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Modified Goldner Trichrome for Non-decalcified Mineralized Tissue Plastinated and Embedded in Resin

Tricrómico de Goldner Modificado para Tejido Mineralizado no Descalcificado Plastinado e Incluido en resina

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SUMMARY: Over time, Goldner's trichrome staining has been essential in paraffin soft tissue research. However, its classic application involves prior decalcification, generating disadvantages in the integrity of the samples and the interpretation of results. This study seeks to overcome the limitations associated with decalcification when applying Goldner's trichrome stain with plastic resins. It focuses on detailed visualization of non-decalcified bone and dental samples in animal models. Samples of jaw and tooth from a dog (*Canis familiaris*) were used, as well as tibia from a rabbit (*Oryctolagus cuniculus*) with a titanium dental implant and bone graft substitute. Adjustments were made to the original protocol, including a surface treatment prior to staining. Plastination and inclusion in specific plastic resins were part of the process. The microplastinated and stained samples showed optimal quality for optical microscopy. Those from dogs allowed detailed observation of the tooth-periodontal tissue relationship, while those from rabbits revealed a clear differentiation between mineralized and osteoid bone tissue. The staining made it easy to examine the precise interface between soft tissues, bone graft, and implant. The successful adaptation of Goldner's trichrome stain to specimens in plastic resins represents a significant advance in histological investigation of hard tissues. This methodology stands out as an effective tool to evaluate implants and biomaterials in animal models, providing detailed visualization without compromising the integrity of the samples. The combination of histochemistry and plastic resins offers a valuable alternative for microanatomical studies, opening new possibilities in hard tissue research and evaluation of bone structures.

KEY WORDS: Trichrome staining; Plastic resins; Hard Tissue histology.

INTRODUCTION

The Goldner trichrome, or Masson-Goldner, histological technique is a staining method used to visualize different components of biological tissues. It was developed by Goldner in the 1930s as a modification of the Masson trichrome technique, with the aim of obtaining a simplified, practical and subtle staining procedure (Goldner, 1938). This histological technique, in addition to its classical application in connective and muscular tissues, has been described as especially useful in the investigation and diagnosis of bone diseases and biomaterials (An & Martin, 2003), although in some cases it can be unpredictable, demonstrating a "reverse" staining in forensic samples of soft connective tissues, being an understudied field (Gentile *et al*., 2021).

The classic formula described by Goldner in his original 1938 article (Table I) could be successfully used for staining paraffin-embedded sections of specimens that have been fixed in formalin (neutral or alcohol-formalin), Zenker's or Bouin's solution. This staining has been tested on decalcified and non-decalcified sections of bone (An & Martin, 2003) using modifications to the original technique (Table II), in bone tissue research such as the evaluation of osseointegration of dental implants (Bumbu *et al*., 2016), and on non-decalcified resin-embedded specimens. Precisely, resin embedding has posed a challenge for histochemical techniques, however, it is possible to apply classic stains such as hematoxylin-eosin, von Kossa and toluidine blue to

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Table I. Solutions and procedure for trichrome staining described by Goldner in 1938 (adapted from his original article).

Solutions	**Components**
1. Hansen's trioxyhaemateine	Solution A: 10 g of ferric ammonium alum and 1.4 g of ammonium sulfate in 150 ml of distilled water. Solution B: 1.6 g of hematoxylin in 75 ml of distilled water.
2. Ponceau Fuchsin (Masson)	Xylidine Ponceau (0.2 g) Acid fuchsin (0.1 g) Distilled water with 0.2 % acetic acid (300 ml)
3. Orange G-phosphotungstic acid	Phosphotungstic acid (3 - 5 g) Orange G (2 g) Distilled water (200 ml)
4. Light green	Light green (0.1 - 0.2 g) Distilled water with 0.2 % acetic acid (100 ml)
Procedure	**Time**
1. Deparaffinization of the sections	
2. Hansen's hematoxylin	1 - 5 minutes
3. Washing with acidified distilled water	5 minutes
4. Masson's Ponceau-Fuchsin solution	5 minutes or more
5. Washing with acidulated distilled water	5 minutes
6. Orange G - phosphotungstic acid (PTA)	15 seconds - 30 minutes
7. Washing with acidified distilled water	5 minutes
8. Masson's light green solution	5 minutes
9. Washing with acidulated distilled water	5 minutes
10. Dehydration in growing alcohols	
11. Rinsing in xylol	
12. Mounting in neutral medium	

these samples (Donath & Breuner, 1982; Horn & Garrett, 2004). With time, as in most histological techniques, some modifications have appeared in their components and their form of presentation, in order to facilitate the technique and amplify its use in different tissues.

Commonly, studies of bone and dental tissue have required submitting the samples to decalcification procedures for subsequent cutting of the paraffin-embedded blocks, a process that could modify the tissue under study, generating dimensional changes, changes in tissue architecture and in the arrangement of the hard and soft tissues analyzed (Cano-Sánchez *et al*., 2005). With the arrival of plastic resin embedding techniques, the study of bone biology has been enhanced without requiring its intervention with traditional techniques. The use of sectioning and polishing methods of the resin blocks used for tissue embedding have allowed the observation of thin and ultrafine bone samples, including those tissues that present metals in their interior. The basic principle of the inclusion of biological tissues in resin consists of infiltrating the previously fixed and dehydrated specimen with reactive monomers of small molecular weight, which once polymerized form a plastic and cross-linked matrix, giving support and protection to the included structures (An & Martin, 2003). The resins used in optical and electron microscopy are classified as epoxy, acrylic or polyester, according to their chemical composition. Some resins can even be mixed with waxes or similar plasticizers in order to obtain a semi-rigid embedding medium that allows ultra-thin sections to be cut for microscopic observation and analysis (Kiernan, 2015).

On the other hand, in the plastination technique described by von Hagens (1979), both the water and the lipids present in the tissue are replaced by certain polymers, which are selected according to the desirable characteristics of the tissue or final structure, such as greater flexibility or rigidity and the degree of transparency desired (von Hagens *et al*., 1987). The process requires a certain sequence of dehydration, degreasing and forced impregnation with the material used, such as epoxy resin (E12) or polyester (P40). This procedure of preservation of biological material allows the study of areas of interest in their intact form, without alteration in the arrangement of tissues, being of great utility in macroscopic, microscopic, and mesoscopic studies (Sora *et al*., 2019; Ottone, 2023).

The proposal of this work arises in response to the need to adapt Goldner's trichrome technique to this specific context, with the aim of taking advantage of the advantages of inclusion in plastination in ultra-thin sections of epoxy resin (Biodur ® E12) and in methyl methacrylate (MMA)-based resin (Technovit® 9100) resin for the observation and microscopic analysis of non-decalcified animal bone and dental samples.

Table II. Trichrome Goldner or Masson-Goldner histological staining described in the literature.

A. Protocol described by An & Martin (2003)	
Solutions	**Components**
1. Weigert's hematoxylin	Solution A - 1 g of hematoxylin 100 ml 96 % ethanol Solution B - 1.1 g of FeCl3 .6H20 + 1 ml 25 % HCl Fill up to 100 ml with distilled water Before use, mix A+B in a 1:1 ratio
2. Xylidine Ponceau	0.75 g of Xylidine Ponceau 0.25 g acid fuchsin 1 ml of glacial acetic acid Fill up to 100 ml with distilled water
3. Azophloxine	0.5 g of azophloxine (or acid red) 0.6 ml of glacial acetic acid Fill up to 100 ml with distilled water
Ponceau-Fuchsin- Azophloxine Working Solution	77.5 ml of Xylidine Ponceau 2 ml of Azophloxine 88 ml of 0.2 % glacial acetic acid
4. Green light	1 g of light green 1 ml of glacial acetic acid Fill up to 500 ml with distilled water
5. Phosphotungstic acid/Orange II	3 g of phosphotungstic acid 2 g of Orange II Fill up to 100 ml with distilled water
6. 1% acetic acid	
Procedure	**Time**
1. Distilled water	3 minutes
2. Weigert's hematoxylin	20 minutes
3. Washing with running water	20 minutes
4. Distilled water	5 minutes
5. Ponceau/fuchsin/azophloxine	5 minutes
6. 1 % Acetic acid	15 seconds
7. PTA Orange II	20 minutes
8. Acetic acid 1 %	15 seconds
9. Light green	5 minutes
10. 1 % Acetic acid	3 minutes
11. Distilled water	5 minutes
12. Dehydration in absolute alcohol	3 x 3 minutes
13. Rinsing in methylcyclohexane	2 x 3 minutes
14. Mounting in neutral medium	
B. Protocol obtained from Technovit – Histotechnology (Kulzerm, Mitsui Chemicals Group)	
Solutions	**Components**
1. Phosphomolybdic acid /Orange G	30 g of phosphomolybdic acid 500 ml of distilled water 20 g of orange G 500 ml of distilled water Mix both solutions and filter
2. Ponceau acid magenta azophloxine	100 ml of Masson's solution 20 ml of azophloxinlsg 880 ml of 0.2 % acetic acid
	Masson's solution: 1 part sol. A + 2 parts sol. B - Solution A: 1 g of acid magenta (magenta-S) ad. 100 ml of aqua dest. Boil and add 1 ml of glacial acetic acid Filter - Solution B: 2 g of Xylidine Ponceau ad. 200 ml of aqua dest. Boil and add 2 ml of glacial acetic acid Filter Azophloxine solution 0.5 g of azophloxin 100 ml of aqua dest. 2 ml of glacial acetic acid
Procedure	**Estimated time***
Deacrylate sections	
Mayer's Hematoxylin[A1]	10 minutes
Tap water	
Ponceau acid magenta azophloxine	45 minutes
1 % acetic acid	
Phosphomolybdic acid /Orange G	7 minutes
1 % acetic acid	
Light green	40 minutes
Ascending alcohol series	
Xylol	
Cover with Eukitt or similar	

MATERIAL AND METHOD

A microplastination protocol was applied to animal samples (*Canis familiaris* and *Oryctolagus cuniculus*), donated for research and teaching purposes to CEMyQ of the Universidad de La Frontera and kept in 4 % buffered formalin. The procedure consisted on the one hand in subjecting the *Canis familiaris* samples to a process of dehydration, degreasing, and forced impregnation by applying the plastination technique using epoxy resin (Biodur® E12/E6/E600) following the protocol described by Ottone *et al*. (2018); and on the other hand, the *Oryctolagus cuniculus* samples were embedded in Technovit® 9100 resin (Kulzer Technik, Germany), following the manufacturer's instructions (sequence of alcoholic dehydration in ethanol, immersion in xylol intermediate, pre-infiltration and infiltration in stages with Technovit® resin, before the polymerization process).

Once the polymerized blocks were obtained, the samples were reduced in thickness by using a diamond saw at high speed (1000 - 1400 rpm) in a precision cutter (PICO155, Pace Technologies, USA), until thin sheets of approximately 2 - 5 mm thickness were obtained. The surface of interest of the cut sample was polished with 600, 1000, 2000 grit sandpaper under a silicon carbide disc P5000 (TrizactTM, 3M) in a manual polishing machine EcoMetTM 30 (Buehler), obtaining a smooth and polished surface that was later glued with a viscous mixture of epoxy resin (E12 and E6, Biodur®) to a glass slide. The resin was allowed to cure in an oven at 60 °C for at least 4 hours before the samples were again thinned with 240, 360, 600, 1000, 2000 grit sandpaper under abundant irrigation

with water, in order to obtain sections of 100 - 150 µm thick (Fig. 1). Each sample underwent a final finishing with a silicon carbide disc P5000 (TrizactTM, 3M) and glycerin in a manual polishing machine EcoMetTM 30 (Buehler) before proceeding with histological staining.

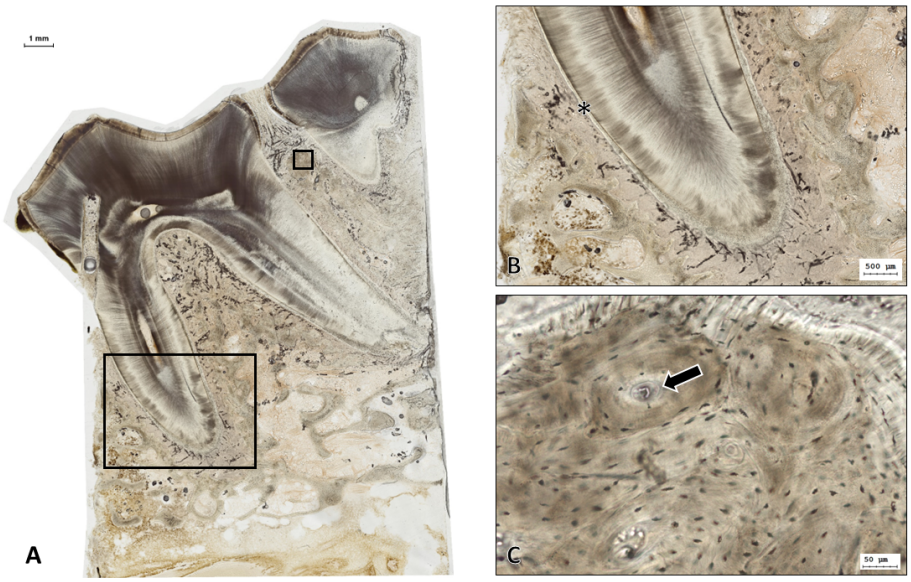

Fig. 1. *Canis familiaris* mandible and molar specimen, microplastinated, after the cutting and polishing protocol; A. Complete section scanned, the integrity of the plastinated tissues is observed, without separation of the tooth from the alveolus, nor loss of dental tissues; B. Close-up of the periapical area of the tooth, the union is observed dentin cement (*); C. Enlarged image of the alveolar bone, with osteone and central canal (black arrow).

The staining protocol applied (Table III) was established using the original procedure described by Goldner, replacing some of its components in order to provide a feasible technique to be performed in the laboratory. Prior to histochemical staining, a surface treatment was applied to the microplastinated samples, applying an "etching" with acetone, hydrogen peroxide and 10% ethylenediaminetetraacetic acid (EDTA), sequentially, in order to eliminate the superficial layer of resin and allow the passage of the dyes into the tissue. We observed that for this particular technique, the surface treatment of the samples included in methyl methacrylate (MMA)-based resin was not necessary to apply to obtain the results presented here.

Table III. Goldner's trichrome staining solutions and procedure used.

Solutions	Components
1. Weigert's iron hematoxylin	-Solution A: 1 g of hematoxylin in 100 ml of 95 % ethanol. -Solution B: 4 ml of 29 % ferric chloride and 1 ml of 37 % hydrochloric acid in 100 ml of distilled water. -Working solution (prepared just before use): mixture of solutions A + B in equal parts.
2. Acid fuchsin - Xylidine Ponceau	-Solution A: 1 g of Xylidine Ponceau in 100 ml of distilled water. -Solution B: 1 g of acid fuchsin in 100 ml of distilled water. -Working solution (prepare fresh): 6 ml of solution A, 2 ml of solution B, 9 ml of 2 % acetic acid and 3 ml of distilled water.
3. Phosphotungstic acid - Orange G	Solución de trabajo: 4 g of phosphotungstic acid and 2 g orange G in 100 ml of distilled water. Filter before use.
4b. Light green	Working solution: 0.2 g of light green and 0.2 ml of acetic acid in 100 ml of distilled water. Filter before use.
4a. Aniline blue	Working solution: 3 g of aniline blue and 2 µl of acetic acid in 100 ml of distilled water.
5. 1% acetic acid	
6. Ethanol	Concentrations of 50 %, 70 %, 80 %, 90 % and 100 %.
Procedure	Estimated time
1. Distilled water	3 minutes
2. Weigert's hematoxylin	25 minutes
3. Washing in running water	15 minutes
4. Xylidine Ponceau	17 minutes
5. 1% acetic acid	15 seconds
6. Orange G/Phosphotungstic acid	7 minutes
7. Acetic acid	15 seconds
8. 0.2 % light green or aniline blue	30 minutes – 1 hour
9. 1 % acetic acid	3 minutes
10. 50% alcohol	1 minute
11. 70% alcohol	1 minute
12. 80% alcohol	1 minute
13. 90% alcohol	30 seconds
14. Alcohol – xylene 1:1	10 seconds

Nuclear staining was performed using Weigert's iron hematoxylin, and continued sequentially with acid fuchsin-Xylidine Ponceau, orange G and light green or 2% aniline blue, the latter step being one of the most important (since it is the step that fixes the dye to the collagen fibers of the bone) and the one that showed the greatest modifications in terms of exposure time. These variations in the exposure times were performed based on the constant visualization of the worked tissue. Subsequently, the sections were dehydrated in a series of alcohols of increasing grade and covered with neutral mounting medium (Entellán New, Merck) and glass coverslips, with light pressure being applied on the samples for 12 hours, prior to visualization and image acquisition.

The digitization process of the samples was performed using TissueFAXS 7.1.139 software together with a PixeLINK camara, divided into two distinct stages. In the first stage, preliminary capture of each slide in a 7 by 14 FOVs (field of view) array was performed using a 2.5x, Air, 0.085, 8800 mm objective. From this preview, the automatic tissue detection option within the software was employed, allowing regions of interest to be flagged based on a set of thresholds. These were determined using the RGB channel information provided by the system. The stage concluded with the validation of the automatic detection process by an individual review of the detected regions, manually adjusting extended areas or excluding related areas by free drawing. In the second stage, the previously defined regions were captured in high resolution using a 20x, Air, 0.5, 2000 mm objective, after performing an illumination correction by white balance adjustment. This correction ensured accurate representation of neutral colors by adjusting the intensities of red, green, and blue in a tissue-free zone on the slide. For the acquisition of these images, a strategy using a multichannel category autofocus algorithm with smart focus points was employed. This approach relied on the preview image to identify areas of high contrast and avoid those without tissue. Finally, the illumination intensity parameters were adjusted to 10.49 V and 0.04 ms exposure to execute the image capture, setting the illumination correction in a matrix of 3 by 3 tissue-free FOVs. Subsequently, through TissueFAXS Viewer 7.1.7.139 software, data extraction was performed. Images were exported with a resolution of 400-1200 DPI in TIFF and JPG format, using 40-50% binding percentages. In addition, areas of cropping and illumination correction were applied to the entire full-region images.

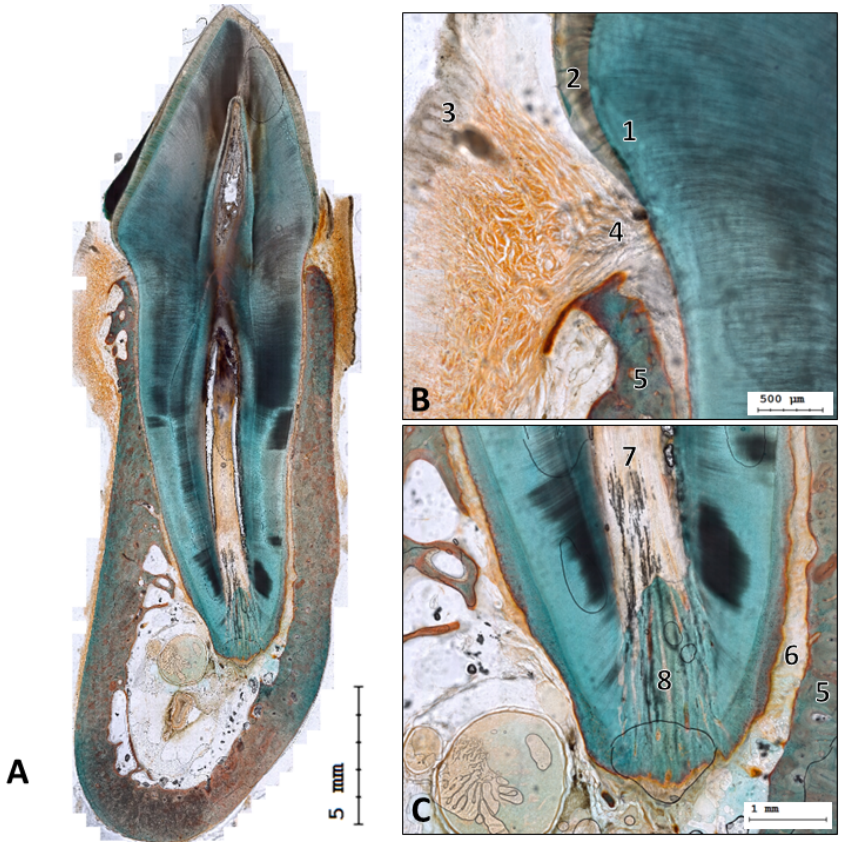

Fig. 2. Tooth and jaw (*Canis familiaris*) microplastinated and stained with Goldner's trichrome. A. Panoramic view of the sagital section (obtained from Panes-Villarroel *et al.*, 2023). B. Dentogingival junction. C. Root apical third. 1. Dentin; 2. Enamel; 3. Keranized stratified squamous epithelim; 4. Gingivoperiodontal fibers; 5. Alveolar bone; 6. Periodontal ligament; 7. Dental pulp) maintain an orange-yellow color. 8. Union of the root canal with the periodontal apical tissues.

RESULTS

The sections obtained from samples *Canis familiaris* (Fig. 2), stained with Goldner's Trichrome, showed good quality for optical microscopy and provided a detailed view of key histological aspects. Not only did they allow the relationship between the tooth and the dentogingival junction to be examined, effectively preserving mineralized tissues, but they also facilitated the meticulous observation of various structures within the periodontal tissue. Among these structures, the essential components of the periodontal ligament stand out, which significantly enriched our understanding of dental microanatomy and tissue interactions in the periodontal context (Fig. 2).

Sections of rabbit tibia (*Oryctolagus cuniculus*) included in Technovit® 9100 resin (Kulzner Germany) and stained with toluidine blue, allowed anatomical orientation of the tissues under observation and analysis of their relationship with the implant (Fig. 3). However, the use of Goldner's trichrome staining allowed us to obtain a contrast between the mineralized bone tissue, identified in green, and the osteoid, highlighted in red. Furthermore, this staining facilitated detailed observation of critical cells such as osteoblasts, osteocytes, and osteoclasts. Significantly, it allowed us to analyze the phases of bone formation and resorption, as well as to examine the precise interface between the surrounding soft tissues, the synthetic bone graft and the implant, as evidenced in Figures 4 and 5.

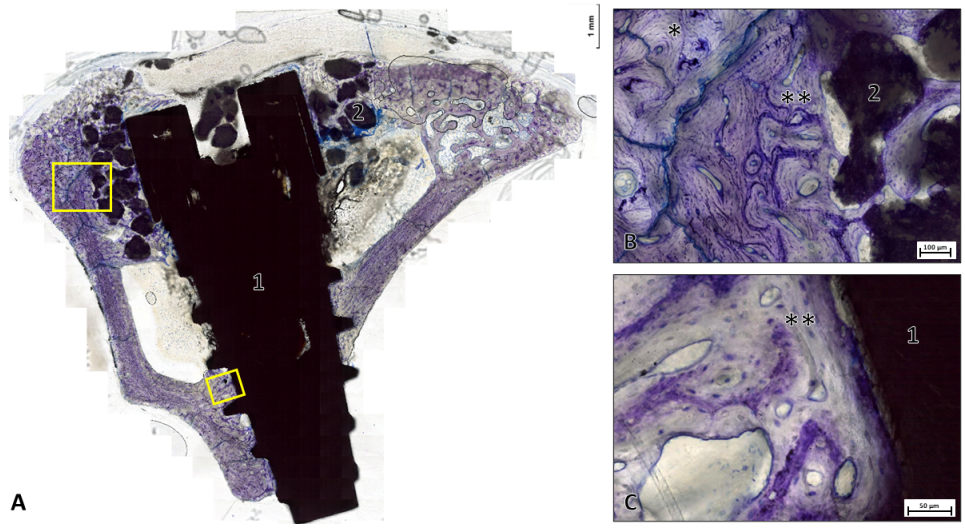

Fig. 3. Rabbit tibia (*Oryctolagus cuniculus*) embedded in Technovit® 9100 resin (Kulzner, Germany) and stained with toluidine blue. A. Panoramic view showing the critical peri-implant bone defect, with insertion of a transcortical titanium implant (1) and synthetic bone graft based on ß-tricalcium phosphate and hydroxyapatite (2). B. Close-up of the bone tissue interface (**) – graft (2). C. Close-up of the bone tissue interface (**) – implant (1).

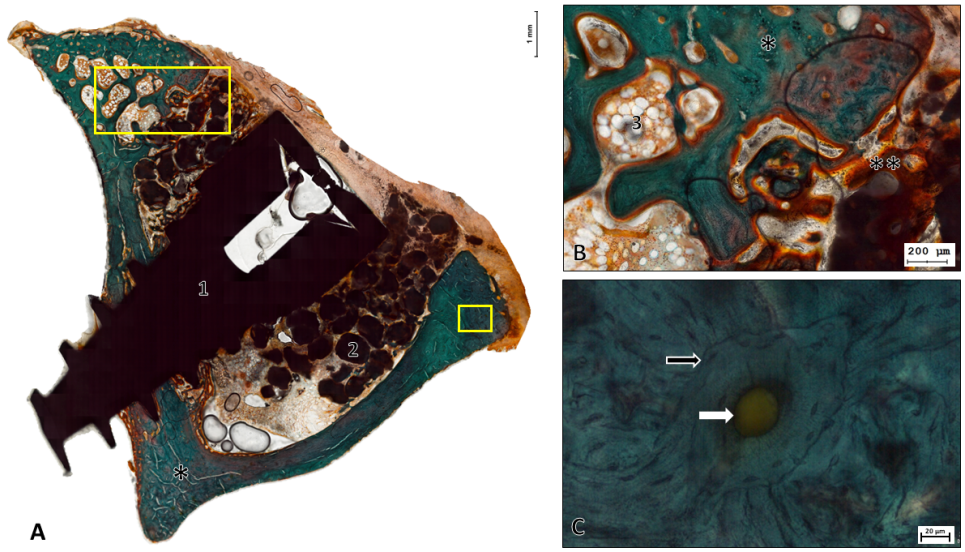

Fig. 4. Rabbit tibia (*Oryctolagus cuniculus*) embedded in Technovit® 9100 resin (Kulzner, Germany) and stained with Goldner's trichrome. A. Panoramic view showing the critical bone defect, with insertion of a transcortical titanium implant (1) and synthetic bone graft based on ß-tricalcium phosphate and hydroxyapatite (2). B. Close-up of the area indicated in A, where a clear differentiation of lamellar bone tissue (*) and osteoid tissue (**) is observed within the defect and around the biomaterial granules. C. Enlarged image of a sector of cortical bone, showing the central canal of an osteon (white arrow) surrounded by circularly oriented lamellae. The boundaries between osteons and interstitial systems are clearly marked by the cement line (black arrow).

Fig. 5. Bone tissue – graft – implant interface in rabbit tibia (*Oryctolagus cuniculus*) included in Technovit® 9100 resin (Kulzner, Germany) and stained with Goldner's trichrome. The staining shows, in a differentiated manner, the ancient cortical tissue of the tibia (*) and the immature osteoid tissue (**) present between the biomaterial granules of the graft (2). In addition, it allows us to observe the neoformation of bone tissue (black arrow) on the surface of the titanium dental implant (1).

DISCUSSION

The advance in histological techniques since the appearance and use of inclusion, epoxy, acrylic or other polymer resins is undeniable, allowing the visualization and study of different types of tissues by conventional, immunohistochemical and electron microscopy techniques (Kiernan, 2015; Pawlina & Ross, 2015; Suvarna *et al*., 2018). Along with this development of better strategies for microscopic observation, anatomical techniques, which preserve body and tissue morphology, have progressed enormously in the last decades, allowing us a stable and practically unaltered preservation of biological tissues, with the obtaining of samples destined for exhibition, teaching and research (Ottone, 2023), even rescuing samples stored for decades in flasks, leaving them in a less toxic state of conservation, resistant over time and easy to manipulate in learning environments (Pirici *et al*., 2023). In this way, with the plastination technique it has been possible to obtain tissue sections with a high degree of transparency, which, in thin and ultrathin sections, can allow microscopic visualization of the treated tissues, reaching sections with a thickness of less than 230 microns, through microplastination (Ottone, 2020; Vargas *et al*., 2020; Ottone, 2023).

Knowledge of the inclusion procedures and the variety of histochemical stains applicable to a given sample is of utmost relevance to the researcher, since the different protocols, commercial brands, times, and concentrations of the products used will influence the optical results obtained and, consequently, the analyses performed on these samples. While the stains in their original formulation were intended for processing paraffin-embedded samples, they were successfully adapted to other embedding materials, including light-curing organic resins, with varying degrees of viscosity, transparency and final properties once cured (Suvarna *et al*., 2018). In the use of these resinous materials, the literature describes different protocols for the cutting and polishing of the sections, obtaining slices of 1 mm - 230 µm in bone microplastinated tissues without decalcifying (Vargas *et al*., 2020), from ~ 5 µm (Donath & Breuner, 1982; Gruber, 1992) to 100 µm (Hillmann *et al*., 1991) for inclusion of bone and/or non-decalcified teeth in resin. Specifically for dental tissue, it has been possible to obtain sections of ~500 µm (Konschake & Fritsch, 2014), up to ~100 µm by Donath & Breuner (1982). Precisely, Donath & Breuner (1982) indicate in their research that the thinning of dental sections to less than 100 µm causes a break in the crystalline structure of the enamel, with the consequent risk of affecting the area of interest, a situation that we confirmed in our experience.

Trichrome stains allow visualization of the collagen fibers of the extracellular matrix, the main component of connective tissues. These techniques use one or more acid dyes and a polyacid to contrast the collagen fibers with the other fibers present and are widely used in pathological analysis. In these multipass techniques, the ionized state of the dyes must be controlled by ensuring an acidic working medium, so that the dyes do not stain the same tissue component. This is also achieved thanks to the principle of mass action, in which the molecular weights of the stains will have different action and affinity in thetissue (Veuthey, 2014). In these techniques, after nuclear staining, the dyes are applied sequentially; the first dye stains the entire section and usually has an intermediate molecular weight; secondly, the polyacid of a higher molecular weight, will have the function of displacing the staining of the collagen fibers; while in the third step, the second acid dye, has the function of a deep staining of all collagen fibers (Veuthey, 2014). Masson's trichrome stain, recognized for its ability to stain collagen fibers blue, and the red contrast created with the rest of the tissue, has proven to be useful in cases of cardiac and pulmonary fibrosis, muscular dystrophies, among other pathologies (Gurina & Simms, 2023); on the other hand, the variation presented by Goldner is especially advantageous

in bone and orthopedic research, due to the affinity of its dyes for bone tissue (either light green or aniline blue) and its differentiation when contrasted with the red of the acid fuchsin that stains the osteoid, in addition to the information reported with the third component (orange G) (Gruber, 1992).

The application of an acetone/alcohol solution to the entire surface of interest in the microplastinated samples, in addition to the EDTA chelating solution, allowed us to obtain a homogeneous staining of the entire tissue, which had not been achieved in previous attempts without etching. In addition, we emphasize that this step did not produce changes or alterations in the cellular architecture, nor in the expected staining with trichrome staining. In addition to this, the previous application of heat to the solutions was avoided, as described for toluidine blue staining, where it is recommended to preheat the solution to 60 °C before applying it (Bhattacharyya *et al*., 2000), or alternatively, during the immersion of the section, to place it in an oven at this temperature. However, this step can be critical for very thin samples, since the sections tend to wrinkle, and as observed by us, it hinders the washing of the dyes and the mounting of the sample, facilitating the entry of air and the permanence of bubbles inside the section. In addition, one must be certain that the EDTA solution has been completely removed from the surface to be stained, since the mixture of both solutions resulted in an oily type of substance, which forced us to re-stain and polish the surface before proceeding with the staining technique.

Currently, *in vitro* studies and animal models are still needed for the development of new drugs, biomaterials, orthopedic devices, among others, as a step prior to human studies, thus making it possible to comprehensively evaluate the responsiveness of tissues and the immune system to these new treatments (Mukherjee *et al*., 2022; Robinson *et al*., 2019). Precisely, in this study, the histochemical staining technique, the correct fixation, and a rapid inclusion in resinous media, allowed an in-situ assessment of tissue cellularity in relation to a bone defect, with preservation of the osteoid and the integrity of the contact with the surface of the titanium implant and of the biomaterial granules. Also, depending on the objective, it will be relevant for researchers to quantify tissues of different characteristics (such as osteoid, lamellar bone and calcified cartilage, enamel, dentin, and dental cement), reporting as much information as possible for each sample. It should be considered, however, that its application in other circumstances may show changes in the affinity of the staining in different connective tissues, which may be due to structural alterations typical of induced disease models, infected areas, or other considerations, which conditions the observed staining, as described by Gentile *et al*. (2021) for forensic samples.

CONCLUSION

Histology is the microscopic study of tissues and organs by means of fixation, inclusion, section, staining and observation under a microscope, allowing the visualization of the tissue structure and possible changes it may have undergone under physiological, pathological, or experimental conditions. The choice of histological staining to be applied to a given sample depends directly on the research objective, considering the characteristics of the tissue and the processing to which it was subjected. On the one hand, although the inclusion and staining of bone and tooth without decalcification has been previously reported, it is the choice of the technique and the reproducibility of its results that are determining factors for its current use. In this case, the tissue inclusion of mineralized tissues without previous decalcification, in different resinous materials, was not an impediment for Goldner's trichrome histochemical staining to allow the observation of the dental, lamellar bone and trabecular tissue architecture, together with the cellularity of these tissues. With this technique it is possible to observe information that cannot be obtained from decalcified samples, such as the dentogingival junction, or obtained with great difficulty, such as bone regeneration patterns around a dental implant, enabling its application in the field of teaching and medical and scientific research.

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PANES, C.; PONCE, N.; OTTONE, N. E.; VALDIVIA-GANDUR I.; BELTRÁN, B. & VÁSQUEZ, B. Modified Goldner trichrome for non-decalcified mineralized tissue plastinated and embedded in resin. *Int. J. Morphol., 42(2)*:516-524, 2024.

RESUMEN: A lo largo del tiempo, la tinción tricrómica de Goldner ha sido esencial en la investigación de tejidos blandos en parafina. Sin embargo, su aplicación clásica conlleva la descalcificación previa, generando desventajas en la integridad de las muestras y la interpretación de resultados. Este estudio busca superar las limitaciones asociadas con la descalcificación al aplicar la tinción tricrómica de Goldner con resinas plásticas. Se enfoca en visualizar detalladamente muestras óseas y dentales no descalcificadas en modelos animales. Se emplearon muestras de mandíbula y diente de perro (*Canis familiaris*), así como tibia de conejo (*Oryctolagus cuniculus*) con implante dental de titanio y substituto de injerto óseo. Se realizaron ajustes al protocolo original, incluyendo un tratamiento superficial previo a la tinción. La plastinación y la inclusión en resinas plásticas específicas fueron parte del proceso. Las muestras microplastinadas y teñidas mostraron una calidad óptima para microscopía óptica. Las de perro permitieron la observación detallada de la relación diente-teji-

do periodontal, mientras que las de conejo revelaron una clara diferenciación entre tejido óseo mineralizado y osteoide. La tinción facilitó examinar la interface precisa entre tejidos blandos, injerto óseo e implante. La adaptación exitosa de la tinción tricrómica de Goldner a muestras en resinas plásticas representa un avance significativo en la investigación histológica de tejidos duros. Esta metodología destaca como una herramienta eficaz para evaluar implantes y biomateriales en modelos animales, brindando una visualización detallada sin comprometer la integridad de las muestras. La combinación de histoquímica y resinas plásticas ofrece una alternativa valiosa para estudios microanatómicos, abriendo nuevas posibilidades en la investigación de tejidos duros y evaluación de estructuras óseas.

PALABRAS CLAVE: Tinción tricrómica; Resinas plásticas; Histología de tejidos duros

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