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Histological and histomorphometric study of human palatal mucosa: Implications for connective tissue graft harvesting

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Abstract

Aims: To analyse the histological structure and histomorphometric characteristics of human hard palatal mucosa in order to determine the donor site of choice for connective tissue grafts from a histological point of view.

Materials and Methods: Palatal mucosa samples from six cadaver heads were harvested at four sites: incisal, premolar, molar and tuberosity. Histological and immunohistochemical techniques were performed, as was histomorphometric analysis.

Results: In the current study, we found that the density and size of cells were higher in the superficial papillary layer, whereas the thickness of the collagen bundles increased in the reticular layer. Excluding the epithelium, the mean percentage of lamina propria (LP) and submucosa (SM) was 37% and 63%, respectively ($p < .001$). LP thickness showed similar values in the incisal, premolar and molar regions, and a significantly greater thickness in tuberosity ($p < .001$). The thickness of SM increased from incisal to premolar and molar, disappearing in the tuberosity ($p < .001$).

Conclusions: As dense connective tissue of LP is the tissue of choice for connective tissue grafts, the best donor site from a histological point of view is tuberosity because it is composed only of a thick LP without the presence of a loose submucosal layer.

KEYWORDS

connective tissue grafts, histology, histomorphometry, human palatal mucosa

Clinical Relevance

Scientific rationale for study: Autogenous soft-tissue graft (connective or epithelium-connective tissue graft) is the standard of care in periodontal plastic surgery when treatment of gingival recession, increase of keratinized mucosa or soft-tissue volume augmentation is demanded. However, there is still some controversy about the histological composition of the palatal mucosa in different zones and about the measurement of each graft compartment.

Principal findings: Two layers are distinguishable in the lamina propria (LP): papillary and reticular. Superficial connective tissue of the papillary layer has greater cell density, whereas collagen

bundles are thicker in the reticular layer. The thickness of the LP is similar throughout the palate except in the tuberosity where it is greater.
Practical implications: Histomorphometrically, tuberosity is the site of choice.

1 | INTRODUCTION

The most common mucogingival defects include the lack of keratinized tissue and gingival recession (Cortellini & Bissada, 2018). The absence of keratinized tissue surrounding the teeth is considered a predisposing factor for the development of gingival recessions and/or inflammation (Kim & Neiva, 2015). Gingival recession is defined as the oral exposure of the root surface caused by displacement of the gingival margin apical to the cemento-enamel junction (Chambrone & Chambrone, 2006) and is highly prevalent worldwide (Merijohn, 2016). Gingival recession occurs frequently in adults and tends to increase with age (Kassab & Cohen, 2003). Several aspects derived from gingival recession make it clinically relevant because root surface exposure is frequently associated with impaired aesthetics, dentinal hypersensitivity and carious and non-carious cervical lesions (Cortellini & Bissada, 2018).

It is therefore very important and clinically relevant to cover the exposed root surface and to recover lost keratinized tissue. To solve this problem, mucogingival surgery came into being in as early as the 1950s, and later Miller (1988) coined the term 'periodontal plastic surgery' to refer to all those surgical procedures performed to prevent or correct anatomical, development, traumatic or plaque disease-induced defects of the gingiva, alveolar mucosa or bone. Among other applications, periodontal plastic surgery techniques demonstrated efficacy in the treatment of gingival recessions (Roccuzzo et al., 2002).

The transition from traditional mucogingival surgery to periodontal plastic surgery also meant the transition from free gingival grafts to subepithelial connective tissue grafts (Zuhr et al., 2014). Free gingival grafts are soft-tissue grafts that come from the palate and are composed of epithelium and connective tissue, while subepithelial connective tissue grafts consist only of connective tissue (Zucchelli et al., 2020). The main indication for free gingival grafts is to increase the width of the gingiva and keratinized tissue in the presence of mucogingival defects (Zucchelli & Mounssif, 2015). Connective tissue grafts emerged as a treatment that could provide significant improvement, especially in terms of better aesthetic results, which is desirable in certain situations such as gingival recessions, alveolar ridge defects or papillae reconstruction (Chambrone et al., 2010; Zuhr et al., 2014).

To carry out soft-tissue replacement grafts, it is essential to know the histology of the donor site, as well as to understand the processes of tissue integration and revascularization. The palatal masticatory mucosa consists of three layers: a keratinized squamous epithelium, the lamina propria (LP) and the submucosa (SM) that joins the periosteum. At present, the anterior and/or posterior palate (including the tuberosity area) are considered as the donor sites of choice (autologous graft). The geometric shape and histological composition of grafts are dependent on the donor site, the choice of which is influenced by the indication they are meant for (Harris, 2003; Zuhr et al., 2014).

Some authors have recently stated that tissue composition and origin may affect the integration and response of tissue grafts (Dellavia et al., 2014). However, there is still controversy regarding the histological composition of connective tissue grafts (Harris, 2003). Moreover, some authors have reported that LP thickness and proportions of fibrous connective tissue and fatty/glandular tissue do not differ significantly among the donor sites (Bertl et al., 2015), whereas other authors have found that the thickness is relatively uniform for the epithelium and the LP and highly variable for the SM (Cho et al., 2013). On the other hand, leaving remains of epithelium in the graft can produce cysts and oedema (De Castro et al., 2007; Gil Escalante & Tatakis, 2015; Parashis & Tatakis, 2007), and so it is important to know the thickness of the different layers of palatal mucosa, in order to obtain a maximum of LP and a minimum amount of residual epithelial tissue (Cho et al., 2013). According to some authors the most appropriate donor site for carrying out grafting procedures is the distal canine to mid-palatal aspect of first molar area (Ramesh et al., 2014), but for others the tuberosity could be an alternative donor site (Sanz-Martín et al., 2019).

The aim of this study was to analyse the histological composition of hard palatal mucosa and the histomorphometric characteristics of its different areas to determine the donor site of choice for connective tissue replacement grafts.

2 | MATERIALS AND METHODS

2.1 | Specimens

Six human cadaver heads (all male) donated to the Faculty of Medicine, University of Santiago de Compostela, for educational and research purpose were used. The mean age was 78.0 years (range 54–95, SD = 19.6). All the cadavers were dentate in at least one side. With respect to periodontal status, all of them presented an attachment level at 4–6 mm (measured with a periodontal probe from the cement-enamel junction to the crestal bone) with 2–3 mm recession. The corpses had been perfused via a carotid artery with 10% neutral buffered formalin and preserved in the same fixative. Causes of death were unknown but no oral pathologies were evident upon physical exploration. The study was conducted in accordance with Spanish law and the 1964 Declaration of Helsinki and its later amendments with approval by the Santiago-Lugo Research Ethics Committee (code 2022/349).

Palatal samples, including the entire thickness of the palatal mucosa, that is, epithelium, LP and complete SM with periosteum (like muco-periosteal techniques of subepithelial connective tissue graft, but including epithelium), were obtained using a 15c blade and

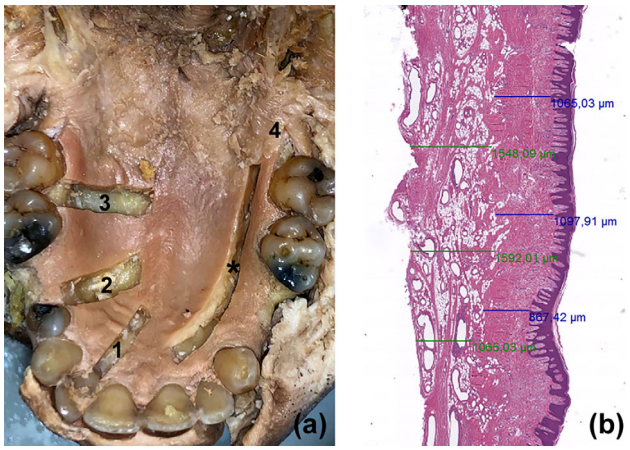


FIGURE 1 (a) Sampling of cadaver hard palate. The samples were collected in the following regions: incisal (1), premolar (2), molar (3) and tuberosity (4). An anteroposterior strip was also collected (*). (b) Examples of image analysis of lamina propria and submucosa (H&E).

periosteotome from the maxilla (perpendicular to the alveolar crest, from the gingival margin to the median raphe). Harvesting areas were standardized. From each palate, rectangular samples (ca. 1.5 cm length × 0.5 cm width × full depth to bone) were harvested at three different sites: (1) incisal (from the lateral incisor), (2) premolar (between the two premolars, from line-angle to line-angle in the interproximal space) and (3) molar (between the first and second molar, from line-angle to line-angle in the interproximal space). We obtained a distal wedge from the tuberosity after second molar (from line-angle to line-angle). The last specimen was a contralateral anteroposterior strip at 0.5 cm from the mesial aspect of the lateral incisor to the distal aspect of the corresponding second molar (Figure 1a).

2.2 | Histological techniques

After extraction, samples were dehydrated in a graded ethanol series, cleared in xylene and embedded in paraffin following routine procedures. Sections of 4 µm thickness were made, mounted on silanized coated slides (Dako-Agilent, Santa Clara, CA) and, after drying, dewaxing and rehydration, were stained with the routine haematoxylin and eosin (H&E) technique and with Masson's trichrome, elastic and reticulin stain kits (Artisan, Dako-Agilent).

2.3 | Immunohistochemistry

Sections of 4 µm thickness were mounted on silanized coated slides (Dako-Agilent). Epitope retrieval was performed in a PT-Link (Dako-Agilent) at high pH for 20 min and then automatically immunostained in an Autostainer-Link 48 (Dako-Agilent), employing the antibodies and protocols listed in Table 1. As detection system, we used EnVision FLEX/HRP (Dako-Agilent) for 30 min and 3,3′-diaminobenzidine tetrahydrochloride, as chromogen, for 10 min. Sections were examined and photographed using an Olympus BX51 microscope equipped with a DP50 digital camera (Olympus, Tokyo, Japan). In two cases where the orientation was not adequate, the paraffin blocks were redone to achieve appropriate orientation.

2.4 | Histomorphometric analysis

For image analysis, H&E sections were scanned with a PathScan combi digital pathology scanner (Excilone, Elancourt, France). All sections were scanned at ×20 magnification at a resolution of 0.274 mm/pixel. From each section (incisal, premolar, molar and tuberosity), the thickness of the epithelium, LP and SM were measured. Although each sample showed a more or less homogeneous histological structure, three measurements per sample were made. Each sample was divided into three (lateral, intermediate and medial) and measurements were performed at the centre of each (Figure 1b).

2.5 | Statistical analyses

Results were expressed as mean ± standard deviation. Comparison of thickness of the different palatal regions was done by analysis of variance. Tukey's multiple comparison test was used to determine which set of means and ratios differed from the rest. Differences were considered statistically significant at $p < .05$.

3 | RESULTS

3.1 | Histological structure and immunohistochemistry

Human hard palate mucosa consists of three layers: (1) surface epithelium, (2) LP (which together constitute the masticatory mucosa) and (3) SM, a loose supporting structure mainly composed of adipose tissue (Figure 2a). In the anterior region of the palate, the mucosa forms a series of ridges (palatal rugae), whereas in the rest of the hard palate the surface is smooth.

Palatal mucosa is covered by a thick, orthokeratinized squamous epithelium (Figure 2b), with occasional areas of parakeratinized epithelium with retained pyknotic nuclei in the keratin squames (Figure 2c). As the palate supports the mechanical forces of chewing, its union is serrated to reinforce the adhesion between the epithelium and the LP, with the epithelium presenting long, rete pegs engaging with complementary evaginations of the LP (papillae) (Figure 2d). Furthermore, the existence of papillae brings the capillaries closer to the most superficial cells of the epithelium, ensuring that oxygen and nutrients reach them by diffusion.

The epithelium is supported by the LP, which is composed of connective tissue with a high proportion of intercellular material (primarily collagen fibres) responsible for its mechanical properties. The LP consists of two layers: a superficial papillary layer (PL), and a deep

TABLE 1 Antibodies and incubation protocols.

Antibody	Source	Clone	Manufacturer	Concentration	Incubation time (min)	Mouse linker
Alpha-Smooth Muscle Actin	Mouse[a]	1A4	Dako-Agilent	Ready to use	20	No
CD31	Mouse[a]	JC70	Dako-Agilent	Ready to use	20	15 min
Factor XIIIa	Mouse[a]	AC-1A1	Gennova Scientific[c]	1:50	20	15 min
Mast Cell Tryptase	Mouse[a]	AA1	Dako-Agilent	Ready to use	30	No
Podoplanin	Mouse[a]	D2-40	Dako-Agilent	Ready to use	20	No
S100 protein	Rabbit[b]	-	Dako-Agilent	Ready to use	30	No
Vimentin	Mouse[a]	V9	Dako-Agilent	Ready to use	30	No

[a]Monoclonal.
[b]Polyclonal.
[c]Gennova Scientific, Sevilla (Spain).

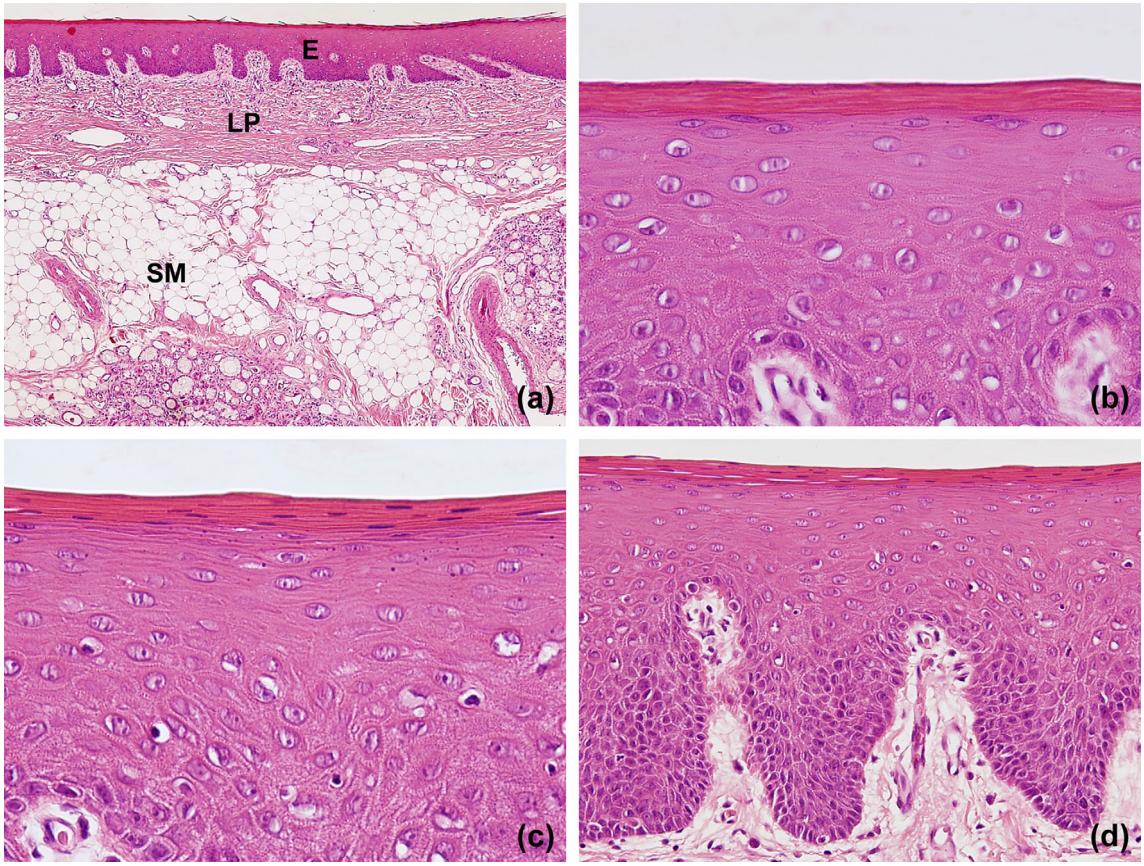


FIGURE 2 Lining of human hard palate. (a) The lining of hard palate consists of three layers: epithelium (E), lamina propria (LP) and submucosa (SM) (H&E, ×4). (b) For the most part, the palate is covered by a stratified squamous keratinized (orthokeratinized) epithelium (H&E, ×40). (c) In some areas, however, nuclei appeared in the stratum corneum (parakeratinization) (H&E, ×40). (d) The junction between the epithelium and the underlying LP is indented, with deep epithelial ridges that articulate with projections of the LP (papillae), containing blood capillaries that vascularize the epithelium by a diffusion mechanism (H&E, ×20).

reticular layer (RL) (Figure 3a). The boundary between them is imprecise because it is not defined by any specific structure. The PL has a pale eosinophilic appearance (Figure 3a) and is made of very fine and short bundles of collagen type I fibres arranged perpendicular to the surface in the papillae, whereas in deep areas collagen bundles are somewhat thicker and oriented parallel to the surface (Figure 3b). Intermingling with collagen bundles in the papillae is a network of fine elastic fibres perpendicular to the surface (Figure 3c) and reticulin fibres (collagen type III) ending at the basement membrane (Figure 3d). In the core of the papillae, there is a rich cell population primarily composed of fibroblasts and dendrocytes. Fibroblasts appear as great stellate cells that express vimentin (Figure 3e), and dendrocytes are smaller and less abundant antigen-presenting cells that express factor XIIIa (Figure 3f). Mast cells are also occasionally found

around blood vessels (Figure 3g). In deep areas of the LP, fibroblast and dendrocytes are less numerous, elongated and parallel to the surface (Figure 3h,i). Blood vessels are small and perpendicular to the surface in the papillae (Figure 3j,k) and larger and parallel to the surface in deeper areas, forming the superficial or papillary vascular plexus that defines the boundary between papillary and RLs

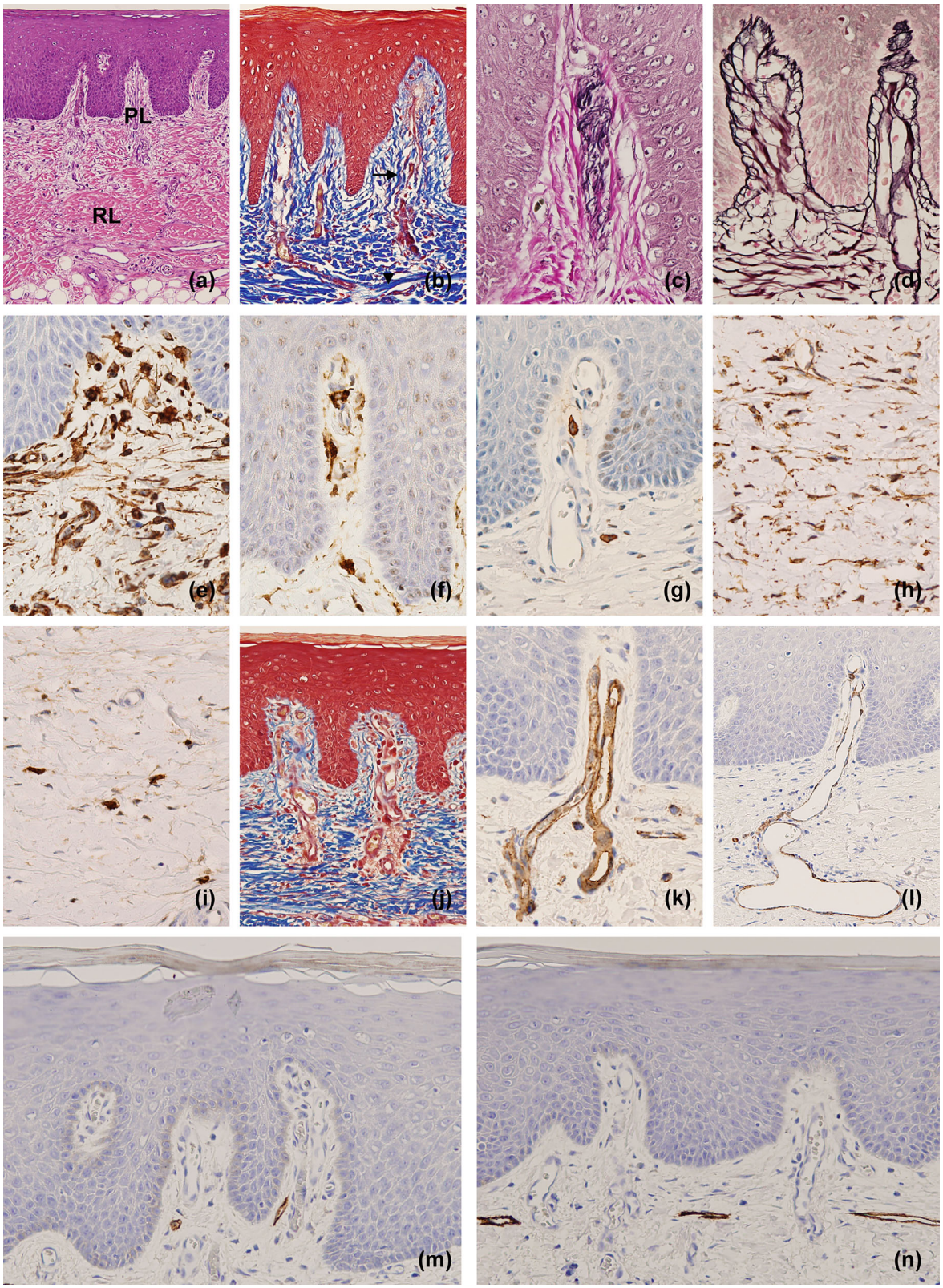


FIGURE 3 Legend on next page.

(Figure 3l). Lymphatic capillaries are also perpendicular to the surface in the core of the papillae (Figure 3m) but parallel in deeper areas (Figure 3n).

The RL is thicker than the papillary one and is made up of closely packed coarse bundles of collagen arranged mostly parallel to the surface, although some may run obliquely (Figure 4a). In this area, the cell population is mainly composed of numerous spindle-shaped fibroblasts arranged parallel to the surface (Figure 4b). A deep reticular vascular plexus, constituted by blood vessels larger than those of the superficial one, separates the RL from the SM (Figure 4c).

In most palate regions, LP rest on a thick SM layer, mainly composed of adipose tissue (Figure 4d) with presence of mucous-type minor salivary glands (palatine glands) which are more conspicuous towards the posterior areas (Figure 4e,f). In this area, blood vessels are prominent (Figure 4g), and large nerve bundles are found (Figure 4h,i). No submucosal layer is present in the maxillary tuberosity in any of the samples studied, as the LP is connected directly to the periosteum of the maxillary bone (Figure 4j).

3.2 | Histomorphometric analysis

All our samples were found to be suitable for image analysis, and 24 sections (four palatal regions of six cadavers) were analysed. The results of the histomorphometric analysis are presented in Tables 2 and 3.

The mean thickness of the palatal mucosa was 4.42 ± 1.72 mm. With respect to each individual layer, the mean thickness of epithelium was 0.24 ± 0.08 mm (6.45% of the total thickness), of the LP was 1.34 ± 1.54 mm (34.31%) and of the SM was 2.84 ± 2.31 mm (59.25%).

The total thickness of the palatal mucosa in the different regions increased from incisal to molar, with an intermediate value in the tuberosity. Comparing the means by region, significant differences were found between region 1 (incisal) and region 3 (molar) ($p = .003$), and between region 3 (molar) and region 4 (tuberosity) ($p = .032$) (Table 2).

The comparative study showed no significant difference for epithelium thickness in the four regions analysed (Table 2). In the LP, significant differences were observed ($p < .001$), with similar values in the first three regions (0.62 ± 0.27, 0.64 ± 0.42 and 0.45 ± 0.18 mm, respectively), and a significantly greater thickness in the tuberosity (3.64 ± 1.46 mm) ($p < .001$). The thickness of the SM was also different between the regions studied ($p < .001$). As for the total palate mucosa, the SM thickness increased from incisal to molar (2.19 ± 1.02; 3.65 ± 1.15, 5.51 ± 1.62 mm, respectively), and with a value 0 in the tuberosity. Consequently, significant differences were found between tuberosity and the other three regions, and also between incisal and molar regions ($p < .001$), as well as between premolar and molar regions ($p = .041$) (Table 2). The ratios between LP and SM thickness was 0.28 in incisal, 0.18 in premolar and 0.08 in molar region. Incisal ratio was significantly higher than premolar ($p = .014$) and molar ratios ($p < .001$), and no differences were found between premolar and molar ($p = .158$).

Excluding the epithelium, the mean thickness of LP and SM was 37% and 63%, respectively ($p < .001$) (Table 3). The percentage of the thickness occupied by the LP decreased from incisal (24.52%) to premolar (15.42%) and molar (8.05%). The maximum was found in the tuberosity (100%), and differences were significant ($p < .001$). As LP represents 100% of the subepithelial layers at tuberosity, significant differences in the percentage of LP were found between tuberosity and the three other sites, but also between incisal and molar regions (Table 3).

4 | DISCUSSION

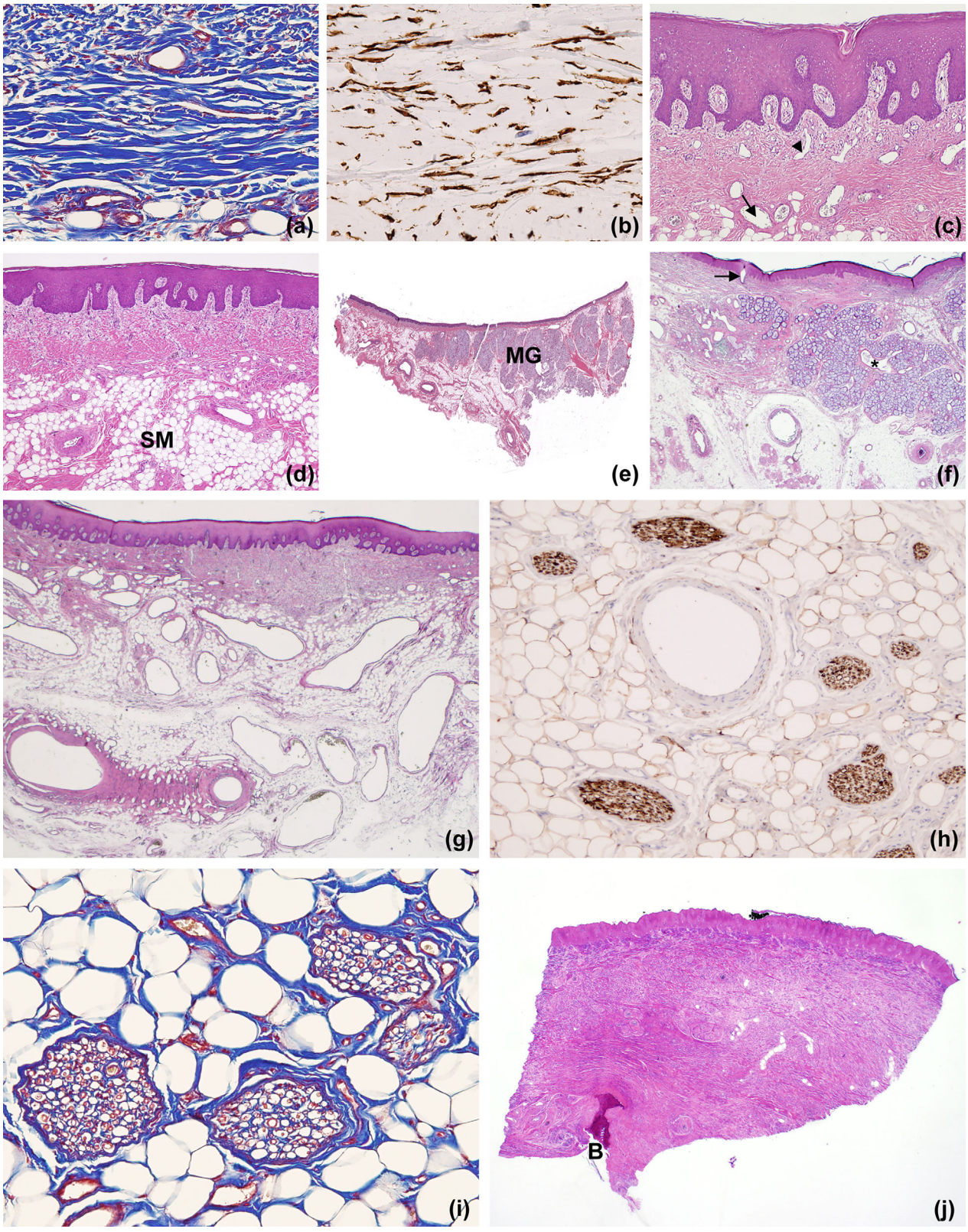
An adequate thickness and width of keratinized tissue is very important for natural teeth as well as dental implants (Chambrone & Tatakis, 2016). The use of clinically predictable periodontal plastic surgery procedures will determine the treatment success in cases of gingival recession (Chambrone et al., 2010). Nowadays, connective tissue graft is the technique of choice in treating localized gingival recessions because it fulfils complete root coverage (Sanz-Martín et al., 2019) and is associated with better biological and aesthetic results (Chambrone & Tatakis, 2015; Zuhr et al., 2014). However, there is a great variability in the histological composition between grafts. In some cases, the entire graft is composed of LP, while in others most of the graft is composed of SM (Harris, 2003). Because of the clinical relevance of periodontal plastic surgery with tissue grafts, it is important to delve into the histological structure of the palatal mucosa.

Palatal mucosa consists of two constant and homogeneous layers (ortho-keratinized squamous epithelium, and LP composed of

FIGURE 3 Papillary layer (PL) of the lamina propria (LP). (a) LP consists in a superficial loose PL and a deep denser reticular layer (RL) (H&E, ×10). (b) The PL is composed by short and thin collagen bundles, oriented perpendicularly to the surface in the papillae (arrow) and parallel in depth (arrowhead) (Masson's trichrome, ×20). (c) Elastic fibres have an undulated course and occupy the core of the papillae (Elastic stain, ×40), deeper and are located primarily around blood vessels (not shown). (d) In the papillae, there is also a dense network of reticulin fibres ascending to the basement membrane (Reticulin stain, ×40). (e–g) The papillae present high cellularity composed of large and irregular fibroblasts with long processes (e, Vimentin, ×40), less numerous dendrocytes (f, FXIIIa, ×40) and scattered mastocytes located around blood vessels (g, Mast cell tryptase, ×40). (h, i) In the deep areas of the PL, fibroblasts and dendrocytes adopt a spindle-shaped morphology and are arranged parallel to the surface (h, Vimentin, ×40; i, FXIIIa, ×40). (j, k) The core of the papillae is occupied by capillary loops arranged perpendicularly to the surface (j, Masson's trichrome, ×20; k, CD31, ×40). (l) Deep in the LP, large blood vessels horizontally arranged constitute the superficial or papillary vascular plexus (Alpha-smooth muscle actin, ×20). (m, n). Fine lymphatic vessels meet very occasionally and are disposed perpendicularly in the papillae (m, Podoplanin, ×20) and parallel to the surface in the subpapillary areas (n, Podoplanin, ×20).

dense connective tissue) and one inconstant and heterogeneous layer (the SM, composed of a variable proportion of adipose tissue and glands).

Histomorphometric analysis has shown that the mean thickness of human palatal mucosa is 4.4 mm with an increase in thickness from incisal to molar, and an intermediate value in the tuberosity. We found



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a higher palatal mucosa thickness than reported by Yu et al. (2013) and Bertl et al. (2015). These differences could be explained by the different sample processing methods used (10% neutral buffered formalin in our case vs. decalcification or fixation in Schaffer ethanolic solution in theirs) and because palatal masticatory mucosa is thicker in older people and in males (Ramesh et al., 2014) (our samples met these two characteristics).

The mean thickness of the epithelium was 0.24 mm (6.45% of the palatal mucosa). This figure is slightly lower than the 0.3 mm reported by Zuhr et al. (2014), and values between 0.30 mm (first molar area) and 0.41 mm (canine area) have also been reported (Cho et al., 2013). With respect to percentage, other authors have reported that the epithelial layer represented only 1.04% of the palatal mucosa (Marques de Mattos et al., 2019). This discrepancy with our result (6.45%) could be explained by the fact the abrasion technique used by the aforementioned authors presented epithelial remnants in only 20% of their samples. Although it has been reported that the mean epithelial thickness decreases significantly towards the posterior teeth (Cho et al., 2013), we did not find significant differences between the four regions studied (0.29, 0.22, 0.21 and 0.24 mm, respectively).

The mean thickness of the LP was 1.34 mm (34.31% of the palatal mucosa). Similar values, between 1.05 mm (first molar area) and 1.36 mm (canine area), have been reported (Cho et al., 2013). Regarding the percentage, similar values have also recently been obtained using the abrasion technique: 32.50% of the total area (Marques de Mattos et al., 2019). Although it has been reported that the LP was significantly thicker in the canine than in premolar and molar areas (Cho et al., 2013), we did not find significant differences between the first three regions studied (0.62, 0.64 and 0.45 mm, respectively). Nevertheless, we found a large increase in the tuberosity (3.64 mm).

Picro-sirius red staining made it possible to demonstrate that the content (total area and percentage) of collagen 1 and 3 in the LP was similar in lateral palate and tuberosity. Immunohistochemistry for matrix metalloproteinases (1 and 2), cytokeratins (10 and 13) and lysine hydroxylase 2 showed no differences between these two areas, except for metalloproteinase 2 in epithelial cells (significantly higher values in the tuberosity) (Sanz-Martín et al., 2019). Molecular studies have shown that collagen in the tuberosity area is more susceptible to crosslinking (hardening) due to the presence of a higher lysine hydroxylase/type 1 collagen ratio and to less probability of being degraded by matrix metalloproteinases. These differences in collagen maturation could act as the main determinants in the hyperplastic response of the tuberosity graft (Dellavia et al., 2014).

We found a mean SM thickness of 2.84 mm (59.25% of the palatal mucosa). Our result is slightly lower than the 66.40% obtained by Marques de Mattos et al. (2019), but it must be remembered that the area of the epithelium reported by these authors was very low (due to the abrasion technique employed), which implies an increase in the percentage of the other areas. SM represents the widest layer in the first three regions studied. In light of this, the overall palatal mucosa thickness increases towards posterior regions in parallel with SM thickness (2.19, 3.65 and 5.51 mm, respectively). No SM was found in our tuberosity samples, although other authors have reported an SM area of 4.89% in the tuberosity (Sanz-Martín et al., 2019).

Excluding the epithelium, the thickness percentage of LP and SM in our samples was 37% (slightly more than 1/3) and 63% (slightly less than 2/3), respectively. The percentage of LP (tissue of choice for volume augmentation in autologous grafts) gradually decreased towards the posterior regions of the palate (24.52%, 15.42% and 8.05%, respectively), and notably reached 100% at the tuberosity. Compared with other regions of the palate, the potential advantages of tuberosity grafts derive from the absence of fatty and glandular tissue, making the graft denser and firmer and, thus, less susceptible to postoperative shrinkage (Zuhr et al., 2014).

Based on samples taken from tissue grafts, Harris (2003) found that LP represented 65.2% of the graft and SM 34.8%. Sanz-Martín et al. (2019) (excluding epithelium) found 51.08% and 25.75%, respectively, in the lateral palate, and 72.79% and 4.89% in the tuberosity. Composition also varied according to the grafting technique employed. Thus, the amount of LP was higher in the group harvested by the mucosal technique than in the group harvested by mucoperiosteal technique (79.86% vs. 58.2%), and adipose tissue was greater by the mucoperiosteal technique than by the mucosal technique (32.64% vs. 11.93%) (Azar et al., 2019).

The subepithelial tissue from the tuberosity is very dense and contains less adipose and glandular tissue (Zuhr et al., 2014), presenting a greater percentage of LP and minimal SM (Sanz-Martín et al., 2019). In grafts from the lateral palate, the areas closest to the gingival margin contain principally LP, whereas more apical areas present increased adipose and glandular tissue (Bertl et al., 2015; Sanz-Martín et al., 2019; Yu et al., 2013). Taking this into account, if a larger

FIGURE 4 Reticular layer (RL) of the lamina propria (LP) and submucosa (SM). (a) RL is composed by thick and long collagen bundles oriented parallel to the surface (Masson's trichrome, ×20). (b) Between them there are numerous elongated fibroblasts that have the same orientation (Vimentin, ×40). (c) The reticular or deep vascular plexus is located on the border with the SM (arrow) and is constituted by vessels with a larger diameter than those of the papillary superficial plexus (arrowhead) (H&E, ×6). (d) The deep submucosal (SM) layer is composed primarily by adipose tissue (H&E, ×4). (e) In some areas, mucous glands (MG) occupy most of the SM and increase progressively towards the posterior areas (below), as seen in the anteroposterior strip (H&E, ×1). (f) At great magnification, mucous glands are constituted by poorly stained tubules that drain into large excretory ducts (*) opening into oral cavity through the surface epithelium (arrow) (H&E, ×1.25). (g–i) In the SM there are prominent blood vessels (g, H&E, ×1.25), and large and numerous nerve bundles (h, S100 protein, ×10; i, Masson's trichrome, ×20). (j) Representative image of tuberosity. Note that in this area there is no SM layer, with the dense connective tissue of the LP continuing to the deepest planes, where a small bone spicule can be seen (B) (H&E, ×1.25).

TABLE 2 Thickness of palatal lining and tissue layers in the different regions analysed (in millimetres).

	Palatal lining, mean ± SD (min–max)	Epithelium, mean ± SD (min–max)	Lamina propria, mean ± SD (min–max)	Submucosa, mean ± SD (min–max)
1. Incisal region	3.10 ± 0.90 (1.95–4.95)	0.29 ± 0.07 (0.17–0.41)	0.62 ± 0.27 (0.24–1.16)	2.19 ± 1.02 (1.19–4.16)
2. Premolar region	4.51 ± 1.18 (2.20–6.31)	0.22 ± 0.06 (0.13–0.33)	0.64 ± 0.42 (0.30–1.99)	3.65 ± 1.15 (1.53–5.69)
3. Molar region	6.17 ± 1.61 (3.64–10.24)	0.21 ± 0.05 (0.14–0.31)	0.45 ± 0.18 (0.19–0.78)	5.51 ± 1.62 (3.21–9.80)
4. Tuberosity	3.88 ± 1.46 (2.24–6.65)	0.24 ± 0.10 (0.09–0.47)	3.64 ± 1.46 (2.11–6.45)	0
	$p = .005$*	$p = .259$	$p < .001$*	$p < .001$*
	1 versus 3: $p = .003$*		1 versus 4: $p < .001$*	1 versus 3: $p < .001$*
	3 versus 4: $p = .032$*		2 versus 4: $p < .001$*	1 versus 4: $p = .014$*
			3 versus 4: $p < .001$*	2 versus 3: $p = .041$*
				2 versus 4: $p < .001$*
				3 versus 4: $p < .001$*

*statistically significant ($p < 0.05$).

TABLE 3 Percent of lamina propria (LP) and submucosa (SM) (excluding epithelium).

	LP (%) mean ± SD	SM (%) mean ± SD	*p*-value
Palatal lining	37.00 ± 37.90	63.00 ± 37.90	<.001*
1. Incisal region	24.52 ± 12.55	75.48 ± 12.55	
2. Premolar region	15.42 ± 8.70	84.58 ± 8.70	
3. Molar region	8.05 ± 3.90	91.95 ± 3.90	
4. Tuberosity	100	0	
	$p < .001$*		
	1 versus 3: $p = .009$*		
	1 versus 4: $p < .001$*		
	2 versus 4: $p < .001$*		
	3 versus 4: $p = .008$*		

*statistically significant ($p < 0.05$).

and deeper graft is taken, the amount of LP remains constant whereas the amount of SM increases considerably (Harris, 2003).

Nevertheless, it is not clear to what extent graft composition affects the outcome of mucogingival surgery (Zucchelli et al., 2020). Some authors (Tavelli et al., 2022) have recently mentioned the two layers of LP. Our study describes these layers in detail: we found that the superficial PL primarily contained a rich cell population (consisting of great stellate fibroblasts) and short bundles of collagen arranged perpendicular to the surface, whereas in deep areas we found that fibroblasts were fusiform and less numerous and collagen bundles were thicker and oriented parallel to the surface. The RL was thicker than the PL and was composed of packed coarse bundles of collagen parallel to the surface. In this area, the cell population was mainly constituted by spindle-shaped fibroblasts. From a histological perspective, both layers are very different, and these differences could be clinically relevant. Thus, the orientation of the tissue graft—whether the papillary or the reticular side is placed towards the root of the tooth—may be of great importance. Of course, this possibility must be demonstrated in randomized controlled trials. Also, the nature of connective tissue graft could determine soft-tissue thickness and keratinized tissue width (Amin et al., 2018). In this sense, some authors suggest that the tuberosity could be an alternative donor site to the lateral palate, because its higher content in LP makes the graft denser and firmer, and therefore better for volume augmentation (Sanz-Martín et al., 2019), and less susceptible to post-operative shrinkage (Zuhr et al., 2014). Moreover, using tuberosity as donor site would also contribute to lower patient morbidity (Amin et al., 2018).

Choice of the harvesting technique could also affect graft quality. The composition of a connective tissue graft derived from a free gingival graft after de-epithelialization (mostly composed of LP) may differ from a conventional connective tissue graft (richer in adipose and glandular tissue) (Bertl et al., 2015; Zucchelli & Mounssif, 2015; Zuhr et al., 2014). The adipose and glandular tissue of the graft may act as barriers during the first phase of healing, preventing revascularization and plasmatic diffusion (Sculean et al., 2014), and avoiding even keratinization (Tavelli et al., 2019). Ouhayoun et al. (1988), after performing histological and biochemical analysis of human samples from the palate, confirmed that the deepest portion of the connective tissue did not produce keratinization, although its clinical relevance has not yet been confirmed (Azar et al., 2019).

The choice of different donor sites has its pros and cons. Subepithelial connective tissue grafts from the anterior palate are of looser consistency but can be more extensive, whereas grafts from the posterior palate are usually denser, but limited in size (Sanz et al., 2014). In line with our histological results, recent papers have concluded that although both soft-tissue graft donor areas (lateral palate and tuberosity) yielded similar clinical results, grafting the tuberosity provided greater volume gain and stability of keratinized tissue as well as improved the gingival phenotype of the recipient area (Ramos-Pilco et al., 2020; Rojo et al., 2018, 2020).

5 | STUDY LIMITATIONS

Our study included a small number of specimens because of the difficulties inherent to the type of samples studied (from human cadaver heads). As we analysed normal palatal histology, striking differences were not expected between individuals. In fact, even with a small sample, the statistical analysis showed significant differences in the means of thickness and relative proportions of the palate layers, which confirms the homogeneity of the samples obtained from the different cadavers and, therefore, the validity of our study. In a recent work by our group on the quantification of human penile innervation in cadaver samples, results were obtained using sample sizes of $n = 3$ (Cepeda-Emiliani et al., 2022), and other authors obtained statistically significant results on the same subject, with sizes of $n = 5$ (Diallo et al., 2015). The advanced age of the specimens used for this study constitutes another limitation, but this is inherent to the type of samples collected.

6 | CONCLUSION

As LP is widely considered to be the tissue of choice for volume augmentation in autologous grafts, from a histological point of view tuberosity seems to be the best donor site because it is the only

palatal region composed solely of LP and has much greater thickness than other areas.

CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest in this study.

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