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MORPHOLOGY AND ULTRASTRUCTURE OF THE TEMPOROMANDIBULAR JOINT DISC IN HUMAN FETUSES 21 TO 28 WEEKS OLD

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ABSTRACT

Ultrastructure of human fetuses temporomandibular joint is poorly understood specially by scanning electron microscopy and cryofracture techniques. In this work, the morphology of the temporomandibular joint (TMJ) articular disc and its insertions was examined in 12 human fetuses. Four TMJs were sectioned in the frontal and sagittal planes and five were cryofractured prior to examination using light and scanning electron microscopy. The three remaining TMJs were fixed, embedded in paraffin, sectioned and stained with HE and NMC trichrome. The articular disc inserted into the mandibular head, temporal bone, joint capsule and upper head of the lateral pterygoid muscle. The fibrous architecture of this disc was complex, with longitudinal fibers predominating in the upper and lower regions, and interposed transverse and oblique fibers predominating in the central region.

Key words: Fetus, light microscopy, scanning electron microscopy, temporomandibular joint disc

INTRODUCTION

In the developing fetus, the region between the cartilage of the mandibular head and the articular surface of the developing temporal bone is filled with ectomesenchyme until about the twelfth week of intrauterine life. At approximately the thirteenth week, the cells in the central region of this ectomesenchyme differentiate into fibroblasts, particularly in the regions adjacent to the mandibular head and temporal bone, and small gaps appear between the ectomesenchymal cells, eventually uniting to form the upper and lower chambers; the newly differentiated fibroblasts of the central region produce collagen that forms the articular disc [7,17].

The articular disc divides the joint cavity into an upper and lower chamber. The lateral and medial margins of the articular disc curve towards the corresponding poles of the mandible. Anteriorly, the disc is divided into upper and lower layers, with some bundles of muscle fibers from the upper head of the lateral pterygoid muscle being attached to these layers. Posteriorly, the articular disc is also divided into upper and lower layers between which there is a gap filled with loose connective, vascularized tissue [1,2,16].

The articular disc contains bundles of type I and III collagen fibers that are arranged anteroposteriorly and obliquely in the anterior and median portions. The posterior portion of the disc is thick, with a fiber bundle ring arranged in laterolateral position. In the upper and lower surfaces of the articular disc, a thin layer of fibers is coated by thick, laterolaterally-oriented fibers arranged perpendicularly [3,4,9,11, 13,18,20,21,26].

In this work, we used light and scanning electron microscopy to examine the morphology of the articular disc and its insertions in the masticatory muscles and TMJ capsule in human fetuses.

MATERIAL AND METHODS

The temporomandibular joints (TMJ) of 12 human fetuses 21 to 28 weeks old were used. The gestational age of the fetuses was determined based on the distance between the skull vertex and the midpoint between the apices of the buttocks (CRL - crown-rump length) [17].

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This study was approved by the Ethics Committee of the Federal University of São Paulo-Paulista School of Medicine (protocol number 0917/04).

For light microscopy, three previously fixed TMJ were rinsed with distilled water, dehydrated in 70% and 100% ethanol, clarified in xylol and embedded in paraffin. Frontal semi-serial (two TMJ) and sagittal (one TMJ) sections 4 µm thick were obtained along the entire extension of the TMJ. The sections were collected on glass slides and stained with hematoxylin-eosin (HE) and Nylceo Marques de Castro´s trichrome [6] and then covered with a coverslip (50% of the slides) prior to examination and photodocumentation.

For scanning electron microscopy, nine TMJ previously fixed in 4% formaldehyde were rinsed in 0.1 M cacodylate buffer, pH 7.2. Four TMJ were sectioned to provide frontal and sagittal sections of the layers (three sections ~4 µm thick per TMJ). The remaining five TMJ were cryofractured in liquid nitrogen. Some of the fragments were treated with decreasing concentrations of sodium hypochlorite (2% to 0.2%), and dehydrated in an increasing ethanol series (50%, 70%, 90% and 100%) [11]. The fragments were critical point dried using carbon dioxide in a Balzers CPD010™ unit and attached to the specimen holder. The samples were then coated with gold in a Balzers SDC050 Sputter Coater™ and examined with a Jeol 5300™ scanning electron microscope in the Electron Microscopy Center at the Federal University of São Paulo-Paulista School of Medicine (UNIFESP-EPM).

RESULTS

Light microscopy

Sagittal sections of the peripheral region of the disc showed that the fibers were arranged longitudinally on the surface and, in the upper and lower portions of the central region of the disc, they were arranged in an anteroposterior fashion (Figs. 1 and 2); frontal sections showed that the upper and lower chambers were separated by the articular disc (Fig. 3).

In the lateral region of frontal sections, the disc curved downwards and its upper surface extended upwards to insert in the joint capsule that merged with the lateral ligament (Fig. 3). Upper and lower medial disc insertions were present (Fig. 4). Sagittal sections confirmed the upper and posterior articular disc insertions. The bilayer zone, which consisted of highly innervated, vascularized loose tissue, was located between the two layers (Fig. 5).

Scanning electron microscopy

Scanning electron microscopy showed the articular disc located between the mandibular fossa and head (Fig. 6). Figures 7 and 8 show the anterior and posterior disc insertions, respectively. The fibrous architecture of this disc was complex and consisted of longitudinal, oblique, and transverse collagen fibers bundles (Fig. 9).

Figure 1. The peripheral portion of the articular disc showing the longitudinal fibers in the upper and lower surfaces of the disc.

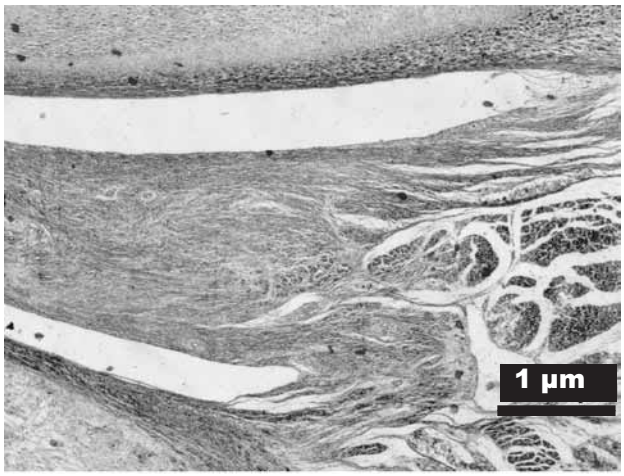

Figure 2. The anterior portion of the disc showing the upper and lower chambers separated by the articular disc.

Figure 3. The articular disc showing the joint capsule (★) and the insertion in the lower surface (●), in the fibrocartilage of the mandibular head, that limits the space of the lower chamber. The (✱) indicates the insertion of the upper surface of the disc in the joint capsule.

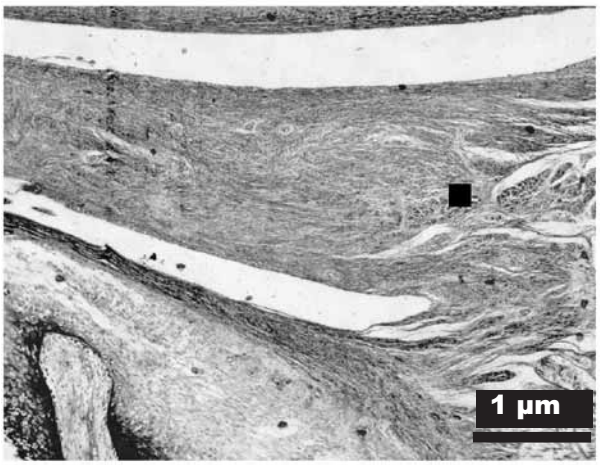

Figure 4. The medial insertions of the articular disc. The (■) indicates the upper insertion.

DISCUSSION

In this study, fetuses 21 to 28 weeks old (corresponding to the end of the 2nd and beginning of the 3rd gestation quarter) were used. Van der

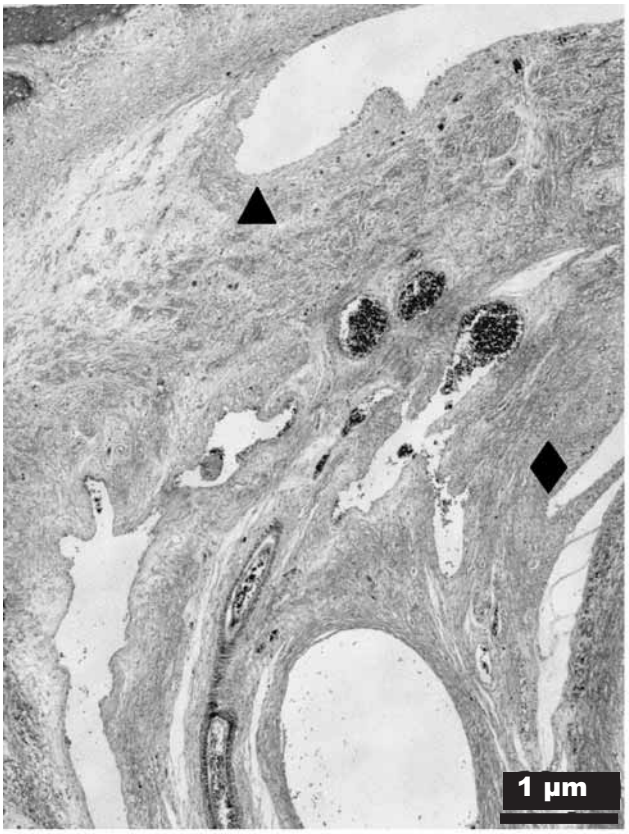

Figure 5. The posterior and upper insertions of the articular disc in the synovial membrane (▲) and the posterior and lower insertions of the disc in the fibrocartilage of the mandibular head (◆). The bilaminar zone with large blood vessels is located between the two layers.

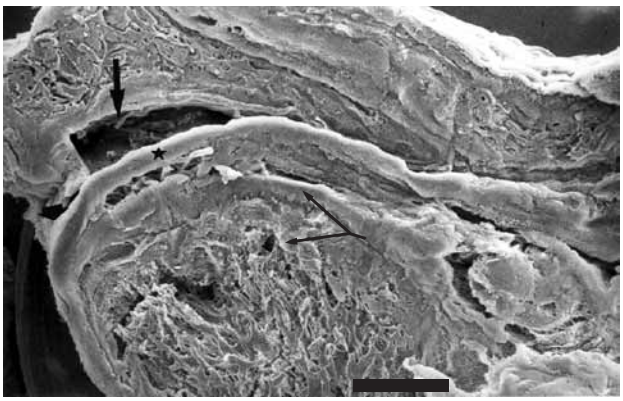

Figure 6. Scanning electron micrograph of a frontal section of the TMJ. The articular disc (★) is interposed between the temporal bone (large arrow) and mandibular head (small arrows). The short arrow indicates the lateral ligament of the articular disc. Bar = 1 mm.

Linden *et al.* [22] used 52 fetuses with ages varying from 32 days to 22 weeks. Caltabiano *et al.* [5] used light microscopy to study 20 human fetuses from 6 to 22 weeks old and 10 neonatal infants. Wong *et al.* [23] examined the articular disc in eight fetuses 13 to 17 weeks old. Other studies have used older fetuses in their analysis. Ohta *et al.* [15] studied 10 fetuses ranging in age from 4 months to full-term, and Minarelli *et al.* [13] used 15 fetuses ranging from 16 to 39 weeks of intrauterine life. As in the present study, all of these investigations used the CRL method [17] to determine the fetal age.

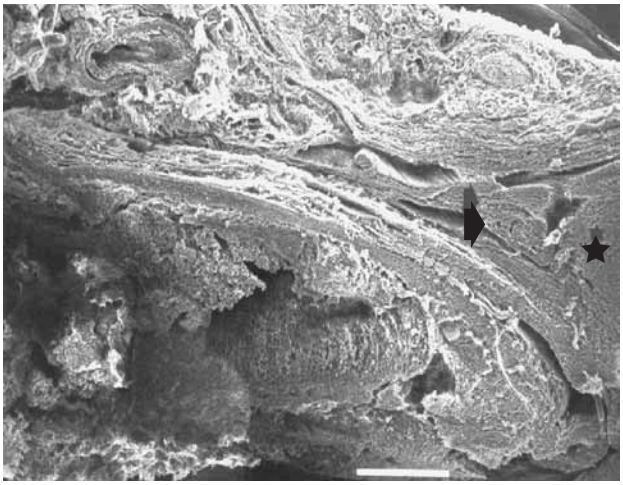

Figure 7. Scanning electron micrograph of a sagittal section of the TMJ showing the anterior insertion of the disc (◗) and the upper head of the lateral pterygoid muscle (★). Bar = 500 μm.

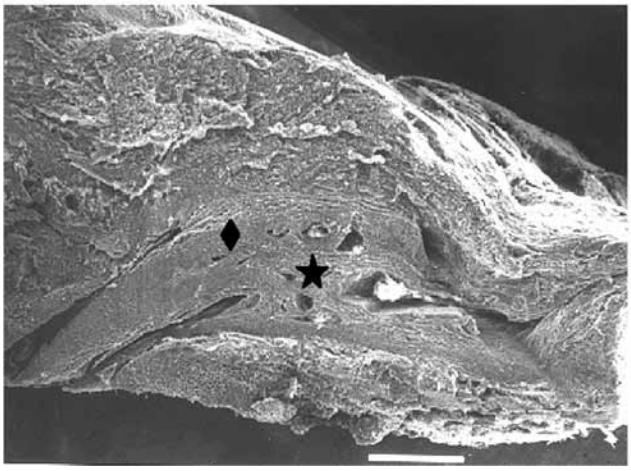

Figure 8. Scanning electron micrograph (cryosection) of a frontal section of the TMJ showing the posterior insertion of the disc (◆) in the mandibular head at the lower end and in the temporal bone at the upper end. The star (★) indicates the bilayer zone with blood vessels of different calibers. Bar = 500 μm.

Van der Linden *et al.* [22] observed that the articular disc differentiates around the 7th week. Around the 10th week, the first collagen fibers appear and, around the 20th week, its fiber structure is fully developed, with a thinner central region. This thinner aspect is already visible in the 12th week, when the disc is still flat and remains so until birth [10,15]. The disc differentiates between the condylar and temporal blastemata, with interposed mesenchymal tissue [19], or exclusively from the condylar blastema [24]. However, the medial portion has been hypothesized to derive from the lateral pterygoid muscle tendon [8].

The articular disc divides the temporomandibular joint chamber into upper and lower chambers that are delimited by the fibrocartilaginous surfaces of the bones and by disc insertions. These insertions in the adjacent structures are represented by the continuity of collagen fibers that form the disc architecture. The fiber arrangement in the fetal articular disc is different from that in adults since in the former there is a large number of cells present between the fiber bundles and blood vessels.

Using light microscopy, Bont *et al.* [4] found that the articular disc in adults contains dense layers of cartilaginous tissue composed of chondrocytes and collagen fiber bundles that run in all directions. However, their observations regarding the surface chondrocytes of adult articular discs were incomplete and probably related to fibrochondrocytes, although

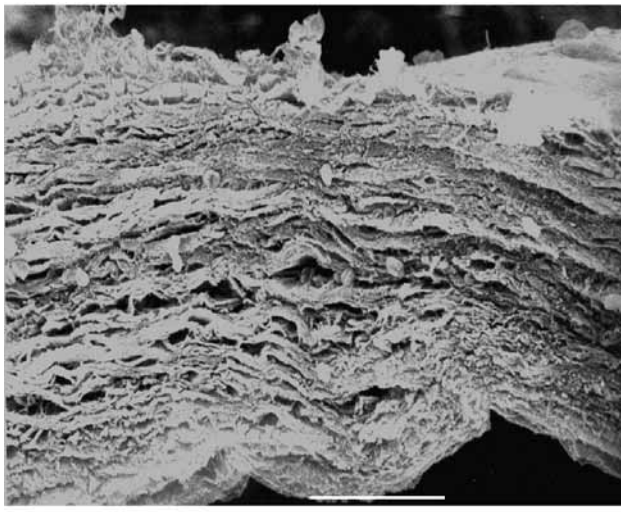

Figure 9. Scanning electron micrograph of a frontal section of the TMJ showing the complex fiber architecture of the articular disc, with longitudinal fibers predominating in the upper and lower portions and oblique and transverse fibers interposed in the central region. Bar = 50 μm.

there are no studies with which to compare their findings [9,13].

In fetuses, children and adults, the collagen fibers have a stratified arrangement and are organized into anteroposterior, laterolateral and oblique bundles that form a mesh. According to Minarelli *et al.* [11], and in agreement with our findings, transverse fiber bundles predominated in the posterior region of the disc and formed a ring that encircled the disc, whereas the central region contained predominantly fibers that were arranged longitudinally in an anteroposterior direction.

As shown here, the articular disc had insertions in the joint capsule, the fibrocartilaginous coating of the TMJ bone structures, and the upper head of the lateral pterygoid muscle, with an insertion also present in the petrotympanic fissure in continuity with the anterior malleolar (mallear) ligament (also known as the discomalleolar or discomallear ligament).

In fetuses, the inferior layer of the disc inserts in the fibrous part of the fibrocartilage around the mandibular head whereas in adults, this insertion is found at a lower position. The upper layer of the disc inserts laterally in the joint capsule and is attached to the lateral ligament medially in the capsule and in the fibrocartilage of the mandibular fossa, as in the anterior and posterior regions, where there is continuity with the capsule in the future articular tubercle and the petrotympanic fissure region, respectively. The disc and its insertions become defined when the joint chambers emerge from small cracks that coalesce to form the lower chamber in the 10th week and the upper chamber around the 11th week. The disc fills this space around the 17th week and accompanies the shape of the mandibular fossa and condyle [22].

The fiber muscles of the lateral pterygoid muscle head in fetuses penetrate the articular disc in an anteromedial direction and insert into the connective tissue. Ohta *et al.* [15] and Mérida-Velasco *et al.* [10] reported that from the 4th month of intrauterine life onwards the collagen fibers of the anterior disc band head towards and insert into the epimysium and endomysium of the lateral pterygoid muscle. Minarelli and Liberti [12] demonstrated that the lateral pterygoid muscle inserts into the anteromedial margin of the articular disc. However, it is uncertain whether fibers from the upper head of the lateral pterygoid muscle insert into the adult articular disc [25]. Other muscles have been reported to insert into the articular disc [10]. However, although the temporalis and masseter muscles were close to the temporomandibular joint in the fetuses studied here, no fibers from these muscles inserted into the disc.

Our results indicated that all of the structures of the temporomandibular joint were present in 21- to 28-week-old fetuses and that despite some differences compared to the adult temporomandibular joint, all of the functional possibilities were also present [14]. The fibrous architecture of the articular disc was complex, with longitudinal bundles of collagen fibers predominating in the upper and lower regions, and interposed transverse and oblique fibers predominating in the central region.

Although our findings were similar to those reported by others, scanning electron microscopy demonstrated that collagen fibers from the lateral pterygoid muscle are inserted into the articular disc. The use of cryofractured specimens to analyze the bilaminar zone and its blood vessels has not been reported by others. An additional novel finding, revealed by scanning electron microscopy, was the arrangement of the fibers of the upper, lower, and central portions of the articular disc.

In conclusion, our results showed the complex morphology of the human fetuses TMJ articular disc, especially in the central portion of the disc (unexplored by others authors). In addition to light and scanning electron microscopy, the use of immunohistochemistry methods to study alterations in morphology and protein expression during embryogenesis should contribute to our understanding of the developmental biology of the TMJ in human fetuses.

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