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Research Article

Scanning Electron Microscopic (SEM) Examination of the Incudomalleal (IM) Joint in Dogs

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Abstract: In this study, the normal incudomalleal (IM) joint surface of dogs was studied by SEM. The animals under the Ketamine + Xylazine (5/2 mg/kg. iv.) anesthesia, were perfused with 2.5% glutaraldehyde solution in 0.1 M sodium potassium phosphate buffer pH 7.2 via left ventricle. Middle ear ossicles were processed with routine histological procedures for SEM and examined under a Philips 505 type SEM. The articular surfaces of malleus and incus showed similar configuration and micromorphology. Characteristic appearance of the collagen fibers on the articular surfaces were not resolved at lower magnifications. On the other hand, at higher magnification, chondrocytes and their relationships with collagen fibers were observed clearly. In conclusion, this research has revealed that the incudomalleal joint in dog resembles the larger joints in human body. However, different configurations were observed in some regions of the joint surfaces. These differences can depend on the sound exposure of the animal over its lifetime.

Key Words: Incudomalleal joint, dog, SEM

Köpeklerde Incudomalleal (IM) Eklemin Scanning Elektron Mikroskopik (SEM) İncelenmesi

Özet: Bu çalışmada köpeklerin normal incudomalleal (IM) eklem yüzeyleri SEM ile araştırıldı. Hayvanlar Ketamin+Xylazin (5/2 mg/kg. iv) anestezisi altında, 0,1 M sodium-potasyum fosfat (pH 7,2) ile tamponlanmış % 2,5 luk glutaraldeyd ile sol ventrikül üzerinden perfüze edildi. Orta kulak kemikçikleri rutin histolojik prosudüre uygun olarak işlemden geçirildi ve Philips 505 type SEM ile incelendi. Malleus ve incus eklem yüzeyleri benzer konfigürasyonlar ve mikromorfoloji gösterdi. Eklem yüzeylerinde kollagen fibrillerin karakteristik görünüşleri küçük büyütmede net olarak gözlenmedi. Diğer taraftan, yüksek büyütmelerde kondrositler ve bunların kollagen fibriller ile olan ilişkileri açık olarak gözlendi. Sonuç olarak, köpeklerde incudomalleal eklemin insandaki büyük eklemlere benzediği görülmüştür. Bununla birlikte, eklem yüzeyinin bazı bölgelerinde farklı konfigürasyonlar tespit edildi. Bu farklılıkların, yaşam boyunca ses etkilerine bağlı olarak meydana geldiği düşünülmektedir.

Anahtar Sözcükler: Incudomalleal eklem, köpek, SEM

Introduction

The morphology of auditory ossicles and their joints has been extensively documented in human beings and several animals (1-16), and the basic data are presented in several textbooks of anatomy and histology (12,17-20). The incudomalleal joint in man was shown to be diarthroidal in nature, making a slide-like movement (1, 9-11). Another report (20) indicated that this joint resembled the primitive lower jaw joint of non-mammalian species. The functional surface of this joint is covered by hyaline cartilage (1,9-11,14-16). Micromorphological structures and configurations seen on the hyaline cartilage occur due to the effect and direction of the force applied to the joint. In this regard, this joint is similar to the other human body joints (4,8-11). The histological appearance of the cartilage and arthrologic changes in the joint have clearly been shown by SEM (10,11).

Structures of bones and cartilage in the diarthrothic big joints of the body have been documented by histological and biochemical methods while surface micromorphology of the cartilage has been revealed by the "Spaltlinien" method. (21-25). The application of SEM has significantly augmented the ability to study bone and cartilage, revealing the fine structure of collagen fibers, lacunae, chondrocytes, synovia and discuss the joints and has made possible more detailed comparison of the appearance of the tissues in normal and disease (10,11,26-28). In this study, we aimed to compare the findings of the incudomalleal joint of the dog by SEM with the data on our previous study on cat (16) and the literature.

Materials and Methods

In the present study we studied morphologically normal middle ear joints of the total 5 adult male dogs. The animal under the Ketamine + Xylazine (5/2 mg/kg. iv), were perfused with 2.5% glutaraldehyde solution in 0.1 M sodium potassium phosphate buffer pH 7.2 via left ventricle. The ossicles were removed utilizing a Zeiss operating microscope and routine microsurgical techniques. The selected specimens were then rinsed thoroughly with distilled water and dehydrated by passage through a graded series of acetone (20%-100%) baths. Ultimately they were dried in critical point (BIO RAD CPD 750), mounted on aluminum stubs and coated (E 5100) with an approximately 400 A° layer of gold. Examination was accomplished by using the Philips SEM 505 type SEM, and photographs were taken of areas of interest.

Results

The ossicles constructing the IM-joint were clearly seen at lower magnification (Figures 1, 2). The remnants of discus, an essential component of the joint, were found on the articular surface of incus (Figure 2). A small part of the discus laid on the convex joint surface of the malleus (Figure 1). The remnants of the joint capsule covered the articular surface entirely (Figures 1, 2). Both of the articular surfaces were perfectly adapted, sliding-type and homogeneous by their similar micromorphological features. Small, spherical processes present on the articular surfaces were dense and prominent on the convex surface and, scattered and compressed on the concave surfaces. They formed column-like structures lying in parallel fashion (Figures 1, 2). Collagen fibers on the articular surface were not resolved at lower magnifications. Chondrocytes were observed as prominent humped structures (Figures 3-6). They were located regularly and smoothly on some areas

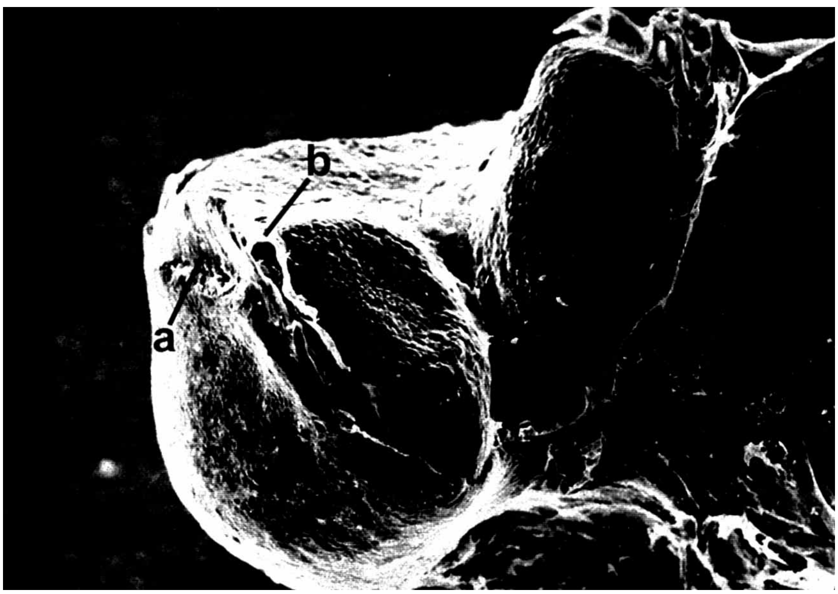

Figure 1. General appearance of the articular surface of malleus with the remnants of the joint capsule (a) and articular disc (b) are seen on the surface of the joint. x 35

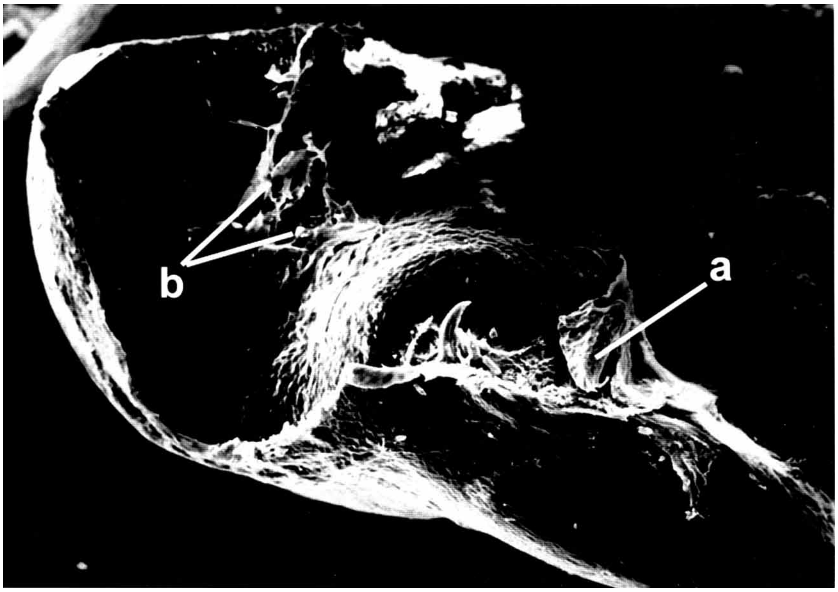

Figure 2. The lower magnification view from the articular surface of incus. The remnants of joint capsule (a) and articular disc (b) can be seen. x 40

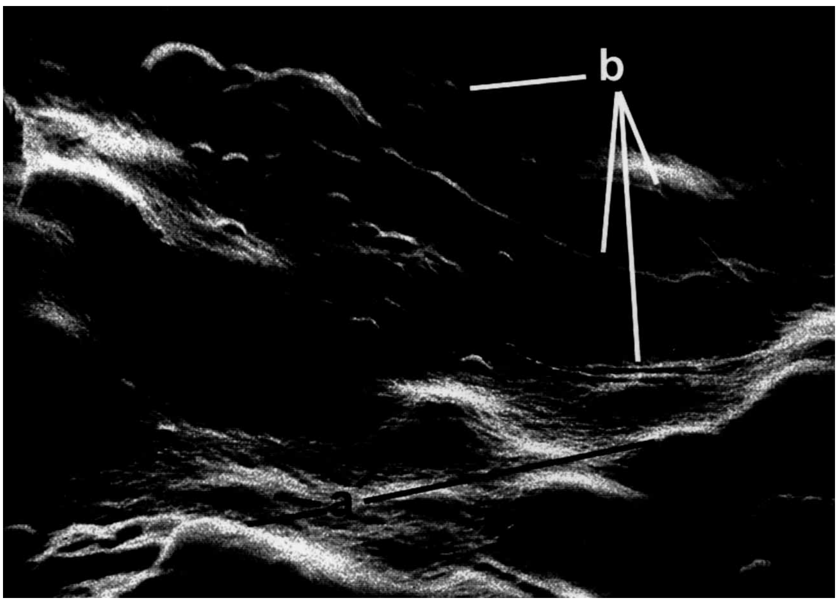

Figure 3. High power view from Fig. 1. Showing numerous protruding of chondrocytes (a). Note the individual collagen fibers and some micromorphological structures (b). x 3240

of the articular surfaces (Figures 3, 6). Chondrocytes engulfed by circular structures on the articular surface of the incus formed prominent humps (Figures 4, 6). Though possessing smooth surfaces, they were interestingly depressed and there were lacunae on some areas (Figures 4, 5). There were wide, flat areas among the prominent humps. Micromorphological structures of different size appeared on these areas (Figures 4, 5).

Figure 4. High power view from articular surface of incus. The circular contours on the top of the humps may correspond to the small rounded protuberances visible in the bottom of some articular surface. x 1550

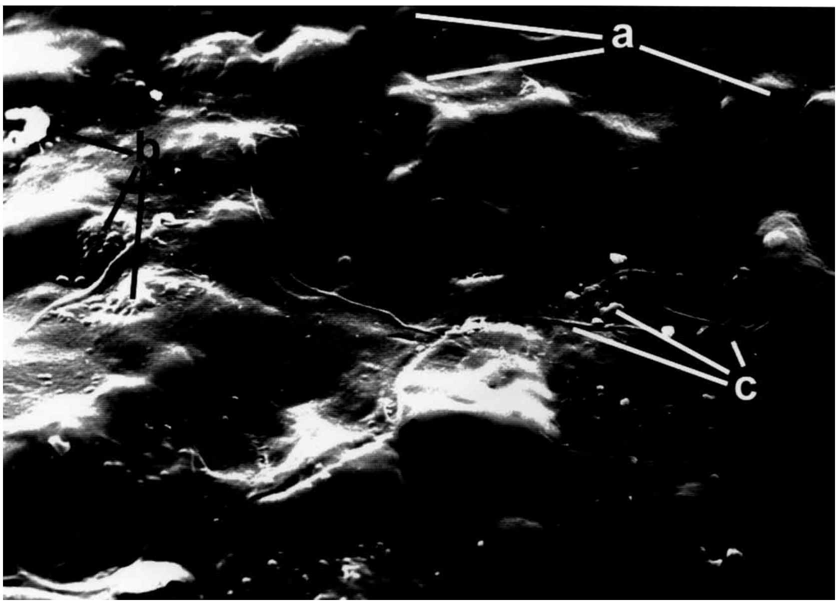

Figure 5. Surface irregularity view at high power at articular surface of incus. The protruding chondrocytes (a) and lacunae (b) are seen in articular surface. Note the collagen fibers (c) lying in different directions and numerous micromorphological structures (d). x 925

While characteristic appearance of the collagen fibers on the articular surfaces were not resolved at lower magnifications, at high magnifications on some areas of the articular surfaces of both the incus and malleus collagen fibers configuration were seen clearly. They were parallel to the cartilage surface, and of varying thickness (Figures 3, 5). These unusual findings may have arisen due to the variability of the forces experienced

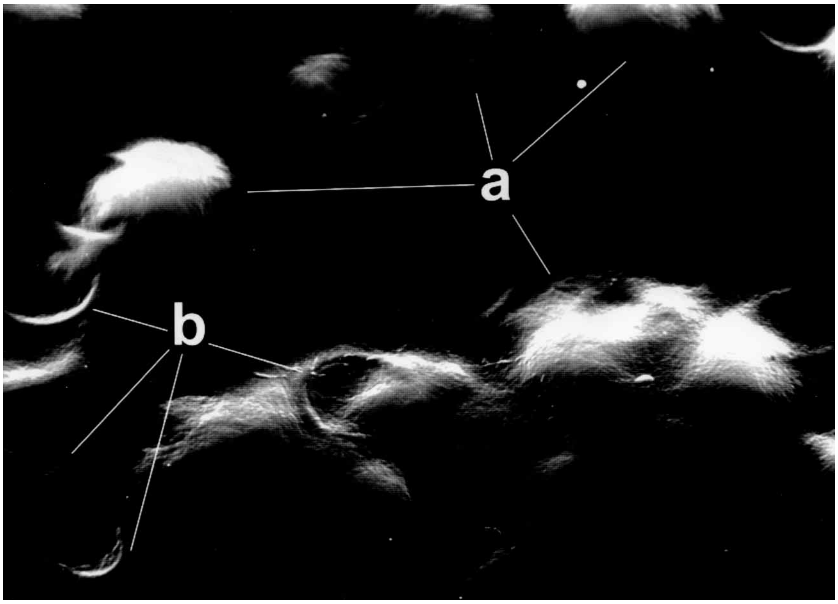

Figure 6. High power view from the specimen shown in Fig. 2. Numerous humps and pits (a) seen on the articular surface of incus. Note also an annulus around a humps (b). x 925

Figure 7. A high power view from the specimen shown in Fig. 2. A zone of cartilage surface from articular surface of incus showing collagen fiber bundles. In this region pits and humps of lacunae and chondrocytes seen (a). x 925

across the joint surface. There were many paired chondrocytes having prominent borders with smooth surface on both of the articular surfaces and they were seen as humps (Figures 3, 4, 6). Collagen fibers on some areas of the articular surface of the incus formed smooth and parallel waves. Among them there were deep and rectangular cavities and scattered lacunas both containing chondrocytes. The fibers enveloped them without interrupting (Figure 7).

Discussion

Basic data on the ear ossicles and their joints are present in several textbooks (12,17-20). However, comparative research on the function, structure and type of the middle ear joints is limited (4,5,7,14-16,29). Several suggestions have been made on the morphology and movement properties of the middle ear joints. Elpern et al. (30) has suggested that the incudomalleal joint is of the symphysis type, having no important role on vibration transmission. Another report showed the auditory ossicles in rhodentia to be attached firmly to each other (11). But, this joint in cats has been documented to be totally movable and of the sellar type (16). The joints of the auditory ossicles in man, too, are totally movable and of the diarthrosis type having a sophisticated and delicate organization (9-11,31,32). Similarly, data on this study indicated that the incudomalleal joint in the dogs was in fact a diarthrosis type joint. Articular surfaces of the joint fit perfectly together and showed sellar type articulation. We think the findings presented here suggest that this joint has the ability to perform flexion-extension and abduction-adduction movements rather than a simple gliding motion. Functional articular surfaces of the diarthrosis type joints are covered by the hyaline cartilage, which regulates contact forces by its high elasticity and viscosity (10). The micromorphology of the articular surface reflects the physiologic forces (1,10,11,14-16,21,26-28,33). Structural and biochemical changes of the hyaline cartilage of the articular surface result as a consequence of a variety of joint malformations, insufficient feeding, limitation and changes in the joint movements, and aging (3,6,9-11,21,26,28,32).

Puhl and Hamacher (28) indicate that not all areas of the functional articular surface of a joint are exposed to same degree of force. Others suggest that different micromorphologic structures on the cartilage surface are due to the variations of the forces applied to the joint (9-11,26-28,32). However, these structures are seen in characteristic configurations only in pathological conditions (3,6,7,26-28,32). Functional articular surfaces of the joints of the auditory ossicles are concealed by typical hyaline cartilage whose morphology resembles that of the larger joints in human body (9-11,14-16). The findings of our study on the incudomalleal joint agree with the literature. Our findings, parallel collagen fibers, spherical humps, lacunae and chondrocytes, were similar to those of in several other SEM studies (9-11,14-16). The lacunae and highly prominent chondrocytes observed on the smooth surface of the hyaline cartilage at higher magnifications show this joint to be of a synovial type. Collagen fibers of the normal hyaline cartilage are scattered homogeneously and deeply in the hyaline matrix (9-11,27). Neither individual fibers nor destruction of the fiber layer and broken fibers are seen in normal cartilage. Atrophic and pathologic changes include atrophy of the fiber matrix and, destruction and breakage of the collagen fibers (2,3,7,11,26,28,33). The present study also supported previous reports on the surface micromorphology of the fiber structure of the cartilage coverings of the joint. Individual collagen fibers were arrayed in different directions and with different thickness in limited areas of both the articular surfaces at high magnification. This led us to suggest that these areas were exposed to different directional forces as has been previously suggested in the literature (3,7,9,21). Some studies have also investigated the topographic relationship of the chondrocytes and the collagen fibers of the hyaline cartilage. Clarke (34) has explained that paired chondrocytes are laid down parallel to the surface. Another report indicates that the chondrocytes in the hyaline cartilage queue, forming columns and elevations (7). Similar results were obtained on the topographic relationship between the collagen fibers and chondrocytes in this research. Again the lacunae and the chondrocytes in them were clearly observed between the collagen fiber waves (27) conducting very intensive research on the hyaline cartilage divides the surface micromorphology into "Primary", "Secondary", and "Tertiary" structures. Primary and secondary structures are seen at low magnifications. Microstructures that can be observed only at high magnifications are designated tertiary, including collagen fibers, lacunae and chondrocytes. Results of this study at all magnifications are similar to those of Puhl (27).

In conclusion this research has revealed that the incudomalleal joint in dog resembles the larger joints in human body. This joint is a diarthrotic and sellar type. Its motion is two-directional, forward and backward, rather than a simple gliding movement.

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