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Visualization of the dentogingival junction using micro-plastination technique

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Abstract

Plastination has revolutionized the field of anatomy and research by providing biosecurity and enabling the long-term preservation of biological material, ranging from entire bodies to individual organs and even micron sections. The dentogingival junction (DGJ) consists of both epithelial and connective tissues that are closely related to the tooth's mineralized tissues. Cutting-grinding techniques are commonly used to visualize DGJ histology. These techniques exclude enamel from preparations and focus on visualizing hard or soft tissues. To improve the micro-anatomical and histological study of this region, we applied micro-plastination technique to obtain micro-thin slices below 150 µm thick from human and animal samples. The DGJ microanatomy was visualized by applying histological stains to the micro-plastinated slices, highlighting the technique's endogenous autofluorescence capacity identifying periodontal tissues, including dentin, enamel, cementoenamel junction, dentinal tubules, connective tissue, and collagen. Based on our results, we confirm that micro-plastination is a useful technique for visualizing anatomical regions that are difficult to access, such as the DGJ. Micro-plastination can be used as an alternative technique, providing a new approach for its application in anatomical and morphological research protocols.

KEYWORDS
dentin, dentogingival junction, enamel, histological stains, micro, plastination, plastinationautofluorescence

1 | INTRODUCTION

E12 sheet plastination invented by Professor Gunther von Hagens (Bickley & Townsend, 1984; von Hagens, 1986; Von Hagens et al., 1987) utilizes epoxy sheet plastination to produce transparent tissue slices. These layers allow for anatomical studies in sections, maintaining their true form without any distortions, alterations, or collapse (Ottone, 2018; Ottone, 2020; Ottone, 2023; Ottone, Baptista, et al., 2018; Ottone, Vargas, et al., 2018; Sora et al., 2007; Sora & Cook, 2007; Sora & Matusz, 2010; Vargas et al., 2020). This method stands out as the optimal choice for creating transparent, odor-free, and dry sections of the body (Aufdemorte et al., 1985; Bickley & Townsend, 1984; Ottone, 2018; Ottone, 2020; Ottone, 2023; Sora et al., 2007; Starchik, 2015; von Hagens, 1986; Von Hagens et al., 1987; Xu et al., 2017). One of the key benefits of epoxy sheet plastination is its minimal shrinkage, making it highly recommended for anatomical and morphological research (Latorre et al., 2019; Minelli et al., 2023; Ottone, 2023; Ottone et al., 2016; Ottone, Baptista, et al., 2018). This technique prevents the sample's decomposition by first preserving the tissue with epoxy and then resulting in significant transparency (Ottone, 2023; Ottone, Baptista, et al., 2018). This clarity arises from the epoxy resin's refractive index, which enhances microscopic examinations, forming an optimal optical connection with the tissue. Furthermore, the sheets produced can be conveniently analyzed using morphometric methods (Ottone, 2020, 2023; Sora et al., 2007).

In epoxy sheet plastination, it is possible to preserve thin slices, between 2 and 5 mm thick, and ultra-thin slices, less than 2 mm thick. Then, the sections can be scanned to perform a detailed analysis of the microstructure, allowing morphometric quantification as well as the possibility of implementing a three-dimensional reconstruction. However, to date there are very few studies that use plastination as a methodology for the identification of morphological changes caused by pathologies (Eckel et al., 1993; Ottone, Baptista, et al., 2018).

Related to the dentogingival junction (DGJ), this anatomical region is a modification of the oral mucosa, and is comprised of both epithelial and connective tissues. This junction acts as the primary protective barrier in our innate immune response against ongoing microbial threats from oral biofilms (Nanci & Bosshardt, 2006). Various terms have been employed to describe this anatomical area: epithelial adhesion, junctional epithelium (Bernick et al., 1951; Soskolne & Bimstein, 1977), and most recently, the term supracrestal gingival tissue (SGT) has been introduced, proposed as a replacement for the older term "biological width."

Within the realm of dental tissue research, it is widely recognized that there is a heightened need for advanced histological techniques and tailored procedures compared to bone studies. Traditional methods, such as routine techniques and the cutting-grinding technique (CGT), face challenges due to the tooth's hard, brittle, and diverse characteristics, along with their associated tissues. As a result, there is an ongoing push for the creation of improved methods that allow for swift, reliable, easily performed research into the intersection of hard and soft tissues. Moreover, these techniques aim to identify specific structures or molecules at both the cellular and subcellular levels (Willbold & Witte, 2010).

Until 1960, bone tissue studies required demineralization of samples for embedding in paraffin for microtome cutting (Bernick et al., 1951; Cano-Sánchez et al., 2005; Correa-Aravena et al., 2023). Thanks to the development of hard embedding media like acrylic and epoxy resins, and the use of advanced microtomes and cutting systems, it is now possible to examine hard dental tissues alongside surrounding soft tissues. This provides valuable insights that cannot be obtained from demineralized tissues (Correa-Aravena et al., 2023). The CGT enables preservation of the mineral phase of the tooth and bone, while maintaining a good structural relationship between the organic and inorganic components, without compromising on histological details (Correa-Aravena et al., 2023; Günhan et al., 1996). Performing bone histology without decalcification is a technical challenge, especially with large specimens (Correa-Aravena et al., 2023). Considerably longer fixation and processing times are required due to the density and lower permeability of bone, often taking several weeks (Correa-Aravena et al., 2023; Goldschlager et al., 2010).

This study aimed to develop the micro-plastination technique (Ottone, 2020) as a promising new option, surpassing the limitations of CGT in mandible samples. This technique allows us to apply micro-plastination to obtain micro-thin slices of the DGJ without decalcification, subsequently apply histological stains, and demonstrate the autofluorescence capacity of the technique in micro-plastinated micro-thin slices below 150 μm. The potential benefits include enhanced visualization and distinction of anatomical and histological structures of this region from micro-plastinated sections, paving the way for significant advancements in anatomy and morphology research.

2 | MATERIALS AND METHODS

We applied the micro-plastination method (Ottone, 2020) (Table 1) on the mandible samples with tooth from a specimen of *Canis familiaris* (two samples, one portion of mandible with incisor teeth, and a portion of mandible with premolar teeth) and a mandible samples with tooth from a human body (one sample, a portion of mandible with incisor teeth). The animal body was donated by its owners for research and teaching purposes, complying with international ethical standards related to the management of laboratory animals, including the three Rs of Russell and Burch (replacement, reduction,

TABLE 1 Steps of the micro-plastination technique.

Steps	Temperature (°C)	Times
Dehydration	−25	10 days
Defatting	20–22	4 days
Forced impregnation	30–65	4 days
Cutting the blocks (200–250 μm)	20–22	12 h
Grinding and polishing (100–150 μm)	20–22	2 h

TABLE 2 Forced impregnation pressures and temperatures.

Days	Pressure (mm Hg)	Temperature (°C)
1	760	22
2	760–20	30–65
3	760–20	65
4	760	65
5	Remove the blocks for cutting	

and refinement) (Russell & Burch, 1959). Likewise, the human sample was obtained from a human body donated to science in compliance with Article 146 of the Chilean Health Code. Furthermore, as the authors of this scientific work, we sincerely appreciate those who donated their bodies to science and their families. We have the highest respect for them. Their generous contributions advance anatomical research, enhancing our overall knowledge. These findings also help improve clinical, imaging, and surgical care for patients (Iwanaga et al., 2022).

All samples underwent the micro-plastination process (Ottone, 2020; Ottone, 2023), which is described below:

2.1 | Stage of the samples

The samples (mandible with teeth) were taken *en-bloc* from the larger sample. In this way, blocks of 1 cm width, 2 cm length, and 2 cm height were extracted from the mandible of the specimens, along with the respective tooth. They were fixed in 10% buffered formalin (1.27 mol/L formaldehyde in 0.1 pH 7.2 phosphate buffer) after a series of increasing fixations, starting at 2% and increasing every 3 days up to 7%.

2.2 | Dehydration

Dehydration of the samples was done in 100% acetone to avoid tissue shrinkage it was done at temperatures between −25 and −20°C. The time depended on the sample size, and the purity of the acetone was checked daily with an acetonometer. Acetone needed changed every 4 days. When acetone purity remained constant at 99%, for two consecutive days, dehydration was considered complete.

2.3 | Defatting

Once dehydration is complete, the samples were defatted to promote greater transparency in the tissues. In this protocol, the samples were immersed in acetone at room temperature, favoring the elimination of fat and benefitting the transparency of the connective tissue. In this sense, at room temperature, acetone is a powerful defatting agent. The samples were subjected to the defatting process for 4 days, with daily changes of 100% acetone.

2.4 | Forced impregnation and curing

The process of forced impregnation took place in a vacuum stove (AccuTemp09s, Across International) at temperatures between 30 and 65°C. The impregnation was performed at high temperatures because this increases the fluidity of the impregnation mixture, making it easier to penetrate the specimen. The impregnation mixture consisted of three components: epoxy resin (Biodur—E12) (100 p.b.w.); Hardener (Biodur—E6) (60 p.b.w.); catalyst (Biodur—E600); total percent (100:60:0.25). The impregnation mixture was poured into an appropriately sized container, like a square plastic container, resembling an ice tray. After placing the samples in the impregnation solution, the forced impregnation step was started (Table 2). Forced impregnation lasted 5 days (see Tables 1 and 2). After being placed in the impregnation mixture, the samples were left at room temperature (22°C) for 24 h at a pressure of 760 mm Hg (day 1). On the following day (day 2), the forced impregnation process began at 30°C. By the fifth hour of impregnation, the temperature was increased to 65°C, reaching a final pressure of 20 mm Hg. On the third day, the resin was replaced with a new mixture, and the pressure was again reduced to 20 mm Hg, maintaining the temperature at 65°C. If the bubbling stops at a maximum pressure of 20 mm Hg, the forced impregnation process can be considered complete, and the pressure inside the vacuum chamber is returned to 760 mm Hg while maintaining the temperature at 65°C. On the fourth day, the samples were kept at a pressure of 760 mm Hg and a temperature of 65°C throughout the day to promote the curing/polymerization of the sample. Finally, on the fifth day, the samples were hardened and could be removed from the vacuum chamber. This stage combines forced impregnation with curing/polymerization, resulting in a hardened epoxy resin block containing the sample within a shorter time frame.

2.5 | Cutting of the blocks

The blocks were cut with a low-speed saw with a diamond blade of 0.35 mm thickness (1000–1400 rpm; PICO155, Pace Technologies, USA), generating slices between 200 and 250 μm thick.

2.6 | Polishing and staining

Before staining, polishing procedure was applied to all 200–250 μm thick slices, until thin slices of approximately between 100 and 150 μm thickness were obtained. In this way, the surface of interest of the cut

TABLE 3 Histological stains applied to the micro-plastinated slices.

Goldner's trichrome	
Procedure	**Times**
1. Distilled water	3 min
2. Weigert's hematoxylin	20 min
3. Washing in running water	20 min
4. Xylidine ponceau	5 min
5. Acetic acid 1%	15 s
6. Orange G/phosphotungstic acid	20 min
7. Acetic acid	15 s
8. Light green 0.2%	5 min
9. Acetic acid 1%	3 min
Hematoxylin and eosin	
Procedure	**Times**
1. Stain in Harris' hematoxylin (filtered)	6–15 min
2. Washing in running water	2–5 min
3. Differentiation in 1% acid alcohol	15 s
4. Washing in running water	15 s
5. Ethanol 80%	1–2 min
6. Eosin	2 min
Toluidine blue 0.5%	
Procedure	**Times**
1. Stain in toluidine blue 0.5%	15–30 min in oven (maximum 60°C)

sample was polished with 600, 1000, and 2000 grit sandpaper under abundant irrigation with water and finished with a P5000 silicon carbide disc (TrizactTM, 3M) in an EcoMetTM 30 (Buehler) manual polisher, to be glued to a glass slide with a viscous mixture of Biodur epoxy resin (E12:E1–50:50). This resin/glue was cured in a 60°C oven for at least 4 h. Then these samples were thinned again with 240, 360, 600, 1000, and 2000 grit sandpaper under abundant irrigation with water, to obtain sections of 100–150 μm thick. Each sample underwent a final finish with a P5000 silicon carbide disc (TrizactTM, 3M) and glycerin in an EcoMetTM 30 (Buehler) manual polisher.

Once each section was polished, a surface treatment was applied sequentially with acetone, 30% hydrogen peroxide, and 10% ethylenediaminetetraacetic acid (EDTA). Subsequently, hematoxylin and eosin, toluidine blue 0.5%, and Goldner's trichrome (xylidine ponceau, orange-g, and light green) stains were applied (Donath & Breuner, 1982; Goldner, 1938; Horn & Garrett, 2004; Panes et al., 2024; Prophet et al., 1995) (Table 3). Once the samples were stained, they were submerged in a series of alcohols of decreasing degree (50%, 70%, 80%, 95%, and 100%), a neutral mounting medium was applied (Entellan® new, Merck), and glass coverslips.

2.7 | Visualization and scanning

The micro-plastinated slices were visualized and scanned using the TissueFAXS i PLUS Cytometer TissueGnostics Axio Observer 7 System Carl Zeiss GmbH (TissueGnostics GmbH, Vienna, Austria). Fluorescence images were obtained at 20× magnification with TissueFAXS 7.1.139 software. Acquisition objective: EC Plan-Neofluar 20×/0.50 M27 (20×, Air).

3 | RESULTS

Micro-plastinated sections between 100 and 150 μm of the DGJ were obtained from one human and two animal samples (*C. familiaris*). In the human sample (Figure 1), the first micro-plastinated sections were obtained, which were only subjected to 488 nm excitation for the manifestation of the endogenous autofluorescence of the technique without performing histological stains. These preliminary results allowed us to refine and improve the technique in *C. familiaris* samples. In this way, micro-plastinated sections could be obtained from two samples of *C. familiaris*, which were stained with Goldner's trichrome (Figure 2), hematoxylin and eosin (Figures 3 and 4C–E) and toluidine blue 0.5% (Figure 4A,B). These sections allowed us to closely examine the relationship between the tooth and the DGJ while preserving the mineralized tissues. Figure 2 displays a dental micro-thin slice that has been stained with Goldner's trichrome, revealing several distinct anatomical features. The image shows parts of the tooth crown, the surrounding periodontal tissue, and the marginal gingiva. Moreover, it displays the initial portion of the tooth root, the periodontium where it inserts, and a section of the alveolar gingiva. Figure 3 displays a dental micro-thin slice of a premolar tooth, along with its alveolus in the mandible; the slice was stained with hematoxylin and eosin to highlight the epithelium of the DGJ and gingival sulcus, enhancing clarity for study and understanding. It depicts a section of the premolar crown, the surrounding periodontal tissue, and the marginal gingiva. Additionally, the image illustrates the beginning of the premolar root, the insertion periodontium, and a portion of the alveolar gingiva. Additionally, in Figure 4 we were able to observe various structures of the periodontal tissue, including the marginal and alveolar gingiva, as well as the underlying connective tissue and components of the periodontal ligament. It is worth noting that the plastination process has given certain elements, such as collagen, autofluorescent properties when excited with 488 nm light, although the enamel showed no autofluorescence. In Figure 4, these sections highlighted stratified squamous epithelium and underlying irregular dense connective tissue. Additionally, the DGJ and dense connective tissue, predominantly composed of obliquely arranged fibers, were clearly visible. A group of fibers from the periodontal ligament specifically highlighted at the gingival ligament.

4 | DISCUSSION

Plastination, created by Gunther von Hagens in Heidelberg, Germany, in 1977 (Bickley et al., 1981; Ottone, 2013; Ottone, 2018; Ottone, 2020; Ottone, Baptista, et al., 2018; Ottone, Vargas, et al., 2018; Vargas et al., 2020; von Hagens, 1979; von Hagens, 1986; Von Hagens et al., 1987) consists of fixation,

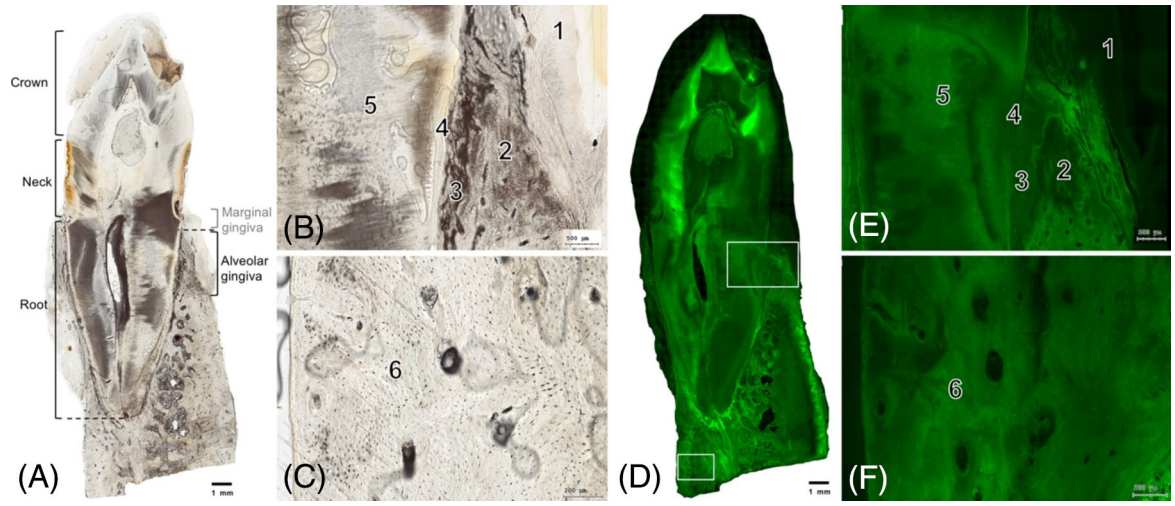

FIGURE 1 Micro-plastination of a human canine tooth in its alveolus in the mandible. (A) Panoramic view of the complete micro-thin dental section without histological stain. Because of alterations in the sample, which could initially be correlated with periodontal disease, the marginal gingiva (marked in gray) appears diminished, leaving the tooth's neck exposed. (B–E) Superior part of the root of the tooth, periodontal tissue and alveolar gingiva are observed. (1) Stratified squamous epithelium, (2) alveolar crest with compact bone tissue, (3) dentoalveolar ligament of the periodontal ligament, (4) acellular cementum, (5) dentin with dentinal tubules. (D) Panoramic view of the complete micro-thin dental section with autofluorescence. (C–F) Compact bone tissue (6) can be observed under autofluorescence.

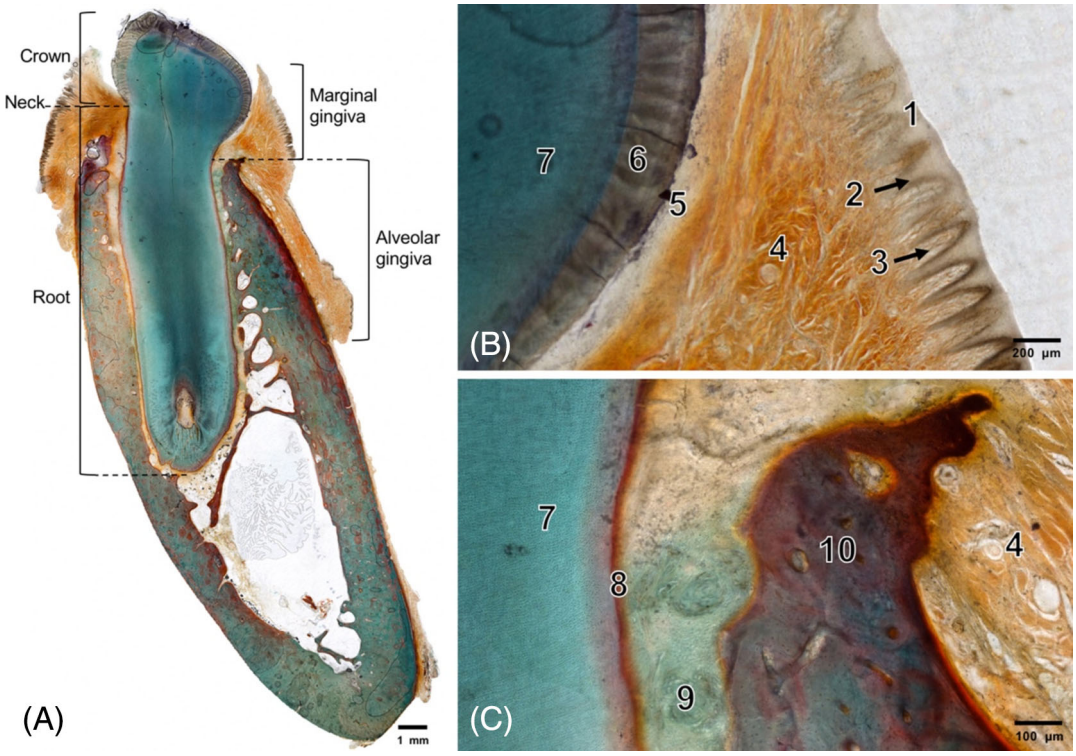

FIGURE 2 Micro-plastination of a *Canis familiaris* incisor tooth in its alveolus in the mandible. (A). Panoramic view of the complete micro-thin dental section stained with Goldner's trichrome. (B) Part of the crown of the tooth, periodontal tissue and marginal gingiva are observed. (C) The beginning of the tooth root, the insertion periodontium and part of the alveolar gingiva are observed. (1) Stratified squamous epithelium, (2) epithelial crest, (3) papillary dermis (loose connective tissue), (4) dense irregular connective tissue with abundant collagen fibers, (5) dentogingival junction, (6) enamel with presence of parazones and diazones, (7) dentin with dentinal tubules, (8) acellular cementum, (9) dentoalveolar ligament of the periodontal ligament, and (10) alveolar crest with abundant compact bone tissue, it is possible to observe osteons and osteocytes.

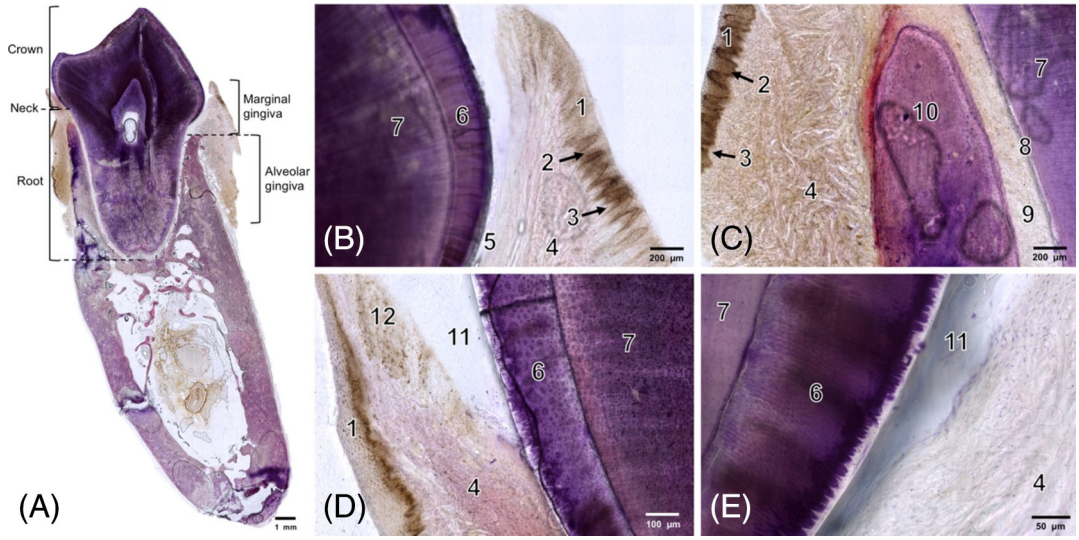

FIGURE 3 Micro-plastination of a *Canis familiaris* premolar tooth in its alveolus in the mandible. (A) Panoramic view of the complete micro-thin dental section stained with hematoxylin and eosin. (B) Part of the crown of the premolar, periodontal tissue and marginal gingiva can be observed. (C) The beginning of the premolar root, the insertion periodontium and part of the alveolar gingiva are observed. (D–E) Part of the premolar crown, periodontal tissue and marginal gingiva can be observed, highlighting the epithelium of the dentogingival junction and gingival sulcus. (1) Stratified squamous epithelium, (2) epithelial crest, (3) papillary dermis (loose connective tissue), (4) stratum reticularis (irregular dense connective tissue with abundant collagen fibers), (5) dentogingival junction, (6) enamel with presence of parazones and diazones, (7) dentin with dentinal tubules, (8) acellular cementum, (9) dentoalveolar ligament of the periodontal ligament, (10) alveolar crest with abundant compact bone tissue, (11) gingival sulcus, and (12) gingival sulcus epithelium.

replacement of water and fat with a curable polymer that are subsequently cured, resulting in dry, transparent, odorless, and durable slices (Bickley & Townsend, 1984; Ottone, 2013; Ottone, 2018; Ottone, Baptista, et al., 2018; Ottone, Vargas, et al., 2018; Sora et al., 2007; Sora & Cook, 2007; Vargas et al., 2020). The fundamental steps of plastination are dehydration, defatting, forced impregnation, and finally curing (Sora et al., 2007; Starchik et al., 2020; von Hagens, 1986; Zhang & An, 2000). But in the case of generating plastinated sections, the standard method of producing plastinated slices from 2 to 5 mm thick (Ottone, 2018; Ottone, Baptista, et al., 2018; Sora, 2007; Sora & Matusz, 2010), is performed by freezing a fresh tissue sample, embedded in gelatin and sectioning it using a band saw, tissue loss is of the order of 0.9 mm per slice (von Hagens, 1986). But there is also a plastination technique to create ultra-thin sections, less than 2 mm, which can reach microns, which would allow descriptive, morphometric and histological analysis of the slices, as well as their three-dimensional reconstruction (Ottone, Baptista, et al., 2018; Sora et al., 2007; Sora & Matusz, 2010; Vargas et al., 2020).

In 1988 (Fritsch, 1988) and 1989 (Fritsch, 1989), Helga Fritsch published detailed findings about the "plastination histology" method. This involved using epoxy resin to create ultra-thin sections, which were then stained with histological stains to visualize anatomical regions studied from macroscopic sections. Fritsch developed this innovative protocol by studying the retrorectal region in human fetuses in 1988 (Fritsch, 1988) and the pelvis of human fetuses in 1989 (Fritsch, 1989). In the latter publication, Fritsch provided a thorough description of the histological staining protocol. Several publications have extensively covered the concept of "plastination histology," with the contribution of Helga Fritsch. Notably, this method combines histological staining techniques with plastination, as expounded in detail in several publications (Fritsch, 1996; Fritsch et al., 2006). Fritsch and Hötzinger (1995) conducted an intricate study on the tomographic anatomy of the pelvis, viscera, and connective tissue, with a keen focus on compartment identification. Fritsch (1996) conducted research on the central ossification of the talus in human fetuses, while Konschake and Fritsch (2014) investigated the human nasal muscles, among other noteworthy investigations.

Continuing with the research started by Fritsch's "histology plastination" (Correa-Aravena et al., 2023; Willbold & Witte, 2010), we are now exploring the concept of micro-plastination (Ottone, 2020; Ottone, 2023). This approach expands the understanding of morphological science from all perspectives. The plastination method allows for the identification of microscopic features within large anatomical specimens. This makes it possible to carry out microscopic and histological studies on macroscopic anatomy, a feat previously unattainable until plastination was introduced. With micro-plastination, a micro-thin sheet plastination technique, we can produce extremely thin sections (less than 0.15 mm/150 µm) that enable the examination of tissue's histological properties. This eliminates the need for traditional histological methods, which only focus on small tissue segments. Micro-plastination, in contrast, offers larger sectional views while retaining the capability for detailed histological analysis. Previous studies by Baygeldi et al. (2022) and Da Silva et al. (2023) have mentioned the use of micro-plastination as a research method in the fields of anatomy and morphological sciences. This technique is

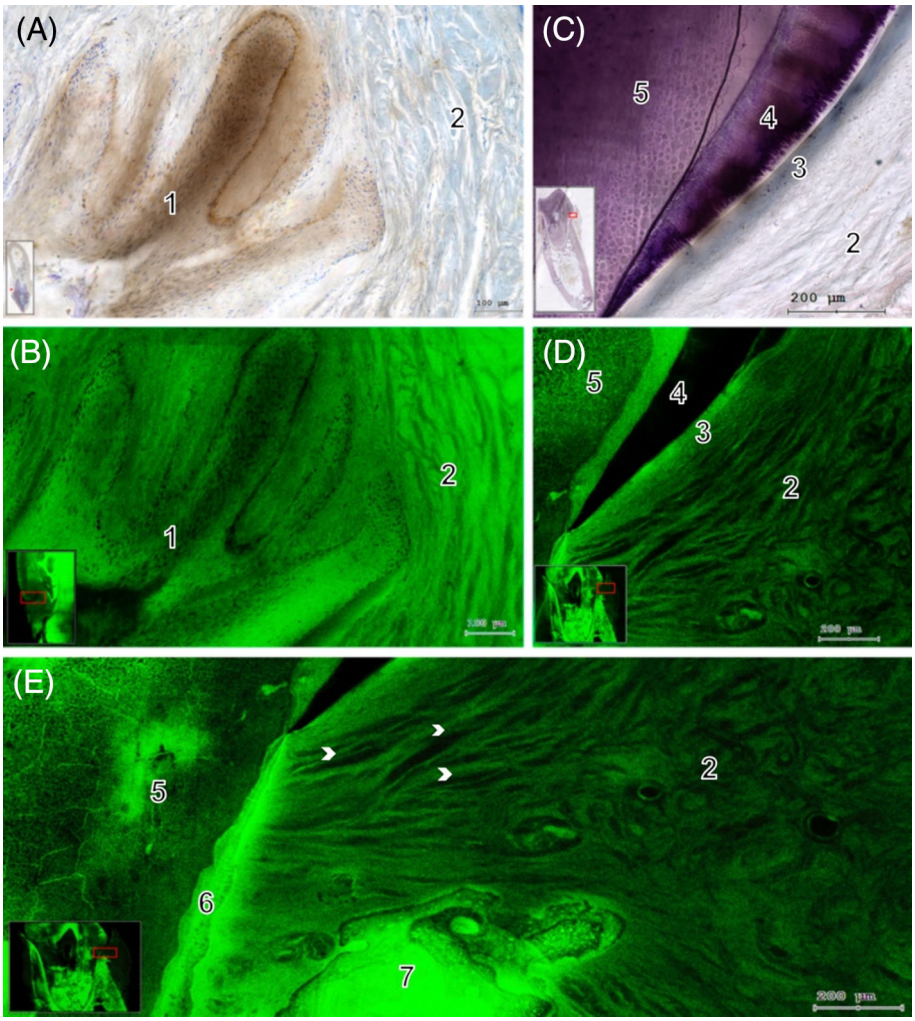

FIGURE 4 Autofluorescence was observed in the micro-plastinated sections, stained with toluidine blue 0.5% (A, B), and hematoxylin and eosin (c–e), upon stimulation with 488 nm light. (A, B). The sections revealed stratified squamous epithelium and underlying irregular dense connective tissue. (C, D) Dentogingival junction and dense connective tissue with predominantly oblique arranged fibers can be observed. (E). A group of fibers belonging to the periodontal ligament, with a focus on the gingival ligament indicated by an arrowhead, were also highlighted (hematoxylin and eosin). The enamel did not exhibit autofluorescence. (1) Stratified squamous epithelium, (2) dense irregular connective tissue, (3) dentogingival junction, (4) enamel, (5) dentin, (6) cementum, and (7) alveolar crest.

valuable for identifying micro-anatomical and histological features in micro-thin sections (less than 150 µm) of biological tissues.

In 2023, our research group reviewed techniques for studying the DGJ, focusing on the CGT applied to nondemineralized mandible specimens (Correa-Aravena et al., 2023). Introduced by Donath and Breuner (1982), CGT has become a reference for histological studies of mineralized teeth and bone with attached soft tissues. The authors utilized human and animal mandibles, ideally not exceeding 1 cm thickness, saturated and fixed in methyl methacrylate. Cutting and grinding were performed using specialized machinery with diamond-tipped blades and water-based cooling systems, resulting in 5–10 µm thin sections suitable for staining. Vacek et al. (1994) used CGT to examine the DGJ dimensions in human cadaver mandibles, achieving thin, nondecalcified sections with an Exakt I machine, followed by staining and examination at 40× magnification. Günhan et al. (1996) validated the technique using animal samples, involving fixation, dehydration, infiltration with resin, and embedding before cutting, grinding, and staining. Silva et al. (2011) detailed a CGT protocol with crystal polyester resin for embedding and power-driven sander-grinder for grinding, also offering a method for processing demineralized samples. Calleja Gómez (2015) performed a complex morphometric analysis of DGJ with methyl methacrylate impregnation, diamond-tipped disc cutting, multiple sanding phases, and staining, observed using a Leica digital optical microscope. Agustín-Panadero et al. (2020) described soft tissue responses in an anterior human tooth with surrounding intact periodontal tissues, using the biologically oriented preparation technique (BOPT) and documenting their findings through microscopy. Collectively, these studies highlight CGT's utility in histological research on dental and periodontal tissues, offering valuable insights into tooth and bone morphology. Nevertheless, performing bone histology without decalcifying the specimen poses a significant technical challenge, particularly when dealing with larger samples. The bone's high density and limited permeability necessitate considerably extended fixation and processing periods, often extending over several weeks, as reported by Goldschlager et al. (2010). Laboratories undertaking such procedures require access to more expensive, specialized equipment, a trained workforce, advanced facilities, and strict safety protocols, as underlined by Karantzoulis et al. (2012). These complexities and associated costs further escalate when conducting enzymatic and immunohistochemical characterization of hard tissue sections processed without decalcification, as underscored by Troiano et al. (2009).

Based on the findings of our study, micro-plastination is a technique that offers benefits compared to CGT for histological investigation in periodontal tissues. The preparation of the impregnation mixture is much easier with micro-plastination, as it provides a longer time for sample incorporation and forced impregnation. This is unlike MMA and PMMA techniques, which have shorter handling times and harden more quickly. CGT techniques are based on inclusions, which do not allow for control over the process, unlike impregnation forced by vacuum in plastination. In micro-plastination, the mixture (epoxy, catalyst, and accelerator) can be diluted during the forced vacuum impregnation process, ensuring a slow and precise introduction of the resin mixture into the sample down to the cellular level.

In our research, we present the potential application of basic histological stains to slices obtained through micro-plastination. This is an emerging area of research, and numerous researchers have made valuable contributions to it: Fritsch (1996) and Fritsch et al. (2006) stained with methylene blue, blue IU, and contrasting with basic fuchsin; Baeres and Møller (2001) applied Mulligan stain; Gruber et al. (2001) applied azure II/methylene and counterstained with basic fuchsine; Sebe, Oswald, et al. (2005); Sebe, Schwentner, et al. (2005) and Lunacek et al. (2005) stained with methylene blue in alkaline solution and counterstained with basic fuchsin; Macchi et al. (2008) stained with methylene blue and periodic acid-Schiff (PAS); Mohsen et al. (2013) stained with Alizarin Red-Alcian Blue; Mooncey and Sagoo (2014) applied Roberts stain; Adds et al. (2017) applied Gomori's trichrome. There is currently no research that applies in situ hybridization, immunohistochemistry, or Picrosirius red techniques. These techniques could further enhance the results of micro-plastination research. However, research on deplastination (the reversal of the plastination process) has shown that it is possible to extract intact DNA from plastinated samples, which can be amplified by polymerase chain reaction in real time. This has significant implications for basic, clinical, epidemiological, and forensic research (Ottone et al., 2020). Therefore, it is important to further investigate in situ hybridization techniques in plastinated samples. Something similar happens with immunohistochemistry, a laboratory technique that uses antibodies to identify specific antigens in a tissue sample. Similarly, research is yet to be conducted on plastinated samples using Picrosirius red (PSR) staining, which is a standard method to evaluate the organization of collagen fibers in tissues. This technique can help distinguish between type I and type III collagens in tissue sections when viewed under a polarized light microscope (López De Padilla et al., 2021). In our research, we used a TissueFaxs I Plus tissue cytometer (TissueGnostics GmbH, Austria) to scan, visualize, and photograph stained micro-plastinated sections. We are also developing immunofluorescence and immunohistochemistry techniques to combine with micro-plastination technique.

Related to autofluorescence, Phillips et al. (2002) demonstrated a breakthrough in the development of plastination, which involves using epoxy resin to preserve tissue samples. Specifically, they found that 488 nm excitation can be used to achieve autofluorescence in this technique, in 2.5 mm thick slices. The authors suggest that the source of autofluorescence is likely to be connective tissue, specifically collagen. They also point out that using confocal laser scanning microscopy together with sequential optical sections can reveal hidden structures and provide improved resolution images, compared to conventional optical microscopy. Several investigations have significantly contributed to the field, developing plastination techniques for ultra-thin sections and detecting autofluorescence in their samples. Some of the investigations with important findings include: Xu et al. (2018) on the prerectal space (2.5 mm thick slices), Xu et al. (2020) on the fascia iliaca (2.5 mm thick slices), and Liang et al. (2014) on the cavernous sinus (2.5–3.0 mm thick slices), among others. As we mentioned before, while examining the micro-plastinated slices that were treated with basic histological stains, we were also able to observe the autofluorescence properties of the technique in micro-plastinated micro-thin slices below 150 µm using the excitation range of 488 nm with the tissue cytometer (TissueFaxs i Plus, TissueGnostics GmbH, Austria) of our lab.

Related the clinical application of the study, micro-plastination is a technique that preserves biological tissues in thin, durable sheets using epoxy resin, offering detailed visualization of anatomical structures. In studies of dental implantology, such as the one conducted by Chiou et al. (2023), it is important to analyze bone quality, implant integration, and the relationship between implants and surrounding tissues. In this context, micro-plastination is an excellent option to apply in this kind of research. Also, micro-plastination serves as an educational tool for teaching implant techniques, enables comparison of implant materials, and aids in research by providing clear cross-sections of bone and tissue. Additionally, it allows for post-implantation analysis, helping to assess tissue response and complications like peri-implantitis, making it a valuable tool for both research and education.

5 | LIMITATIONS OF THE STUDY

In this work, the micro-plastination technique, combined with histological stains, was applied to a small number of samples. The aim was to describe in detail this plastination protocol, an alternative to the CGT, and described a detailed step-by-step protocol, making it possible for other researchers to apply it. In this sense, the technique's development began in a human sample, in which the epoxy resin's autofluorescence capacity was evident. Subsequently, the results were improved in animal samples, in which autofluorescence was also evidenced, in addition to performing the indicated histological stains on them. In this way, in addition to previously untested histological stains, we also demonstrated the technique's autofluorescence capacity in micro-thin sections less than 150 µm thick. Finally, the results obtained highlight the possibility of implementing the micro-plastination technique in a large number of samples in future anatomical and morphological research protocols.

6 | CONCLUSION

We established that micro-plastination is effective in studying the DGJ. We were able to stain periodontal tissues in micro-plastinated sections using two histological staining techniques: Goldner's trichrome, hematoxylin and eosin, and toluidine blue 0.5%. Through

these sections, it was possible to identify the DGJ and its several associated structures, including dentin, enamel, cementoenamel junction, dentinal tubules, connective tissue, and collagen. Furthermore, the micro-plastination process triggered autofluorescence, for example of collagen, when excited with 488 nm light. This is the first documented instance of such discoveries made using an alternative plastination technique called "Micro-plastination." This method is a valuable research tool for creating micro-thin sections of biological tissue to identify and analyze micro-anatomical and histological features using tissue cytometer and confocal microscopy technologies. As demonstrated in this work, this technique can also be combined with histological stains, which allows even more evidence of the tissues' micro-anatomical characteristics, and offers a new approach for its application in anatomical and morphological research protocols.

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