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Original Research Article

Temporomandibular Joint Histology

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ABSTRACT

Background: The temporomandibular joint plays a crucial role in daily oral functions like mastication. A comprehensive understanding of the histological components of these joints proves valuable in deciphering their intricate anatomical structures.

Purpose of the study: This study aimed to compare the histochemical stains of hematoxylin-eosin and Goldner's trichrome applied to a temporomandibular joint sample using the Wistar Rat as a biomodel.

Results: Complete sagittal slices of murine biomodel hemicranium were evaluated and the comprehensive histological technique involved various steps, including perfusion, post-fixation, decalcification, dehydration, clarification, inclusion, cutting, staining, and assembly. The temporomandibular joint specimens were stained using H&E and Goldner's trichrome. The histological description included structures such as the external auditory canal, mandibular fossa and condyle, pterygoid muscles, joint capsule, synovial membrane, articular disc, and articular surface. Special cell types like chondrocytes and synovial cells were also identified.

Conclusion: Goldner's trichrome, an underutilized technique, exhibited superior chromatic richness in differentiating the cellular and tissue components of the temporomandibular joint.

KEYWORDS: histology; joint capsule; synovial membrane; temporomandibular joint; temporomandibular joint disc; trichrome stain; Wistar rats.

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INTRODUCTION

Morphologically, the temporomandibular joint (TMJ) is a complex structure pivotal for mammalian jaw movement [1].

At a macroscopic level, it comprises the mandibular condyle and the articular fossa of the temporal bone, referred to as the glenoid cavity or mandibular fossa. Additionally, it encompasses the articular disc, synovial membrane, joint capsule, key ligaments, and muscles [2,3].

Histologically, the TMJ showcases diverse tissues enclosed by connective tissue [4].

Its uniqueness lies in the absence of hyaline cartilage on articular surfaces, replaced by a resilient layer of fibrocartilaginous tissue capable of withstanding pressure [2]. (Table 1). Hematoxylin and eosin (H&E) staining impart a basophilic hue to nuclei, while cytoplasmic and extracellular matrix components exhibit varying degrees of eosinophilic staining [5]. Conversely, Goldner's trichrome stain, a modification of Masson's trichrome technique [6], highlights different cells and tissues in red, black, orange, and green [7].

Table 1: Temporomandibular joint Histology.

Structure	Tissue	Associated cells
Mandibular condyle	Compact bone on the surface	Fibroblasts and chondrocytes
	Spongy bone on the inside	
	Fibrocartilage on articular surface	
Glenoid cavity (temporal articular fossa)	Thin fibrous layer on joint surface	Chondrocytes
	Compact bone on the surface	
	Spongy bone on the inside	
Articular disc	Fibrous connective tissue	Fibroblasts, mesenchymal cells and surface proteoglycans
Superior retrodiscal lamina (Top part of the articular disc)	Collagenous and elastic fibers	Fibroblasts
Inferior retrodiscal lamina (Lower part of the articular disc)	Collagenous fibers	
Bilaminar region of the articular disc	Diffuse connective tissue	
Synovial cavity	Areolar connective tissue	
Fluid or synovial fluid	Mainly hyaluronic acid	Monocytes, lymphocytes, free synovial cells and occasionally polymorphonuclear leukocytes
Synovial membrane	Loose connective tissue	White adipocytes, stromal cells, macrophage-like cells and fibroblast-like cells
Subintimal layer of the synovial membrane		Fibroblasts, macrophages, mast cells, white adipocytes and elastic fibers
Intimal layer of the synovial membrane		Synovial cells embedded in a cell-free and amorphous intercellular matrix
Joint capsule	Non-elastic fibrous membrane	

Given the scarcity of articles detailing TMJ histology, this paper compares the histochemical stains of hematoxylin-eosin and Goldner's trichrome in a rat TMJ.

METHODS

In this study, adult female Wistar strain rats (*Rattus norvegicus*) sourced from the Intermediate Laboratory of Preclinical Research at Valle University were utilized. The experiment received approval from the Biomedical Experimentation Animal Ethics Committee under the internal code 013-017.

The biomodels underwent perfusion, a sample fixation method [8]. Prior to this, rats were anesthetized in an induction chamber (Hallowell EMC; USA) using Isoflurane. Perfusion, conducted with an automatic pump (Cole-Parmer; USA); was intracardiac and involved saline solution and formaldehyde. Hemicranium samples were obtained through surgical resection [9].

Skulls were preserved in a 10% buffered formalin solution for 24 hours [10-12].

Decalcification was achieved using 10% ethylenediaminetetraacetic acid (EDTA) with a pH of 7.22 [1,13-14]. The samples were kept at room temperature on a rotator (DSR 2100V; Digisystem; COL), with chelating agent changes on days three, five, seven, 10, 14, 18, and 21.

Dehydration using increasing alcohol concentrations and clarification with xylol followed. Impregnation with soft paraffin (Histoplast PE; Thermo Fisher Scientific; USA) at 60°C occurred in an automatic tissue processor (Sakura Finetek; Spain) over twelve and a half hours. Given the sample size, steel forms and angles were employed during the soft paraffin step at 62°C, utilizing an inclusion center (Histocentre 3; Leica; USA).

Blocks containing hemicranium samples were cut at 4µm using a microtome (Leica RM2245; USA); [1,13]. Subsequently, these were immersed in a bath (Boekel Scientific; USA) at 65°C, placed on slides, and stored between 35 and 38°C for a minimum of 24 hours.

H&E staining involved twenty-three steps lasting approximately one hour [12-13,15]. The

first eight steps focus on deparaffinization and rehydration of the samples: two immersions in xylene, two immersions in 100% alcohol, two immersions in 95% alcohol, one immersion in 70% alcohol, and a tap water wash. Staining in fifteen steps, which included: a hematoxylin immersion for 9 minutes, a tap water wash, an acidic alcohol step to eliminate the affinity of hematoxylin for the acidic components of the nucleus; one wash in tap water, one step in lithium carbonate, one wash in tap water, one immersion in eosin for 1 minute, one step in 95% alcohol, three immersions in 100% alcohol, and finally two immersions in xylol for clearing and a last xylol in which the samples were left until the moment of assembly. This process was carried out in a type II biological safety cabinet.

Staining with the Goldner trichrome technique was performed based on the Lee G. Luna protocol [16].

A process of twenty-seven steps was carried out with an approximate duration of five hours. Like H&E staining, a deparaffinization in two immersions in xylene for two minutes each and a hydration process in two immersions in 100% alcohol for two minutes each, two immersions in 95% alcohol for two minutes each, and wash in distilled water for three minutes. With Bouin's solution, samples were baked at 56°C for one hour. After reaching room temperature, the samples were washed with 70% alcohol until the yellow color was eliminated. Nuclei were stained with Weigert's iron hematoxylin, prepared with solution A: 1.16 grams of iron chloride, 1 milliliter of iron chloride, and 99 milliliters of distilled water; and a solution B: 1 gram of hematoxylin and 100 milliliters of 96% ethanol, for ten minutes, the excess of hematoxylin was washed with running water for ten minutes and distilled water for three minutes. The cytoplasm was stained with ponceau acid fuchsin prepared with 0.175 grams of ponceau, 0.075 grams of acid fuchsin, 0.5 milliliters of glacial acetic acid, and 250 milliliters of distilled water, for 5 minutes, the erythrocytes and the muscle were stained with orange G prepared with 1 gram of orange g, 1.5 grams of phosphomolybdic acid, 1 crystal of sodium azide and 250 milliliters of distilled water, for 13 minutes and finally the bone was stained with light green [10] prepared with 0.5 grams of light green, 0.5 milliliters of glacial acetic acid and 250 milliliters of distilled water, for 5 minutes. Excesses of each dye were washed with 1% glacial acetic acid. Subsequently, the samples were dehydrated in three 95% alcohols each for three minutes and in three 100% alcohols for three minutes each, they were rinsed in two xylols each for three minutes and left in a last xylol until the mounting process began.

After staining, slides were individually removed from xylol, and a coverslip was applied using a resin-type mounting medium (Consul-Mount 9990440; Thermo; Spain) compatible with xylol and left to self-cure at room temperature.

The Wistar rat TMJ samples were observed using an optical microscope (Leica DM750; USA), and digital photographs were obtained through an attached camera (Leica DFC295; USA) and Leica Application Suite software (USA).

RESULTS

The hemicranium sagittal section displays the rat's nose on the left side, the crania on the right, and, with Goldner trichrome stain, a portion of the brain on the upper right of the skull. The oral cavity surrounds the TMJ, bordered by muscles; teeth are visible on the bottom.

The TMJ tissues exhibit eosinophilia due to H&E stains (Figure 1A). TMJ stained with Goldner trichrome shows bone structures like the external auditory canal, mandibular fossa, and mandibular condyle in purple. Pterygoid muscles stain orange, the joint capsule presents a purple stain, and the articular disc displays a peripheral purple stain and a central red stain (Figure 1B and 1D). The articular surface of this joint is also visible (Figure 1C and 1D).

The histological trichrome stain reveals diverse tissue and cellular characteristics on the TMJ. The mandibular condyle appears as fibrocartilage, filled with chondrocytes. These cells

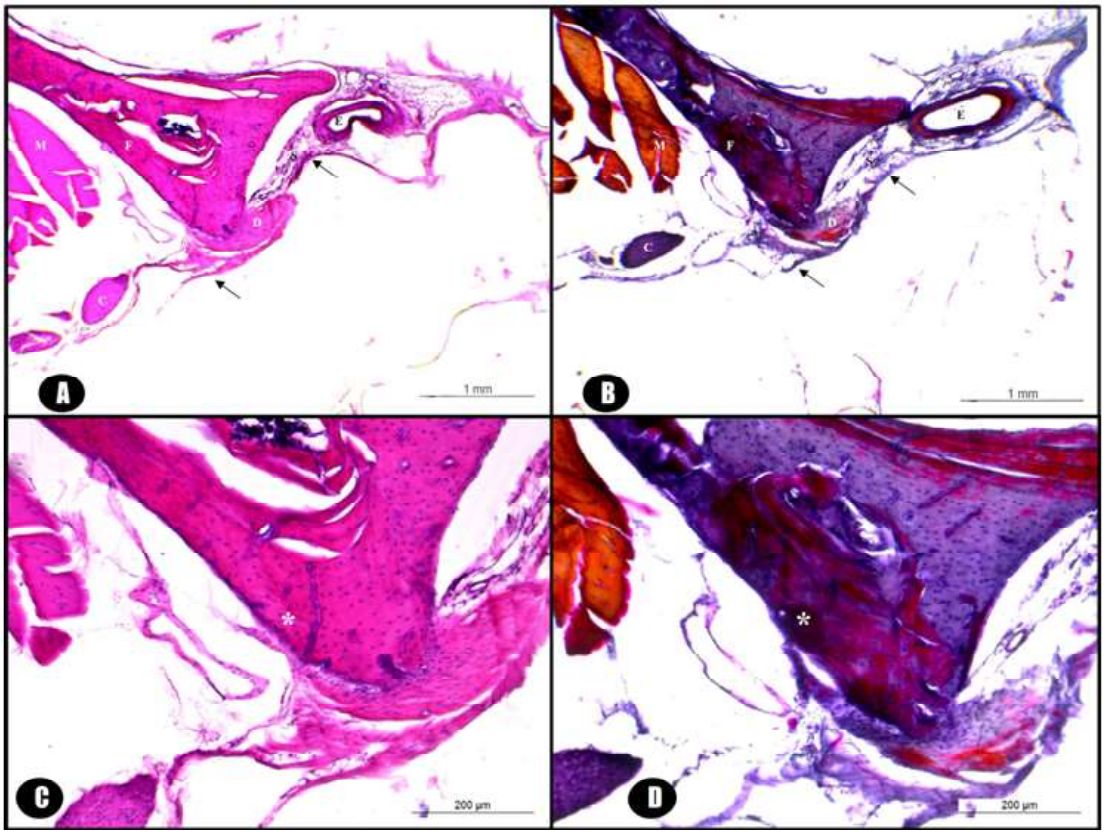

Fig. 1: TMJ sagittal section photomicrographs. A. H&E at (X4). B. Goldner's trichrome technique at (X4). Black arrow: joint capsule; C: mandibular condyle; D: articular disc; E: external auditory canal; F: mandibular fossa; M: pterygoid muscles; S: synovial membrane. C. H&E at (X10). B. Goldner's trichrome technique at (X10). White asterisk: articular surface.

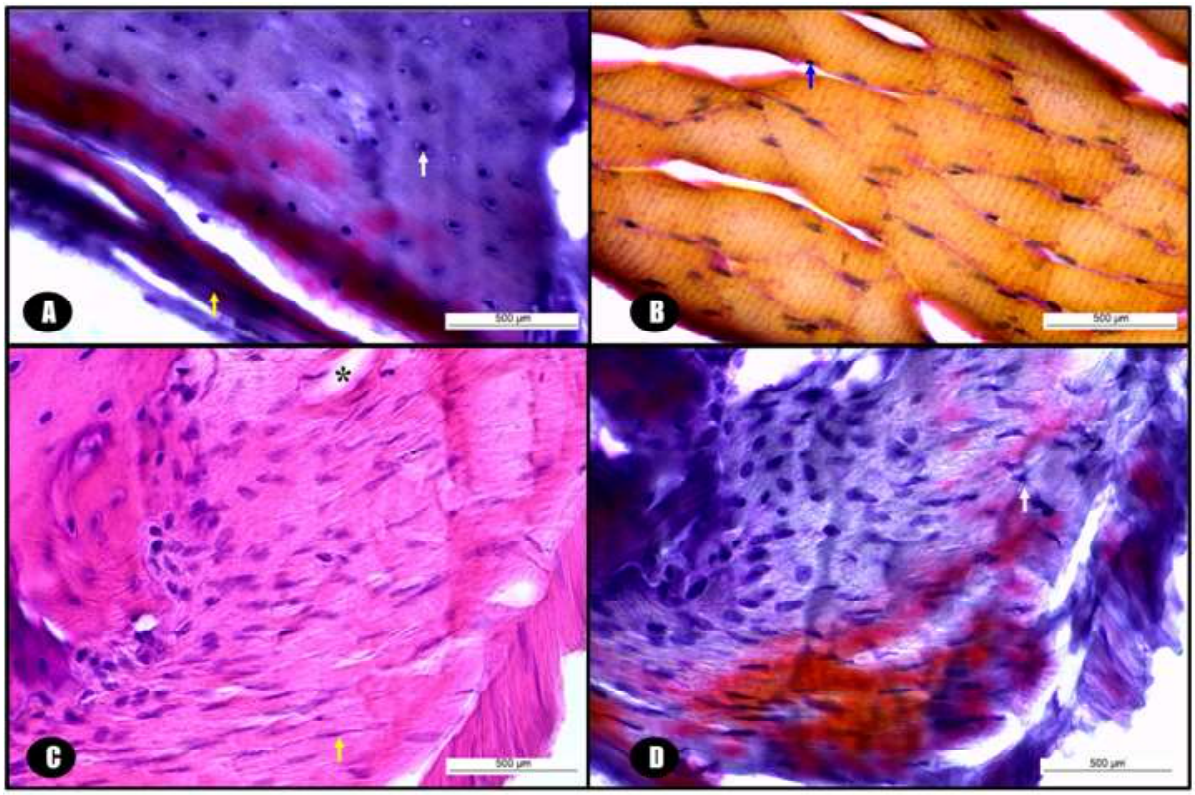

Fig. 2: Cellular detail on sagittal section photomicrographs at (X40). A. Mandibular fossa with Goldner's trichrome technique. Yellow arrow: fibroblast; white arrow: chondrocyte. B. Lateral pterygoid muscle with Goldner's trichrome technique. Blue arrow: satellite cell. C. H&E. Black asterisk: blood vessel; yellow arrow: fibroblast. D. Goldner's trichrome technique. White arrow: mesenchymal cell.

have an oval basophilic cytoplasm within pale lacunae. The mandibular fossa, also fibrocartilage, is stained purple for older tissue and red for new cartilage deposits, with chondrocytes presence. A dense connective tissue on the periphery includes fibroblasts with basophilic spindle-shaped cytoplasm and elongated nucleus (Figure 2A). Nearby, the pterygoid muscles, skeletal or striated muscle, consist of multiple muscle fibers appearing as long orange cytoplasm and cylindrical basophilic multinucleated structures. Muscle satellite cells display a round and very dark nucleus (Figure 2B).

Between the mandibular condyle and fossa, the articular disc exhibits a dense regular connective tissue with numerous fibroblasts aligned with collagen fibers, and a few mesenchymal cells. A couple of blood vessels are also visible (Figure 2C and 2D). The joint capsule, a fibrous membrane encapsulating the synovial membrane, showcases loose connective tissue and blood vessels in two layers: the intimal synovial, which borders the cavities of the joint, and the subintimal attached to the fibrous connective tissue of the capsule. The subintimal layer, includes fibroblasts, white adipocytes, and collagen fibers, while the intimal layer displays synovial cells. These cells present a basophilic stain, polygonal to elongated cytoplasm, and oval to elongated central nuclei (Figure 3).

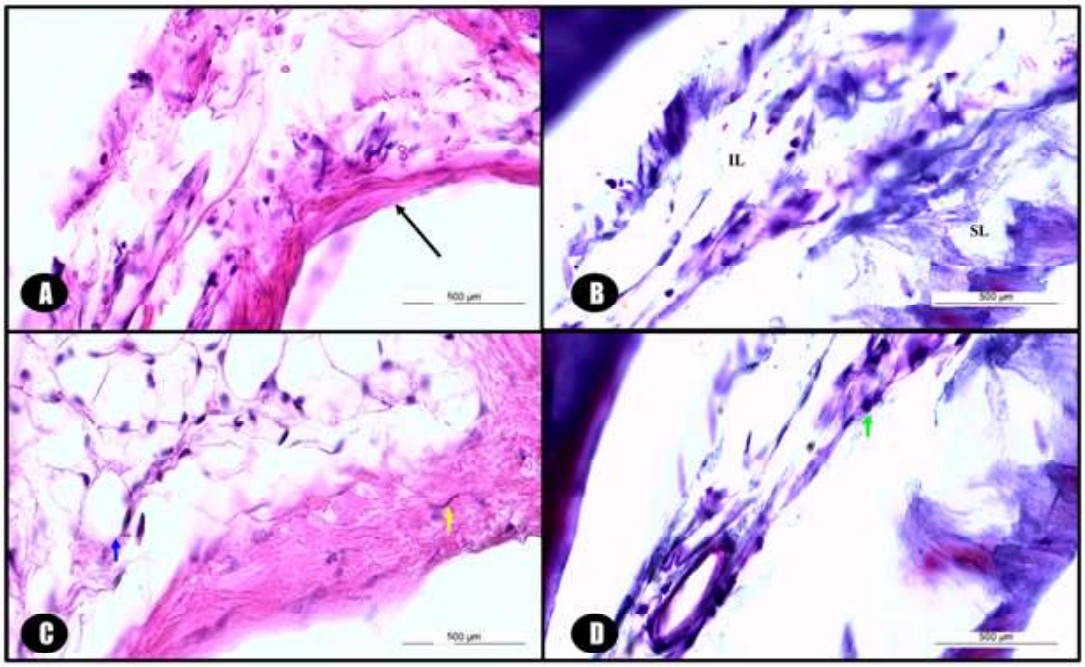

Fig. 3: Joint capsule and synovial membrane at (X40). A. H&E. Black arrow: joint capsule. B. Goldner's trichrome technique. IL: intimal layer; SL: subintimal layer. C. H&E. Yellow arrow: fibroblast; blue arrow: white adipocyte. D. Goldner's trichrome technique. Green arrow: synovial cell.

DISCUSSION

The temporomandibular joint is a collection of anatomical structures that, with the participation of special groups of muscles, allows the jaw to perform various movements during chewing [15]. Classically, it has been described as a bilateral synovial joint of the ginglymo-arthroid type, which allows rotation and displacement of the mandibular condyle [17,18] in addition to opening, closing, protrusion, retrusion and laterality movements of the mandible [19,20].

Due to the above, the reconstruction of the TMJ, both morphologically and functionally, after its surgical extraction is extremely difficult [21]. Thanks to the fact that this was known in advance, the articulation could be preserved and it was not disarticulated in any of the steps of the applied histological technique. Therefore, on the sagittal section of the joint, the capsular ligament that wraps the structure was able to be preserved.

Histological studies of the TMJ are fundamental to determining the morphological and

anatomical structure of this rat joint. According to Basting et al., this kind of paper allows correlating, and detection anomalies based on cellular morphological alteration in tissues. Consequently, the histological protocol followed guaranteed the good quality of the histological analysis [22]. The fixation of a fresh sample due to perfusion reduced the tissue changes and was according to the bone processing protocol mentioned by Sanderson [23]. Decalcification was also an imperative step to accomplish bone cutting to shelter the specific area of interest. And in agreement with Basting et al., acid decalcification velocity is associated with integrity of morphological structures [22]. Microtome cuts of 4µm together with the histological stain and the correct mounting technique, secured the tissue and cell morphology as well.

In consonant with Goldner [6], chondrocytes cytoplasm stain in red, nuclei in black, collagenous matrix in orange, and bone tissue and cartilage in green [7]. However, the cartilage extracellular matrix was stained red and purple, being the red the one that is more exposed to mechanical load. Chondrocyte cytoplasm was red and also purple and, in fact, had black nuclei. Bone tissue stained purple, and the muscle tissue stained orange. Nevertheless, this stain offers exceptional clarity and detail in revealing tissue cells: osteoclasts, osteoblasts, fibroblasts [10], also chondroblasts, chondrocytes, satellite cells, mesenchymal cells, white adipocytes, epithelial and synovial cells.

As reported by Farfán et al., the bilaminar zone of the synovial membrane generally consists of loose connective tissue characterized by the presence of a network of collagen fibers and abundant elastic fibers. These elastic fibers are distributed in both layers [24], but only one of these fibers was on the subintimal layer, which stained purple with the trichrome stain.

The limitations of this paper were not only the limited sample size but also the ambitious protocol used to guarantee an accurate anatomical sagittal section of the TMJ. Further studies should consider using the cutting protocol used by Basting et al. to determine the anatomical portion of the TMJ [22], obtaining a small and easier-to-work-with sample. Working with both sagittal and coronal sections of the joint could enrich future discussions.

CONCLUSION

Both histological stains showed cellular and tissue components of the temporomandibular joint. A detailed protocol for obtaining complete sagittal histological samples of rat TMJ was also established. Goldner's trichrome stain allows simpler tissue identification and detailed cellular morphology observation.

ABBREVIATIONS

H&E: Hematoxylin and eosin
TMJ: Temporomandibular joint

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Author Contributions

Effective scientific and intellectual participation in the study, technical procedures, image acquisition, data interpretation, preparation and draft of the manuscript, critical review and final approval: DBG.

Conflicts of Interests: None

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