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REVIEW ARTICLE

Wound healing dynamics, morbidity, and complications of palatal soft-tissue harvesting

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1 | INTRODUCTION

Mucogingival deformities affecting the natural dentition and dental implants, particularly recession and keratinized mucosal deficiency, are prevalent.[1–4] A demand for soft-tissue esthetics and stability compels the search for predictable and less burdensome techniques to treat these conditions. Although various graft materials, including acellular dermal or collagen matrices, biologic agents, and living cellular constructs, have been used for root coverage procedures and for augmenting soft tissue around teeth,[5–9] autogenous grafts—connective-tissue grafts and free gingival grafts—remain the superior treatment option,[8,10–12] as further evidenced by a network meta-analysis from our group evaluating 105 randomized controlled trials.[13]

The health, esthetics, and comfort around dental implants hinge on adequate soft tissue as well.[14–17] Most effective for widening the band of keratinized mucosa around implants, free gingival grafts significantly reduce probing depth, plaque index, and mucosal recession.[14] Regarding enhancing mucosal thickness, soft-tissue graft substitutes, such as collagen or acellular dermal matrices,[7,9,18,19] may be comparable to connective-tissue grafts;[14,20,21] grafting with either connective-tissue graft or collagen matrix appears to maintain the peri-implant marginal bone levels.[14] Still, connective-tissue graft is the current treatment of choice for correcting peri-implant soft-tissue dehiscences[2,12,22] and is often performed simultaneous to implant placement, peri-implant papillae reconstruction, or alveolar ridge preservation.[22–25]

The palate is the standard donor site for harvesting autogenous grafts, and treatment protocols have been and continue to be developed to curtail intraoperative and postsurgical morbidity. The aims of this article are as follows: (1) to evaluate wound healing following palatal harvesting, including the effects of adjunctive biologic agents and photobiomodulation; (2) to review clinical errors in and complications of palatal harvesting techniques; and (3) to discuss methods for minimizing morbidity.

2 | CHARACTERISTICS OF THE PALATAL MASTICATORY MUCOSA

The masticatory mucosa is composed of three distinct histological layers: epithelium, lamina propria, and submucosa (Figure 1A).[26] The epithelium is orthokeratinized and approximately 0.36 mm thick.[27,28] Epithelial thickness differs significantly between canine

[Correction added on August 10, 2023, after first online publication: The affiliation for the author William V. Giannobile has been updated.]

and posterior teeth; it is thicker around canines than around premolars and molars.[29] Located immediately below the epithelial layer, the lamina propria is a dense bilayered connective tissue rich in type I and III collagen fibrils. Its superior papillary layer interlocks with the adjacent epithelium, and its deeper layer consists of thick and dense reticular fibers.[26,28] Beneath the lamina propria, the submucosa is a connective tissue band overlying the periosteum. Surrounding the palatal neurovascular bundles, the submucosa contains a large concentration of glandular and adipose tissue. The central and anterior palatal regions may not feature a submucosa—there, dense lamina propria binds directly and intimately to the periosteum,[26,29] which covers the bone. The periosteum has three zones: zone 1, the innermost cambium, or osteogenic layer, attached to the bone; zone 2, a highly vascularized fibrous layer containing fibroblasts and fibroblast progenitors; and zone 3, an outermost fibrous layer composed of dense collagen fibers (Figure 1B).[30] Mesenchymal stem cells that can be harvested and expanded in culture to become bone, cartilage, and fat cells exist in zone 1 throughout the palatal periosteum, congregating proximal to neurovascular regions.[30]

Palatal mucosal thickness varies at intra- and interpatient levels.[31–34] Relative to other regions, thin mucosa (1.8-2.7 mm) occurs at the first and second maxillary molars palatal root prominences,[32,33] whereas the maxillary tuberosity has the thickest soft tissue (4-6 mm).[31–33,35] Palatal thickness increases directly with distance from the gingival margin[31–33] and age.[31,35] Women may have thinner palatal tissue than men, though conflicting reports on this subject exist.[31–35]

The thickness and composition of the lamina propria differs depending on palatal mucosal thickness; thicker palatal mucosa has thinner lamina propria and higher proportions of fatty and glandular tissue.[36] As such, the lamina propria is significantly thicker near the canine and progressively decreases posteriorly. The thickness of the lamina propria decreases as the distance from the gingival margin increases,[29,36] ranging 1.41-1.99 mm and 0.86-1.39 mm at the marginal and apical regions, respectively.[36] A wide range of palatal mucosal composition has been observed in a cadaver study, with dense connective tissue and fatty/glandular tissue ranging 47-67% and 22.5-46.5%, respectively.[36] Despite lacking statistical significance, the

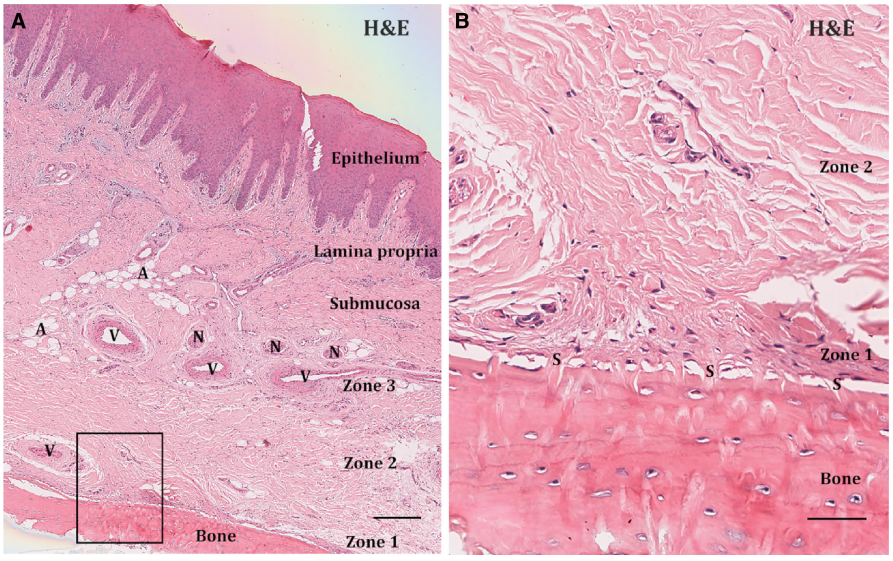


FIGURE 1 A, Histologic section of the hard palate showing the epithelium, lamina propria, submucosa, vessels (V), nerve bundles (N), and adipose cells (A). Scale bar: 200 μm. B, Magnified view of the rectangular box in A, displaying the periosteum, its layers, and its attachment to the bone via Sharpey's fibers (S). Scale bar: 50 μm. H&E: hematoxylin and eosin stain. Reproduced with permission from John Wiley and Sons[30]

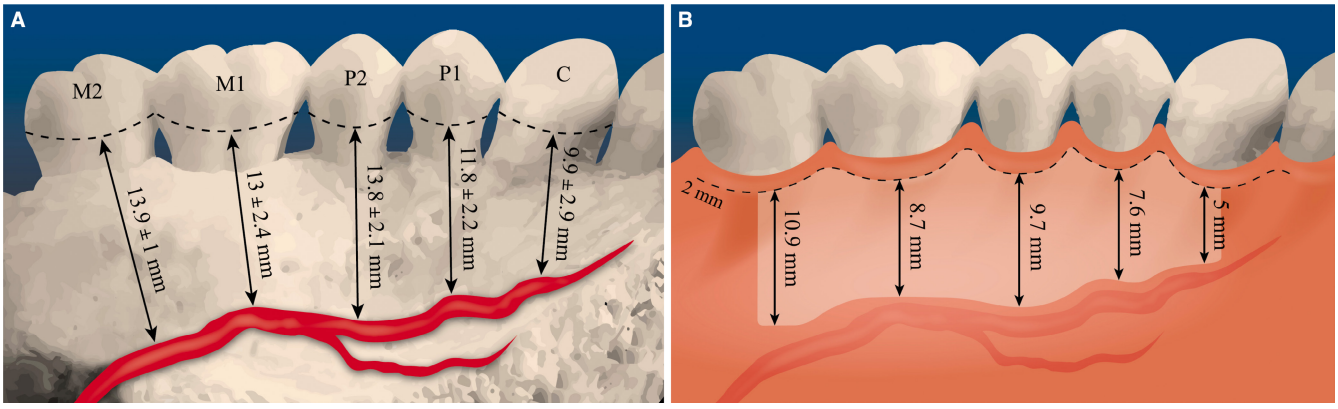


FIGURE 2 A, Schematic illustration showing the average distance between the cemento-enamel junction of the maxillary teeth and the greater palatine artery (C: canine; P1: first premolar; P2: second premolar; M1: first molar;M2: second molar). B, Safety zone for palatal harvesting in a healthy periodontium. Reproduced with permission from Elsevier[37]

second molar region tends to have an overall higher proportion of fatty/glandular tissue than the second premolar area.[36]

3 | THE COURSE OF THE GREATER PALATINE ARTERY

Originating from the maxillary artery, the greater palatine artery—the main vessel supplying the hard palate—descends with its nerve via the greater palatine canal, emerging at the inferior surface of the hard palate through the greater palatine foramen, which is commonly located near the midpalatal aspect of the third molar.[37–39] From there, the greater palatine artery travels anteriorly—paralleling the medial and lateral grooves of the hard palate, which are separated by a bony prominence[40,41]—and leaves the palate superiorly through the incisive canal, terminating in the nasal cavity.[38,41]

Several attempts have been made to evaluate the course of the greater palatine artery and its mean distance from maxillary teeth.[39,42–45] A fabricated cast-based study that assumed rather than measured the course of the vessel found average distances from the neurovascular bundle to the gingival margins of the canine and the second molar to be 12 mm and 14.7 mm, respectively.[42] The shape of the palatal vault influences the mean position of and potential injury to the neurovascular bundle.[46] Mean distances from the cemento-enamel junction to the bundle are 7 mm, 12 mm, and 17 mm in shallow/flat, average, and high/"U-shaped" vaults, respectively.[46]

A series of cadaver studies provided further anatomical details,[39,43–45] which our group summarized in a systematic review.[37] The distance from the greater palatine artery to the maxillary dentition progressively decreases from the second molar region (13.9 ± 1 mm) to the canine area (9.9 ± 2.9 mm), the exception being at the second premolar region (Figure 2A); a safety zone for palatal harvesting was proposed based on our analysis (Figure 2B),[37] though any recommendation must be followed with caution because the greater palatine artery exhibits highly variable branching patterns and anastomoses (Figure 3).[45,47] As per Yu et al,[45] the most common vascular pattern is one in which the lateral branch runs anteriorly in the lateral groove of the bony prominence and diverges into medial and canine branches after the prominence (type I). Although less prevalent, type III and type IV patterns demonstrate a canine or lateral branch in closer proximity to the cemento-enamel junctions of the maxillary teeth compared with type I or II.[45]

4 | HARVESTING APPROACHES

Soft-tissue palatal harvesting was introduced in the late 1960s to obtain epithelialized free gingival grafts, which left the donor site to heal by secondary intention.[48,49] To achieve primary closure, subepithelial connective-tissue harvesting was developed; in 1974, Edel[50] (Figure 4A,B) documented a trapdoor technique involving two vertical incisions to retain an epithelial flap at the donor site. In 1985, Langer and Langer[51] developed a method that sidestepped extensive vertical incision making and instead facilitated connective-tissue extraction by including a small band of epithelium during removal.

Modifications to these approaches have been proposed. The single-incision (envelope) technique employs one horizontal cut and harvests deeper connective tissue (which may include periosteum) with a more consistent thickness[52,53] (Figure 4C,D).

Connective-tissue grafts obtained from scalpel-, bur-, or laser-assisted de-epithelialization[54–57] of free gingival grafts are mainly composed of lamina propria, contain less fatty and glandular tissue than deeper-harvested conventional connective-tissue grafts, and have similar morbidity to grafts harvested using the trapdoor approach (Figure 4E,F).[36,58]

The maxillary tuberosity is a valid alternative to the palate as a donor site owing to its comparatively minimal postoperative morbidity.[12,59] A connective-tissue graft derived from the tuberosity can be harvested via external gingivectomy or distal wedge; tissue obtained in this manner is de-epithelialized (Figure 4G,H). The limited tuberosity width can be compensated for by creating accordion graft slits that allow the tissue to be expanded to cover multiple sites.[59–62] Maxillary tuberosity grafts have unique characteristics[63] and tend to become hyperplastic, a phenomenon better suited for soft-tissue volume augmentation or papilla reconstruction rather than for recession coverage.[12,59,60,64]

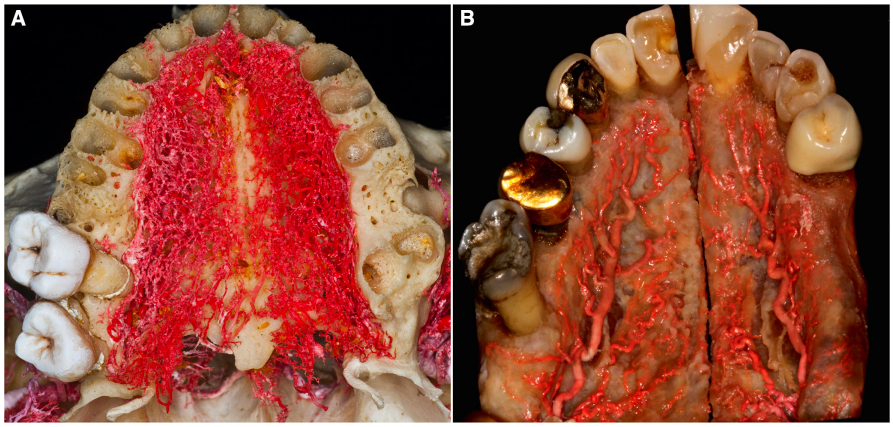


FIGURE 3 Course of the greater palatine artery, as described by Shahbazi et al.[47] A, Highlighted by corrosion casting. B, Highlighted by latex milk injection. A highly complex network of anastomoses exists between the bilateral greater palatine, nasopalatine, and lesser palatine arteries. Injury to one of these anastomoses can cause excessive intra-surgical bleeding (Figure 12). Reproduced with permission from Springer Nature[47]

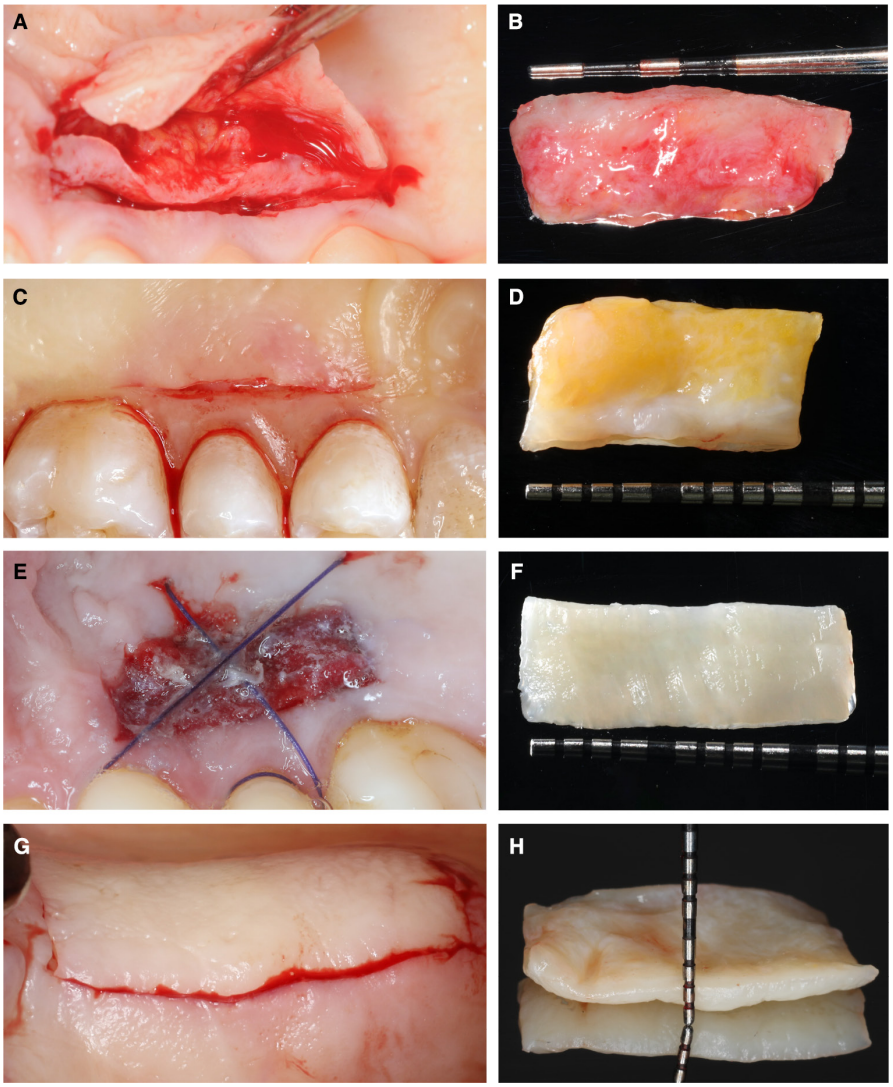


FIGURE 4 Palatal harvesting approaches. A, B, Trapdoor technique and harvested subepithelial connective tissue graft. C, D, Single-incision technique and harvested subepithelial connective tissue graft. E, F, Free gingival graft technique and harvested graft after its de-epithelialization and trimming. A hemostatic collagen sponge was stabilized with sutures and an additional layer of cyanoacrylate tissue adhesive was applied over the sponge. G, H, Harvesting an epithelialized gingival graft from the maxillary tuberosity

5 | PALATAL SOFT TISSUE WOUND HEALING

5.1 | Palatal wound healing physiology

Oral mucosa and skin wounds follow a similar healing pattern in terms of temporal sequence of wound healing events and environmental/biological changes, although oral mucosa appears to heal faster and with less scar tissue.[65,66] A recent study by Iglesias-Bartolome et al[66] characterized the molecular, microscopic, and macroscopic wound healing dynamics of oral mucosa and skin in healthy human participants, demonstrating that oral wounds have accelerated wound closure and re-epithelialization when compared with skin wounds. It was observed that oral mucosa and skin have distinct transcriptional identities, with oral wounds demonstrating rebounds of gene expression to basal conditions at earlier time points, increased keratinocytes activation, and heightened antimicrobial defenses. Interestingly, the transcriptional regulatory network responsible for the accelerated healing in the oral mucosa was found to be expressed in the basal, unwounded state, with oral keratinocytes activation and reduced differentiation as the main mechanism driving acute wound repair and rapid re-epithelialization.[66]

Following soft-tissue harvest, the palatal wound heals in four partially overlapping phases: hemostasis, inflammatory, granulation, and maturation (Figure 5).[67–70] First, a blood clot forms at the injury site and seals the wound against dehydration and infection as it provides a matrix for cell migration. A few hours after the injury, inflammatory cells, such as neutrophils and macrophages, are recruited to debride the wound and ward off microbial invasion and proliferation. Macrophages secrete growth factors and cytokines that stimulate fibroblasts and other cells, promoting connective tissue biosynthesis.[71,72] A provisional wound-covering fibrin clot is formed by aggregated platelets, neutrophils, and red blood cells.[67,68] Fibroblasts proliferate and produce granulation tissue and extracellular matrix components, including fibronectin, collagen, and hyaluronic acid. Vascular endothelial growth factor drives angiogenesis and vascular permeability at this stage. About 7-10 days after injury, select fibroblasts differentiate into myofibroblasts that contract the wound (although minimal in the palate compared with oral, nonkeratinized mucosa).

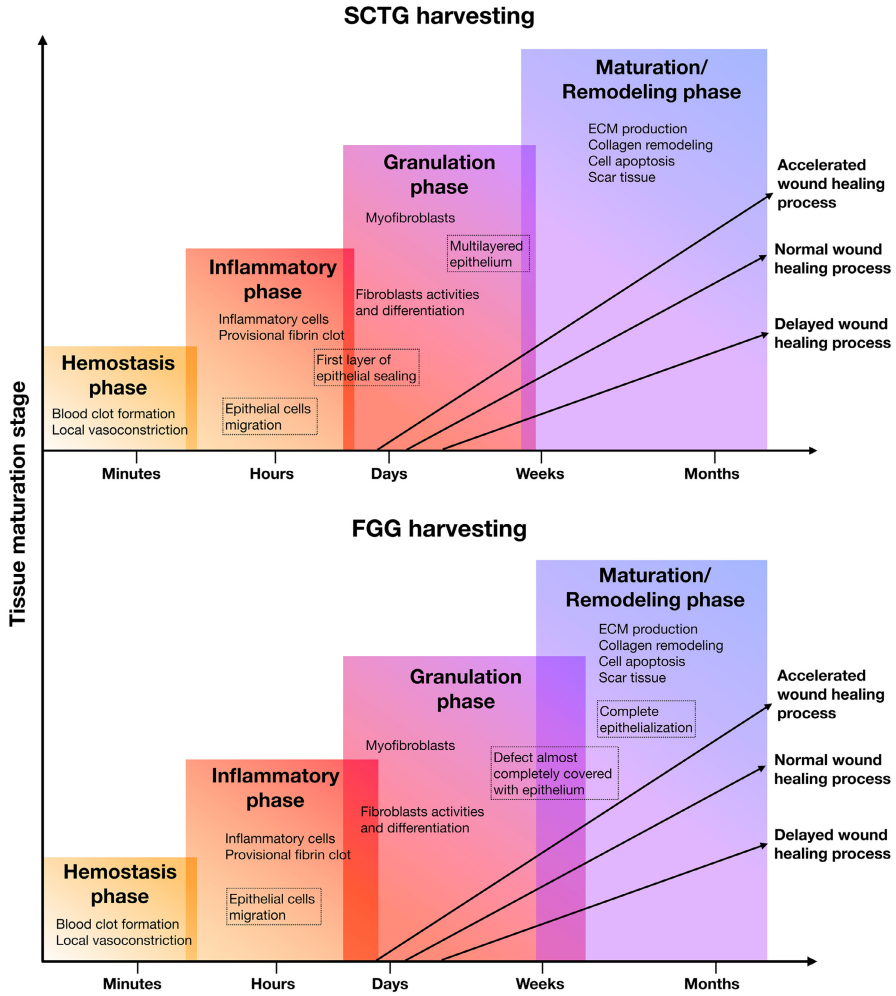


FIGURE 5 Phases of wound healing following subepithelial connective-tissue graft (SCTG) and free gingival graft (FGG) harvesting techniques. Accelerated wound healing process may occur with the use of biologic agents or topical drugs, and delayed wound healing is associated with patient characteristics, postoperative trauma, smoking habit, medications, and systemic conditions. ECM: extracellular matrix

For donor site healing by primary intention, re-epithelialization occurs within a few hours after injury. Epithelial cells migrate from the wound edges and from the base toward the incisional region, sealing the laceration within 24-48 hours. The approximating wound edges encourage clot formation and eschar formation, repelling microbial ingress; a new multilayered oral mucosa takes shape by day 5, if complications do not occur[69,71,73,74] (Figure 6A-K).

Palatal soft-tissue healing by secondary intention is characterized by a larger tissue deficit that requires a longer healing time and is susceptible to a greater risk of infection or scarring[73,75–78](Figure 6B-L). During the hemostatic phase, more pronounced inflammation is noted in wounds healing by secondary than by primary intention because more necrotic debris, exudate, and fibrin must be removed. Secondary intention wound healing predominantly involves granulation tissue formation; epithelialization only occurs when enough granulation tissue fills the injury site.[69,75–77] After 5 days, an inflammatory infiltrate persists, but there is active migration of cells from the basal epithelia. Typically not observed in primary intention healing, myofibroblast-dependent wound contraction prevails in sites healing by secondary intention.[69,75–77] Complete epithelialization is usually achieved in 3-5 weeks[79–83]. A common way to assess complete epithelialization is by applying hydrogen peroxide to the palatal wound area; the absence of bubbling suggests that hydrogen peroxide has not been able to diffuse into the connective tissue and liberate oxygen, meaning that total epithelialization is present.[84–87]

The last healing phase, tissue maturation and remodeling, is typified by a reduction in blood vessels and apoptosis of fibroblasts, myofibroblasts, epithelial cells, and macrophages (Figure 6). Alternating synthesis and degradation of extracellular matrix proteins take place and may result in fibrous scar tissue that has reduced biomechanical capacity compared with the original mucosa.[65,67,69,71]

A recent pilot study from our group described the ultrasonographic tissue perfusion changes occurring at palatal sites following free gingival graft harvesting, showing a substantial increase of blood flow at the donor site area at 1 week and 1 month compared with baseline.[88] Interestingly, increased blood volume was also observed at the greater palatine foramen area at both 1 week and 1 month, suggesting that adjacent regions are also affected from palate harvesting procedures, with changes occurring in the adjacent vascular network for redirecting blood supply to the wounded area.[88,89]

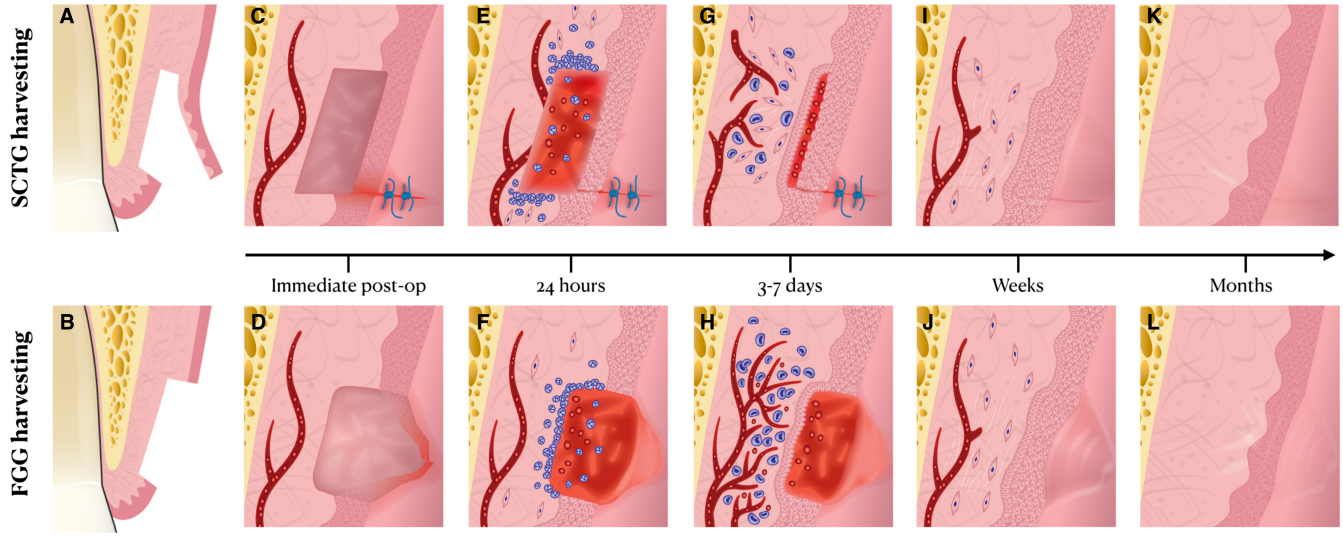


FIGURE 6 Wound healing events after subepithelial connective-tissue graft (SCTG) harvesting using the single-incision or free gingival graft (FGG) technique. A, Subepithelial connective-tissue graft harvesting technique using the single-incision approach. B, Free gingival graft harvesting approach. C, Immediately post-op with the primary flap repositioned over the donor site aiming for a primary intention healing. D, Immediately post-op following free gingival graft harvesting with the donor site healing by secondary intention. E, During the first 24 hours, inflammatory cells, mainly neutrophils and monocytes, are recruited and populate the clot. Their main function is cleansing the wound from bacteria and necrotic tissue. The process of epithelialization is initiated within a few hours, starting from the basal layer. F, The inflammatory phase is more enhanced compared with donor-site healing by primary intention. Granulation tissue formation is initiated and will represent the main bulk for the proliferation of epithelia. G, Macrophages migrate into the wound area and secrete several growth factors and cytokines that stimulate the other cells, in particular fibroblasts. Platelets, neutrophils, and red blood cells aggregate to form a provisional fibrin clot. Epithelial cells migrating from the basal layer through the fibrin clot seal the laceration within 24-48 hours, with a new multilayered oral mucosa that is formed by day 5. H, The inflammatory infiltrate is still present, but cells from the basal layer of the epithelium had been actively migrating to close the wound. I, Tissue maturation and remodeling with reduction in blood vessels and cell population and alternating of extracellular matrix synthesis and degradation. J, Myofibroblasts plays a key role in wound contraction, facilitating the complete epithelialization of the wound, usually observed after the third week. K, L, Complete healing of the wound that may result in fibrous scar tissue

5.2 | Preclinical animal models of wound healing by first and second intention

Rodents are one the most common experimental models for excisional and incisional wound healing studies.[90,91] Although these models are cost effective, there are several limitations that need to be considered when interpreting the results obtained, including the fact that healing in rodents is mainly promoted by myofibroblast-mediated wound contraction and structural differences between male and female rodents.[91,92] Early studies evaluated the palatal wound healing after excisional biopsies including all the soft tissue to the level of the palatal bone.[76,77,93–95] Nevertheless, the current harvesting techniques do not involve secondary intention healing with denuded bone. In a recent study, the palatal wound healing after an excisional wound was found to progress from the most anterior and posterior wound borders, with minimal changes in the medio-lateral dimension at the early stage of healing.[96] It was also observed that the inflammatory phase decreased in a time-dependent manner at the lateral and mid aspects of the wound regions, whereas in the central part of the wound it remained high until the third week. A significant increase in the number of myofibroblasts in the central portion of the wound was found during the third week.[96] The authors speculated that healing of the palate follows a "zipper" pattern, where one zipper is closing the wound from the anterior portion and the other from the posterior.[96] In line with this theory, a human study confirmed that the periphery of the palatal wounds filled earlier and to a greater extent than the central region of the wounds.[97]

A study evaluating the healing following a single-incision harvesting technique in a rat model showed relatively low inflammatory reaction and vascular density after the injury.[78] Interestingly, myofibroblasts' activity was found not to be influenced by the surgery, corroborating that palatal wound contraction promoted by myofibroblasts is more enhanced in healing by secondary rather than primary intention.[78]

Ginestal et al[73] evaluated the differences between first and second intention palatal wound healing in rats. After 21 days, all the incisional wound showed completely closure, whereas the group healing by secondary intention exhibited contracted edges of the defect with a small open surface in the central region of the wound. The histologic analysis revealed that after 7 days the incisional defects were characterized by scar tissue rich in inflammatory cells distributed along the axis of the incision, together with small newly formed vessels and immature collagen. After 21 days, this tissue had a similar appearance to healthy tissues; the reparative process of the excisional defects was not complete at 21 days. Fibrin clot and inflammatory exudate were observed in the central

portion of the excisional wound. Incisional wounds presented a large amount of macrophages in the granulation tissue at day 7, mainly located in the most superficial areas and near the edge of the incisions. After 14 days, a decrease in macrophage populations was observed in the wound healing by primary intention, while the excision defects exhibited macrophagic cells forming clusters throughout the scar tissue, both in the most superficial and deeper areas.[73]

5.3 | Genes implicated in palatal soft-tissue wound healing

Targeting specific genes related to growth factors and cytokines appears to affect palatal wound healing and contraction,[98–101] but the genetic framework and expression profiles of palatal wound healing is largely unknown. Wang and Tatakis compared human transcriptomes between intact palatal mucosa and contralateral post–free gingival graft harvest sites.[102] Between groups, 700 genes were differently expressed ($P < 0.05$), 80% of which were upregulated in the healing group; gene set enrichment analysis identified focal adhesion, cytokine-cytokine receptor interaction signaling, and extracellular matrix receptor interaction pathways to be most affected. As per Gene Ontology term analysis, the top three regulated processes in palatal healing are signal transduction, immune system process, and multicellular organismal development (Figure 7). In a follow-up study, the same group found that smokers express 830 contrasting genes compared with nonsmokers. These differentially expressed genes were associated with innate immunity and antimicrobial response and were downregulated in smokers.[103]

5.4 | Local and systemic factors affecting palatal soft-tissue wound healing

Oxygenation, venous sufficiency, infection, and foreign bodies are local factors that can modify palatal wound healing.[70,104] Oxygenation, essential for cell metabolism and wound healing, promotes angiogenesis, stimulates keratinocytes and fibroblasts, and contracts wounds. Hypoxia, ischemia, and venous stasis disease have been well documented to impair healing.[70,105,106] Infection or the presence of foreign bodies prolongs the inflammatory phase, delaying wound repair.[69,70]

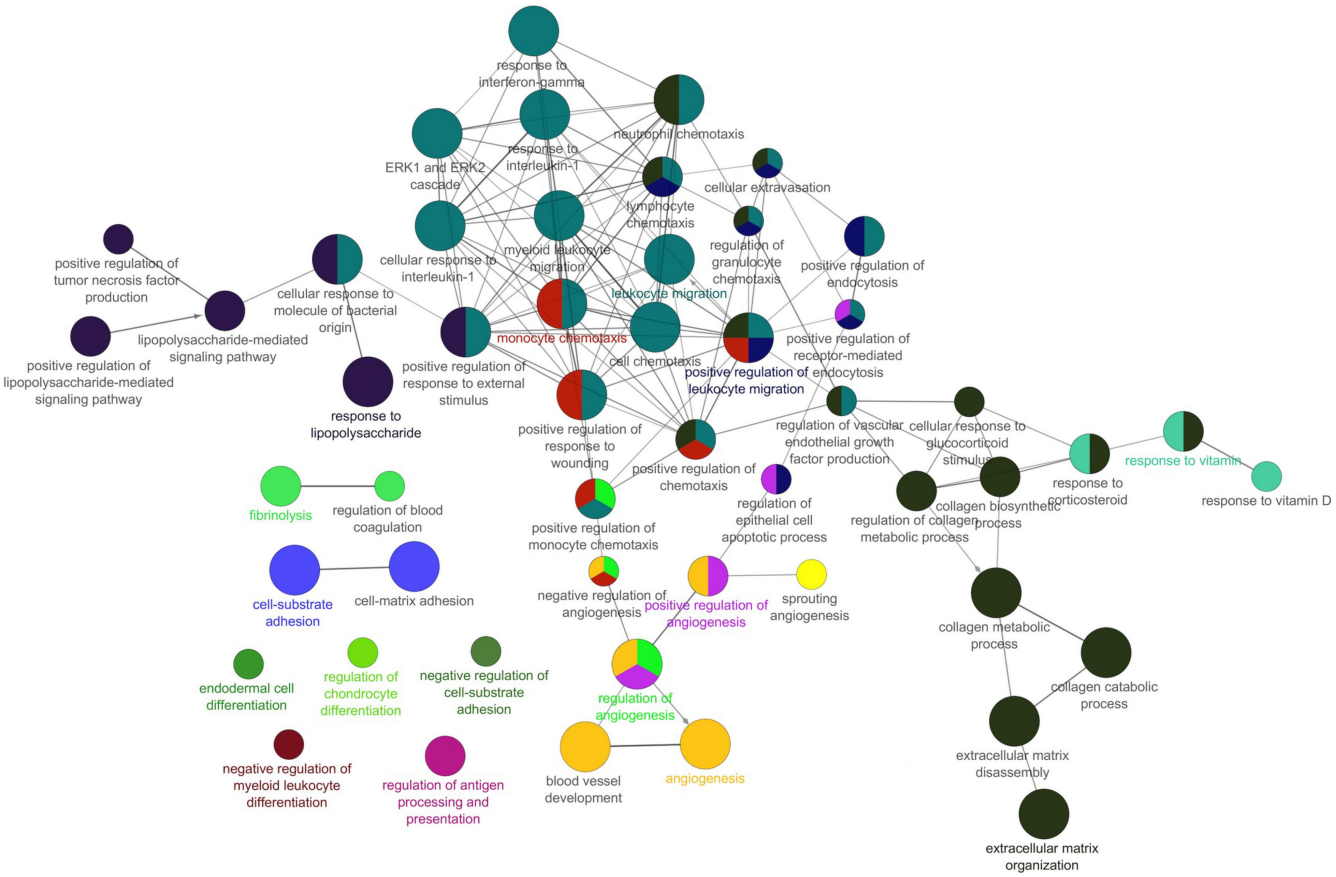


FIGURE 7 The functionally grouped network of differentially expressed genes (fold change greater than 5) in palatal wound healing. The nodes represent Gene Ontology terms, their size represents the significance of term enrichment, and the lines represent shared genes between connected Gene Ontology groups from the considered dataset as described by Wang and Tatakis.[102] Reproduced with permission from John Wiley and Sons[102]

TABLE 1 Systemic factors negatively affecting wound healing.

Factors impairing/delaying wound healing	Mechanisms	Results	References
Increased age	• Delayed T-cell infiltration • Reduced macrophage phagocytic capacity • Poor tissue oxygenation	• Delayed re-epithelialization • Delayed collagen synthesis • Delayed angiogenesis	Swift et al 1999,[189] Quan and Fisher 2015,[190] Guo and DiPietro 2010[70]
Gender	• Different effects of sex hormones • Estrogens upregulate several genes implicated in wound healing, whereas androgens impair the regulation of these genes	• Delayed healing of acute wounds in males compared with females • Possibly delayed proliferative phase in males	Engeland et al 2009,[191] Gilliver et al 2007,[192] Hardman and Ashcroft 2008[193]
Stress	• Increased levels of glucocorticoids • Reduced expression of crucial cytokines at the wound sites	• Impaired cell-mediated immunity at the wound site • Delayed healing	Boyapati and Wang 2007[194] Godbut and Glaser 2006[195]
Nutritional deficiencies of proteins, carbohydrates, vitamin A, vitamin C, vitamin E, magnesium, copper, zinc, or iron	• Decreased production of proinflammatory cytokines • Decreased leukocyte phagocytosis • Decreased cell metabolism • Decreased fibroblast function • Impaired angiogenesis • Impaired wound remodeling	• Greater risk for infection • Delayed healing	Campos et al 2008,[196] Guo and DiPietro 2010[70]
Diabetes	• Hypoxia • Fibroblast and epithelial cell dysfunction • Poor vascularization and impaired angiogenesis • High levels of metalloproteases • Damage from reactive oxygen species and advanced glycation end-products	• Reduced host immunity • Greater risk for infection • Delayed healing	Guo and DiPietro 2010,[70] Woo et al 2007[197]
Obesity	• Decreased vascularity • Increased wound tension and tissue pressure • Venous hypertension • Increased production of adipokines, proinflammatory cytokines, and chemokines	• Delayed healing	Guo and DiPietro 2010,[70] Wozniak et al 2009[198]
Smoking	• Vasoconstriction • Impaired leukocyte activity • Decreased macrophage and neutrophil activity • Decreased fibroblast function • Impaired epithelial regeneration • Hypoxia • Decreased angiogenesis • Increased proteases	• Poor immune response • Increased risk of infection • Delayed inflammatory phase • Higher chances of flap necrosis, wound dehiscence, and infection	Ahn et al 2008,[199] Anderson and Hamm 2014[104]
Alcohol intake	• Increased insulin resistance • Higher blood sugar levels • High risk of protein malnutrition • Decreased inflammatory and immune response • Decreased fibroblast migration and collagen production • Impaired angiogenesis	• Delayed healing • Increased risk of infection	Anderson and Hamm 2014,[104] Ranzer et al 2011[200]

(Continues)

TABLE 1 (Continued)

Factors impairing/delaying wound healing	Mechanisms	Results	References
Immunocompromised conditions (cancer, radiation therapy, acquired immune deficiency syndrome, etc) and medications (glucocorticoid steroids, nonsteroidal anti-inflammatory drugs, and chemotherapy drugs, etc)	• Impaired blood clot formation, platelet function, inflammatory response, and cell proliferation • Reduced fibroblast proliferation and collagen production • Suppressed cellular response, fibroblasts proliferation, and collagen synthesis • Impaired cells functions, angiogenesis, and collagen production	• Delayed healing • Increased risk of infection	Anderson and Hamm 2014,[104] Fowler 2018,[201] Guo and DiPietro 2010[70]

Table 1 describes the main systemic factors that can impede palatal soft-tissue healing, including increased age, nutritional deficiencies, obesity, alcohol use, smoking, diabetes, and stress.[70,104] Silva et al[84] demonstrated that patients who smoke display wounds that undergo delayed epithelialization; at 15 days after graft harvest, 20% of smokers and 92% of nonsmokers exhibit complete epithelialization. Diabetes generates advanced glycation end products, which slow cell turnover, decrease circulation, and alter inflammatory cell function.[107] Psychologic factors, such as stress and depression, may also negatively affect palatal mucosal healing.[86,108]

5.5 | Effect of biologic agents and photobiomodulation in accelerating palatal wound healing

Certain biologic agents have been suggested to accelerate palatal wound healing by stimulating particular cells, cytokines, or genes, though some of these recommendations are based on in vitro or preclinical studies, which indicate promising effects of epithelial growth factor, enamel matrix derivative, and synthetic proline-rich peptide on re-epithelialization, wound closure, and inflammatory response suppression (Table 2).[109,110] Clinical studies reported faster re-epithelialization using topical erythropoietin, hyaluronic acid, and platelet-rich fibrin.[111–113] Platelet-rich fibrin elicits the continuous release of platelets, leukocytes, and growth factors[114,115] critical to palatal wound healing.[80,85,113,116,117] One randomized controlled trial suggested that platelet-rich fibrin may improve feeding habits (ie, ability to eat hard or warm foods) during the 2 weeks following free gingival graft harvesting.[85] However, another randomized controlled trial described no improved clinical healing with platelet-rich fibrin application to the palatal donor site except for decreased patient morbidity.[116]

Figure 8 summarizes palatal re-epithelialization rates after using various adjunct biologic modifiers at donor sites in randomized controlled trials.[80–83,85,111–113,116–125] No treatment protocol obtained complete re-epithelialization after 1 week. Regardless of the treatment (or nontreatment) performed, complete re-epithelialization of the wound was observed in nearly all cases after 4 weeks. Ozone therapy, cyanoacrylate tissue glue, and photobiomodulation generated more cases with complete epithelialization than spontaneous healing did within the first month.

Photobiomodulation, the biostimulation of tissue with low-level laser irradiation, is relatively new to dental medicine.[126] Photobiomodulation may accelerate wound healing by stimulating fibroblasts, reducing production of reactive oxygen species, facilitating angiogenesis, and promoting provisional matrix formation.[126–128] A randomized controlled trial showed that photobiomodulation resulted in smaller wounds at palatal connective tissue donor sites.[128] The same group demonstrated that the power density of photobiomodulation alters palatal wound healing.[127] Photobiomodulation may boost endogenous growth factor expression and release beyond levels found in spontaneous healing,[129] which may explain the accelerated complete re-epithelialization rate found following laser irradiation.[81,120]

Low-level microcurrent electrotherapy may also expedite palatal wound healing after free gingival graft harvesting via fueling cell migration and proliferation and modulating growth factor release.[130] A randomized controlled trial observed faster re-epithelialization and wound closure in donor sites treated with electrotherapy compared with controls.[130]

The magnitude of effect and cost/benefit ratio of adjunct biologic agents and photobiomodulation on palatal donor site healing remains ambiguous; further study is warranted, considering that some of these potential modifiers are not inexpensive or readily available.

5.6 | Timing for soft-tissue graft reharvesting from the same donor region

Harvesting a soft-tissue graft from the same donor site after a few months is at times required.

A minimum 9-week interval between palatal harvesting procedures has been recommended based on a study by Soileau and Brannon,[131] who monitored patients undergoing two harvesting procedure with a parallel incision technique at different intervals and observed significantly better soft-tissue maturation after

TABLE 2 Biologic agents and topical drugs accelerating palatal healing after excisional wounding

Biologic agent	Mechanisms	Outcomes on palatal wounds
Enamel matrix derivatives	• Increased amount of granulation tissue • Increased levels of growth factors and proteinases • Downregulation of genes and cytokines related to inflammation • Stimulation of growth factors related to wound repair	• Faster re-epithelialization and wound closure • Reduced early marked inflammation in the connective tissue • Lower inflammation score and higher angiogenesis score at day 1 compared with placebo • Decreased leukocyte infiltration at day 1 compared with placebo
	References: Mirastschijski et al 2004[c], Parkar and Tonetti 2004[c], Myhre et al 2006[a,202–204]	Reference: Villa et al 2015[a,109]
Epithelial growth factor	• Stimulation of epithelial cells, endothelial cells, and fibroblasts • Increased epidermal proliferation rate • Accelerated wound contraction by enhancing myofibroblast proliferation • Accelerated collagen synthesis	• Epithelial growth factor group showed faster wound closure and more granulation tissue than control group at day 8 • Enhanced keratinocytes proliferation, decrease in granulation tissue and inflammatory response, with more deposition of collagen in the epithelial growth factor group at day 16[a,110]
	References: Kwon et al 2006[a], Broughton et al 2006[d,205,206]	Reference: Ben Amara et al 2019[a,110]
Erythropoietin	• Stimulation of angiogenesis • Promotion of extracellular matrix and collagen synthesis • Reduction of inflammatory mediators	• More patients with complete epithelialization at the end of week 3 compared with placebo
	References: Buemi et al 2002[a], Hamed et al 2010[a,207,208]	Reference: Yaghobee et al 2018[b,111]
Hyaluronic acid	• Stimulation of polymorphonuclear leukocyte migration and function • Induction of proinflammatory cytokines that initiate the inflammatory response • Increased proliferative and migration abilities of fibroblasts • Promoting the expression of genes characterizing "scarless" wound healing	• No differences in blood flow variation between hyaluronic acid and control group • Reduced morbidity and burning sensation • Accelerated epithelialization compared with the control group
	Reference: Asparuhova et al 2019[c,209]	References: Cankaya et al 2020[b], Yildirim et al 2018[b112,210]
Phenytoin gel	• Increased protein synthetic activity and collagen production • Increased fibroblast proliferation • Stimulation of the connective-tissue metabolism • Increased vascularization	• Decreased patient morbidity • Less swelling • Accelerated healing of granulation tissue
	References: Hasamnis et al. 2010[a], Sayar et al. 2014[a], Doshi et al. 2020[b,211–213]	Reference: Doshi et al. 2020[b,212]
Platelet-rich fibrin	• Stimulation of cells migration and proliferation • Antimicrobial activity • Stimulation of cell chemotaxis, intracellular matrix deposition, remodeling	• Decreased patient morbidity • Accelerated epithelialization • Better healing indices • Lower postoperative bleeding
	References: Dohan Ehrenfest et al 2010[b], Jain et al 2012[b], Tavelli et al. 2020[d6,214,215]	References: Bahammam 2018[b], Femminella et al 2016[b], Kiziltoprak and Uslu 2020[b,80,113,166]
Simvastatin gel	• Anti-inflammatory and antibacterial actions • Enhancement of vascular endothelial growth factor and promotion of angiogenesis	• Significant reduction in wound-healing scores and postoperative pain
	References: Rego et al. 2007, Bitto et al. 2008, Asai et al. 2012[216–218]	Reference: Madi and Kassem 2018[b,219]

(Continues)

TABLE 2 (Continued)

Biologic agent	Mechanisms	Outcomes on palatal wounds
Synthetic proline-rich peptide	• Promotion of mesenchymal stem cells differentiation • Antimicrobial properties • Enhancement of the immune response • Induction of angiogenesis References: Ramis et al 2012[c], Zanetti 2004[d], Li et al 2020[a,220–222]	• Promotion of early wound closure • Increased angiogenesis • Significant decrease in leukocyte infiltration at days 3 and 7 compared with placebo Reference: Villa et al 2015[a,109]
Tyrosine-rich amelogenin peptide	• Stimulation of differentiation of different cell populations • Stimulation of endothelial cell migration[109,223] References: Villa et al 2015[a], Jonke et al 2016[c,109,223]	• Reduced inflammation at days 3 and 7 compared with placebo Reference: Villa et al 2015[a,109]

[a]Animal study.
[b]Human study.
[c]In vitro study.
[d]Review.

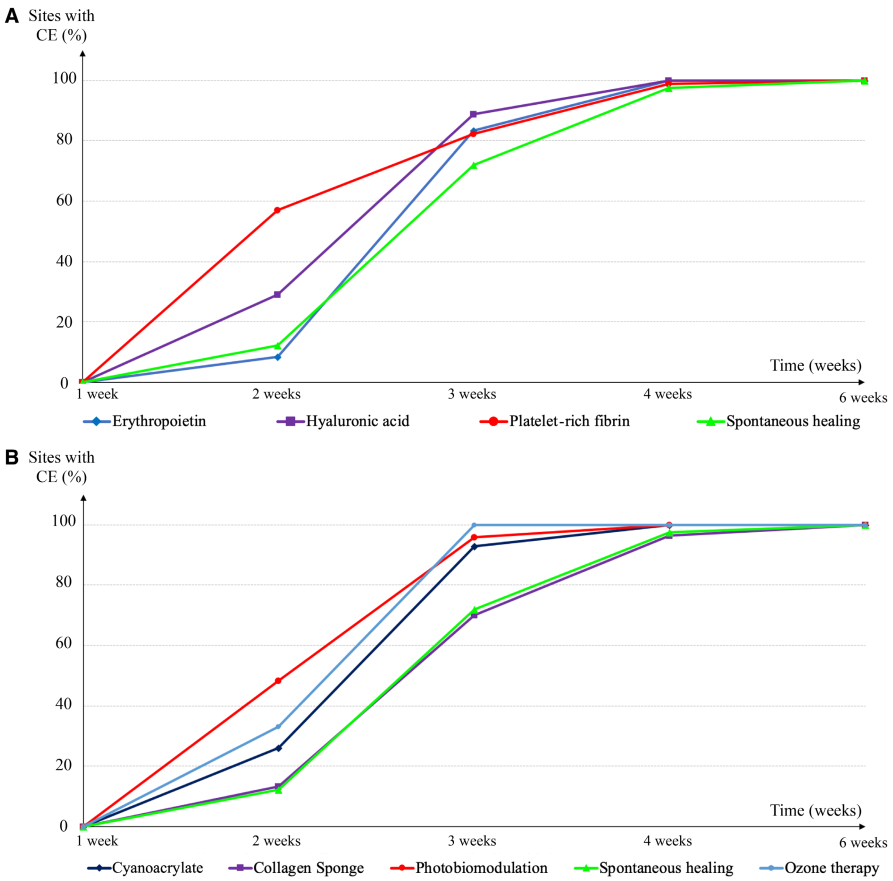


FIGURE 8 Rate of complete epithelialization (CE) of the palatal wound following free gingival graft harvesting. A, The effect of biologic agents on the rate of complete epithelialization. B, The effect of hemostatic agents, photobiomodulation, and ozone therapies on the rate of complete epithelialization

63 days (9 weeks) compared with after 48 or 54 days postsurgery. A longer healing interval may be more sound, as affirmed by a clinical study from our group that showed volumetric donor site loss during the first 3 months following free gingival graft harvesting.[132] After an initial volume loss observed at the 1- and 3-month visits, the donor site regained its presurgical volume at the 6-month follow-up (Figure 9).[132] After 6 months, the regenerative capabilities of the healed palatal connective tissue may also be restored.[133] Bearing in mind volume and tissue quality, a 6-month interval between harvesting from the same area is recommended.

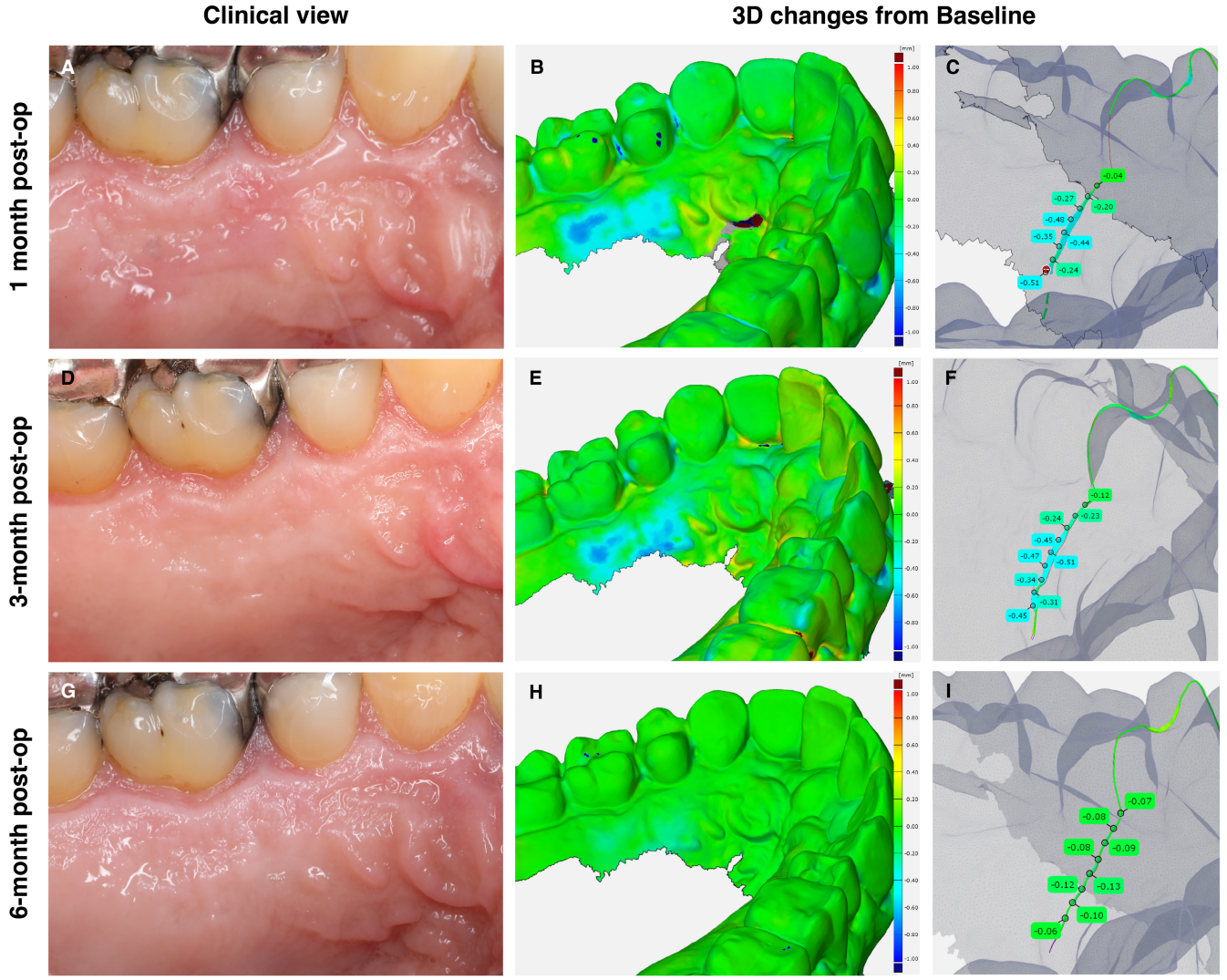


FIGURE 9 Three-dimensional digital analysis assessing volumetric changes of the palatal donor site following free gingival graft harvesting at 1, 3, and 6 months. A, D, G, Clinical view at the 1-, 3-, and 6-month follow-up, respectively. B, C, Digital analysis from the superimposition of the STL digital files at baseline and 1-month follow-up evaluating volumetric changes. E, F, Digital analysis from the superimposition of the STL digital files at baseline and 3-month follow-up. H, I, Digital analysis from the superimposition of the STL digital files at baseline and 6-month follow-up

6 | INTRAOPERATIVE COMPLICATIONS AND MANAGEMENT DURING PALATAL HARVESTING

6.1 | Injury to the greater palatine artery

Excessive intraoperative bleeding is a very common complication of palatal harvesting.[134,135] A thorough knowledge of palatal anatomy is fundamental to avoid severing the greater palatine artery and its main branches (see Section 3).[37,45,47] Noninvasive technologies, such as ultrasonography, magnetic resonance imaging, and near-infrared vein visualization, can locate the greater palatine foramen and the course of the greater palatine artery presurgically[88,136–138] (Figures 10 and 11); however, apart from ultrasound, none of these other approaches are commercially available or practical for daily use. Respecting the guidelines provided by the literature and identifying the greater palatal foramen by palpation are therefore recommended.

Injuries to the greater palatine artery should be first managed by applying pressure on the wound for several minutes, using a local anesthetic with a vasoconstrictor, or electrocauterizing the vessel. If the bleeding persists, performing deep compression sutures distal to the palatal donor site followed by electrocauterizing the vessel is recommended.[139,140] However, electrocoagulation requires special equipment and training, and there is limited evidence of its safety or efficacy at the palatal donor site.

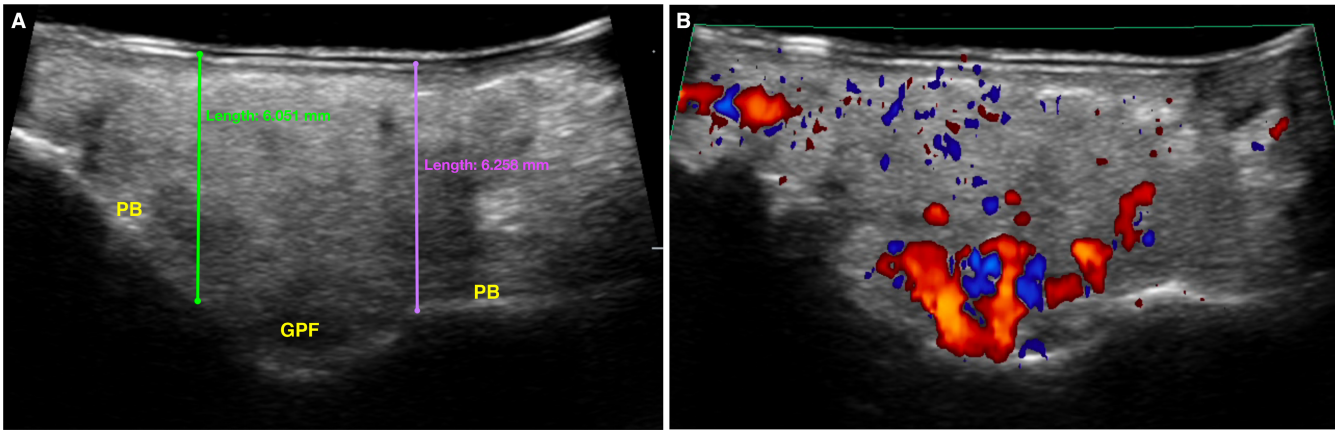


FIGURE 10 Nonionizing real-time ultrasonography for assessing the position of the greater palatine foramen (GPF). A, "B-mode" imaging showing the thickness of the palatal mucosa and the depth of the greater palatine foramen. The palatal mucosa presented as a hypoechoic (dark) region between two hyperechoic (bright) bands, which were the ultrasound probe (in the upper part of the scan) and the palatal bone (PB) (in the lower part of the scan). Measurements of palatal mucosa thickness were performed using a commercially available software package (Horos, version 3.3.6, Horos Project) as previously described by Chan and Kripfgans.[225] B, "Color velocity" mode showing blood flow in the region examined. The displayed color velocity visualized the speed at which blood flows, and it was performed using a constant velocity scale (±2.3 cm/s), with the color red indicating blood flow towards the transducer and the blue color denoting blood flow in the opposite direction. Note that blood volume was visualized and measured as videos generated from collected consecutive images; a still image is shown here. The location of the greater palatine foramen was identified by palatal bone morphology in the second molar region and by the increased blood flow in this area

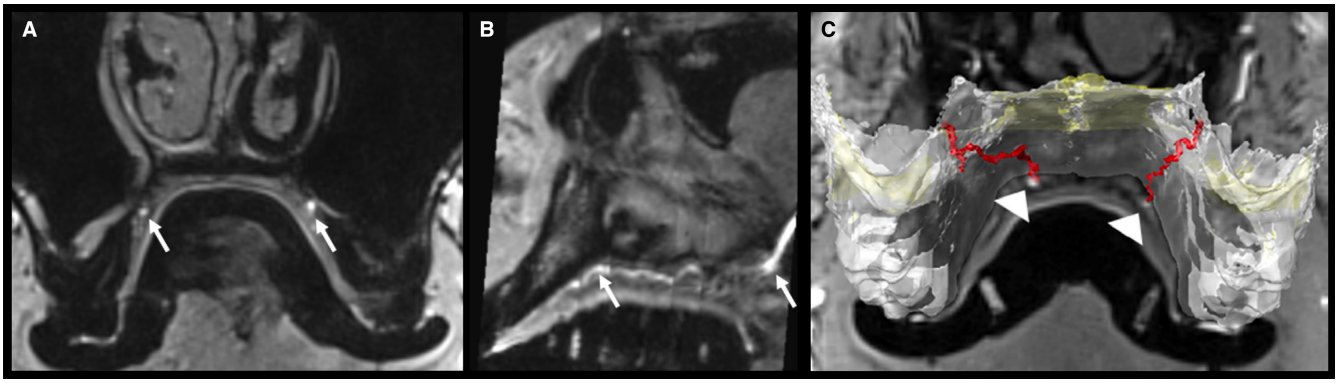


FIGURE 11 High-resolution dental magnetic-resonance imaging acquired without the use of contrast agents for identifying the course of the greater palatine artery using time-of-flight angiography. A, Coronal image showing the greater palatine artery (white arrows). B, Imaging showing the greater palatine artery course in an oblique sagittal direction (white arrows), where it is possible to appreciate the descent of the artery through the greater palatine foramen and its course in the palatal masticatory mucosa (Courtesy of Dr Tim Hilgenfeld). C, Three-dimensional segmentation of the maxilla (white) with the course of the grater palatine artery highlighted in red by using the time-of-flight angiography and MSVAT-SPACE sequences, as described by Hilgenfeld et al.[137] Readapted and reproduced with permission from John Wiley and Sons[137]

6.2 | Excessive bleeding unrelated to direct greater palatine artery injury

Aside from direct severing of the greater palatine artery, prolonged or excessive bleeding at the donor site may occur in patients with bleeding disorders or using anticoagulants or from certain flap designs.[134,135,141] According to a cadaver study, the harvesting approach may play a role in intraoperative bleeding.[135] Trapdoor connective-tissue harvesting generates approximately four times more "leakage"—defined as the amount of injected betadine solution that exited from the harvested areas—than free gingival graft harvesting, suggesting that more or larger vessels are injured when a subepithelial connective-tissue graft is withdrawn. Histologic analysis revealed a greater number of medium and large vessels in the graft obtained with the trapdoor technique (Figure 12). Leakage also occurs more frequently in palates with thin mucosa or shallower vaults.[135] Based on that study, harvesting a free gingival graft, which is more superficial, circumvents injury to the deeper palatal vessels, whereas collecting a deeper connective-tissue graft risks damaging vascular structures, resulting in more bleeding.

Regarding immediate postoperative bleeding, a randomized controlled trial comparing free gingival graft, single-incision connective tissue, and trapdoor connective-tissue harvesting techniques reported that the percentages of patients with bleeding following the trapdoor approach and free gingival graft harvesting were 33% and 25%, respectively.[79] However, no protective materials were applied over donor sites after free gingival graft harvesting, which may explain the relatively high percentage of patients with bleeding.[79] Cases using the single-incision method had no postoperative bleeding.[79]

Excessive bleeding interferes with accurate palatal harvesting and suturing. Notably, patients who smoke obtain hemostasis in nearly half the time as nonsmokers do, possibly because nicotine and its by-products are vasoconstrictors.[142] Hemostasis may be achieved by packing the palatal wound with wet gauze and applying pressure on the site for a few minutes.[134] Microfibrillar collagen hemostat, oxidized regenerated cellulose, and absorbable gelatin sponge can be applied instead of gauze and are effective within a few minutes.[142,143] Alternatively, hemostatic agents (eg, aluminum chloride, ferric sulfate), local anesthetic with epinephrine, or an acrylic palatal stent can be used.[134]

Using a tissue glue such as cyanoacrylate may be helpful, as it has hemostatic, bacteriostatic, and tissue-compatible properties (Figure 13).[85,144] Applying cyanoacrylate alone results a mean bleeding time of 1.65 minutes, whereas wet gauze compression produces a mean bleeding time of 3.18 minutes. Combining platelet-rich fibrin with cyanoacrylate shortens the mean bleeding time to 0.57 minutes.[85]

6.3 | Primary flap laceration

Harvesting a subepithelial connective-tissue graft involves preparing a split-thickness flap (the primary palatal flap), elevating and withdrawing the connective-tissue graft without direct visualization, and closing the palatal wound by flap repositioning. During this delicate procedure, inadvertent flap laceration may occur; lacerated or excessively thin primary palatal flaps associate with increased patient morbidity and analgesic consumption.[58] The integrity and a specific thickness of the overlying palatal tissue must be maintained to combat sloughing or necrosis resulting from these surgical issues (see Section 7.3) (Figure 14).

Minor flap lacerations may not affect the healing of the palatal wound and do not require suturing or additional treatments. However, a major laceration jeopardizes the chance of obtaining a primary intention healing. Applying a hemostatic collagen sponge underneath the tear flap is advocated to prevent bone exposure in the case that the flap undergoes necrosis.

To prevent complications, a thickness of at least 3 mm palatal fibromucosa (1 mm of the overlying palatal flap and 1-2 mm for the graft) is needed when subepithelial connective-tissue graft harvesting approaches are performed.[12,58,145] Free gingival graft harvesting, which aims to withdraw the superficial epithelial layer along with a thin layer of connective tissue, can be executed in thin palatal fibromucosa—secondary intention is self-evident. In thin palatal donor sites, de-epithelialization of free gingival grafts may be the most conservative way to collect connective tissue.[146–148]

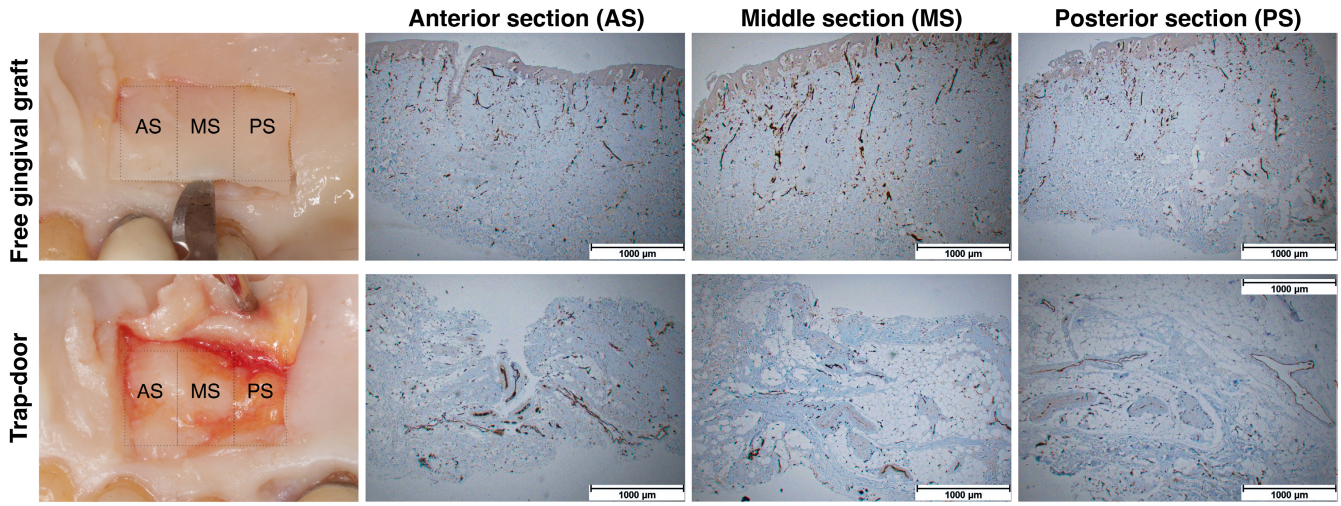


FIGURE 12 Histologic analysis from a single human head comparing soft-tissue graft composition between free gingival graft and trapdoor harvesting. CD31 immunohistochemistry was performed to highlight vessel density. The graft obtained from free gingival graft harvesting showed a lamina propria composed mainly of dense connective tissue fibers with a minimal number of vessels. Adipose and glandular tissue was present in the middle and posterior sections. The graft obtained with the trapdoor technique displayed a large amount of fatty and glandular tissue, with small- and large-diameter vessels that were severed during harvesting. The middle and posterior sections seem to contain more fatty tissue than the anterior one; a large-diameter vessel of approximately 1 mm is observed in the posterior section. Readapted with permission from John Wiley and Sons[135]

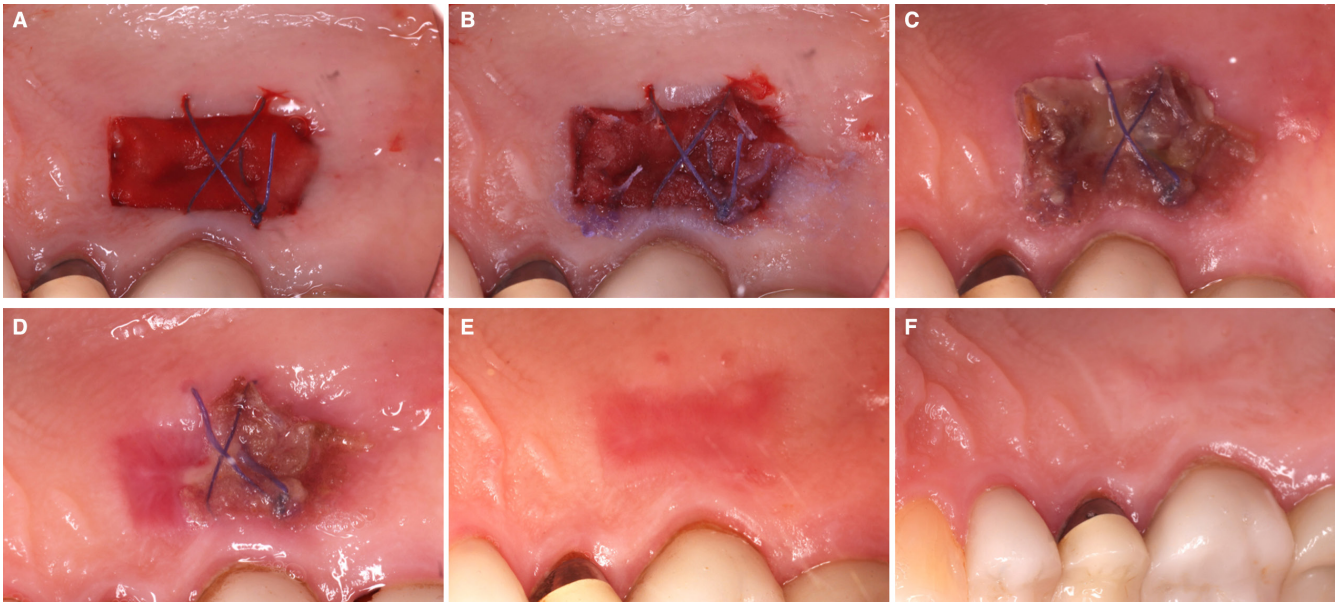


FIGURE 13 Hemostasis of the donor site following free gingival graft using a hemostatic collagen sponge and superficial layer of cyanoacrylate tissue glue. A, Collagen sponge was applied to the donor site, and a retaining suture was placed. B, Cyanoacrylate tissue glue was applied over the collagen sponge. C, 1 week post-op; D, 2 weeks post-op; E, 1 month post-op; F, 3 months post-op

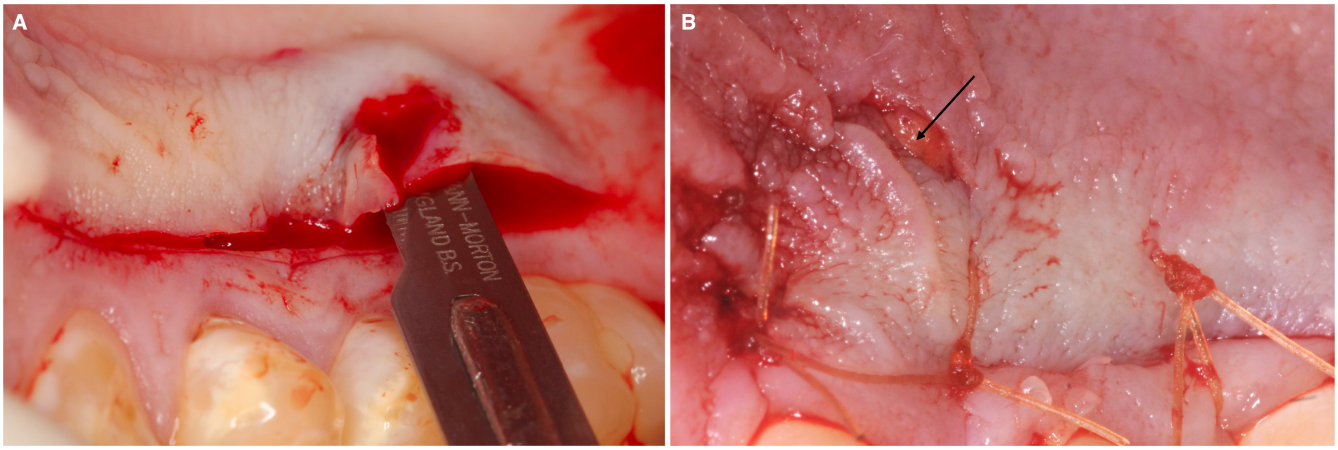


FIGURE 14 Flap laceration occurring in primary flap during single-incision harvesting. A, In the most coronal part. B, In the most apical part Courtesy of a colleague

6.4 | Inadequate graft dimension

Owing to anatomical discrepancies, obtaining a dimensionally insufficient subepithelial connective-tissue graft is not uncommon. A connective-tissue graft thickness of approximately 1 mm is needed for root coverage procedures and to decrease postoperative pain.[149] The palatal fibromucosa is thinner in the first molar area, where palatal exostoses pose additional challenges.[32,33,37,150] The palatal thickness must be measured using transgingival probing or ultrasonography to determine the best location and flap design for harvesting (Figure 15). Bearing in mind that the initial incision of the harvesting should be placed 1 to 2 mm from the cemento-enamel junction,[28,37] a greater palatal thickness is usually observed in the first and second premolar areas than in the canine and first molar regions.[31–33]

Free gingival graft harvesting is preferred when thin palatal mucosa is present (less than 3 mm) to preclude primary palatal flap overthinning or obtaining a suboptimal connective-tissue graft. If the residual soft tissue underlying the primary flap is less than 1 mm thick, then the periosteum should be elevated with the connective-tissue graft instead of dissected out with a blade.[28] Including the periosteum with the graft has no clearly documented clinical advantages, but Zuhr et al[28] suggested that such grafts may have superior mechanical stability relative to nonperiosteal grafts, though removing the periosteum from bone may potentially delay wound healing.

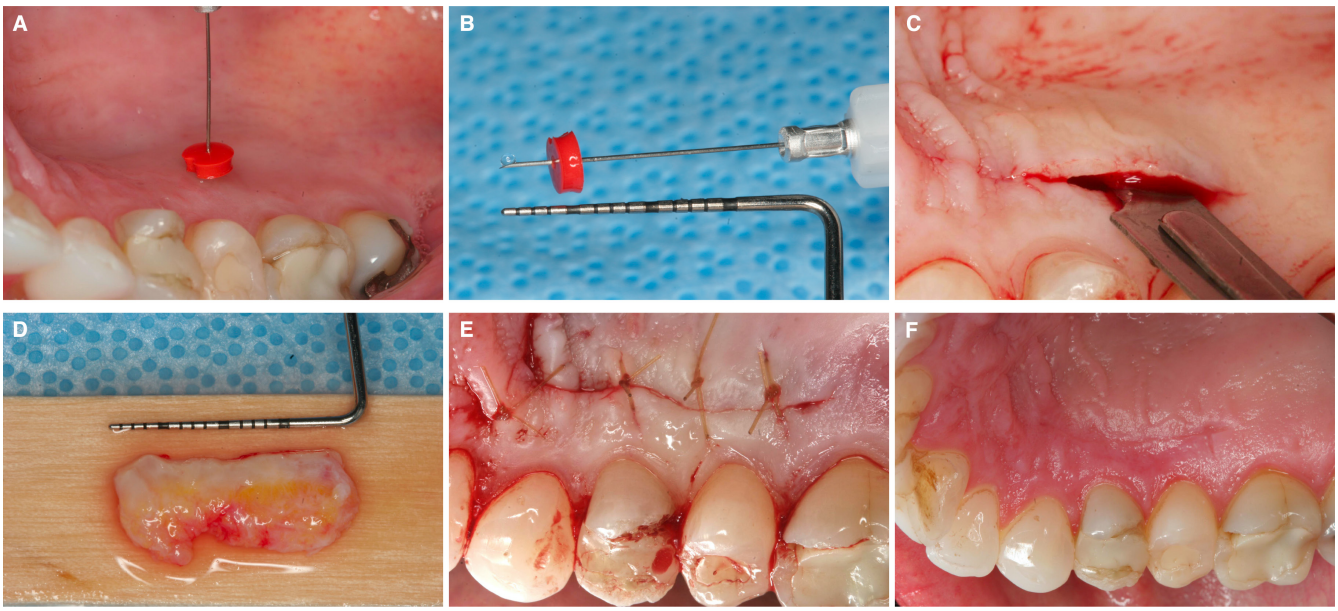
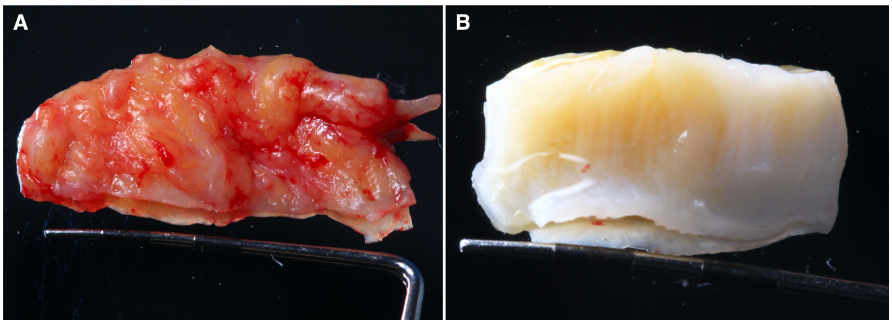


FIGURE 15 Subepithelial connective-tissue graft harvesting performed in a 4-mm thick palate. The palatal thickness allowed for the harvesting of an adequate and uniform subepithelial connective-tissue graft and for healing by primary intention. A, B, Palatal thickness was measured with a silicon stop positioned over an injection needle. C, Single-incision harvesting technique. D, Subepithelial connective-tissue graft. E, Suturing of the palatal flap. F, 2 weeks post-op. Courtesy of Dr Ho-Young Byun

FIGURE 16 Harvesting of connective tissue grafts from the same subject. A, From left palate using the single-incision technique. B, From right palate using the free gingival graft approach. Both harvested connective-tissue grafts were rich in fatty and glandular tissue, regardless of the harvesting technique performed



Harvesting an additional autogenous graft from the maxillary tuberosity or the contralateral palate or using a collagen or acellular dermal matrix is recommended when an inadequate graft is obtained.

6.5 | Inadequate graft quality

A tissue graft abundant in fibrous connective tissue is firmer, more stable, and easier to manipulate than a graft rich in fatty or glandular tissue, though clinical differences stemming from graft composition have not been confirmed.[12,58,151] Improved handling properties may explain the greater mean root coverage obtained with de-epithelialized gingival grafts (91.7%) compared with subepithelial connective-tissue grafts (84.7%) 1 year after tunneling.[152]

As mentioned, the harvesting technique affects connective-tissue graft composition. A connective-tissue graft derived from de-epithelialization of a free gingival graft is mainly composed of lamina propria; a connective-tissue graft harvested conventionally (ie, deeper) incorporates submucosa, in which glandular and adipose structures are more profuse.[12,28,58,145,151] A cadaver study determined that, relative to conventional harvesting, connective-tissue grafts acquired from free gingival graft de-epithelialization contained significantly more fibrous connective tissue (73-81% versus 53-56%) and less fatty and glandular tissue (18-27% versus 40-46%); however, wide interindividual variability was reported (Figure 16).[36]

These results were supported by a histologic human study that noted that connective tissue derived from free gingival grafts is primarily composed of dense connective tissue (89%) with a minimal contributions from adipose tissue (1%), vascular tissue (3%), and epithelial remnants (6%).[153] Histology revealed that conventional subepithelial connective-tissue grafts contain 59% dense connective tissue 32% adipose tissue, and 8% vascular tissue.[154] In this view, the maxillary tuberosity represents a valid alternative to the palatal side, as it is mainly composed of lamina propria with a minimal percentage of submucosa.[63]

7 | POSTOPERATIVE COMPLICATIONS

7.1 | Pain

Pain is the most common postoperative complication following palatal harvesting,[58,144,155–157] with some patients recalling discomfort associated with this procedure a decade after undergoing it.[158]

Subepithelial connective-tissue harvesting techniques aim for primary closure, which has been linked to less discomfort than free gingival graft harvesting; Table 3 summarizes randomized clinical trials evaluating patient-reported outcomes following subepithelial extraction methods.[79,156,157] As per analyses by Zucchelli et al[58] and Fickl et al,[74] the trap-door technique scores approximately 2.7 out of 10 on a pain visual analogue scale after 1 week; single-incision approaches range 2.16-3.49 after 1 week.[74,159] Other subepithelial connective-tissue harvesting approaches, including those described by Bruno[160] and Langer and Langer,[51] exhibit postoperative morbidity ranging 2.3-4.8 out of 10 on a visual analogue scale.[128,161,162] No statistically significant difference in postoperative pain based on visual analogue score between trap-door and single-incision techniques is reported, even though the single-incision technique corresponds to significantly lower painkiller intake and fewer incidences of secondary wound healing.[74] Application of platelet-rich fibrin following single-incision harvesting technique seems to improve wound healing and patient comfort.[163]

Free gingival graft donor sites heal by secondary intention and have been assumed to associate with postoperative pain.[79,156,157] However, a randomized clinical trial by Zucchelli et al[58] demonstrated that no difference in reported pain exists between trap-door connective tissue and free gingival harvesting when the donor site is protected with an absorbable collagen matrix in the latter procedure.

Use of protective materials (ie, Essix, stent, retainers, periodontal dressing),[112,122,164,165] hemostatic agents (ie, collagen matrix, gelatin sponge, cyanoacrylate),[58,85,116,144,155] and wound-healing enhancers (ie, platelet-rich fibrin, autogenous fibrin glue, platelet-rich plasma, laser photobiomodulation, ozone therapy, hyaluronic acid)[80,81,83,112,113,165–167] have been explored for reducing postsurgical donor-site discomfort. Biologic agents are usually stabilized with sutures, cyanoacrylate tissue glue, or acrylic stents to the palatal donor site. Though the use of a palate-covering retainer alone does not seem to reduce patient postoperative pain,[164,165] it has been shown that hemostatic collagen or gelatin sponge can significantly decreased patient morbidity compared with spontaneous secondary intention healing, especially if combined with cyanoacrylate tissue glue.[58,85,144,155] Similarly, compared with spontaneous healing, platelet concentrates (such as platelet-rich plasma, platelet-rich fibrin, and autogenous fibrin glue) were found to be associated with lower postoperative pain scores.[85,113,167] Laser photobiomodulation seems also to be effective in reducing patient morbidity following free gingival graft harvesting compared with placebo laser therapy.[81,83,125]

Figure 17 plots weighted mean visual analogue scale scores for postoperative pain following various harvesting approaches and is based on individual patient-level data from three studies.[58,144,155] The median pain scores following trap-door and collagen sponge–applied free gingival graft harvesting were 2 and 1.63, respectively. The median pain scores were 1.4 and 0.2 for cyanoacrylate alone and cyanoacrylate combined with collagen sponge, respectively. Cyanoacrylate has strong adhesive, bacteriostatic, and hemostatic properties[85,144,168] and allows for donor-site isolation during secondary intention healing, qualities that may explain its analgesic properties.

Patient- and surgical-related factors that can potentially affect postoperative discomfort were not accounted for in Figure 17. Smoking, palatal thickness less than 4 mm and graft thickness greater than 2 mm have been correlated with greater postoperative pain following free gingival graft harvesting.[169] Similarly, analgesic consumption was higher for increased apico-coronal graft height and reduced residual palatal thickness.[58] Though studies conflict regarding the exact effect of certain graft dimensions (width, thickness, and surface area) on patient morbidity, apico-coronal graft height has been shown to predict postoperative pain in three randomized controlled trials.[58,144,155] Age, gender, and patient anxiety do not significantly impact patient morbidity.[85,119,166,169] Postoperative pain can also be a result of accidental trauma to a donor site healing by secondary intention during the first few days (Figure 18).

On the other hand, a thin palatal flap and its dehiscence/necrosis during the healing period are the factors that have been correlated with increased morbidity following subepithelial connective-tissue graft harvesting.[58,74,170] It has been suggested to preserve, when possible, a residual palatal flap thickness of at least 1 mm for reducing patient discomfort and enhancing the healing.[170]

Recommendation for minimizing postoperative pain includes reducing, when possible, the size of the graft, applying protective materials on the donor site, using wound-healing enhancers, and prescribing painkiller medication (ibuprofen 600 mg). Lastly, it has to be mentioned that a recent study advocated that harvesting from the maxillary tuberosity was associated with significantly less morbidity compared with the lateral palate.[171]

7.2 | Prolonged postoperative bleeding

Postoperative donor-site bleeding is a common adverse event that arises from inadequate closure of the primary palatal flap, mismanagement of the de-epithelialized mucosa, nonachievement of complete hemostasis immediately following surgery, accidental postsurgical trauma, bleeding disorders, or anticoagulant use. Several studies have evaluated the incidence of self-reported postoperative bleeding.[58,79,85,142,157,172] Rossmann and Rees[142] reported that donor-site postoperative bleeding occurs during the 7 days after free gingival graft harvesting in 40% of patients who received oxidized regenerated cellulose or gauze at the time of surgery; no patients who received absorbable gelatin sponge experienced bleeding. Zucchelli et al[58] observed no statistically significant differences in postoperative bleeding between trapdoor and free gingival graft harvesting. However, in a prospective study

TABLE 3 Randomized control trials investigating patient-related outcomes of subepithelial connective-tissue graft harvesting techniques

Study	Comparison	Visual analogue scale[a] (mean plus/minus standard deviation)	Main findings
Trapdoor harvesting technique			
Del Pizzo et al 2002[79]	Trapdoor vs single incision vs free gingival graft	Not reported	Trapdoor and single incision had similar levels of postoperative discomfort; both were lower than that following free gingival graft Single incision had a faster recovery rate than trapdoor and free gingival graft
Fickl et al 2014[74]	Trapdoor vs single incision	2.67 ± 2.28 (trapdoor) 2.16 ± 1.18 (single incision)	Trapdoor showed lower healing scores, higher incidence of secondary wound healing, and greater painkiller intake than single incision
Zucchelli et al 2010[58]	Trapdoor vs free gingival graft	2.65 ± 2.18 (trapdoor) 3.1 ± 1.99 (free gingival graft)	No statistically significant differences for patient morbidity and postoperative bleeding. Trapdoor showed less inability to chew and stress than free gingival graft
Single-incision/envelope harvesting technique			
Del Pizzo et al 2002[79]	Trapdoor vs single incision vs free gingival graft	Not reported	Single incision showed faster recovery than trapdoor in terms of absence of pain, feeding habits, and normal sensibility (but no statistically significant differences)
Fickl et al 2014[74]	Trapdoor vs single incision	2.16 ± 1.18 (single incision) 2.67 ± 2.28 (trapdoor)	Single incision showed better healing scores, fewer cases with secondary wound healing, and less painkiller intake than trapdoor
Isler et al 2018b[121]	Single incision + flurbiprofen spray vs single incision + placebo (control group)	1.07 ± 2.04 (flurbiprofen spray) 1.23 ± 1.98 (control)	No statistically significant differences between the two groups in terms of postoperative pain, discomfort, changes in dietary habits, and healing scores
Lektemur Alpan and Torumtay Cin 2020[163]	Single incision + platelet-rich fibrin vs single incision (control group)	0.65 (platelet-rich fibrin) 2.89 (control)	Platelet-rich fibrin group exhibited less pain scores, lower analgesic intake, and better healing scores than the control group
Maino et al 2018[170]	Single incision with interlocking suture vs single incision with crisscross suture	0.75	No statistically significant differences between the two suture techniques in terms of wound healing scores
Stähli et al 2020[159]	Single incision	2.95 (group 1) 3.49 (group 2)	/
Stavropoulou et al 2019[224]	Single incision with sutures vs single incision with cyanoacrylate tissue glue	1.42 ± 1.18 (sutures) 1.27 ± 1.92 (cyanoacrylate)	No statistically significant differences in terms of pain, analgesic intake, and wound healing scores
Other techniques			
Dias et al 2015[128]	Harvesting technique described by Bruno[160] with laser photobiomodulation or sham (control)	4.8 ± 1.7 (laser photobiomodulation) 4.6 ± 1.5 (control)	No statistically significant differences between the two groups in terms of pain and painkiller intake
da Silva Neves et al 2016[127]	Harvesting technique described by Bruno[160] with laser photobiomodulation or sham (control group)	Not reported	Higher number of analgesics in the control group compared with the laser photobiomodulation groups, but no statistically significant differences
Harris 1997[174]	Parallel incisions technique vs free gingival graft knife method	Not reported	More pain and analgesic prescriptions for patients in the free gingival graft knife method

(Continues)

TABLE 3 (Continued)

Study	Comparison	Visual analogue scale[a] (mean plus/minus standard deviation)	Main findings
Pandit et al 2016[161]	Langer and Langer technique[51] vs free gingival graft knife method	2.5 ± 1.6 (Langer and Langer technique) 3.4 ± 2.3 (free gingival graft knife method)	No statistically significant differences in terms of pain, painkiller intake, and delayed bleeding at 1 week Faster re-epithelialization in the Langer and Langer technique group
Yen et al 2007[162]	Harvesting technique described by Bruno[160] with or without the addition of platelet concentrate	2.3 (platelet concentrate) 2.3 (control)	No statistically significant differences in wound healing, complications, and pain.

[a]Visual analogue scale from 1 to 10 during 7-10 days.

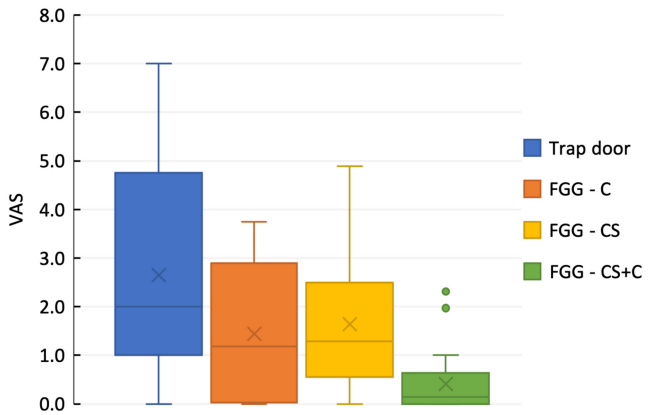


FIGURE 17 Postoperative patient morbidity as evaluated by a visual analogue scale (VAS), ranging 1-10, during the first week following trapdoor or free gingival graft harvesting. The free gingival graft donor site received a layer of cyanoacrylate tissue adhesive (FGG - C), a hemostatic collagen sponge (FGG - CS), or a combination of these two materials (FGG - CS+C). Individual patient data from three previously published clinical trials[58,144,155] were utilized for presentation in this figure. Note that statistical inferences are not made due to the variability in its assessment time among the trials mentioned

including more than 300 palatal donor sites, Griffin et al[157] described a threefold higher incidence of postoperative bleeding in the free gingival graft group than in the subepithelial connective-tissue cohort; donor-site bleeding was not distinguished from recipient-site bleeding.

To prevent postoperative hemorrhage, complete hemostasis of the donor site must be ensured immediately following surgery; the patient cannot be dismissed without fulfilling this condition. The use of hemostatic agents, such as collagen/gelatin sponge, can be further combined with cyanoacrylate tissue glue and/or a customized acrylic stent. Patients must be provided detailed oral and written postoperative instructions that include adherence to a soft food diet and avoidance of excessive physical exertion, brushing, flossing, or other trauma adjacent to the surgical sites, vigorous mouth rinsing, smoking, negative pressure (ie, suction or expectoration), and anticoagulant therapy (if discontinuance is physician approved) during the 2-3 weeks following surgery. Self-management of bleeding is performed by applying wet gauze pressure to the palatal wound for at least 10-20 minutes.

Patients on anticoagulant therapy may need to take a drug holiday prior to oral grafting surgery; a physician consult is required to dictate this action. Pseudoaneurysm of the greater palatine vessel following subepithelial connective tissue grafting has been described in a patient who did not discontinue anticoagulant therapy preoperatively.[173] Bleeding persisted for 6 days and was eventually managed by vessel embolization using cyanoacrylate glue.[173]

7.3 | Flap sloughing or necrosis

Visually and physically disturbing to patients, flap sloughing or necrosis is correlated to higher patient pain and analgesic consumption.[58] Sloughing or necrosis of the primary flap following subepithelial connective-tissue graft harvesting occurs in 20-92% of cases, most often presents centrally, and is influenced by the harvesting approach (Figures 19 and 20).[58,74,79,147,172,174] Palatal flap dehiscence can occur with or without necrosis (Figure 21).[74,134] Bone exposure is more likely to occur at exostoses,[134] especially if a deep connective-tissue graft has been harvested and a thin primary palatal flap is present. Fickl et al[74] described complete flap necrosis and bone denudation after trapdoor harvesting in one subject. An additional intervention was necessary to remove sharp bone ledges.[74]

Current use of minimally invasive surgical approaches, such as microsurgery or flap designs that preserve blood supply and ease tissue manipulation, may partially explain the higher incidence of flap sloughing observed in early studies relative to more recent ones.[58,74,79,147,172,174] In 1974, Edel observed a sloughing or necrosis of the primary flap in the majority of the patients 1 week after trapdoor graft harvesting;[50] Harris,[174] in 1997, reported similar results after using trapdoor or parallel incision techniques. In 2002, Del Pizzo et al[79] described secondary wound healing in 11 out of 12 patients for both single-incision and trap door techniques.Articles published in or after 2010 report lower incidences of healing by secondary intention (20-40%), possibly due to use of minimally invasive methods and surgical magnification.[58,74,147,172] Maximizing

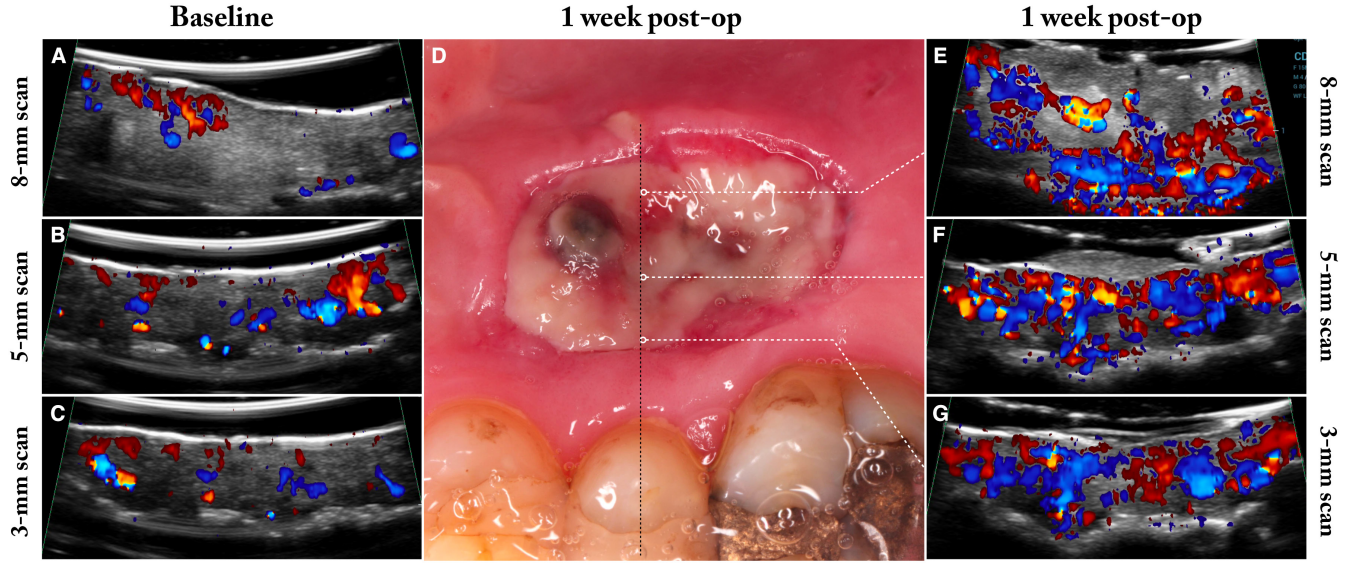


FIGURE 18 Ultrasound color velocity imaging comparison of vascularization. A, B, C, Presurgical baseline. E, F, G, At 1 week following palatal graft harvesting that had trauma during healing. The patient reported accidental donor-site injury while eating. Image D shows the clinical presentation of the palatal wound at the 1 week post-op. The ultrasonographic color flow mode at 1 week post-op revealed increased perfusion compared with baseline; the 5-mm and 8-mm scans showed a lack of superficial wound vascularization, which may be impeded by inflammatory infiltrate and cell debris. In this case, revascularization of the area started from the deepest layers

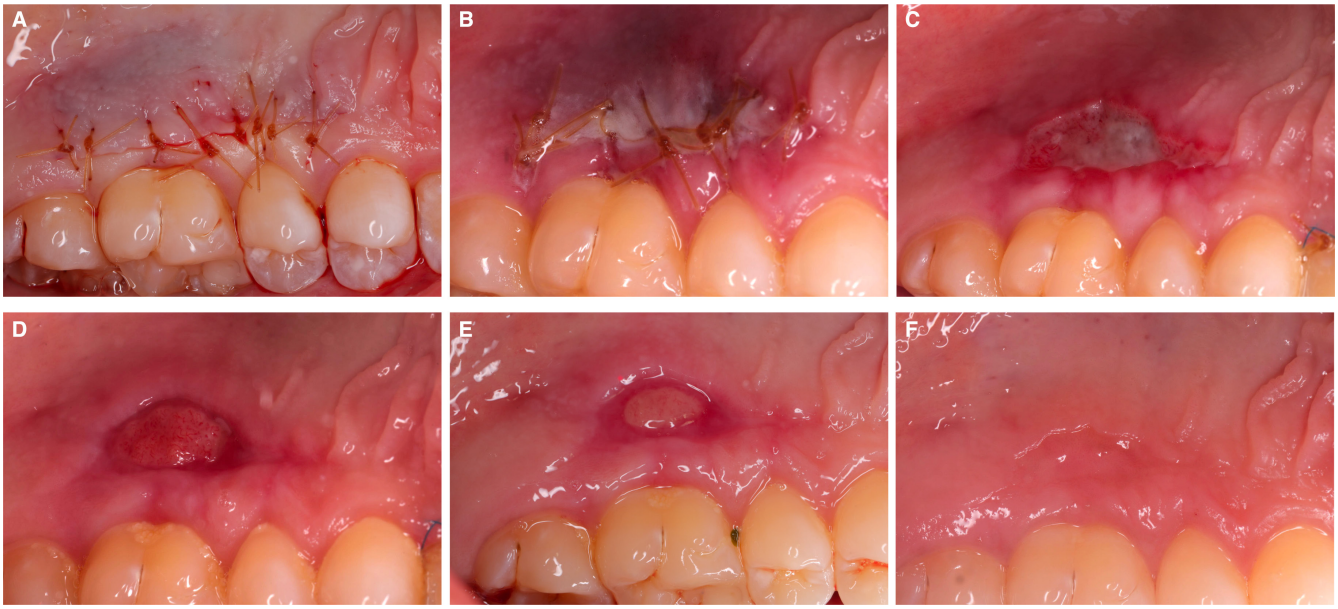


FIGURE 19 Single-incision harvesting technique performed in a thin palate, resulting in postsurgical flap sloughing. A, Primary closure of the donor site; B, At 2 days post-op showing initial sloughing of the wound; C, At 5 days post-op showing sloughing/necrosis of the primary flap, in particular in the molar region. The patient referred to increased discomfort in the last days and additional painkillers were prescribed. D, At 9 days post-op. E, At 2 weeks post-op. No discomfort was reported by the patient at this time point. F, At 6 weeks post-op

blood supply of the palatal donor site may also play a role in the incidence of flap sloughing or necrosis, with a 2014 evaluation reporting flap sloughing in 66.7% of trapdoor cases compared with 20.8% of single-incision cases.[74]

A thin, lamina propria–poor primary flap may heighten the risk of flap dehiscence or necrosis; thin flaps lack intact vascular structures, diminishing perfusion, and may be unduly perforated during surgical handling (see Section 6.3) (Figure 22).[58,170] A randomized controlled trial determined that thin palatal flaps, not the suturing technique employed, negatively affect healing outcomes.[170]

Recommendation for preventing flap sloughing or necrosis includes performing a single-incision harvesting approach ensuring a minimal palatal thickness of 1 mm and the use of the free gingival graft harvesting technique in the presence of thin palatal mucosa

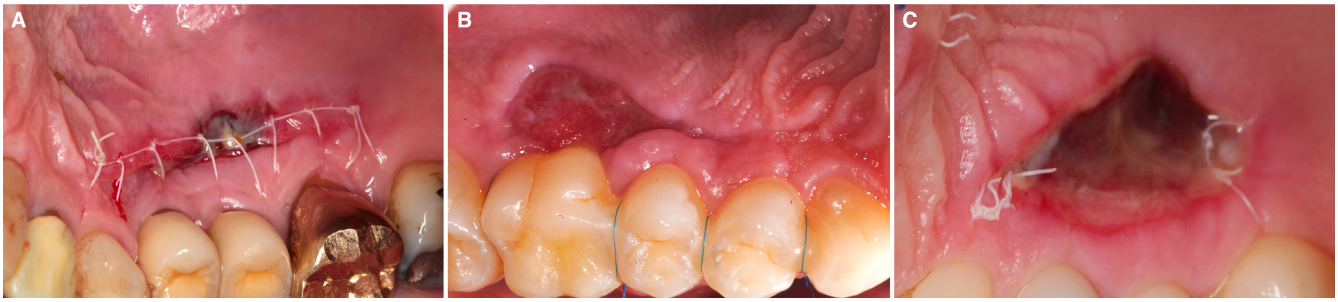


FIGURE 20 Primary flap sloughing/necrosis 2 weeks following single-incision harvesting technique

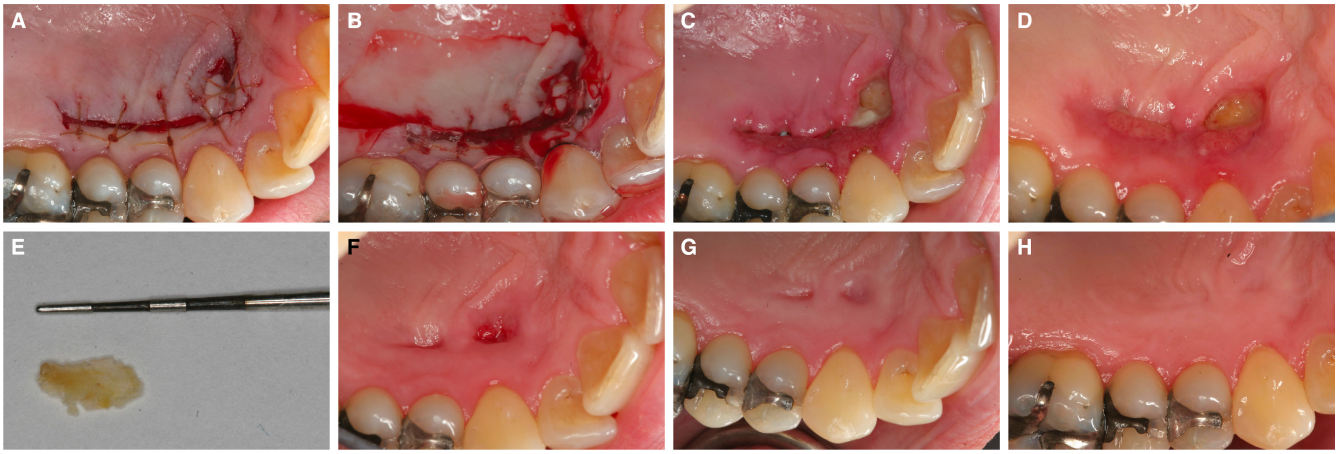


FIGURE 21 Bone exposure following subepithelial connective-tissue graft harvesting using one vertical incision (L-technique). A, At suturing. B, Patient was dismissed wearing an acrylic palatal stent. C, Bone exposure in the canine area was present at 2 weeks. D, At 1 month post-op with persistent bone exposure. E, F, Removal of necrotic bone at 2 months post-op. G, At 3 months post-op showing healing of the palate within normal limits following the removal of necrotic bone. H, At 6 months post-op

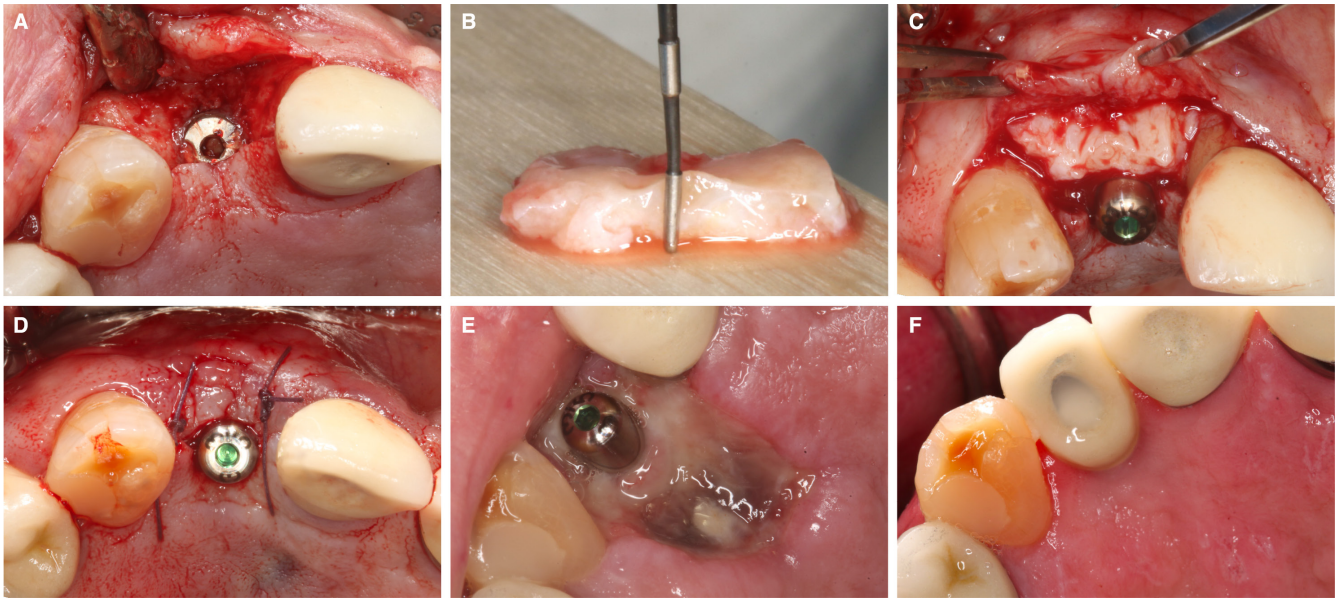


FIGURE 22 Palatal necrosis from the harvesting of a thick connective-tissue graft adjacent to an implant that resulted in minimal primary flap thickness. A, Implant with buccal deficiency requiring soft-tissue augmentation. B, Connective-tissue graft was harvested from the inner aspect of the palatal flap. C, D, Graft stabilization and flap suturing. E, At 1 week post-op showing palatal flap necrosis. F, At 3 months post-op Courtesy of a colleague

(less than 3 mm). Additional painkiller medication and chlorhexidine rinsing are suggested in the presence of this complication.

7.4 | Infection

Postoperative infection is an atypical complication following periodontal surgery and occurs in less than 2% of cases using assorted soft-tissue grafting procedures per one investigation.[175] Harris et al[176] reported a 0.8% incidence of donor-site infection following subepithelial connective-tissue grafting (one case out of 500); in the affected patient, the infection was at the palatal wound sutures. A retrospective study noted a 7.5% incidence of donor-site infection after subepithelial connective-tissue graft harvesting (three cases out of 40).[172] The treatment of postoperative infections includes oral or topical antibiotics, chlorhexidine rinsing, and wound irrigation with saline.

Ulcers from herpetic reactivation (herpes simplex virus type 1) can develop on the palate following tissue harvesting and may relate to surgical stress or the administration of local anesthetic.[134] Such lesions are self-limiting, lasting 7-14 days on average, but may be initially painful. There is no clinical standard regarding prevention of recurrent herpetic lesions, though the practitioner may consider prescribing a prophylactic antiviral medication (ie, acyclovir or valacyclovir) to patients who have persistent herpetic recurrence.[134]

7.5 | Sensory dysfunction

Patients may experience temporary sensory dysfunction following palatal harvesting, as free nerve endings might be severed intraoperatively. According to Del Pizzo et al, sensory disorders were present in all patients 2 weeks after free gingival graft harvesting; all patients regained normal sensation after 8 weeks.[79] There are no differences between harvesting methods (free gingival, trapdoor connective tissue, or single-incision connective tissue) with respect to sensory dysfunction incidence.[41] Harris et al[176] observed temporary donor-site paresthesia in 0.2% of the patients who had subepithelial connective-tissue graft harvesting. Buff et al[177] reported that 14.3% of patients (two cases out of 14) experienced palatal anesthesia following subepithelial connective-tissue grafting. In one patient, paresthesia persisted 20 months postoperatively, and another patient reported an altered (rough) palatal surface morphology. Figure 23 shows altered tissue perfusion of the donor site of a patient reporting temporary paresthesia following free gingival graft harvesting.

7.6 | Epithelial cyst formation

Though rare, epithelial cyst development after subepithelial or de-epithelialized connective-tissue grafting has been documented, occurring several months after the surgical procedure and resulting in an esthetically concerning bump at the grafted site (Figure 24);[178–182] fluid may emanate from a punctured cyst, distressing the patient.[179,182,183] Gordon et al[184] speculated that invagination of epithelial tissue between the graft and recipient bed—via surgical introduction or auto-marsupialization—following free gingival grafting could result in cyst formation. Residual epithelium left on a connective-tissue graft, especially one derived from free gingival graft de-epithelialization, may seed a cyst.[179,182,183,185] Despite high levels of epithelial remnants in connective-tissue grafts—up to 80-100% in biopsied samples[153,186]—this complication does not typically manifest.[153,187] An animal study that purposefully introduced

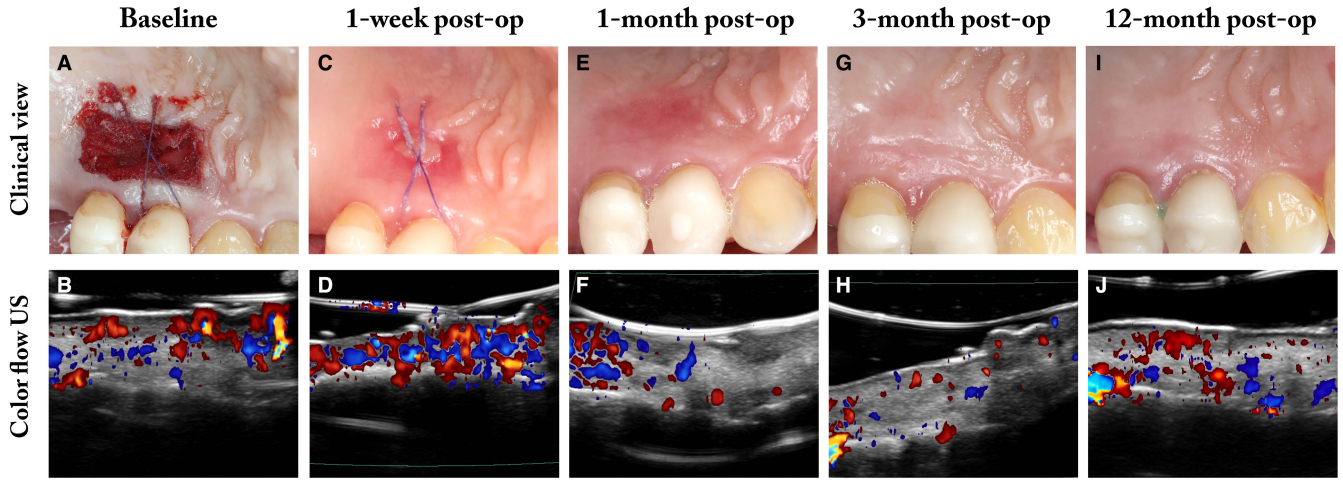


FIGURE 23 Temporary palatal sensory dysfunction following palatal harvesting. A, B, Baseline clinical view and ultrasound (US) scan. C, D, Clinical view and ultrasound scan 1 week after the surgery. The patient did not report any concern at this follow-up visit. E, F, Clinical view and ultrasound scan 1 month after the surgery. The patient complained regarding a sensory dysfunction in the anterior area of the palate, in the proximity of the canine and palatine rugae. The ultrasound scan showed an abnormal vascularization of the area with no blood flow visible in the anterior palate. G, H, Clinical view and ultrasound scan 3 months after the surgery. The patient reported that the sensory dysfunction of the anterior palate was completely resolved. The ultrasound scan showed a more uniform blood flow in the palate. I, J, Clinical view and ultrasound scan 12 months after the harvesting. It is possible to appreciate a uniform tissue perfusion of the donor site

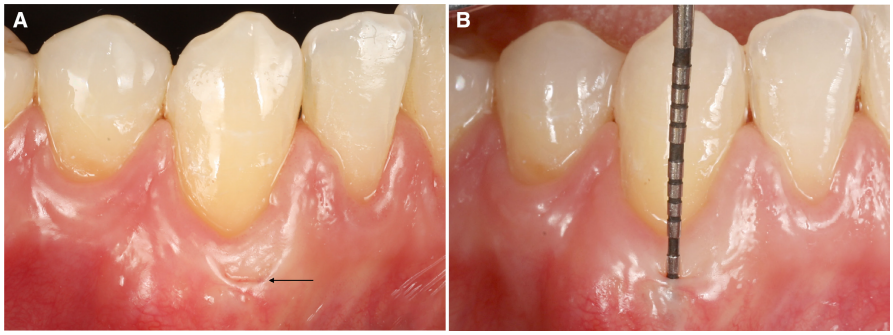


FIGURE 24 Epithelial cyst following a root coverage procedure using tunnel technique in combination with a de-epithelialized connective tissue graft. A, A black arrow is pointing the invagination of the mucosa due to the epithelial cyst on the midfacial aspect of the right mandibular canine. B, The periodontal probe shows the pseudo-pocket caused by the epithelial cyst, with exudate that was also observed upon "probing"

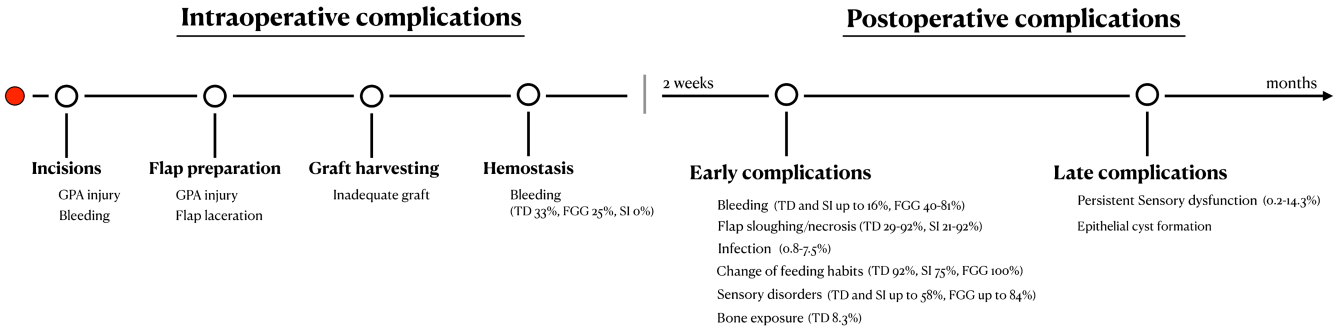


FIGURE 25 Characteristics, chronology, and incidence of intraoperative and postoperative complications of palatal harvesting. FGG: free gingival graft; GPA: greater palatine artery; TD, trapdoor; SI, single incision

FIGURE 26 Decision-making for donor site selection based on risk for complications according to the authors' experiences. GPA: greater palatine artery; SCTG: subepithelial connective tissue graft; FGG: free gingival graft; MT: epithelialized gingival graft from the maxillary tuberosity; −: low risk for complications; +: moderate risk for complications; ++: high risk for complications

	Harvesting technique		
	SCTG	FGG	MT
Risk for intraoperative complications			
Risk for GPA injury	++	+	-
Risk for excessive bleeding	++	+	-
Risk for inadequate graft size	+	-	++
Risk for inadequate graft quality	++	-	-
Risk for postoperative complications			
Risk for prolonged bleeding	-	++	+
Risk for high patient discomfort	+	+	-
Risk for significant change in feeding habits	+	++	-

epithelium underneath a full-thickness flap observed no negative histologic consequences, such as cyst formation, ankylosis, or epithelial attachment to roots.[188] Few reports described the occurrence of epithelial cysts, with gingivoplasty seemingly effective in removing the bulkiness of the soft tissue and in preventing recurrence of the complication.[179,182,183]

Figure 25 summarizes the intraoperative and postsurgical complications after palatal harvesting, and Figure 26 elucidates decision-making for choosing a donor-site location and technique for soft-tissue harvesting based on risk for complications, according to the authors' experiences.

8 | CONCLUSIONS

Autogenous grafts are routinely performed for periodontal and peri-implant soft-tissue reconstruction. Understanding the dynamics of palatal soft-tissue wound healing reduces patient discomfort and other complications. Although complications of palatal harvesting are usually not severe, they may negatively impact patient's quality of life and willingness to undergo Future surgical procedure. Various factors, including graft height, residual flap thickness, and intraoperative trauma, affect postoperative discomfort; the harvesting protocol, per se, is not one of them. Significant evidence exists to support the use of hemostatic agents and wound-healing enhancers for managing patient morbidity. Cyanoacrylate tissue adhesive—alone or with a hemostatic collagen sponge—platelet concentrates, hyaluronic acid, photobiomodulation, and ozone therapy can diminish patient morbidity following graft harvesting. The findings presented in this chapter support the use of the free gingival graft harvesting technique from the palate or tuberosity (rather than the conventional subepithelial connective-tissue graft approach) in several clinical scenarios, with appropriate donor-site management.

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CONFLICT OF INTEREST

The authors declare they have no relevant or material financial interests that relate to the research described in this chapter.

DATA AVAILABILITY STATEMENT

Data sharing not applicable—no new data generated.

ETHICS APPROVAL

Not applicable.

PATIENT CONSENT

Not applicable.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

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