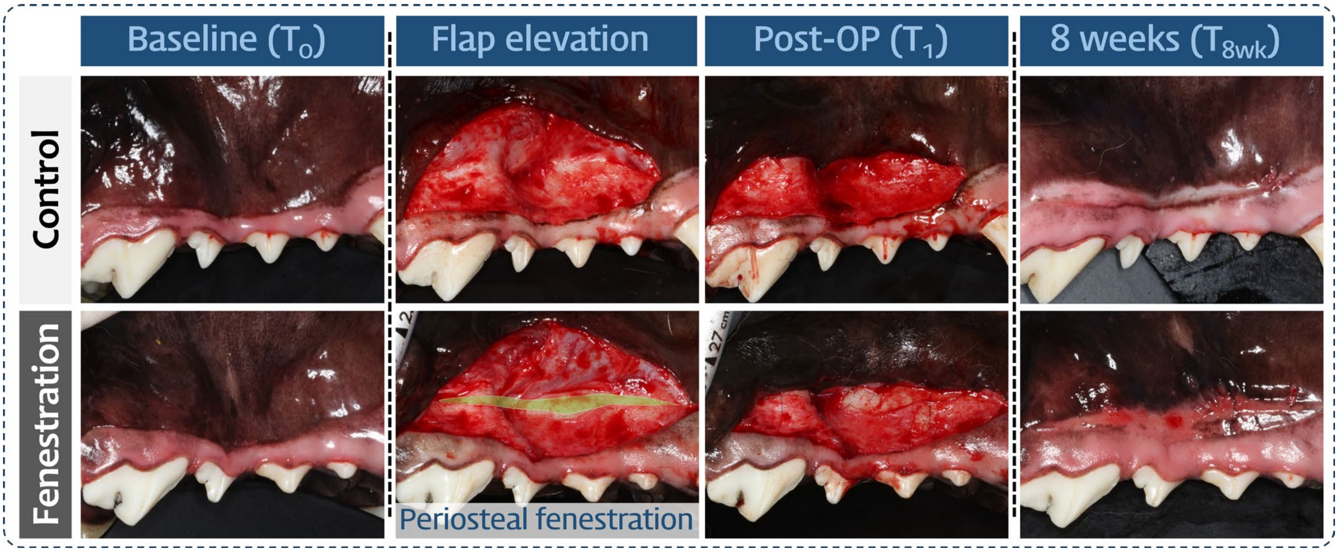


**FIGURE 1** | Study design. Vestibuloplasty with apically positioned flap (APF) was performed on the unilateral control side, while vestibuloplasty with APF and periosteal fenestration was conducted on the contralateral test side. Clinical measurements were taken at baseline ($T_0$), postoperatively ($T_1$) and 8 weeks after healing ($T_{8wk}$). Intraoral scans were taken at $T_0$ and $T_{8wk}$. At the end of 8 weeks of healing, animals were euthanised for histological analysis.

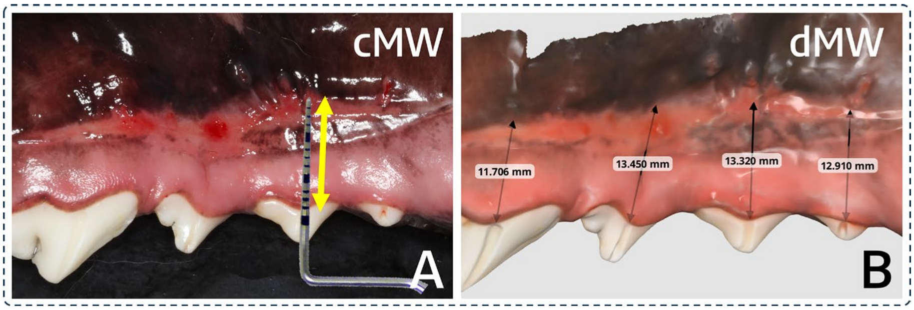


**FIGURE 2** | Measurement of masticatory mucosal width. (A) Clinical measurement of masticatory mucosal width (cMW). (B) Digital measurement of masticatory mucosal width (dMW). To standardise clinical, digital and histological measurements, a positional groove was created on each crown, serving as a reference for periodontal probe placement, digital measurement and histological sectioning.

### 2.6 | Histomorphometric Analysis

The histological slides were evaluated under a light microscope (DM6000, Leica-Mikrosysteme, Wetzlar, Germany) and then digitally scanned at ×200 magnification and observed using a computer software (CaseViewer, 3DHISTECH, Budapest, Hungary). The measurement of the examiner (S.J.) was calibrated with randomly selected five histologic slides under the supervision of an expert (J.S.L.) to ensure accuracy and reliability in the analysis. According to a previous study, the endpoint of the keratinised epithelium was considered to be the end of the squamous keratin layer (Karring et al. 1975). At low magnification, the presumptive area was identified where the prominent rete ridges tapered off and the underlying dense connective tissue appeared. Subsequent high-magnification observation delineated the histological boundary of the keratinized epithelium at the point where the continuity of the coronal keratin layer ceased and the basal cells first displayed well-defined nuclei. This interface was designated as the endpoint of the keratinized epithelium for measurements.

The following measurements were recorded:

- The distance from the free gingival margin to MGJ (FM–MGJ);
- The distance from the free gingival margin to the end of keratinized epithelium (FM–eKe);
- The width of non-keratinized attached mucosa (NKAM): the mucosa between the end of keratinized epithelium and MGJ, defined as NKAM. The NKAM width was calculated as 'FM–MGJ' – 'FM–eKe'.

### 2.7 | Statistical Analyses

Descriptive statistics, presented as means (with standard deviations), were calculated for all metric parameters. Changes in measurements (e.g., cMW, dMW) and histometric parameters (FM–MGJ, FM–eKe and NKAM) between the groups were analysed using a linear mixed model. This model accounted for repeated measures within animals and teeth. The fixed factors included in the model were the treatment group, time and their interaction, allowing the estimation of treatment effects at each time point. Random factors in the model were the animals and tooth locations. Mean differences between the two treatment groups, along with their 95% confidence intervals (CIs), were calculated. To ensure the validity of the model, its assumptions were visually assessed using residual plots, including Q–Q plots, histograms and residual versus fitted value plots.

All statistical analyses were performed using Stata v18.0 (Stata, College Station, TX, USA) and SPSS v26.0 (IBM, Chicago, IL, USA). A significance level of $p<0.05$ was applied, and all tests and reported $p$-values were two-sided.

## 3 | Results

All animals healed well without complications (Figure 3). In the fenestration group, a reddish, scar-like attached mucosal area was observed at the APF site. It exhibited a distinct texture in contrast to the movable mucosa in the vestibule (Figure 3B).

### 3.1 | Changes in MM Width: Clinical Measurements (cMW)

In both groups, the cMW increased significantly from baseline (Table 1). The mean gain in cMW was 3.17 (1.63) mm in the control group and 4.27 (1.91) mm in the fenestration group, with a significant difference in favour of the fenestration group. The estimated mean difference was 1.45 mm (95% CI: 0.57–2.33; $p=0.002$) (Table 2).

### 3.2 | Changes in MM Width: Digital Measurements (dMW)

Digital measurements (dMW) similarly showed significant gains in both groups compared to baseline (Table 1). The mean gain in dMW was 3.05 (1.39) mm in the control group and 4.37 (1.87) mm in the fenestration group (Table 2). Again, the fenestration group exhibited a significantly greater increase, with an estimated mean difference of 1.46 mm (95% CI: 0.59–2.33; $p=0.002$).

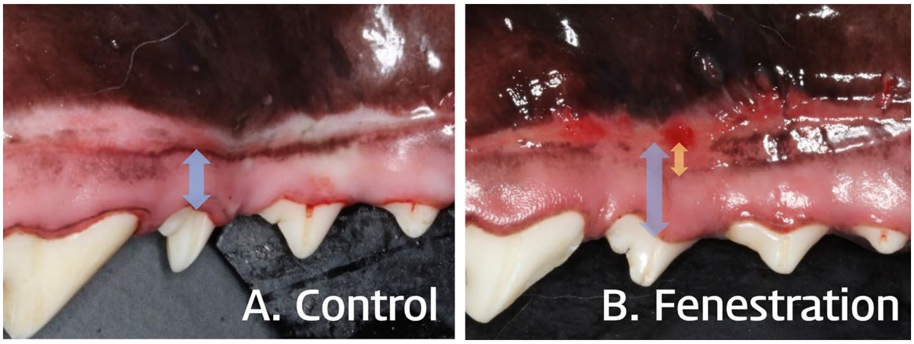


**FIGURE 3** | Representative clinical photographs from control (APF alone) and the fenestration group (APF with periosteal fenestration). Fenestration group shows additional reddish scar-like non-keratinized attached mucosal area, which is distinct from the movable vestibular mucosa. Blue arrows, clinical masticatory mucosal width; yellow arrow, non-keratinised attached mucosal (NKAM) area.

**TABLE 1** | Mean masticatory mucosal width (in mm) in both treatment groups at different time points measured clinically (cMW), digitally (dMW) and histometrically.

| Time point | APF, mean (SD) | APF + periosteal fenestration, mean (SD) | Adjusted treatment difference (95% CI) | *p* |
|---|---|---|---|---|
| cMW $T_0$—Pre-operative (mm) | 5.30 (1.41) | 5.65 (1.66) | 0.35 (−0.52 to 1.23) | 0.430 |
| cMW $T_1$—Post-operative (mm) | 16.98 (2.06) | 16.69 (1.69) | −0.29 (−1.17 to 0.58) | 0.516 |
| cMW $T_{8wk}$ – 8 weeks follow-up (mm) | 8.46 (2.08) | 9.92 (2.17) | 1.45 (0.57 to 2.33) | 0.002 |
| dMW $T_0$—Pre-operative (mm) | 5.18 (1.30) | 5.32 (1.47) | 0.14 (−0.25 to 0.52) | 0.473 |
| dMW $T_{8wk}$—8 weeks follow-up (mm) | 8.23 (1.87) | 9.69 (2.18) | 1.46 (0.59 to 2.33) | 0.002 |
| Histometric | | | | |
| FM–MGJ (mm) | 8.43 (1.92) | 9.95 (2.10) | 1.52 (0.83 to 2.21) | 0.000 |
| FM–eKe (mm) | 6.66 (1.64) | 7.21 (2.08) | 0.54 (−0.22 to 1.32) | 0.157 |
| NKAM width (mm) | 1.77 (0.80) | 2.75 (1.50) | 0.97 (0.28 to 1.67) | 0.007 |
| NKAM proportion (%) | 20.88 (9.19) | 27.58 (12.42) | 6.70 (0.44 to 12.96) | 0.036 |

*Note:* Linear mixed-effects model including terms for treatment group, animal and tooth location. Values of cMW and dMW are in mm for each treatment arm across time ($T_0$, $T_1$ and $T_{8wk}$). Effect sizes are adjusted mean difference (95% CI).
Abbreviations: cMW, clinical masticatory mucosal width; dMW, digital masticatory mucosal width; FM–eKe, distance from the free gingival margin to the end of keratinized epithelium; FM–MGJ, distance from the free gingival margin to mucogingival junction; NKAM, non-keratinized attached mucosa (NKAM width was calculated as 'FM–MGJ' – 'FM–eKe').

**TABLE 2** | Mean change of masticatory mucosal width (in mm) in both treatment groups between pre-operative ($T_0$) and 8 weeks of follow up ($T_{8wk}$).

| Changes in masticatory mucosal width | APF | APF + periosteal fenestration |
|---|---|---|
| Gained cMW ($T_{8wk} - T_0$) (mm) | 3.17 (1.63) | 4.27 (1.91) |
| 95% CI | | 2.87–5.67 |
| Intragroup *p*-value | | 0.007 |
| Gained dMW ($T_{8wk} - T_0$) (mm) | 3.05 (1.39) | 4.37 (1.87) |
| 95% CI | | 3.15–5.59 |
| Intragroup *p*-value | | 0.001 |

## 3.3 | Histological Analysis

### 3.3.1 | Apically Positioned Flap Without Periosteal Fenestration (Control Group)

In the control group, the free gingiva and attached gingiva that were not included in the partial-thickness flap retained a mature keratinized epithelium with well-developed rete peg and dense CT firmly attached to the alveolar surfaces (Figure 4A). The periosteum bed area, exposed during the APF procedure, healed without signs of inflammation and became epithelialized but did not develop keratinization. Beneath the epithelium, thick layers of horizontally oriented CT fibers originating from the movable vestibular mucosa were observed (Figure 4B). On the alveolar side, CT fibers running parallel to the upper periosteum were observed (Figure 4C). The considerable thickness of the horizontally running CT fibers contributed to the convex and bulging shape of the epithelialized area (Figure 4).

### 3.3.2 | Periosteal Fenestration Group

Similar to the control group, in the fenestration group the coronal part of the horizontal incision, which was not included in the partial-thickness flap, retained mature KM with well-developed rete pegs (Figure 5B). The exposed area healed completely and became epithelialized. Near the coronal KM, some areas exhibited keratinized epithelium (Figure 5C), while the exposed areas in the apical were covered with para-keratinized epithelium (Figure 5D). Beneath the epithelium, a dense scar-like CT was observed at the site of the periosteal fenestration (Figure 5A). This dense CT obstructed the horizontally running loose CT fibers originating from the movable vestibular mucosa. Below the dense scar-like CT, parallel-running CT fibers were observed on the alveolar side, connecting to the coronal periosteum.

## 3.4 | Histomorphometric Analysis

Histomorphometrically, FM–MGJ was significantly greater in the fenestration group (9.95 vs. 8.43 mm) with an estimated mean difference of 1.52 mm (95% CI: 0.83–2.21; $p = 0.000$) (Table 1). FM–eKe was 6.66 mm in the control group and 7.21 mm in the fenestration group, with no significant differences between the groups (estimated mean difference: 0.54 mm [95% CI: 0.59–2.33]; $p = 0.157$). As for the width of

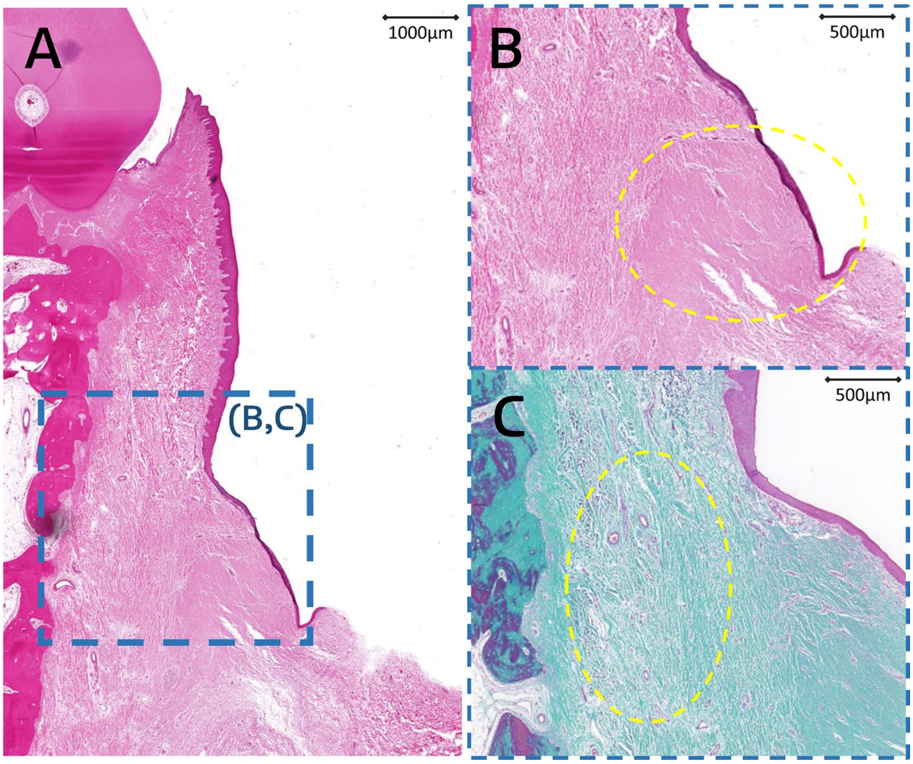


**FIGURE 4** | (A) Representative histological views of the control (APF alone) group. (B) Connective tissue fibers originating from the movable vestibular mucosa run horizontally (H&E staining). These connective tissue fibers from the vestibule induce a condition in which the gingival tissue becomes easily movable, potentially leading to compromised stability. (C) Inner connective tissue fibers connected to the periosteum run parallel (Masson's trichrome staining).

NKAM, this was significantly higher in the fenestration group, with a mean of 2.75 versus 1.77 mm in the control group (estimated mean difference = 0.97 mm [95% CI: 0.28–1.67]; $p = 0.007$). Consistently, the proportion of NKAM was significantly higher in the fenestration group than in the control group (estimated mean difference: 6.7% [95% CI: 0.44–12.96]; $p = 0.036$).

## 4 | Discussion

The present study, comparing the efficacy and the histological properties of APF with or without periosteal fenestration for increasing the MM, predominantly revealed the following:

- Periosteal fenestration increased the width of attached MM compared to APF alone;
- The increase in the MM was characterised by an NKAM rather than orthokeratinized epithelium;
- The NKAM width after APF stabilized the mucogingival line, preventing its coronal migration.

### 4.1 | Clinical Findings

The periosteal fenestration following APF increased the gain in mucosal width by approximately 1.5 mm compared to APF with no fenestration. These higher gains in mucosal width when performing fenestrations are likely attributed to the denudation of the alveolar bone, which stimulates the formation of granulation tissue regenerating new attached gingiva (Kon et al. 1978; Staffileno et al. 1966). While MGJ tends to migrate coronally on non-fenestrated sides, it remains in a more apical position on fenestrated sides (Carranza et al. 1966). The fenestrated periosteum heals with scar-like tissue, preventing the coronal relapse of mucosal and muscular attachments, enhancing the MM gains after APF (Lee et al. 2021). It has been indicated that the fenestration of periosteum provokes an osteogenic response and a fibroblastic proliferation that joins with the granulation tissue of the soft-tissue wound and maintains the MGJ in a more apical position (Carranza et al. 1966).

Vestibuloplasty is a surgical procedure to restore vestibular depth and regenerate the MM that may have been compromised due to periodontal destruction. This restoration is crucial for facilitating effective prosthetic reconstruction. The MM, characterised by dense fibrous tissue and keratinized epithelium, provides stable sealing and resistance to trauma (Nanci 2013). Vestibuloplasty using an APF provides a stable seal and barrier by promoting healing of the exposed CT into keratinized gingiva with properties similar to the surrounding tissue (Staffileno et al. 1966). However, previous studies have shown that APF alone is prone to relapse (Ko et al. 2020; Lim et al. 2018; Montero et al. 2022). To address this limitation, the addition of periosteal fenestration to the APF—a relatively simple modification—has been shown to significantly reduce relapse rates, achieving results comparable to FGG, which is considered the gold standard for securing MM and vestibular depth (Yadav et al. 2014). In a retrospective study, APF, APF with periosteal fenestration and

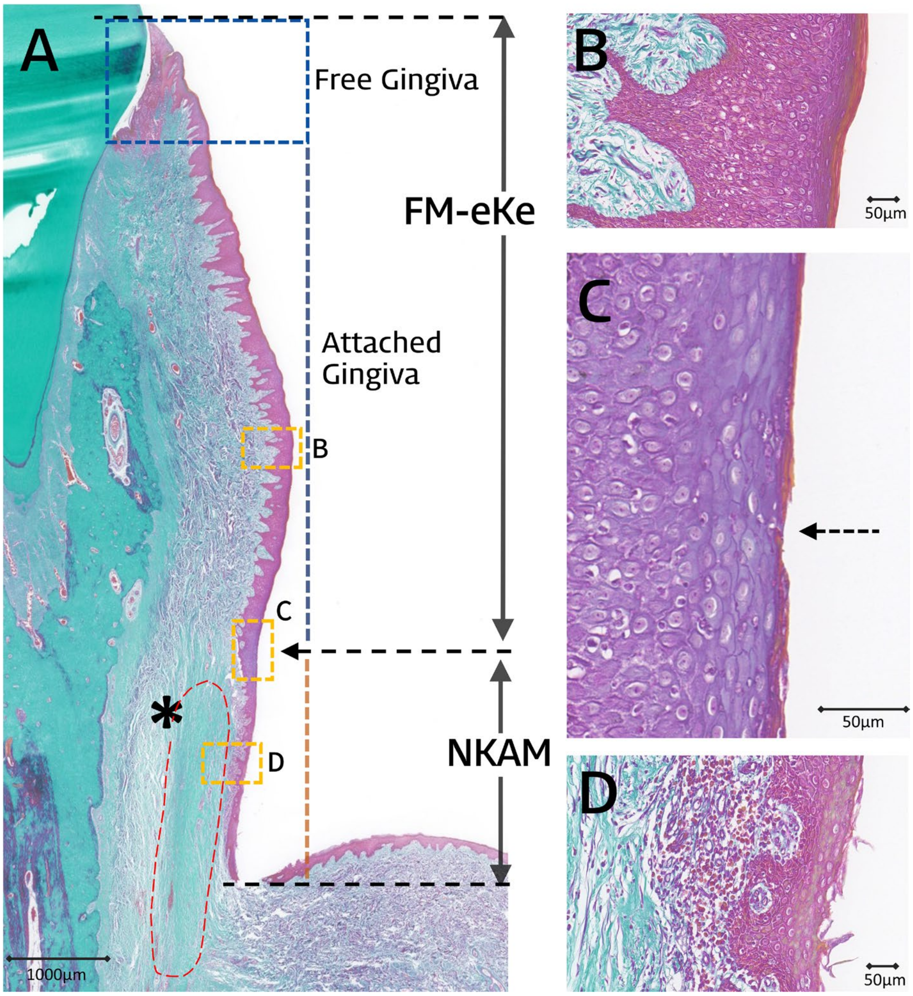


**FIGURE 5** | (A) Representative histology of APF with periosteal fenestration group. (B) Coronal part of horizontal incision maintains the keratinized epithelium. (C) Boundary between keratinised mucosa and NKAM (marked with arrow). (D) NKAM area covered with non-keratinized epithelium. The NKAM area increases the masticatory mucosal width of the fenestration group. Dense scar-like connective tissue area (*) blocks loose connective tissue fibers from the movable vestibular mucosa and offers immobile and stable condition to NKAM area. FM–eKe, distance from the free gingival margin to the end of keratinised epithelium; NKAM, non-keratinised attached mucosa. *Dense scar-like connective tissue.

FGG were found to initially produce approximately 5 mm of KT width immediately after surgery. However, after 1 year, the MM width in the APF decreased to 0.5 mm, whereas the APF group with periosteal fenestration and FGG groups retained 2.5 mm of MM width (Lee et al. 2021). In other words, this finding suggests that the scar-like tissue formed by periosteal fenestration stabilizes the MGJ, thus preventing the coronal migration of mucosal and muscular attachments.

## 4.2 | Histological Findings

The present study revealed that the exposed periosteum and fenestration healed with NKAM. When periosteal fenestration was combined with an APF, it not only secured greater vestibular depth but also resulted in a higher proportion of NKAM within the secured vestibular area. Although NKAM is non-keratinized, it possesses a dense CT layer with fibers running parallel to the alveolar surface and connecting with the firm connective layer of the attached gingiva. Additionally, the embedding of horizontal CT fibers originating from the movable vestibule is inhibited within NKAM. As a result, NKAM secured through periosteal fenestration clinically contributes to achieving wider tissues and providing immobile soft-tissue conditions.

In the control group, the exposed periosteal area exhibited a convex shape, likely due to the influence of horizontally running CT fibers originating from the vestibule. In contrast, the fenestration group healed with higher NKAM, characterized by exclusively parallel-running, dense CT fibers. This variation can be attributed to the periosteal fenestration, which physically separates soft-tissue healing with the immobile attached gingiva (coronal) from that of the mobile vestibule (apical). Wound healing occurs through the migration of epithelial cells from the surrounding tissues (Wennstrom 1983). In the fenestration group, the coronal part heals with epithelial cells derived from the firm, immobile attached gingiva, while periosteal fenestration obstructed the migration of epithelial cells from the loose mobile vestibular tissue in the apical area. Consistent with this, a previous study reported that more scar-like tissue developed in areas where the periosteum was removed compared to areas where the periosteum was preserved (Cho et al. 2011). The dense scar-like CT formed at the periosteal fenestration site seems to block the embedding CT fibers from the mobile vestibular mucosa and inhibits coronal migration of muscle and frenum attachment.

Areas with wider attached gingiva show higher resistance to plaque-induced inflammation (Wennstrom and Lindhe 1983). This resistance is due to the firm collagen fibers that anchor the gingiva to the alveolar bone and periodontal ligament (PDL), providing biomechanical stability (Schroeder and Listgarten 1997). Conversely, movable mucosa facilitates biofilm penetration into the gingival crevice, leading to biofilm accumulation (Lang and Loe 1972; Warrer et al. 1995). Once biofilm accumulates, inflammation begins at the junctional epithelium—the inner surface of the free gingiva—regardless of the keratinization of the outer gingival surface (Bosshardt and Lang 2005; Squier 1981). The attached gingiva plays a critical role in providing an immobile and mechanically stable environment, effectively blocking the continuous penetration of biofilm into the sulcus.

Based on Karring's study, which reported that keratinization of gingiva is determined by the underlying CT, APF and FGG have been performed to secure KM around the teeth (Karring et al. 1971). However, recent studies have confirmed that these procedures are less effective in securing KM at the implant site compared to tooth sites (Lim et al. 2024). The lack of regenerative potential from PDL is thought to have a negative effect on the keratinization around implants (Linares et al. 2022). As previously mentioned, in the case of APF with periosteal fenestration, the scar-like tissue at the fenestration site blocks the CT fiber insertions from the movable mucosa. Since scar-like tissue formation is closer to repair than to regeneration, the absence of PDL cells may not hinder the establishment of a firm and immobile gingival condition through APF with periosteal fenestration.

In the present study, the MM width was measured in two ways: using a periodontal probe and an intraoral scanner. Digital intraoral scanning provides higher reproducibility and reliability for measuring MM width than using periodontal probes (Lee et al. 2020). However, in the present study, the presence of the NKAM area made it challenging to clearly distinguish the boundaries between the keratinized and non-keratinized tissue as well as between movable and attached areas. This ambiguity hindered achieving agreement between clinical and digital measurements.

This animal study has some limitations. First, mucosal movement due to oral behaviour cannot be controlled, which could lead to coronal repositioning of muscle and frenum attachment, contributing to the relapse. Additionally, the absence of vertical incisions in the APF design resulted in the apical shift being smaller at the first and fourth premolars than at the second and third premolars, and the amount of exposed periosteum therefore varied along the wound. The 8-week follow-up period may have been insufficient to fully assess long-term relapse, which can continue beyond 12 months.

Also, caution should be taken when applying these findings in clinical situations. One limitation is the lack of a positive control, namely FGG, which is considered the standard of care for increasing KM. Without it, a direct comparison of the efficiency of APF with periosteal fenestration is not possible. Additionally, the baseline KM of the present study was sufficient (> 5 mm). A sufficient amount of surrounding soft tissue provides favourable conditions for wound regeneration (Smith 1970). However, in clinical settings, the implant sites with insufficient KM (< 2 mm) may show different healing characteristics. Lastly, the clinical relevance of MM is an important factor in peri-implant health. However, the present study did not include implants. Further studies are needed to confirm whether an increase in MM can be achieved at implant sites and to evaluate its potential clinical benefits.

## 5 | Conclusion

APF with periosteal fenestration enhances the width of attached mucosa; however, this increase is primarily characterised by NKAM rather than orthokeratinized mucosa.

### Author Contributions

All authors made substantial contributions to this study. J.S.L. conceived and designed the whole experiment and approved the article submission. J.S.L. and H.J.S. performed the experiments, and I.H., S.J. and F.J.S. interpreted the data and performed the statistical analyses. I.H drafted the original manuscript, and F.J.S., D.S.T. and J.S.L. critically reviewed and revised the manuscript.

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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

### References

Bosshardt, D. D., and N. P. Lang. 2005. "The Junctional Epithelium: From Health to Disease." *Journal of Dental Research* 84, no. 1: 9–20. https://doi.org/10.1177/154405910508400102.

Carranza, F. A., Jr., J. J. Carraro, C. A. Dotto, and R. L. Cabrini. 1966. "Effect of Periosteal Fenestration in Gingival Extension Operations." *Journal of Periodontology* 37, no. 4: 335–340. https://doi.org/10.1902/jop.1966.37.4.335.

Cho, E. H., J. C. Park, J. K. Cha, et al. 2011. "Dimensional Change of the Healed Periosteum on Surgically Created Defects." *Journal of Periodontal & Implant Science* 41, no. 4: 176–184. https://doi.org/10.5051/jpis.2011.41.4.176.

Halperin-Sternfeld, M., H. Zigdon-Giladi, and E. E. Machtei. 2016. "The Association Between Shallow Vestibular Depth and Peri-Implant Parameters: A Retrospective 6 Years Longitudinal Study." *Journal of Clinical Periodontology* 43, no. 3: 305–310. https://doi.org/10.1111/jcpe.12504.

Isler, S. C., M. Romandini, G. Akca, et al. 2024. "Soft-Tissue Phenotype as a Risk Indicator of Peri-Implantitis and Peri-Implant Soft-Tissue Dehiscence—A Cross-Sectional Study." *Journal of Clinical Periodontology* 51, no. 11: 1443–1457. https://doi.org/10.1111/jcpe.14059.

Karring, T., N. P. Lang, and H. Loe. 1975. "The Role of Gingival Connective Tissue in Determining Epithelial Differentiation." *Journal of Periodontal Research* 10, no. 1: 1–11. https://doi.org/10.1111/j.1600-0765.1975.tb00001.x.

Karring, T., E. Ostergaard, and H. Loe. 1971. "Conservation of Tissue Specificity After Heterotopic Transplantation of Gingiva and Alveolar Mucosa." *Journal of Periodontal Research* 6, no. 4: 282–293. https://doi.org/10.1111/j.1600-0765.1971.tb00619.x.

Ko, K. A., J. S. Lee, J. H. Kim, J. M. Park, R. Gruber, and D. S. Thoma. 2020. "Changes in Mucogingival Junction After an Apically Positioned Flap With Collagen Matrix at Sites With or Without Previous Guided Bone Regeneration: A Prospective Comparative Cohort Study." *Clinical Oral Implants Research* 31, no. 12: 1199–1206. https://doi.org/10.1111/clr.13665.

Kon, S., F. E. Pustiglioni, A. B. Novaes, M. P. Ruben, and N. S. de Araujo. 1978. "Split Thickness Flap, Apically Replaced, With Protected Linear Periosteal Fenestration: A Clinical and Histological Study in Dogs." *Journal of Periodontology* 49, no. 4: 174–180. https://doi.org/10.1902/jop.1978.49.4.174.

Lang, N. P., and H. Loe. 1972. "The Relationship Between the Width of Keratinized Gingiva and Gingival Health." *Journal of Periodontology* 43, no. 10: 623–627. https://doi.org/10.1902/jop.1972.43.10.623.

Lee, J. S., Y. S. Jeon, F. J. Strauss, H. I. Jung, and R. Gruber. 2020. "Digital Scanning Is More Accurate Than Using a Periodontal Probe to Measure the Keratinized Tissue Width." *Scientific Reports* 10, no. 1: 3665. https://doi.org/10.1038/s41598-020-60291-0.

Lee, W. P., K. H. Lee, S. J. Yu, and B. O. Kim. 2021. "A Retrospective Comparison of 3 Approaches of Vestibuloplasty Around Mandibular Molar Implants: Apically Positioned Flap Versus Free Gingival Graft Versus Modified Periosteal Fenestration." *Journal of Periodontal & Implant Science* 51, no. 5: 364–372. https://doi.org/10.5051/jpis.2007320366.

Lim, H. C., S. C. An, and D. W. Lee. 2018. "A Retrospective Comparison of Three Modalities for Vestibuloplasty in the Posterior Mandible: Apically Positioned Flap Only vs. Free Gingival Graft vs. Collagen Matrix." *Clinical Oral Investigations* 22, no. 5: 2121–2128. https://doi.org/10.1007/s00784-017-2320-y.

Lim, H. C., F. J. Strauss, S. I. Shin, R. E. Jung, U. W. Jung, and D. S. Thoma. 2024. "Augmentation of Keratinized Tissue Using Autogenous Soft-Tissue Grafts and Collagen-Based Soft-Tissue Substitutes at Teeth and Dental Implants: Histological Findings in a Pilot Pre-Clinical Study." *Journal of Clinical Periodontology* 51, no. 5: 665–677. https://doi.org/10.1111/jcpe.13949.

Lin, G. H., H. L. Chan, and H. L. Wang. 2013. "The Significance of Keratinized Mucosa on Implant Health: A Systematic Review." *Journal of Periodontology* 84, no. 12: 1755–1767. https://doi.org/10.1902/jop.2013.120688.

Linares, A., A. Rubinos, A. Punal, F. Munoz, and J. Blanco. 2022. "Regeneration of Keratinized Tissue Around Teeth and Implants Following Coronal Repositioning of Alveolar Mucosa With and Without a Connective Tissue Graft: An Experimental Study in Dogs: Fifty Years After Karring's Landmark Study: Fifty Years After Karring's 71 Landmark Study." *Journal of Clinical Periodontology* 49, no. 11: 1133–1144. https://doi.org/10.1111/jcpe.13673.

Mancini, L., F. J. Strauss, H. C. Lim, et al. 2024. "Impact of Keratinized Mucosa on Implant-Health Related Parameters: A 10-Year Prospective Re-Analysis Study." *Clinical Implant Dentistry and Related Research* 26, no. 3: 554–563. https://doi.org/10.1111/cid.13314.

Monje, A., J. Y. Kan, and W. Borgnakke. 2023. "Impact of Local Predisposing/Precipitating Factors and Systemic Drivers on Peri-Implant Diseases." *Clinical Implant Dentistry and Related Research* 25, no. 4: 640–660. https://doi.org/10.1111/cid.13155.

Montero, E., A. Molina, P. Matesanz, A. Monje, I. Sanz-Sanchez, and D. Herrera. 2022. "Efficacy of Soft Tissue Substitutes, in Comparison With Autogenous Grafts, in Surgical Procedures Aiming to Increase the Peri-Implant Keratinized Mucosa: A Systematic Review." *Clinical Oral Implants Research* 33, no. Suppl 23: 32–46. https://doi.org/10.1111/clr.13751.

Nanci, A. 2013. "Oral Mucosa." In *Ten Cate's Oral Histology*, edited by A. Nanci, 278–310. Mosby. https://doi.org/10.1016/b978-0-323-07846-7.00012-4.

Percie du Sert, N., V. Hurst, A. Ahluwalia, et al. 2020. "The ARRIVE Guidelines 2.0: Updated Guidelines for Reporting Animal Research." *Journal of Cerebral Blood Flow and Metabolism* 40, no. 9: 1769–1777. https://doi.org/10.1177/0271678X20943823.

Perussolo, J., A. B. Souza, F. Matarazzo, R. P. Oliveira, and M. G. Araujo. 2018. "Influence of the Keratinized Mucosa on the Stability of Peri-Implant Tissues and Brushing Discomfort: A 4-Year Follow-Up Study." *Clinical Oral Implants Research* 29, no. 12: 1177–1185. https://doi.org/10.1111/clr.13381.

Ramanauskaite, A., F. Schwarz, and R. Sader. 2022. "Influence of Width of Keratinized Tissue on the Prevalence of Peri-Implant Diseases: A Systematic Review and Meta-Analysis." *Clinical Oral Implants Research* 33, no. Suppl 23: 8–31. https://doi.org/10.1111/clr.13766.

Schroeder, H. E., and M. A. Listgarten. 1997. "The Gingival Tissues: The Architecture of Periodontal Protection." *Periodontology 2000* 13, no. 1: 91–120. https://doi.org/10.1111/j.1600-0757.1997.tb00097.x.

Smith, R. M. 1970. "A Study of the Intertransplantation of Alveolar Mucosa." *Oral Surgery, Oral Medicine, and Oral Pathology* 29, no. 3: 328–340. https://doi.org/10.1016/0030-4220(70)90130-1.

Squier, C. A. 1981. "Keratinization of the Sulcular Epithelium—A Pointless Pursuit?" *Journal of Periodontology* 52, no. 8: 426–429. https://doi.org/10.1902/jop.1981.52.8.426.

Staffileno, H., S. Levy, and A. Gargiulo. 1966. "Histologic Study of Cellular Mobilization and Repair Following a Periosteal Retention Operation via Split Thickness Mucogingival Flap Surgery." *Journal of Periodontology* 37, no. 2: 117–131. https://doi.org/10.1902/jop.1966.37.2.117.

Strauss, F. J., J. Y. Park, J. S. Lee, et al. 2024. "Wide Restorative Emergence Angle Increases Marginal Bone Loss and Impairs Integrity of the Junctional Epithelium of the Implant Supracrestal Complex: A Preclinical Study." *Journal of Clinical Periodontology* 51, no. 12: 1677–1687. https://doi.org/10.1111/jcpe.14070.

Tavelli, L., S. Barootchi, G. Avila-Ortiz, I. A. Urban, W. V. Giannobile, and H. L. Wang. 2021. "Peri-Implant Soft Tissue Phenotype Modification and Its Impact on Peri-Implant Health: A Systematic Review and Network Meta-Analysis." *Journal of Periodontology* 92, no. 1: 21–44. https://doi.org/10.1002/JPER.19-0716.

Tavelli, L., S. Barootchi, S. S. Namazi, et al. 2020. "The Influence of Palatal Harvesting Technique on the Donor Site Vascular Injury: A Split-Mouth Comparative Cadaver Study." *Journal of Periodontology* 91, no. 1: 83–92. https://doi.org/10.1002/JPER.19-0073.

Thoma, D. S., F. J. Strauss, L. Mancini, T. J. W. Gasser, and R. E. Jung. 2023. "Minimal Invasiveness in Soft Tissue Augmentation at Dental Implants: A Systematic Review and Meta-Analysis of Patient-Reported Outcome Measures." *Periodontology 2000* 91, no. 1: 182–198. https://doi.org/10.1111/prd.12465.

Warrer, K., D. Buser, N. P. Lang, and T. Karring. 1995. "Plaque-Induced Peri-Implantitis in the Presence or Absence of Keratinized Mucosa. An Experimental Study in Monkeys." *Clinical Oral Implants Research* 6, no. 3: 131–138. https://doi.org/10.1034/j.1600-0501.1995.060301.x.

Wennstrom, J. 1983. "Regeneration of Gingiva Following Surgical Excision. A Clinical Study." *Journal of Clinical Periodontology* 10, no. 3: 287–297. https://doi.org/10.1111/j.1600-051x.1983.tb01277.x.

Wennstrom, J., and J. Lindhe. 1983. "Plaque-Induced Gingival Inflammation in the Absence of Attached Gingiva in Dogs." *Journal of Clinical Periodontology* 10, no. 3: 266–276. https://doi.org/10.1111/j.1600-051x.1983.tb01275.x.

Yadav, N., B. P. Khattak, S. Misra, and A. Sharma. 2014. "Comparative Evaluation of the Relative Efficacy of the Free Mucosal Graft and Periosteal Fenestration for Increasing the Vestibular Depth – A Clinical Study." *Contemporary Clinical Dentistry* 5, no. 3: 366–370. https://doi.org/10.4103/0976-237X.137951.