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Rat Gingiva Histology: Comparison of two Histological Stains

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Research Article

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ABSTRACT

Objectives: Describe rat gingiva histology with a traditional hematoxylin-eosin stain and non-traditional Goldner trichrome stain technique.

Materials and Methods: Samples of gingiva of adult Wistar rats were processed for histological purposes. The classic hematoxylin and eosin stain and Goldner trichrome stain technique were applied to the tissue. Digital microphotography of the three parts of the gingiva were obtained.

Results: For the free gingiva its oral, sulcular and junctional epitheliums were described. For the attached gingiva, its oral epithelium and for the interdental gingiva, its particular epithelium was detailed as well. Each layer of the stratum of the five epitheliums were specified and the cellular morphology explained. The gingiva lamina propria was also detailed. Among others, structures such as the gingival groove, the collagen fibers corresponding with the transseptal fibers, the bone on the alveolar crest, the enamel space, the dentin and the pulp were also identified.

Conclusions: Goldner's trichrome stain allows simpler tissue identification, easier epithelium stratum establishment and detailed cellular morphology observation for the rat free, attached and interdental gingiva.

Keywords: Gingiva; Histological Techniques; Histology; Trichrome stain; Wistar rat

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Introduction

Gingiva is a part of the oral mucosa. It functions as an effective barrier for microorganisms' entry, protects the deeper tissues from mechanical forces and from abrasive nature of foodstuffs. Additionally, gingiva as a part of the digestive tract, is sensitive to touch, pressure, pain, and temperature.[1]

Embryologically, the gingival component of the periodontium is derived from the ectoderm of the pharyngeal arches.[2] Anatomically is the mucosa covering the alveolar bone ridge surrounding the cervical section of the tooth[3] and has being divided into three regions: free or marginal gingiva, attached gingiva, and interdental zone.[4] Histologically it is surrounded with keratinized stratified squamous epithelium on its masticatory surface and non-keratinized stratified squamous epithelium on its crevicular and junctional surface.[2,5]

Free gingiva microanatomy can be understood according to the gingival epithelium, which is divided into three different sections: oral, sulcular, and junctional epithelium. The last one being the closest to the tooth surface.[2] The attached gingiva presents keratinized stratified squamous epithelium and is the oral mucosa that intermingles directly with the mucogingival junction and the frenum, and indirectly to the inner surface of the cheeks, the hard palate and the mouths floor. On the other hand, the interdental gingiva exhibits a nonkeratinized stratified squamous epithelium[6,7] (Table 1).

The aim of this paper was to describe rat gingiva histology with a traditional hematoxylin-eosin stain and non-traditional Goldner trichrome stain technique.

Table 1. Gingiva histology

Region	Tissue	Stratum	Cell	Cell junction
Free gingiva	Oral epithelium or keratinized stratified squamous epithelium	Corneum	Keratinized cell without cell nuclei	Desmosomes
		Granulosum	Granular cell	Desmosomes and gap junctions
		Spinosum	Prickle cell and Langerhans cell	
		Basale	Basal cell, Merkel cells and melanocyte	Gap junctions and hemidesmosomes
	Sulcular epithelium or parakeratinized stratified squamous epithelium	Corneum	Keratinized cell with cell nuclei and with highly condensed chromatin	Desmosomes
		Granulosum	Granular cell and transmigrating leukocytes	Desmosomes and gap junctions
		Spinosum	Prickle cell and Langerhans cell	
		Basale	Basal cell, Merkel cells and melanocyte	Gap junctions and hemidesmosomes
	Junctional epithelium	Suprabasal	Suprabasal cell	Hemidesmosomes and adherens junction
		Basale	Basal cell and transmigrating neutrophils	
	Dense irregular connective tissue	None	Fibroblast, mast cell, tissue macrophage, blood cells, plasma cell and T cells	None
Attached gingiva	Oral epithelium or keratinized stratified squamous epithelium	Corneum	Keratinized cell	Desmosomes
		Granulosum	Granular cell	Desmosomes and gap junctions
		Spinosum	Prickle cell and Langerhans cell	
		Basale	Basal cell, Merkel cells and melanocyte	Gap junctions and hemidesmosomes
Interdental gingiva	Nonkeratinized stratified squamous epithelium	Distendum	Flattened cell	Desmosomes and adherens junction
		Filamentosum	Polyhedric cell	
		Basale	Basal cell, Merkel cells and melanocyte	Gap junctions and hemidesmosomes
	Dense irregular connective tissue	None	Fibroblast, mast cell, tissue macrophage, blood cells and plasma cells	None

References: Autor & [4,6,8–10]

Materials and Methods

For this observational research, three adult Wistar female rats (*Rattus norvegicus*) sourced from the Intermediate Laboratory of Preclinical Research at Universidad del Valle were utilized. The experiment received approval from the Biomedical Experimentation Animal Ethics Committee under the internal code 013-017. To achieve the purpose of the investigation, rats underwent perfusion with a saline solution and formaldehyde[11] prior to anesthesia. Hemicranium sagittal samples were obtained through surgical resection.[12] Samples decalcification was achieved using 10% ethylenediaminetetraacetic acid in which the dental enamel dissolves.[13] Histological slices were acquired and cut at 4µm with a microtome. Slices underwent the classic hematoxylin and eosin (H&E) stain, and Goldner trichrome stain technique based on the Lee G. Luna protocol.[14] Sagittal sections of the gingiva, around the molars, was observed using an optical microscope with camera (LaboMed LB-239; USA), and ScopeImage Advanced Software (USA) was used for digital microphotography acquisition.

Results

Microphotography showed free, attached and interdental gingiva. Regarding the free gingiva and related to its coronal portion, the gingival groove can be seen as an apical tissue located towards the incisal/occlusal surface of the tooth. It is the transition between the keratinized stratified squamous epithelium and the parakeratinized stratified squamous epithelium; last one being adjacent to the enamel space. The intermediate portion of the free gingiva displays a lamina propria among the two prior epitheliums. Capillaries and defense cells, such as plasmacyte and lymphocytes are also present among the lamina propria tissue. The cervical portion of the free gingiva exhibits the gingival sulcus towards the tooth, where the junctional epithelium becomes attached to the dentin on the neck of the tooth (Figure 1).

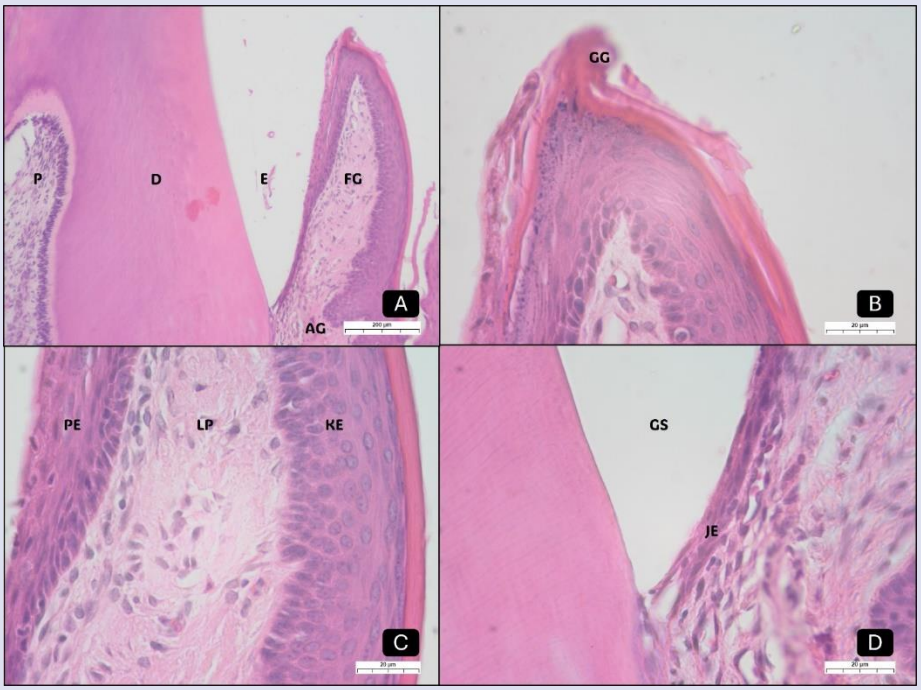

Figure 1. Gingiva with H&E stain. ***A.*** *Tooth and gingiva at (X10). P: Dental pulp; D: Dentin; E: Enamel space; FG: Free gingiva; AG: Attached gingiva.* ***B.*** *Coronal portion of the free gingiva at (X40). GG: Free gingiva groove.* ***C.*** *Intermediate portion of the free gingiva at (X40). PE: Parakeratinized epithelium; LP: Lamina propria; KE: Keratinized epithelium.* ***D.*** *Cervical portion of the free gingiva at (X40). GS: Gingival sulcus; JE: Junctional epithelium.*

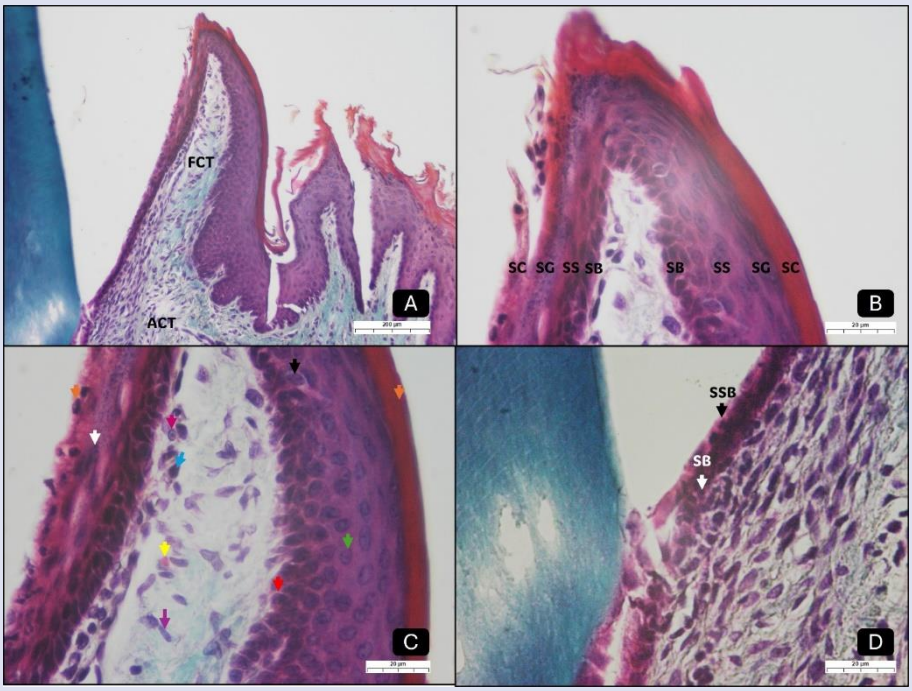

Figure 2. Gingiva with Goldner´s trichrome stain. ***A.*** *Gingiva at (X10). FCT: Free gingiva connective tissue; ACT: Attached gingiva connective tissue.* ***B.*** *Coronal portion of the free gingiva at (X40). SC: Stratum corneum; SG: Stratum granulosum; SS: Stratum spinosum; SB: Stratum basale.* ***C.*** *Intermediate portion of the free gingiva at (X40). Orange arrow: Keratinized cell; White arrow: Granular cell; Green arrow: Pickle cell; Black arrow: Langerhans cell; Red arrow: Basal cell; Pink arrow: Lymphocyte; Blue arrow: Plasma cell; Yellow arrow: Erythrocyte; Purple arrow: Fibroblast.* ***D.*** *Cervical portion of the free gingiva at (X40). SSB: Stratum suprabasal and suprabasal cell; SB: Stratum basale and basal cell.*

The oral epithelium exhibits a keratinized stratified squamous epithelium with four stratums. The most outer stratum, stratum corneum, presented keratinized cell without cell nuclei and filled with keratin filaments. The stratum granulosum showed granular cells, and the stratum spinosum had prickle and Langerhans cells. The most inner stratum, stratum basale, features melanocytes and basal cells. The sulcular epithelium presented the same stratums as the oral epithelium. Cellularly, transmigrating leukocytes were found on the stratum granulosum additionally to the resident granular cells. The keratin cell on the stratum corneum presented cell nuclei and highly condensed chromatin (Figure 2).

The cervical portion of the free gingiva presents the junctional epithelium. This is a less thick epithelium with only two stratums. The outer stratum, stratum suprabasal, has cells called after this layer. The same as for the stratum basale, which also contains transmigrating neutrophils (Figure 2).

The lamina propria of the free and attached gingiva differs in location. Being the first the most coronal tissue, and the second the most cervical one. They are composed of the same dense irregular connective tissue which displays collagen fibers, extracellular matrix proteins, small blood vessels and nerves, fibroblast, blood cells and immune system cells (Figure 1 and 2).

Concerning the interdental gingiva, it contains a nonkeratinized stratified squamous epithelium adjacent to booth teeth. The coronal surface of this epithelium corresponded to the free gingiva groove. This epithelium presented the stratum basale as the inner stratum with basal cells, followed by the stratum filamentosum with polyhedric cells and the most apical stratum, stratum distendum with flattened cells. Basal to this epithelium a dense irregular connective tissue appears accompanied by many capillaries and postcapillary venules. Undernet, transseptal fibers which are collagen fibers, pass horizontally from the root of one molar, above the alveolar crest; to be inserted into the root of the adjacent molar. The bone on the alveolar crest showed an extracellular matrix and cells consistent with this special connective tissue (Figure 3).

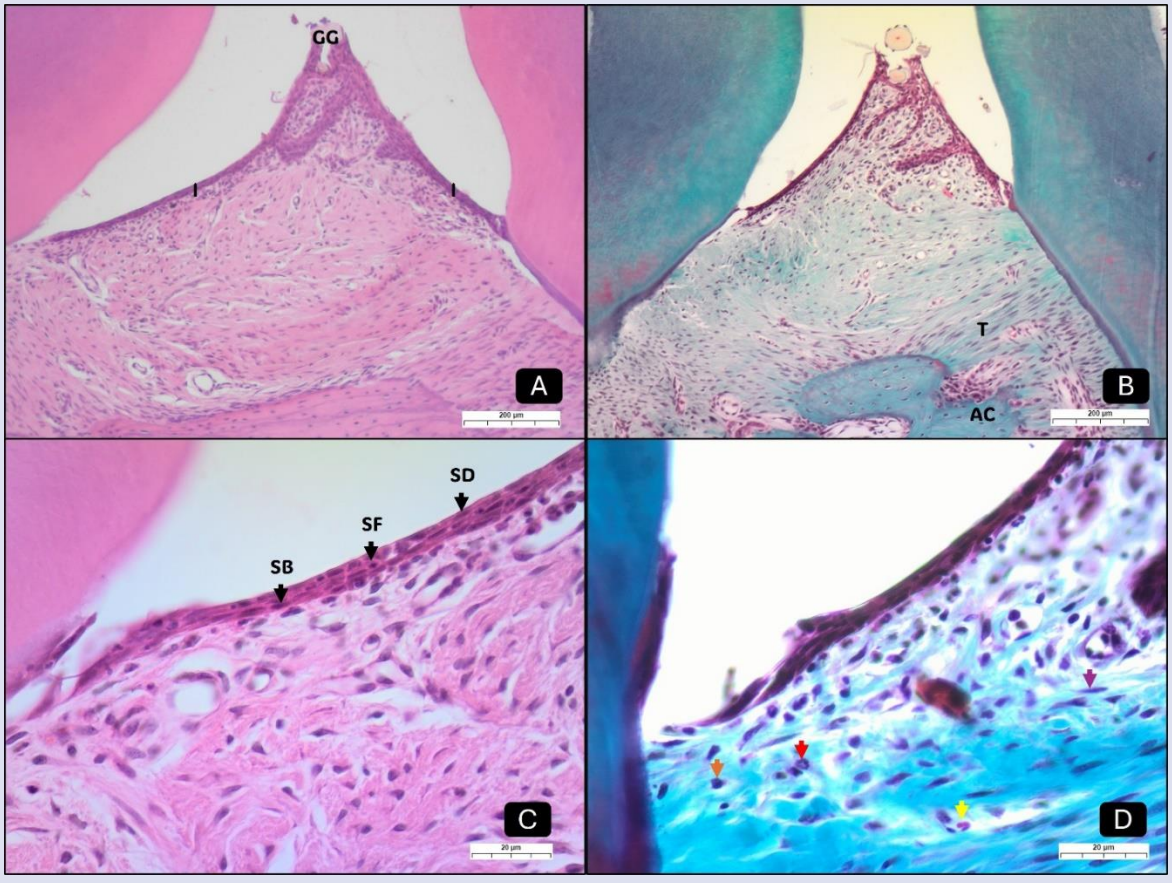

*Figure 3. Interdental zone. **A.** Interdental gingiva with H&E stain at (X10). GG: Free gingiva groove; I: Interdental epithelium. **B.** Interdental gingiva with Goldner´s trichrome stain at (X10). T: Transseptal group of gingival fibers; AC: Alveolar crest. **C.** Interdental gingiva with H&E stain at (X40). SD: Stratum distendum and flattened cell; SF: Stratum filamentosum and polyhedric cell; SB: Stratum basale and basal cell. **D.** Interdental gingiva with Goldner´s trichrome stain at (X40). Red arrow: Lymphocyte; Orange arrow: Plasma cell; Yellow arrow: Erythrocyte; Purple arrow: Fibroblast.*

Discussion

In anatomical and biomedical research, specifically morphologists are often in need of establishing the complex topographical relationships between tissues and structures in a body. Beyond that, under magnification, tissues and cells can be appreciated and thus describe their microanatomy in detail. Microscopy augmentation is essential to understand the organization of epitheliums, connective tissues, extracellular matrix fibers, all types of cells and arrangements such as bonding a certain tissue with another one.[15] Therefore, histological techniques offered unique opportunities in dentistry research and in this case, oral mucosa description.

Nevertheless, Widbiller et al., proposed that the histological methods for dental tissues pose challenges, consequently to the proximity of various soft and mineralized tissues. Therefore, standard histological procedures had to be modified accordingly.[16] Understanding the anatomy of the tooth, the gingiva and its intimate connection with the dentogingival junction was essential. And having in mind the purpose of this paper, the technical methods for this research were modified; which included hard tissues samples that underwent decalcification and paraffin embedded. Inevitable and due to decalcification, enamel was lost, however, the space of this tissue was preserved. Unlike what it reported by Panes et al., the trichrome stain on a decalcified sample, allowed the clear observation and description of the dentogingival junction.[17]

In relation to the histological stains, H&E is a broader used histological technique used on rat´s oral mucosa.[18] It consists of a nuclear stain obtained by an alum-mordanted hematoxylin and a cytoplasmic stain achieved with an alcoholic or aqueous solution of eosin Y.[19] This stain results are a range of purple for nucleus or other basophilic structures, and a spectrum of pink shades for acidophilic cellular or extracellular components. And as Golbert et al., stated this histochemical method is not always sufficient.[15] Therefore, special stains with higher substance selectivity became a valuable method in this specific research topic. In this regard and almost ninety years ago, Goldner presented a modification of Masson's trichrome stain. The author explains that the modification provides exceptionals results on microphotography, better quality of detail and fewer color artifacts.[20] This staining method is more accurate to observe nuclear features on a range from brown to black colors. Cell cytoplasm and apical specializations such as keratin, appear in a gradient of red to orange. While tissues like connective ones, with the presence of collagen fiber turn out in green.[15]

Widbiller et al., on its dental histology research, and as it also resulted in the present investigation, H&E dyed nuclear chromatin on blue and purple shades. Eosin as its counterstaining helped visualize red blood cells in red, cell cytoplasm in pink, and collagen fibers in connective tissue and hard tissues, like the tooth and bone; in different saturations of pink.[16] Furthermore, Panes et al., establish that the main gold of a trichrome stain is to allow visualization of different colors and shades. The authors also declared that any trichome stain is perfect for the dyed of collagen fibers in the extracellular matrix, the main component of connective tissues.[17] Which results relevant for the gingival lamina propria description, or for other morphologist and researchers to quantify oral cavity tissues of different characteristics.

Panes et al., also affirmed that Goldner's trichrome histochemical stain allows the observation of the dental, lamellar bone and trabecular tissue architecture, together with the cellularity of these tissues.[17] Likewise, the selected trichrome staining was used by Widbiller et al., with a very similar protocol to the one used for this research, authors stated that the specific stain was good to visualized the microanatomy, the collagen fibers and the cell perceptibility.[16] This paper results showed that additionally to be a great stain for the already named tissues, it´s a great dyed to showed the different stratums and cells of the keratinized, parakeratinized and nonkeratinized epithelium.

As explained on the results section, histologically the rat gingiva displays different epitheliums and the same tissue on its lamina propria. Hassan et al., on its investigation with albino rats, agreed with these findings. Investigators explained that the gingival mucosa of this type of biomodels is covered with keratinized stratified squamous epithelium. Which showed short columnar basal cell layer resting on a well-defined basement membrane, polygonal prickle cells and flattened granular cell layer covered by thin and regular eosinophilic keratin layer, with a H&E stain. The same research also showed that some clear cells were detected within epithelium, corresponding with Langerhans cell. Additionally, the researchers made clear that the gingiva lamina propria appeared dense and fibrous. Long and slender connective tissue papillae and spindle shaped fibroblasts were observed[21] similarly to this paper results with both histological techniques.

The results presented on this paper, contribute to the field of oral histology by showing how the gingiva in all its regions presents different types of epithelia, with their respective strata and associated cellularity. Furthermore, the comparison of both histological stains in the figures, enables the trichrome stain to showed its superiority for appreciating the morphological detail of the epithelial cells within the free and attached gingiva, as well as of the different connective tissues and their cells. Most importantly, it allowed a very detailed appreciation of the strata of the coronal portion of the free gingiva. Furthermore, in very thin tissues such as the epithelium of the interdental gingiva, the H&E technique allowed detailed observation showing the morphological detail of the cell nuclei and cytoplasm.

For future studies, the results shown with both stains can be extrapolated to research on gingiva in other species. Specifically, the use of trichrome staining could allow for detailed histological descriptions of human gingiva. It would be particularly useful for observing cellular differences in epithelia and connective tissues, since, unlike in rats, these tissues would be thicker.

The limitation of the study is that once sagittal sections of the hemicranium were surgically obtained, coronal sections of the gingiva could not be acquired and therefore the histology in this anatomical position could not be described.

Conclusions

Rat gingiva histology was described with the classic hematoxylin and eosin stain and the less used, Goldner trichrome stain technique. Both histological stains showed cellular and tissue components of the free, attached and interdental gingiva. These parts of the oral mucosa have different epitheliums with multiple stratums and rich cellular morphology, which can be deeply appreciated with the trichrome stain.

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Conflicts of Interest Statement

The author declares no conflict of interest.

References

1. Kumar GS. Oral mucous membrane. In: Kumar GS. Orban's Oral Histology & Embryology. Hayyana, India: Elsevier 2019:191-233.
2. Torabi S, Soni A. Histology, Periodontium. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing 2023
3. Lowe JS, Anderson PG. Alimentary Tract. In: Lowe JS, Anderson PG. Stevens & Lowe's Human Histology. Internet: Elsevier 2015:186-224.
4. Chiego DJ. Oral mucosa. In: Chiego DJ. Essentials of oral histology and embryology: a clinical approach. St. Louis: Elsevier 2023:172-188.
5. Sasaki A, Yamada T, Inoue K, Momoi T, Tokunaga H, Sakiyama K, et al. Localization of Heat Shock Protein 27 (Hsp27) in the Rat Gingiva and its Changes with Tooth Eruption. ACTA Histochem Cytochem 2011;44(1):17-24.
6. Groeger S, Meyle J. Oral Mucosal Epithelial Cells. Front Immunol 2019;10:208.
7. Berkovitz BKB, Holland GR, Moxham BJ. Oral mucosa. In: Berkovitz BKB, Holland GR, Moxham BJ. Oral anatomy, histology and embryology. Edinburgh: Elsevier 2018:261-292.
8. Hassell TM. Tissues and cells of the periodontium. Periodontol 2000 1993;3(1):9-38.
9. Puzhankara L, Rajagopal A, Kedlaya MN, Karmakar S, Nayak N, Shanmugasundaram S. Cell Junctions in Periodontal Health and Disease: An Insight. Eur J Dent 2024;18(02):448-457.
10. Gómez de Ferraris ME, Campos Muñoz A. Periodonto de protección. In: Gómez de Ferraris ME, Campos Muñoz A. Histología, Embriología e Ingeniería Tisular Bucodental. Madrid: Editorial Médica Panamericana 2019:247-266.
11. García R. Fundamentos del proceso de fijación tisular. In: García R (eds). Laboratorio de anatomía patológica. Madrid: Interamericana-McGraw-Hill 1993:654.
12. Gage GJ, Kipke DR, Shain W. Whole Animal Perfusion Fixation for Rodents. J Vis Exp 2012;(65):3564.
13. Sterchi DL. Bone. In: Suvarna SK, Layton C, Bancroft JD. Bancroft's Theory and Practice of Histological Techniques. Internet: Elsevier 2019:280-305.
14. Luna LG. Histopathologic Methods. In: Luna LG. Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts. Michigan University: American Histolabs 1992.
15. Golberg M, Kobos J, Clarke E, Bajaka A, Smędra A, Balawender K, et al. Application of histochemical stains in anatomical research: A brief overview of the methods. Transl Res Anat 2024;35:100294.
16. Widbiller M, Rothmaier C, Saliter D, Wölflick M, Rosendahl A, Buchalla W, et al. Histology of human teeth: Standard and specific staining methods revisited. Arch Oral Biol 2021;127:105136.
17. Panes C, Ponce N, Ottone NE, Valdivia-Gandur I, Beltrán V, Vásquez B. Modified Goldner Trichrome for Non-decalcified Mineralized Tissue Plastinated and Embedded in Resin. Int J Morphol 2024;42(2):516-524.
18. Dönmez YZ, Akpınar A, Göze ÖF. Effect of topical humic acid on excisional palatal wound healing: a histopathological and histomorphometric study in rats. Cumhuriyet Dental Journal 2021;24(4):326-336.
19. Titford M. Progress in the Development of Microscopical Techniques for Diagnostic Pathology. J Histotechnol 2009;32(1):9-19.
20. Goldner J. A modification of the Masson trichrome technique for routine laboratory purposes. Am J Pathol 1938;14(2):237.
21. Hassan R, Ahmed NF, Hussein SI. Histological, immunohistochemical and radiographic evaluation of Amitriptyline administration on the periodontium of albino rats (An experimental study). Saudi Dent J 2022;34(6):449-457.