Ultrastructural evidence of vascularization of the central zone of the temporomandibular joint disc in human fetus

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SUMMARY

The present study examined the ultrastructural features of the articular discs of the temporomandibular joint (TMJ) of nine human fetuses aging from 21 to 28 weeks. The specimen of the fetal articular discs was sectioned frontally and sagitally and examined under the scanning electron-microscope. The ultrastructural survey of the architecture of the articular disc revealed three bands composed of fibrocartilaginous tissues in the articular disc: namely, 1) anteromedial longitudinal fibers, the dominant component in the superior and inferior portions; 2) anteroposterior transversal fibers, 3) central diagonal fibers. The central portion of the articular disc showed scattered blood vessels containing erythrocytes. To our knowledge, this is the first description of the presence of blood vessels in the central articular of the human TMJ articular disc. This evidence is also lacking in classic textbooks of Anatomy and Developmental Biology.

Key words: Temporomandibular joint disc – Vascularization – Human fetus – Scanning electron microscopy

INTRODUCTION

Many authors have addressed the morphogenesis of the articular disc pertaining to the

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temporomandibular joint (TMJ) in adult humans. In human fetuses, in fourteenth week (14th) of fetal gestation the endochondral ossification process is initiated in the cartilage of the mandibular condyle and forms a secondary centre of mandibular ossification. The layer of cartilaginous tissue facing the infradiscal cavity covered by dense conjunctive tissue generates the remaining portion of the TMJ articular disc. Between the eighteenth (18^{th}) and twentieth (20^{th}) , the mandibular fossa and the articular tubercle of the temporal bone acquire a profile similar to that of the mature form. The synovial membrane appears and, finally, the articulation becomes functional. During intrauterine life, gentle movements of the mandibular joint help to shape the final articular disc. During the last trimester of intrauterine life, small modifications can be seen such as the appearance of radial slits in the articular cartilages. The latter are covered by the vascularized, dense conjunctive tissue. These slits extend from the articular surface to the central part of the mandibular condyle, and separate the areas that are undergoing active endochondral ossification (Caltabiano et al., 1990; Bach-Petersen et al., 1994; Katchburian and Arana, 2004). The articular capsule delimitates the articular cavity into two compartments by a disc formed by a dense conjunctive tissue.

The main histology of the articular disc is fairly consistent in most reports but there is a discrepancy in the presence of vascularization in terms of articular disc tissues (Mérida-Velasco et al., 1993). According to the descriptions of Katchburian and Arana (2004), the articular disc is composed of three bands consisting of dense conjunctive, nonvascularized tissue. This can also be seen in the tissue covering the mandibular condyle and the mandibular fossa of the temporal bone, which has fibroblasts and fibrochondrosteal and undifferentiated cells sparsely scattered among interlacing collagen fibers. The posterior region, by contrast, consists of denser conjunctive tissues without vascularization. According to Zarb et al. (2000), the articular disc has abundant peripheral vascular supplies, but there are no vascularized signs in the central region.

The aim of this study is to demonstrate the presence of blood elements in the central region of the articular disc of human fetuses by scanning electron microscopy.

MATERIALS AND METHODS

The protocol of the study was reviewed and approved by the Committee of Ethics in Research of UNIFESP-EPM. The protocol number of the approval is 329/01.

The articular discs of the TMJ of nine human fetuses (aged 21 to 28 weeks) were obtained from collections at the UNIFESP-EPM and UFSM (Universidade Federal de Santa Maria). The ages of the fetuses were estimated by measuring the dimensions between the external occipital protuberance and the coccyx – (CRL, crown-rump length) (Sadler, 2005) and were 230 to 270 mm. The fetuses were preserved in 10% of formalin solution.

In the preparation for examination under the scanning electron microscope (SEM), the samples were rinsed in 0.1M cacodylate, pH 7.2. Then, they were sectioned frontally (n=4)and sagittally (n=5) in three sections of approximately 4 mm thickness. Freeze-fractured from liquid nitrogen were also done in five joints. Some sectioned samples were immersed in diluted sodium hypochlorite (from 2% to 0.2%) (Minarelli et al., 1997). Following dehydration an increasing gradient of ethanol (50%, 70%, 90% and absolute), the specimens were critical point-dried with liquid carbon dioxide in a Balzers' apparatus (CPD 010^{TM}) and fixed in the specimen holder. The samples were gold-sputtered in the Balzers apparatus (SDC 050 Sputter Coater[™]) and examined under a SEM (Jeol, 5300TM) at the Electron Microscopy Center of UNIFESP-EPM.

Results

Scanning photomicrography revealed that the articular disc is disposed between the mandibular fossa and the mandibular condyle. The latter showed two type of textures: an osseous tubercle, which was thinly trabeculated, and another composed of dense conjunctive tissue that covered the osseous texture (Figure 1).

Lateral insertion of the disc was seen in the dense conjunctive tissue of the mandibular condyle, binding to the infradiscal space, and in the articular capsule, where the disc was fused to bind the supradiscal space (Figure 1). The posterior insertion of the disc was inferiorly seen in the mandibular condyle, through a fiber lamina that ran inferiorly and superiorly to the temporal bone. In this region, the



Fig. 1. Scanning electron microscopy, frontal section, supradiscal cavity (\downarrow); articular disc interspaced between the mandibular fossa and mandibular condyle (\Leftrightarrow); lateral insertion of the disc (\downarrow); mandibular condyle with two textures: osseous, thinly trabeculated (\checkmark) and fibrocartilaginous (\aleph). Scale bar = 1 mm.



Fig. 2. Cryofracture. Observe the petrotympanic fissure (\Rightarrow) and bilaminar zone containing different calibre blood vessels (\Rightarrow). Scale bar = 500 µm.



Fig. 3. Scanning electron microscopy, frontal section; note the complex longitudinal fibril architecture of the articular disc in the longitudinal, transversal and oblique directions. Scale bar = $50 \ \mu m$.

compact aspect of the disc fibers changed progressively in the posterior region to assume a looser aspect. Another observation in this region was the presence of blood vessels, of different calibers; these were interspaced between the laminae (bilaminar region, Figure 2).

This region was oblique freeze-fractured and its electron micrographs showed the aspects of the complementary faces: the insertion of the inferior lamina of the disc into the mandibular condyle; the blood vessels, and the relationship of the superior lamina to the temporal bone, laterally to the petrotympanic fissure (Figure 2). The supra- and infradiscal spaces were seen to be limited by the synovial membrane lying over the disc insertions (Figure 2).

The fibrillary architecture of the articular disc proved to be complex. The fibers were disposed differently: 1) in the longitudinal direction (lateral-medial), prevailing in the superior and inferior regions; 2) in the transversal direction (anterior-posterior); and 3) in the oblique direction, interspaced in the central region (Figure 3). In the central region, erythrocytes were also noted inside the blood vessel (Figure 4).

DISCUSSION

The presence of blood vessels in the TMJ articular disc of the human fetuse has been described by many authors. Van der Linden et al. (1987) noted that capillaries were distributed in the periphery of the anterior and posterior portions of the articular discs (9 to 230 mm, CRL) of 52 fetuses aged 33 days to 33



Fig. 4. Scanning electron microscopy, frontal section showing blood vessels in the central region of the articular disc containing erythrocytes (\mathbf{I}). Scale bar = 10 μ m.

weeks. However, they failed to notice any blood vessels in the central region of the articular disc. Wong et al. (1985) observed that in fetuses (intrauterine age 13 to 17.5 weeks, 100 to 155 mm of CRL) blood vessels are mainly located anteriorly and posteriorly, but no evidence of vascularization was found at the center of the articular discs. Thilander et al. (1976) studied 61 specimens of articular discs aged 2 days to 26 years and concluded that vascularization only exists in the first year of life, after which it disappears. They also reported that there are no references that describe in which region of the disc this vascularization is found.

Interestingly, in the present study we observed that the central portion of the articular disc displayed blood vessel containing red blood cell in the TMJ of human fetuses. If these cells do not originate in the central zone of the disc, another possibility is that they might possibly diffused in from the surrounding the area. Using histological approaches, for example immunohistochemistry, the source of the erythrocytes could possibly be confirmed, hence clarifying the presence of blood vessel in the central portion of the articular disc.

In our present study, we not only confirm the existence of blood vessels in the anterior and posterior positions of the articular disc, but report that the central portion of the articular disc contains evidence of vascularization. To our knowledge, this is the first study showing evidence of the presence of blood vessel in central portion of the TMJ articular disc of human fetuses.

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