Qualitative Study of Young, Adult, and Aged Wistar Rats Temporomandibular Synovial Membrane Employing Light, Scanning, and Transmission Electron Microscopy

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ABSTRACT The aim of this study was to analyze the rat temporomandibular joint (TMJ) synovial membrane at different ages using light, scanning, and transmission electron microscopy. Under light microscopic analysis, the TMJ structures were observed such as condyle, capsule, disk, the synovial membrane collagen type, and cells distribution. In the scanning electron microscopy, the synovial membrane surface exhibited a smooth aspect in young animals and there was an increase with ageing in the number of folds. The transmission electron microscopic analysis showed more synoviocytes in the synovial layer in the young group and still a great number of vesicles and cisterns dilation of rough endoplasmic reticulum in the aged group. In the three groups, a dense layer of collagen fibers in the synovial layer and cytoplasmic extensions were clearly seen. It was possible to conclude that synovial membrane structures in aged group showed alterations contributing to the decrease in joint lubrication and in the sliding between disk and joint surfaces. These characteristic will reflect in biomechanics of chewing, and may cause the TMJ disorders, currently observed in clinical processes. Microsc. Res. Tech. 75:1522–1527, 2012. © 2012 Wiley Periodicals, Inc.

INTRODUCTION
Temporomandibular joint (TMJ) disorders in humans have controversial treatment among healthcare professionals and hence the normal structural and ultrastructural morphology in different stages of life is necessary to propose more effective treatments.

Studies in humans are usually carried out from autopsy samples but the clinical conditions and the articular pre-existing illnesses cannot be accurately evaluated through the familiar and clinical histories (Lekkas et al., 1988). Considering these facts and other difficulties such as standardized operational protocols of age, gender, race, and period of treatment or illness, animal specimens are being used for the TMJ study and the existence of anatomical and functional differences between animal species must be considered (Ogi et al., 1997).

Several structural and ultrastructural articular aspects were studied using methods of light, transmission, and scanning electron microscopy, such as ultrastructure of articular cartilage of the mouse TMJ (Yoshida et al., 2004), calcification of articular cartilage in monkeys (Luder and Schroeder, 1992), the normal morphology and steroids use in rat knee synovial membrane (Murashige et al., 1999), the ultrastructure of articular cartilage of the chicken knee (Graf et al., 1993), and synovial membrane vascular architecture of the rat knee joint (Funk et al., 1995).

Ageing is a natural process, which causes several morphological and physiological changes in different tissues and organs. These changes predispose tissues to various diseases, and the temporomandibular disorders have shown a drastic increase in dentistry offices in the last years (Nozawa-Inoue et al., 2003).

Although it is widely known the structures and functions of synovial membrane, little is known about their structural and ultrastructural aspects during the ageing process. The aim of this study was to analyze the rat TMJ synovial membrane at different ages, using the methods of light, scanning, and transmission electron microscopy.

MATERIALS AND METHODS
In this descriptive study, 27 male Wistar rats used were divided into three groups: Young (1 month of age), Adult (4 months of age), and Aged (24 months of age). In each group, three animals were used for light microscopy, three for scanning electron microscopy, and three for transmission electron microscopy.

The procedures were approved by the Ethics Committee on Animal Experiments of the Federal University of São Paulo (Protocol 0233/08). The animals were kept in cages with light/dark cycle of 12 h, room temperature (25 ± 2°C), chow, and water ad libitum.

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Light Microscopy

For light microscopy analysis, animals were anesthetized intraperitoneally with urethane overdose (3 g/kg, i.p.) (Ciena et al., 2010). TMJs were removed in a block containing the skull base, the mandible, and adjacent soft tissues and fixed in Bouin’s solution. After fixation, they were decalcified using 10% ethylenediaminetetraacetic acid solution. The specimens were dehydrated, embedded in paraffin, cut transversal to the condyle with 5 μm thick and stained with Hematoxylin–Eosin and Picrosirius red for the examination under polarized light (Nikon Eclipse E1000, Software Image-Pro Plus 4.5).

Scanning Electron Microscopy

Animals were anesthetized using the same conditions, perfused, and fixed with modified Karnovsky solution, containing 2.5% glutaraldehyde and 2% paraformaldehyde in sodium phosphate buffer 0.1M for 12 h at 4°C (Watanabe and Yamada, 1983). The tissues were dissected under a surgical microscope, and removed the condylar process of mandible, containing the capsular joint membrane. After the mandibular condyle section, the synovial membrane was reversed exposing the articular disk. Samples were fixed in the same solution for 8 h at 4°C. After rinsed in distilled water, the samples were postfixed in 1% buffered osmium tetroxide for 2 h at 4°C. Dehydrated in an increasing series of ethanol, and dried in a critical point apparatus, Balzers CPD-030. These samples were mounted on metal stubs using double-sided carbon tape and coated with gold on ions sputter, Balzers SCD-040. The samples were examined in a scanning electron microscope, Jeol-JSM 6100, in the Institute of Biomedical Sciences, University of São Paulo.

Fig. 1. Light microscopy images of the TMJ synovial membrane. Young (A and B), adult (C and D), and aged groups (E and F). Sections stained by Hematoxylin–Eosin (A, C, and E) reveal the characteristics of the cellular distribution in the synovial membrane surface (arrows), the condylar articular surface (arrowheads). Sections stained by Picrosirius red and examined under polarized light microscopy (B, D, and F) reveal the distribution of collagen type I (reddish) and III (greenish) in the synovial membrane (arrows) and articular surface (*). Scale Bars: 100 μm.
Transmission Electron Microscopy

Animals were anesthetized using the same conditions, perfused, and fixed with modified Karnovsky solution containing 2.5% glutaraldehyde and 2% paraformaldehyde in sodium phosphate buffer 0.1M (Ciena et al., 2011). Tissues were removed and sectioned in small blocks, fixed in the same solution for overnight at 4°C. Then, the samples were rinsed in sodium phosphate buffer during 15 min and postfixed in 1% buffered osmium tetroxide solution for 2 h at 4°C. The dehydration was performed in an increasing series of ethanol and embedded in Spurr® resin. Thin sections (0.5 μm) were cut in ultra-microtome Reichert Ultra Cut® and stained with 1% toluidine blue and ultrathin sections (90 nm) were obtained using a diamond knife and collected in a 200-“mesh” grid. The grids were counterstained using 4% uranyl acetate solution and 0.4% lead citrate solution (Watanabe and Yamada, 1983). The tissues were examined in a transmission electron microscope, Jeol-1010, in the Institute of Biomedical Sciences, University of São Paulo.

RESULTS

Observations under light microscopy of young, adult, and aged animals exhibited the structural characteristics related to articular surface of the mandibular condyle covered by hyaline cartilage, inferior joint space, the articular disk, and the spatial distribution of synoviocytes nuclei on the surface of synovial membrane inserted in the margins (Figs. 1A–1F). At higher magnification, in the young group the smooth synovial membrane surface can be noted (arrows, Fig. 2B). However, in adult group there are evident folds (arrows, Fig. D) and in the aged group, an irregular aspect in face of several deeper folds in the surface (arrows, Fig. F) can be seen. Scale Bars: (A and F) 100 μm; (B and D) 10 μm; (C and E) 1 mm.
under polarized light showed heterotypical fibril networks and prevalence of different types of collagen fibers in the synovial membrane of three groups, whereas in the young group it was visualized collagen I (reddish) and collagen III (greenish) (Fig. 1B). In adult group, it was observed a predominance of collagen type I (Fig. 1D), whereas in the aged group, it was observed the predominance of collagen type III (Fig. 1F).

The surface of synovial membrane under scanning electron microscopy showed the general appearance of articular surface of the disk and synovial membrane of the three groups in three-dimensional aspects (Figs. 2A, 2C, and 2E).

Under higher magnification, a smooth synovial membrane surface was observed in the young group (Fig. 2B); however, in adult group some folds were observed (Fig. 2D) and in the aged group an irregular aspect in face of several deeper folds in the surface (Fig. 2F).

Images obtained using the transmission electron microscopy exhibited the intimal and subintimal layers of the synovial membrane where it was evident a great number of synoviocytes disposed in monolayer or multilayer in young and adult groups (Figs. 3A and 3E), and the heterogeneity on the synovial membrane surface in aged group characterized by a diminished number of lining cells (Fig. 3I). In young group an organization of parallel synoviocytes showing euchromatin nuclei between collagen fiber bundles was observed (Figs. 3A and 3B), with long cytoplasmic projections (Fig. 3B), rough endoplasmic reticulum, and oval mitochondria (Figs. 3C and 3D).

It was observed in adult group that synoviocytes euchromatin nuclei showed its nucleoli and marginal heterochromatin. In addition, adjacent capillary vessels with the endothelial cell nuclei and pericytes were seen (Fig. 3E). In the long cytoplasmic portions, the rough endoplasmic reticulum, oval mitochondria, some cytoplasmic vesicles, and caveolae were observed (Figs. 3F–3H). In aged group, distribution and reduction of synoviocytes heterochromatic nuclei and a large area of connective tissue were noted (Figs. 3I–3K). It is important to observe that there was still a great number of vesicles and cisterns dilation of rough endoplasmic reticulum (Fig. 3L).

**DISCUSSION**

This study demonstrated qualitatively, structurally, and ultrastructurally, the TMJ synovial membrane surface, in young, adult, and aged rats. Over the past decades, experimental models were employed to describe the synovial membrane in the embryonic period, training, and in different stages of life (Nozawa-Inoue et al., 2003). Topographical and ultrastructural studies of the trigeminal nerve endings in
rat TMJ reported joint structures such as articular surfaces, articular disk, cavities, and synovial membrane (Kido et al., 1995) as evidenced in our light microscopic analysis in all studied groups.

The present data showed that the connective tissue in young and adult groups differed from the ageing group as reported by other authors (Jilani and Ghadially, 1986; Stravino, 1972). In young animals, the collagen fibers under polarized light microscopy showed a reddish–greenish predominance, indicating the collagen types I and III; in adult group, it was observed a reddish predominance, indicating the presence of collagen type I; and in the ageing group, it was detected the greenish predominance, indicating the presence of collagen type III. These findings corroborate with other studies that suggested the collagen tissue increase with ageing owing to a decrease in the number of synovial membrane cells and the occupation of this space by collagen fibers (Pasquali-Ronchetti et al., 1992). It can also be speculated that an increase in collagen type III which is characterized by thinner and weaker fibrils may be related to disorders in TMJ like disk displacement.

Adult and aged groups demonstrated folds in the synovial membrane surface but the ageing group ones were more pronounced in number, size, and depth. These folds were not observed in young animals and probably occurred after the decrease in the cell number near the synovial membrane surface. Folds of the human TMJ synovial membrane surface were present in the back side, and with the sliding in front of the disk but these wrinkles were likely to disappear according to the data reported by Murakami and Ito (1981). Small and delicate synovial microvilli in the synovial joint of dogs and rabbits were reported mainly in adjacent areas of articular cartilage margin and in ageing animals, and they were increased in number, size, and thickness (Curtiss, 1964; Pasquali-Ronchetti et al., 1992). The increase in the number and size of the folds are important because Piette and Lametsch-Younger (1995) reported that the number of subdivisions of the vascular supply to the synovial membrane depends on the size of the folds.

The synovial membrane cells were disposed in rows, facing the articular cavity (Nozawa-Inoue et al., 2003; Tong and Tideman, 2001) and it was composed by a synovial layer supported by a loose connective tissue layer densely vascularized and innervated, called sub-synovial layer (Cascone et al., 1999; Piette, 1993). According to the present data, it was observed a synovial layer with cells aligned in one to three rows interposed by bundles of collagen fibers and there was a reduction in the number of these cells with ageing. The increase of collagen tissue arranged between cells also occurred in the subsynovial layer as reported by Jilani and Ghadially (1986). The increase of collagen tissue, observed in several structures, occurs in pathological and in physiological conditions like ageing (Hitchcock et al., 2008). It can be also speculated that a decrease in the number of these cells and the collagen increase with ageing can be important to synovial circulation as it enters in the cavity near capillaries and is drained through intercellular pathways distant from capillaries (McDonald and Levick, 1992).

The development of synovial membrane in the embryonic period and after birth showed the presence of fibroblasts associated to the joint cavity formation at the beginning of mandibular movements (Ikeda et al., 2004). These cells were found in abundance in our young group characterized by large amounts of rough endoplasmic reticulum, suggesting active protein synthesis (Iwanaga et al., 2000) and decreased thickness in aged group (Stravino, 1972). These cells with cytoplasmic processes, a relative large nucleus compared to the small amount of cytoplasm and usually located deeper in the synovial intima were called type B cells by Iwanaga et al. (2000).

We also identified mitochondria, rough endoplasmic reticulum, caveolae, vacuoles, cytoplasmic extensions, and nuclei of the synoviocytes which were similar to those reported by Jilani and Ghadially (1986), Iwanaga et al. (2000), and Barland et al. (1962). The synovial membrane also contains specialized cells with phagocytic function, immune, and synovial fluid synthesis that provides nutrition and facilitate the gliding (Tanaka et al., 2008). The decrease in the synovial fluid may cause cartilage damage and in severe degree, its degeneration and osteoarthritis. These round cells located in the superficial layer rich in vacuoles were called type A cells by Iwanaga et al. (2000).

Finally, after observation of light and electron microscopy images, we can speculate that the decrease of the number superficial cells showing vacuoles and microvilli possibly associated with its macrophage function in the joint cavity and the relative increase in the number of cells with developed endoplasmic reticulum with ageing are related to morphological and functional aspects like disk displacement, cartilage damage, and its clinical consequences described in temporomandibular disorders.

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