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COLLAGEN FIBER ARRANGEMENT IN TEMPORO-MANDIBULAR JOINT (TMJ) DISKS FROM HUMAN SUBJECTS WITH FUNCTIONAL DISEASES. SCANNING ELECTRON MICROSCOPY INVESTIGATIONS

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Abstract

Twelve articular disks from patients with temporomandibular joint (TMJ) arthropathy were studied and compared with two normal disks. Scanning electron microscopy (SEM) examination of the surfaces and of longitudinal and cross-sections of the disks allowed the observation of the arrangement of the collagen fiber component in different parts of the disk. The superficial part of the articular disks appears to be formed by rather compact fibers. The internal portion is usually formed by bundles of collagen fibers in sheets, alternating with isolated fibers arranged in a parallel or irregular way.

In some samples, blood vessels were observed. Our investigations suggested that the appearance of vascularization is the first remarkable histological change that can be observed in functionally abnormal articular disks.

Key Words: Temporo-mandibular joint (TMJ), articular disk, collagen fibers, scanning electron microscopy.

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Introduction

The temporo-mandibular joint (TMJ) is characterized by the presence of a dense fibrous tissue coating the articular surfaces and constituting the articular disk structure.

The presence of layers of fibrous tissue both on the articular surfaces and in the disk of the TMJ has recently led some authors to focus their attention on the "functionally active" role performed by this particular tissue (Bibb and Pullinger, 1993). As far as the articular disk is concerned, light microscopy and, in particular, scanning electron microscopy (SEM) investigations highlighted a varied and complex arrangement of the collagen fibers in the disk structure (Jagger, 1980; Taguchi *et al.*, 1980; de Bont *et al.*, 1985; Shengyi and Yinghua, 1991; Berkovitz *et al.*, 1992 a,b; Berkovitz and Robertshaw, 1993; Rohlin *et al.*, 1993). However, literature data on the arrangement of the collagen component were obtained by examining disks from corpses or different experimental animals, and such data are not always comparable.

It has been shown that in conditions of altered functionality of the joint, the disk undergoes some serious structural modifications with the appearance of vascularization and proliferation in the synovial coating (Helmy *et al.*, 1989; Kurita *et al.*, 1989; Bibb and Pullinger, 1993). Recent investigations have attempted to find correlations between the structural arrangement of the disk fibers, possible changes in this arrangement, and the different functional conditions of the disk as a whole and of its various parts. Alterations in the weave of the collagen fibers have been indicated as possible primary causes of the degenerative process of the disk structure (Shengyi and Yinghua, 1991).

However, data on possible structural modifications or degeneration of the fibrous component of the articular disk are, so far, not available. Therefore, the objective of this study was the SEM examination of the structural characteristics of articular disks in abnormal functional conditions in humans.

Figures 1 and 2. Control disk. **Figure 1.** The stereo-microscope picture shows the smooth superior surface and the subtle profile. Bar= 4 mm. **Figure 2.** The regular undulated configuration of the superficial bundles of fibers. Bar = 10 μm.

Figure 3. Stereo-microscope feature of the surface of a pathological non-adhered disk. Bar = 4 mm.

Figure 4. Scanning electron micrograph of the disk surface, shown in Figure 3, showing the undulated configuration of the fiber bundles. Bar = 10 μm.

Figure 5. The irregular, undulated configuration of a disk surface bordering the perforated area. Bar = 10 μm.

Figure 6. Superficial bundles of fibers in a pathological non-adhered disk at higher magnification. Bar = 1 μm.

Figure 7. The variable interlacing of the fibers in a perforated disk. Bar = 0.5 μm.

Figure 8. A flat cellular layer covers the peripheral part of the disk surface that shows an undulated configuration under the cellular layer (> >). Bar = 20 μm.

Materials and Methods

In a group of 115 human subjects affected by arthropathy of the TMJ, after functional therapy, physiotherapy and specific radiological tests (computer tomography, CT; and nuclear magnetic resonance, NMR), 50 patients were submitted to arthroscopy and 12 of them to arthrotomy providing us with the disks for our study. Such disks, which were removed because they interfered with the condylar dynamics because they were dislocated, also showed adhesions in 3 cases and perforations in 7 cases. Those cases were, therefore, representative, in their different degrees of alteration, of the evolution of the arthropathy. The surgical removal was very carefully performed in order to preserve the integrity of the disks during the excision of the surrounding tissues. Control samples were obtained from two disks collected at autopsy from subjects free of TMJ symptoms.

After removal, all disks were carefully washed in saline buffer, observed and photographed with a stereo-microscope, and dissected into smaller samples. The anterior and posterior portions were discarded.

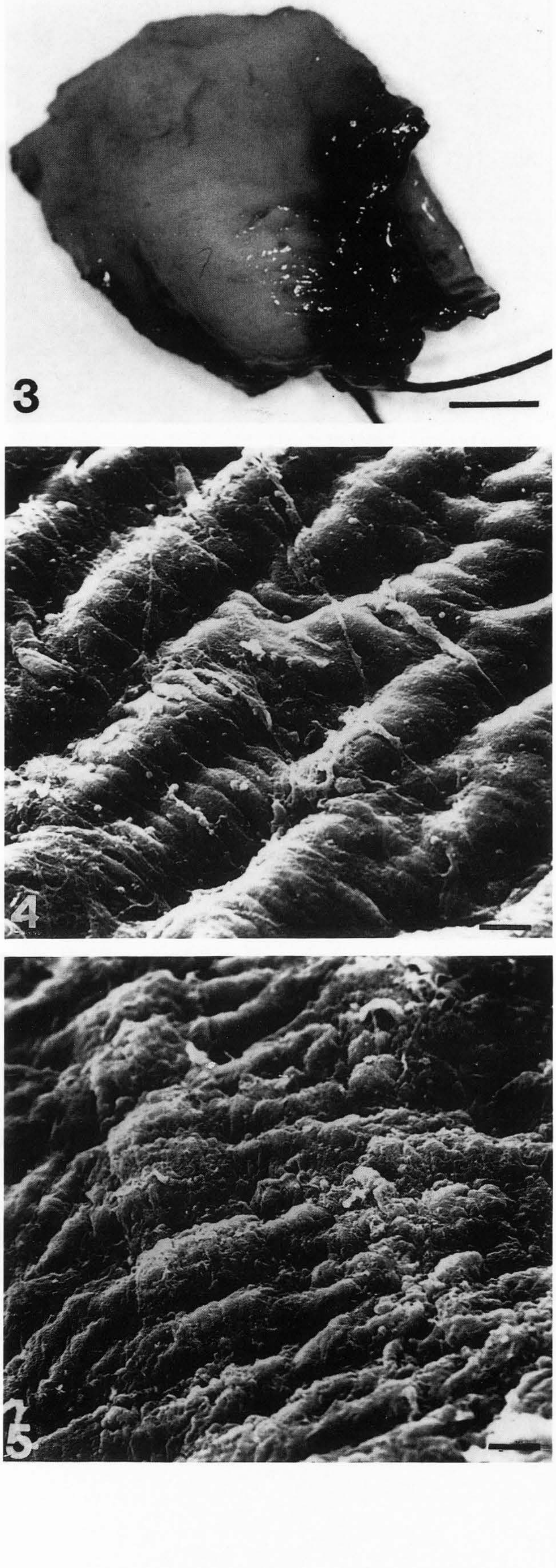
The samples were immediately immersed in a fixative solution consisting of glutaraldehyde (2.5%) and paraformaldehyde (2%) in 0.1 M sodium cacodylate buffer (pH 7.4) for 5 hours at 4°C. They were then briefly rinsed in the same buffer solution, post-fixed in 1% OsO_4 in 0.2 M collidine buffer (pH 7.4) for 2 hours at 4°C. Then they were dehydrated in ethanol and critical point dried. The surfaces chosen for observation were sputtered with gold and observed in a Philips 515 SEM.

Results

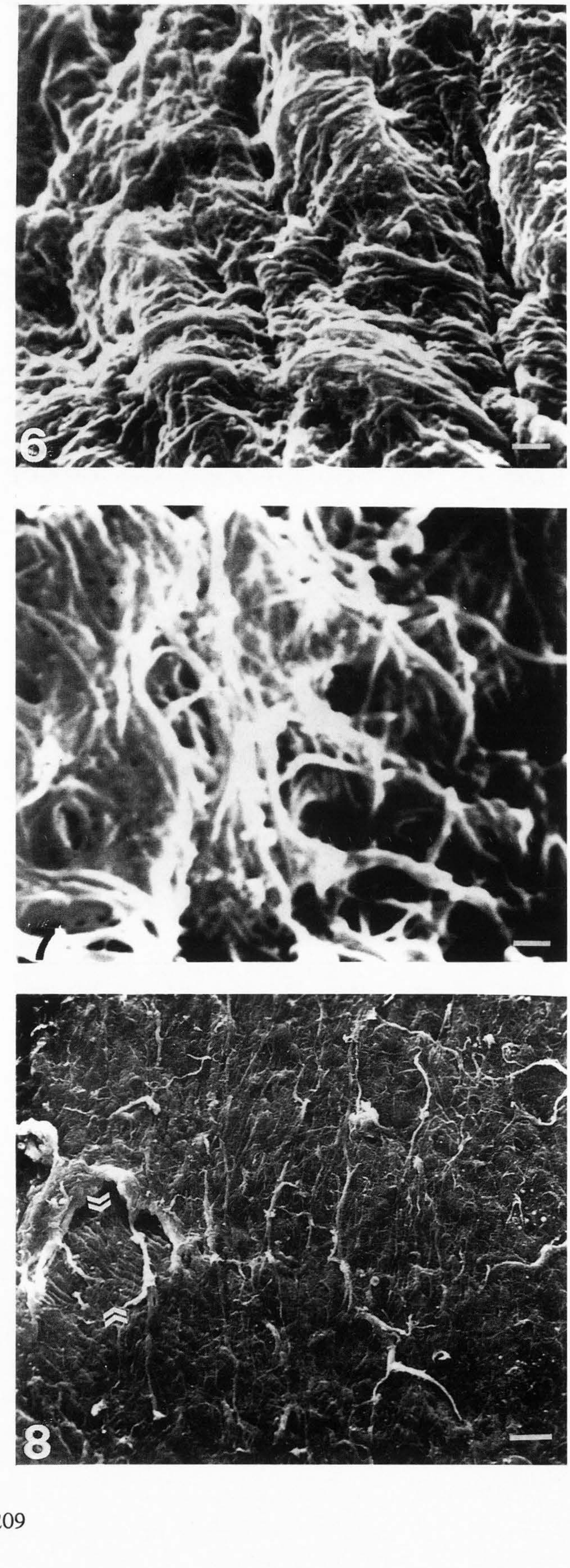
The control disks had a biconcave shape (Fig. 1). SEM observation showed that their surfaces appeared regularly undulated with regularly distributed peaks and valleys, usually with medio-lateral orientation (Fig. 2). The surface of the pathological disks without functional immobility appeared smooth at macroscopic examination (Fig. 3). SEM examination showed that such surfaces consisted of a rather compact tissue, with a regular, undulated configuration (Fig. 4).

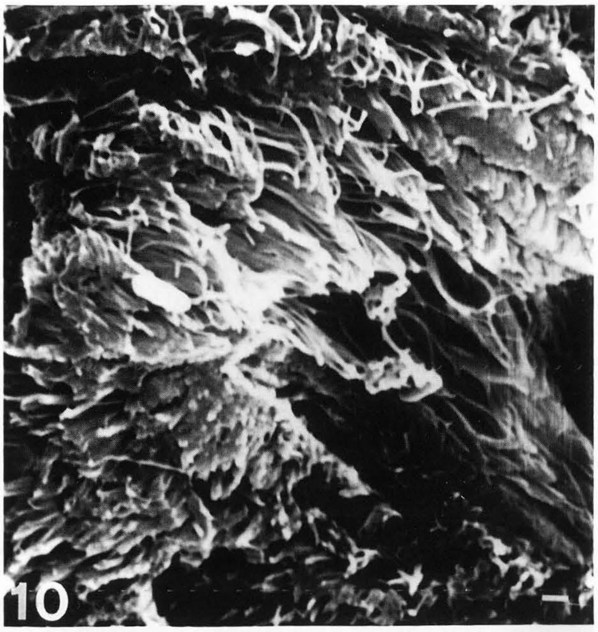
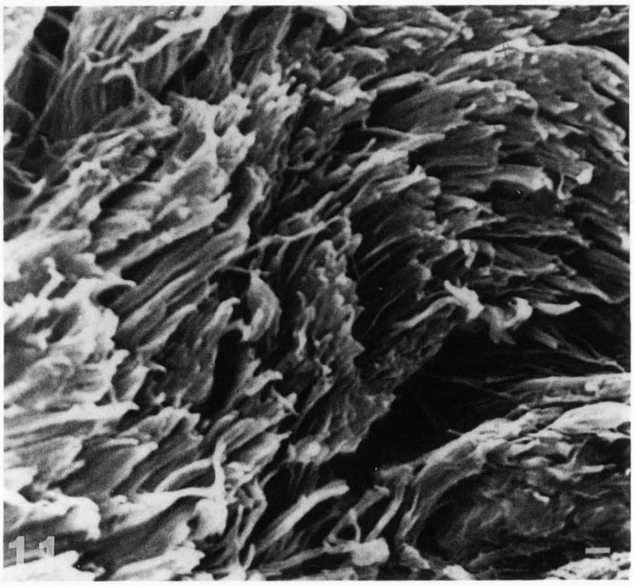
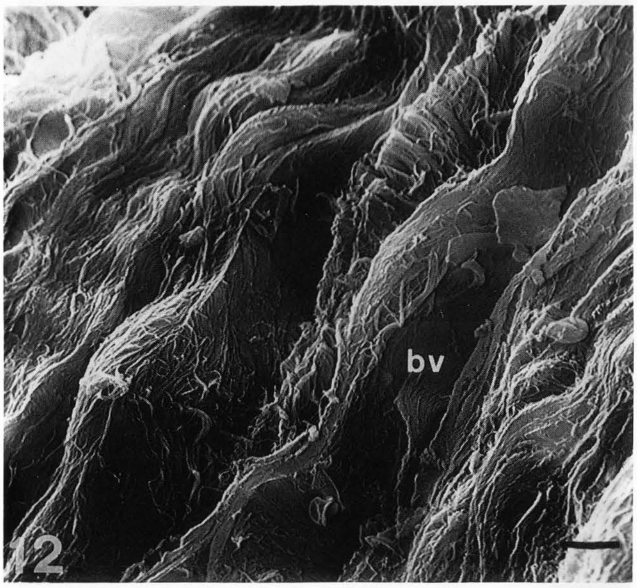
In the disks with perforations, the sequence of peaks and valleys on the surface appeared to be more irregular closer to the perforation areas (Fig. 5). At high magnification, it was possible to observe that the undulated configuration of the surfaces was due to the arrangement and the orientation of the bundles of collagen fibers

Figures 3-5



Figures 6-8



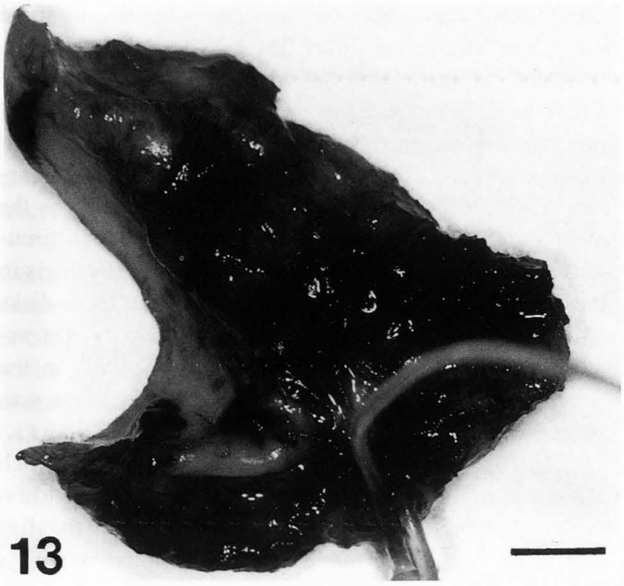
Figures 9 and 10. Control disks. Cross-section of peripheral (**Fig. 9**) and internal (**Fig. 10**) bundles of collagen fibers. Bars = 0.5 μm.

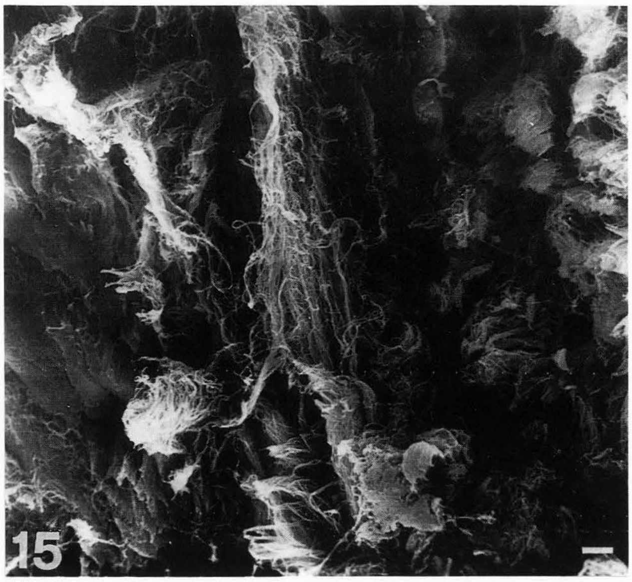
Figure 11. Cross-section of a bundle of collagen fibers arranged in parallel in the superficial region of a non-adhered disk. Bar = 0.5 μm.

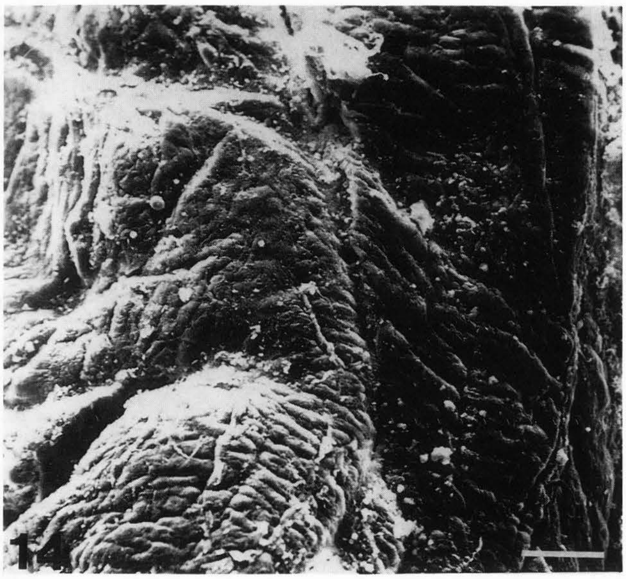
Figure 12. Longitudinal section of a disk. A blood vessel (bv) runs parallel to the fiber sheets. Bar = 10 μm.

(Fig. 6). In those areas in which the surface appeared more irregular, bundles of collagen fibers and single collagen fibers intertwined and showed a more varied configuration (Fig. 7).

The surfaces of the samples corresponding to the most peripheral parts of the disks were often coated by a sheet of flat polygonal cells, at times linked by thin and short extensions. At those points where the cell sheet was torn or raised, the underlying intertwined bundles of collagen fibers could be seen. The fibrous surface showed the same undulated configuration observed in the superficial parts of the disks which were not coated by cells. No other structures were observed between the cellular layer and the fibrous surface (Fig. 8).







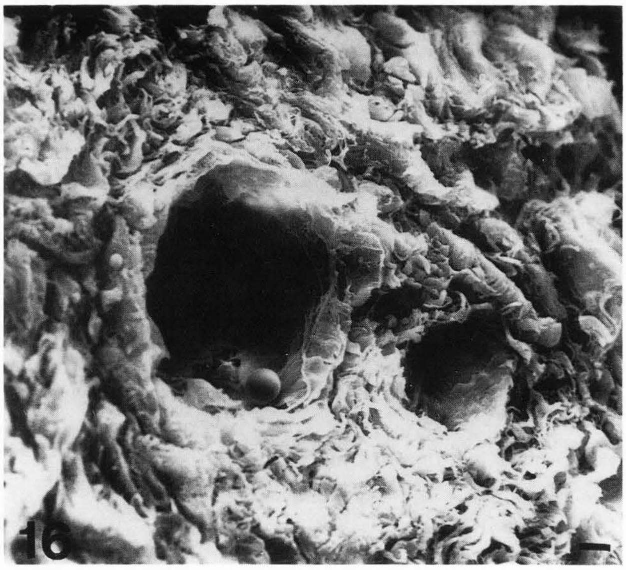


Figure 13. Stereo-microscope feature of the superior surface of a disk adhered to the glenoid fossa. Bar = 4 mm.

Figure 14. The disk surface presents reliefs and furrows. Bar = 0.1 μm.

Figure 15. Interior part of an adhered disk. Small bundles of fibers interlace with waved configuration. Bar = 5 μm.

Figure 16. Some blood vessels in cross-section. Bar = 10 μm.

In the disk sections it was possible to observe the disposition and the internal arrangement of the bundles of fibers. In the control disk sectiond, bundles of collagen fibers were regularly packed in the superficial layer (Fig. 9) and ran in an ordered way with a parallel or perpendicular arrangement inside the disk (Fig. 10). In the pathological samples without adhesions, at the edge of the disks, the fibers formed large bundles overlapping in parallel layers (Fig. 11) or with an irregular arrangement. The internal fibrous tissue appeared to be less compact than that at the surface and that in the control disks. The fibers formed bundles or sheets which were thinner than those on the surface. Also isolated fibers with an irregular and varied configuration were observed. In three of these disks, blood vessels between collagen fibers were present (Fig. 12).

The disks with adhesions (all to the glenoid fossa) appeared to be rather thick (5 mm, on average) and the adhering surface was very irregular due to the presence of remarkable protrusions (Fig. 13). At higher magnification, their adhering surface did not appear to be covered by a cellular layer but rather formed by compact fibrous tissue. The arrangement of the bundles could not be discerned (Fig. 14). The opposite condylar surface of these disks (towards the head of the condyle) was smooth and in some peripheral areas, it was covered by a sheet of flat cells resembling those observed on the smooth surface of the other disks.

The internal part of the adhered disks is formed by overlapping bundles of fibers. In some areas, the cross-sectioned bundles showed an undulated configuration, in other zones, particularly in the central part of the disk, they showed a more varied arrangement (Fig. 15). Inside the disks, between bundles of collagen fibers, a network of blood vessels was present. This network was more extended in the proliferated part that adhered to the glenoid fossa; the blood vessels, which according to size and wall structure could be considered arteries and veins, were also more numerous in this part (Fig. 16).

Discussion

Recent investigations by SEM have described the complex arrangement of the collagen fibers in the articular disk structure of the TMJ. The different orientation of the fibers and of the bundles in the various areas of the disk has been related to the functional stimulus to which these parts are subjected (Shengyi and Yinghua, 1991). A primary involvement of the collagen fiber network in the functional alterations undergone by the articular disk has also been proposed (Freeman and Meachin, 1979; Shengyi and Yinghua, 1991).

Our observations were carried out on disks from patients affected by serious functional diseases of the TMJ. In most of the samples examined, no serious or extended modifications in the arrangement of the fibrous component were found despite the remarkable alterations in the form of the disk itself. In general, the surfaces of the pathological disks had kept their undulated appearance, usually with the medio-lateral orientation observed in the normal disks. Only the surfaces close to the perforated edges of the disks and particularly those close to the adhered surfaces had an irregular appearance.

The investigation on sections allowed us to compare the surface arrangement with the internal arrangement of the bundles of fibers. Observations on sections of control disks show that there is a difference in the orientation of the bundles of fibers inside the disk and on the surface. Both surface layers of the disks consisted of a layer of bundles of fibers, more compact than the one constituting the inside of the disk. Inside, in some areas, the bundles of fibers formed parallel sheets with different orientation of the fibers; in other areas, the fibers were intertwined in various ways. Such an arrangement is also maintained in the majority of the pathological disks. Therefore, this three-dimensional arrangement of the bundles of collagen fibers might be the most suitable in respect to the normal functional stimulus, but it might also be able to bear the modifications in the functional activity of the TMJ. Only in those disks which remained adhered to the glenoid cavity, the arrangement of the bundles of fibers, particularly in the adhering part, was more irregular and the tissue was vascularized.

The appearance of vascularization, in areas which are not normally vascularized, seems to be the first histological change affecting the fibrous tissue of the disk which no longer has its normal functional mobility. This is confirmed by the presence of functionally inactive (adhered) areas with vascular proliferation; these areas were adjacent to others which were still partially functional and which did not present vascularization. The presence of a vascular supply suggests an aetiology in the change of the disk structure which is not only mechanical but also linked to vascular inflammatory factors.

Acknowledgements

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Discussion with Reviewers

L.G.M. de Bont: Are you sure that the articular surface irregularities observable by SEM are not shrinkage artifacts caused by dehydration during tissue processing for SEM? Also the described cell extensions may be the results of shrinkage artifacts.

Authors: The undulations observed on the surface of the disks have an arrangement, an orientation, and a regular or irregular configuration in the different areas and in various disks. Such characteristics, in our opinion, correspond to real differences in morpho-functional patterns between the pathological situation and the normal situation.

The images at high magnification, clearly show that the undulated surfaces are formed by well recognizable and well-preserved bundles of collagen fibers. If we compare our observations with the literature data, it appears that the irregularities of the disk surface are no artifacts due to sample preparation. Also, the observation of a flat cell layer which covers part of the surfaces of some disks is not artefactual, but seems to be in agreement with previous light-microscopic observations (please see the **References** cited in the third paragraph of the **Introduction**).

Reviewer I: Are your control samples really normal disks?

Authors: The disks that we used as control disks were taken from corpses of young persons; they were chosen with care and did not show either morphological defects or defects of the position of the teeth. Moreover, the case-histories did not show that the donors had suffered from functional pathology of the TMJ. The morphological results that we obtained from these control samples are, in our opinion, comparable and in agreement with the analogous cases present in the literature.

Reviewer I: Can you explain in more detail the functional therapy and the NMR the patients underwent?

Authors: Our therapeutic procedure includes the use of bite-planes and of repositioning bites; the bite planes basically have the aim of reprogramming the muscle function; the repositioning bites use the muscle action in order to modify the functional stress and demands inside the articular space. This first therapeutic moment is often associated with physiotherapeutic treatment aiming at restoring a correct range of movement of the mandibular condyle and at relaxing the masticatory muscles and the muscles of the cervical rachis. If the patients reach a sufficient level of functional stability with this therapy, they can follow a therapeutic program which includes the correction of dentoskeletal defects and the use of bite-planes during the night.

In contrast, the patients who do not obtain a good result with the bite (approximately 5 to 30% of the cases in the literature) follow a diagnostic and therapeutic program that includes various investigations. Among these, NMR can show the morphology and the position of the articular disk and its position with regard to the functional surface of glenoid cavity and the condylar surface during the movement.

Moreover, if we change the signal, we can show the presence of articular shedding and of exudate.

M. Albertsson: Do you have any idea about the mechanism of vascularization and also of the event preceding the appearance of blood vessels?

Authors: The absence of vascularization is a morphological characteristic of fibrous tissues submitted to heavy compression. We think that the vascularization that we saw in the central region of some disks, which, normally, is not vascularized, may be the consequence of modified functional conditions including a decrease of compression forces on the disk itself. The neogenesis of the vessels could probably occur starting from the bilaminar zone which is richly vascularized.

W.L. Wilborn: Please summarize: which Figures are from the control disks and which are from the pathological disks?

Authors: Figures 1, 2, 9 and 10 from control disks. All remaining Figures are from pathological disks.