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C. Piacentini
Universita di Pavia

C. Marchetti
Universita di Pavia

A. Callegari
Universita di Pavia

M. Setti
Universita di Pavia

G. Bernasconi
Universita di Pavia

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ENDOARTICULAR LOOSE BODIES AND CALCIFICATIONS OF THE DISK OF THE TEMPOROMANDIBULAR JOINT: MORPHOLOGICAL FEATURES AND CHEMICAL COMPOSITION

C. Piacentini*, C. Marchetti[1], A. Callegari[2], M. Setti[2], G. Bernasconi, U. Baciliero[3], P. Menghini and C. Brusotti

Istituto di Discipline Odontostomatologiche, [1]Istituto di Istologia e Embriologia Generale, [2]Dip. Scienze della Terra, Università di Pavia, [3]Ospedale S. Bortolo, Vicenza, Italy

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Abstract

We studied articular disks and endoarticular loose bodies taken from patients suffering from different types of temporomandibular joint (TMJ) pathology. The scanning electron microscopy (SEM) analysis of the disks and the endoarticular loose bodies was followed by a chemical-compositional analysis using an energy dispersive spectrometer (EDS) and by characterization of the crystalline phases by X-ray powder diffraction (XRD). The articular disks were composed of a central radio-opaque area lacking any evident structural features, surrounded by compact bundles of collagen fibers. EDS and XRD analyses showed that endodiscal radio-opaque areas were hydroxyapatite. By SEM, we observed a fibrous network only in circumscribed areas of the endoarticular loose bodies. The chemical-compositional analysis showed that the loose bodies were composed of calcite ($CaCO_3$). The results of this investigation, along with the clinical history of the patients, allow us to formulate some hypotheses regarding the etiopathogenesis of these structural anomalies. The endodiscal calcifications could be the result of a chronic inflammatory process that produces displastic alterations of the articular disk. Moreover, an acute inflammatory process with modifications in the mechanisms of the synovial fluid turnover seems to be the event that leads to the formation of endoarticular loose bodies.

Key Words: Temporomandibular joint, disk, calcification, endoarticular, loose bodies, scanning electron microscopy, energy dispersive spectrometer, X-ray powder diffraction.

*Address for correspondence:
Cesare Piacentini
Istituto di Discipline Odontostomatologiche,
Università di Pavia,
Ospedale Policlinico S. Matteo,
Piazzale Golgi 2,
27100 Pavia, Italy
Telephone number: +39-382-526221
FAX number: +39-382-423029

Introduction

The fibrous tissue of the temporomandibular joint (TMJ) articular disk undergoes some structural modifications due to functional alterations and aging. Functional alterations lead to modifications in the relationship between the articular surfaces and to variations in the amount of pressure exercised on the disk. These events alter the disk's shape, or cause its dislocations and perforation, and its adhesion to one of the articular surfaces.

Macroscopic modifications correspond to variations in the disk morphology at the microscopic and ultrastructural levels. The variations mainly consist of the disaggregation of the compact collagen fiber bundles, the appearance of vascularization and innervation in the non-innervated and non-vascularized central part of the disk, and an increased number of the cellular component (Carlsson *et al.*, 1967; Freeman and Meachin, 1979; Sharawy *et al.*, 1987; Helmy *et al.*, 1988, 1989, 1990; Kurita *et al.*, 1989). Therefore, the pathological patterns which may be observed in this kind of situation are extremely variable. They depend on the developmental stage of the pathology, on specific characteristics due to individual reactivity as a response to pathogenous noxae, and on the morphological adaptation which the articulation undergoes as a result of functional changes (Folke and Stallard, 1966; Moller, 1966; Takenoshita, 1982).

With aging, cells similar to chondrocytes begin to appear in association with the modification of the extracellular matrix, as described by various authors (Oberg and Carlsson, 1979; Bhussry *et al.*, 1991; Berkovitz *et al.*, 1992). Foci of mineral precipitates were also found (Shaw and Molineux, 1994). Modifications in the synovial membrane with synovial chondromatosis or osteochondromatosis and formation of loose bodies have been studied by Tasanen *et al.* (1974).

As part of an extensive investigation on the morphological characteristics of disks removed from patients affected by various forms of TMJ pathologies, we used scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to examine different structural modifications undergone by these disks (Piacentini

et al., 1994; Marchetti *et al.*, 1995).

In this part of the study, we considered some articular disks presenting a calcified central area and endoarticular loose bodies.

Materials and Methods

For the purpose of this study on discal calcifications and endoarticular loose bodies, we used biopsies from subjects belonging to a group of cases of 156 patients treated with conservative therapy for TMJ arthropathy. Of these patients, 53 underwent arthroscopy and arthrotomy.

Endodiscal calcifications were diagnosed by radiological examinations (orthopantomography, tomography, computerized tomography, three dimensions computerized tomography), whereas endoarticular loose bodies were diagnosed by arthroscopy.

Endodiscal calcifications were found in 3 female subjects who were about 50-years-old, with clinical histories of functional defects and generalized articular pain, and who did not present any sign of rheumatoid or autoimmunological diseases. In particular, 1 of the 3 patients had already undergone an operation for pathology of the cervical rachis, and at orthopantomography showed a considerable lengthening of the left styloid process.

These subjects had been affected by TMJ arthropathy for several years. With time, the pathology had become increasingly disabling, with pain of variable intensity, articular crepitus and serious limitation of motion. Radiological examinations revealed the presence of calcifications and pathological remodeling of the articular bone heads. These subjects underwent arthrotomy, diskectomy and intra-articular shaving. The post-operative recovery was positive for all 3 patients but the woman who had already been operated for another pathology, showed pain relapse and functional limitation three months after the operation.

Endoarticular loose bodies were found in a young male subject whose only symptom was acute and relapsing articular pain, and who did not respond to any conservative treatments. Radiological examination of the subject did not reveal any sign of pathology either of the articular bony surfaces (mandibular condyle and glenoidal eminence) or of the disk, which, in nuclear magnetic resonance (NMR) investigation, had a regular shape and size and was normally located. The only pathological finding, which was given by a signal referring to the articular cavity and capsule during NMR examination, was the presence of synovial oedema and endoarticular effusion. Arthroscopy performed on the patient allowed the identification of loose bodies showing a mild hyperemic synovitis. After removal of the loose bodies and the intra-articular cleaning performed during the arthroscopy, the patient did not show any pain relapse in the six months following the operation.

Figures 1-5. Scanning electron micrographs of a calcified disk.

Figure 1. The surface of the calcified disk. In the central region a dense and compact area is identifiable (arrows). Bar = 0.2 mm.

Figure 2. At higher magnification, the compact area presents a granular superficial pattern. Bar = 2 μm.

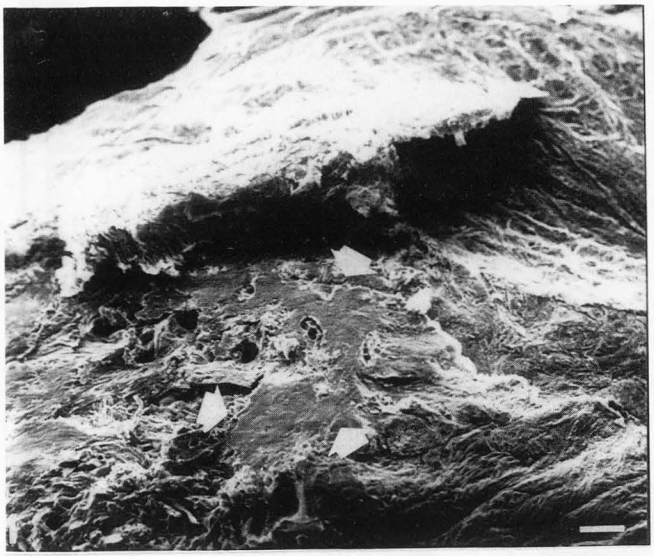
Figure 3. The border between the compact portion and the surrounding fibrous tissue of the disk. Bar = 10 μm.

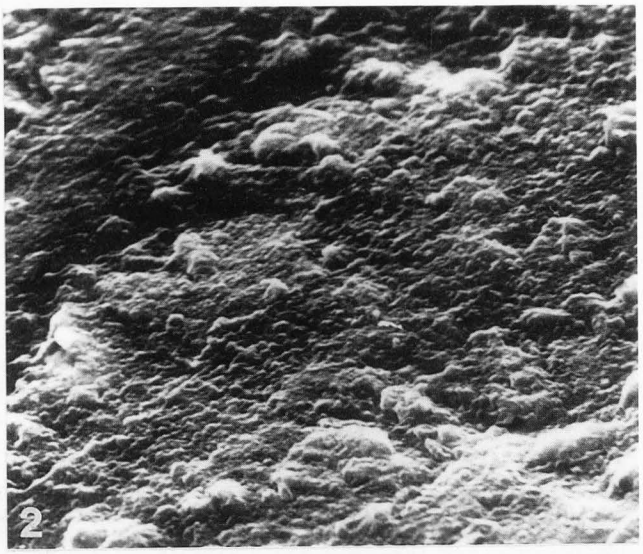
Figure 4. Bundles of fibers variously-arranged characterize the remaining surface of the disk. Bar = 20 μm.

Figure 5. SE (a) and BSE (b) images of the central region of the disk. The BSE image highlights the different compositions of the central area (clear zone) and the fibrous tissue. Bar = 100 μm.

After removal, the excised disks and the loose bodies were washed in saline buffer and fixed in a solution of glutaraldehyde (2.5%) and paraformaldehyde (2%) in 0.1 M Na cacodylate buffer (pH 7.4) for 5 hours at 4°C, rinsed in the same buffer solution and post-fixed in 1% OsO_4 in 0.2 M collidine buffer (pH 7.4) for 2 hours at 4°C. We performed SEM studies on the morphology of the samples by JEOL JXA 840A electron microscope using secondary (SE) and back-scattered electrons (BSE). Characterization and identification of the chemical composition were performed with the same microscope, equipped with an Tracor Northern energy dispersive X-ray spectrometer (EDS). The samples were dehydrated in alcohol and critical-point dried and sputtered with gold film. We undertook the qualitative analysis by using the "IDENT" program. This program performs a qualitative analysis of a spectrum in data memory of all the chemical elements from Na to U. The conditions for microprobe analysis were: an accelerating voltage of 15 kV, a probe current of 6 x 10^{-9} A, a working distance of 39 mm and a counting time of 30 seconds. In order to avoid the possible chemical-physical manipulation of the samples, we did not replace the gold film with carbon, so that, in all the spectra, the Au signal appears.

The same samples used for morphological and chemical studies were analyzed with X-ray powder diffraction (XRD) for the characterization of the crystalline phases (Bonucci and Graziani, 1975; Formenti *et al.*, 1993, personal communication). Samples were analyzed in the natural condition, previous grinding in agate mortar, using a Philips PW1800/10 diffractometer, equipped

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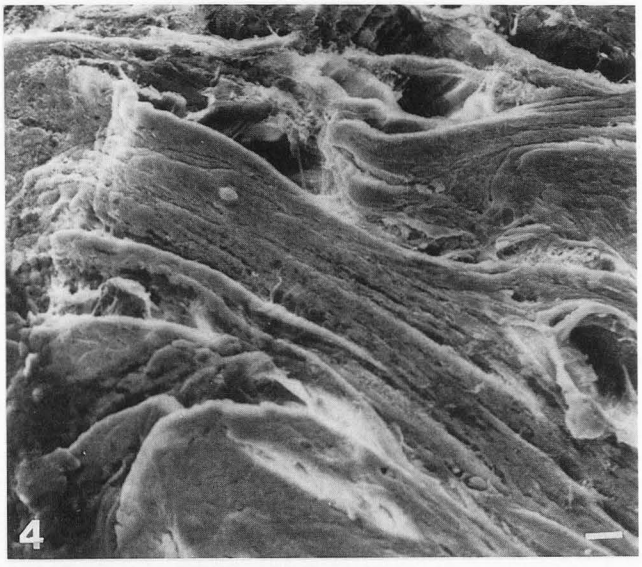
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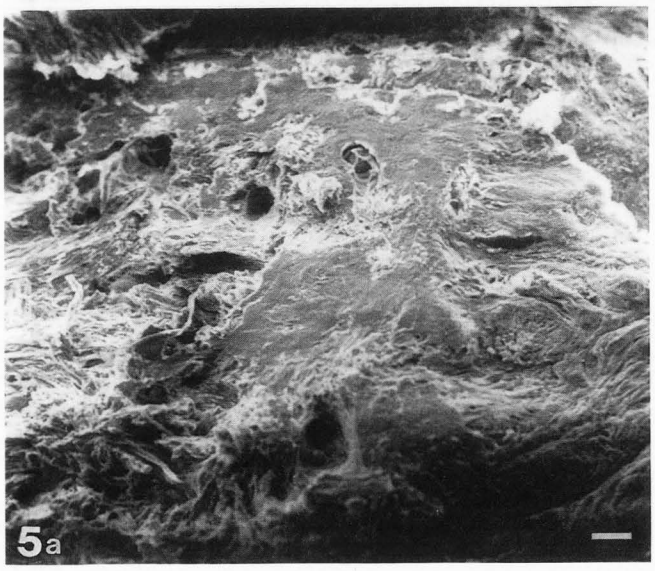
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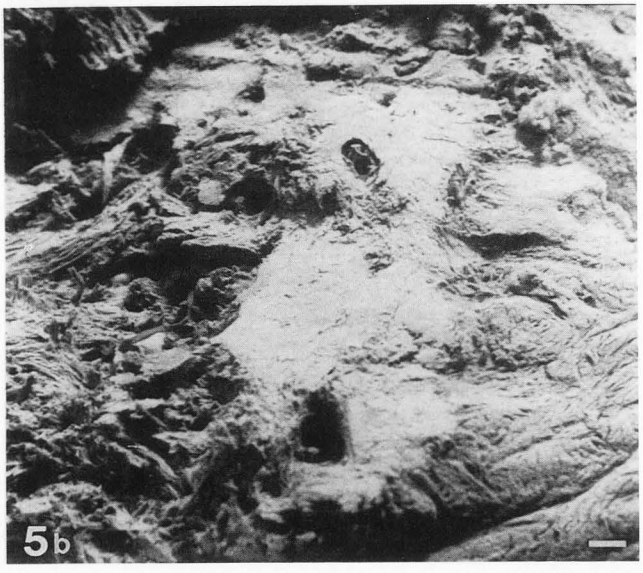
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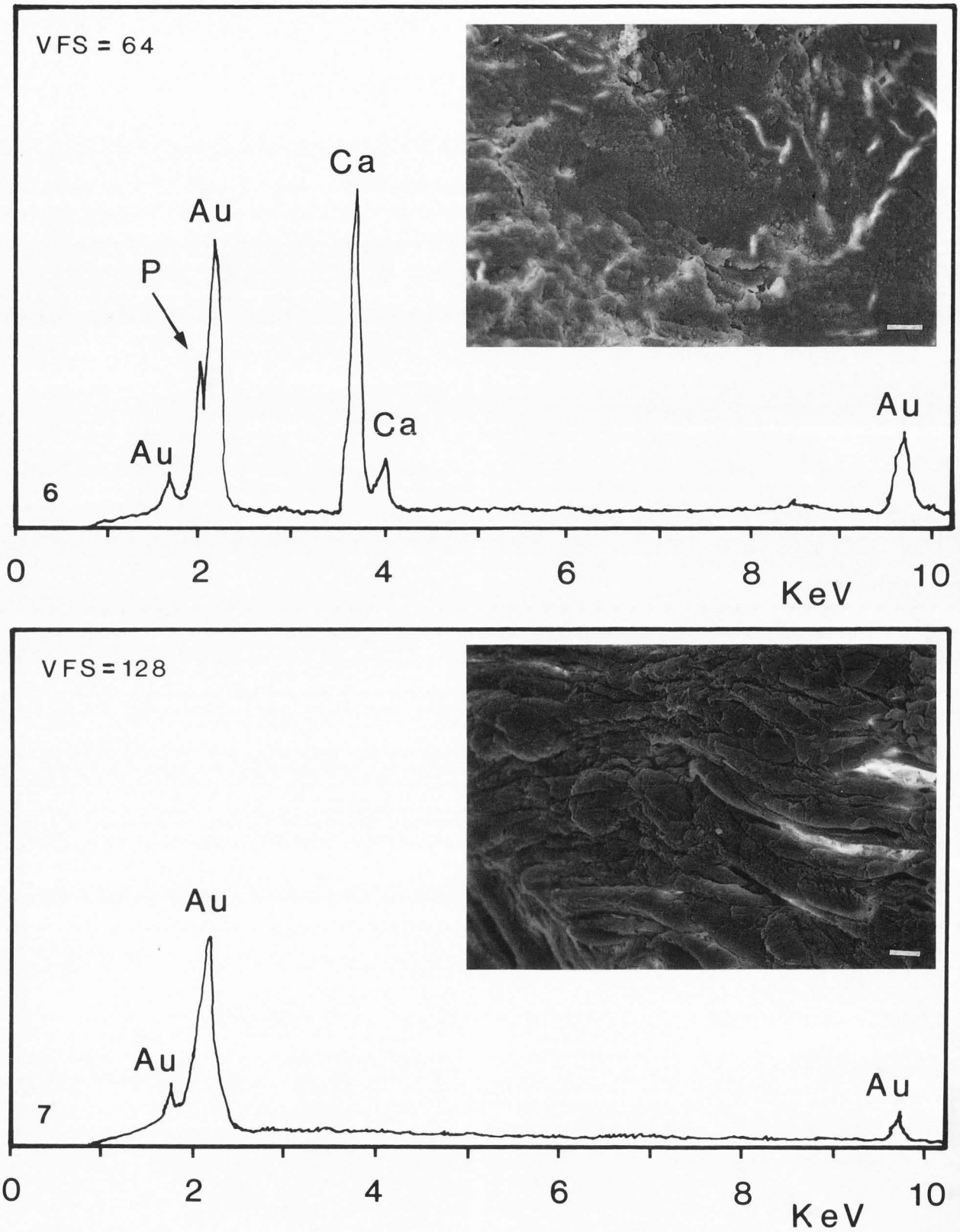

Figures 6 (top) and 7 (bottom). Energy dispersive X-ray spectra of internal mineralized zone (**Fig. 6**) and external fibrous portion (**Fig. 7**) in the calcified disk (area scanning). Vertical full scale (VFS) = 64 (Fig. 6) and 128 (Fig. 7). Bars (in inserts) = 20 μm (Fig. 6) and 10 μm (Fig. 7).

with Digital Microvax 2000 (with software APD-1700). The main characteristics and setting parameters of the diffractometer are: radiation Cu Kα, 50 kV, 30 mA, graphite monochromator, range 2-50° 2Θ.

Results

Calcified disks

SEM investigation At low magnification (Fig. 1), the surfaces of the disks appeared irregular and with non-homogenous features. Centrally-located areas, which appeared more compact compared with the remaining parts of the disks, were present in each disk. The compact areas did not show the presence of bundles of collagen fibers even at higher magnification (Fig. 2). They appeared to be composed of variously-shaped globular aggregations with rather homogenous features.

At the border of these compact areas (Fig. 3), the surfaces of the disks appeared to be formed by bundles of collagen fibers, with a parallel arrangement or with various other orientations. More externally (Fig. 4), the network of fibrous bundles was much more evident.

Secondary (Fig. 5a) and backscattered (Fig. 5b) electron (BSE) images were obtained simultaneously on identical areas of the disk surfaces. BSE images show that the central areas are more luminous than the surrounding areas.

EDS investigation Two analyses were carried out: one (Fig. 6) in the mineralized areas and one (Fig. 7) in the external areas of the disks. In mineralized areas, EDS spectrum, showed presence of the calcium (Kα and Kβ) and the phosphorous signal (at 2.01 keV, partially overlapping the gold Mα peak at 2.12 keV). We also observed that (Figs. 6 and 7) these signals had low intensities, this is mainly due to the SEM operating conditions used so as to avoid evaporation of organic matter of the sample.

XRD investigation To prepare the mineralized portion, we separated the hard central part of the sample from the surrounding soft tissue using a scalpel under a stereo-microscope. The XRD pattern of these samples (Fig. 8a) was characteristic of a mineral with low crystallinity given the low resolution of the peaks. In order to increase the crystallinity, we heated the samples in air to 750°C for 2 hours (Fig. 8b). With this procedure, we increased the number of diffraction peaks and obtained a number of peaks in perfect agreement with peaks characteristics of hydroxyapatite (JCPDS 9-432).

Endoarticular loose bodies

SEM investigation At low magnification the endoarticular loose bodies showed globular formations. One of the loose bodies showed a superficial hollow (Fig. 9). The collagen fiber bundles in the central part of the disk

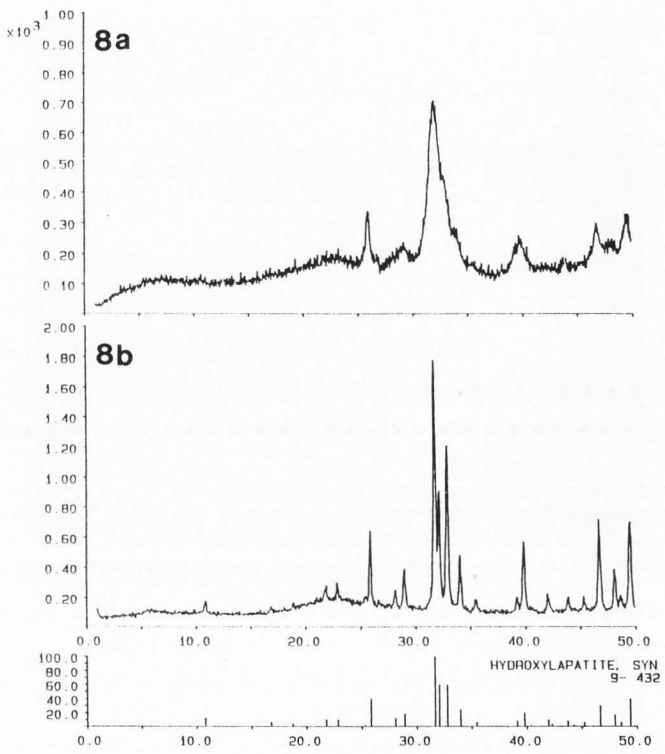

Figure 8. XRD patterns from the calcified disk: (**a**) untreated sample; and (**b**) sample heated at 750°C for 2 hours.

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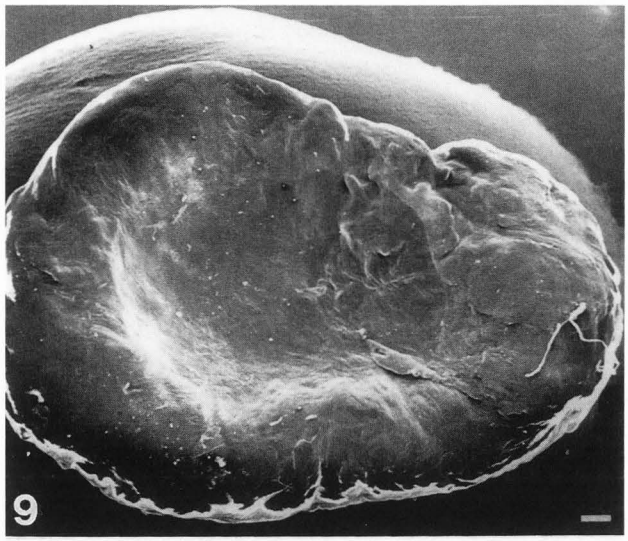
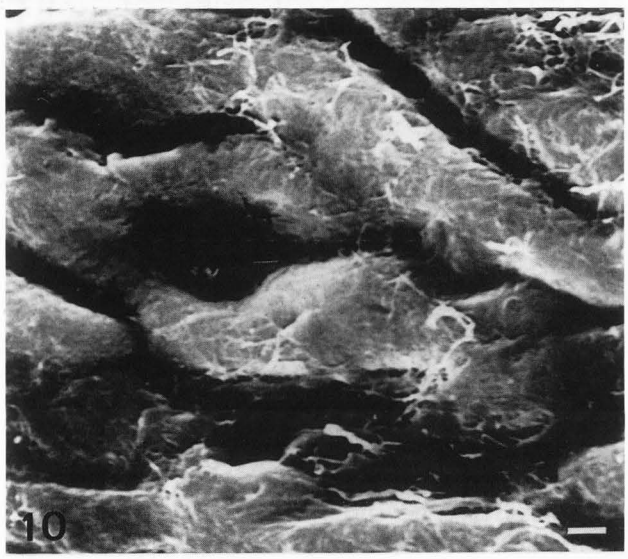
are arranged irregularly and are clearly separate from each other (Fig. 10). The border of this portion of the loose body surface was formed by denser, parallel bundles whose network of fibrils was more visible (Fig. 11). The remaining surface of this loose body and the whole surface of the second loose body appeared compact, and no fibers were detected.

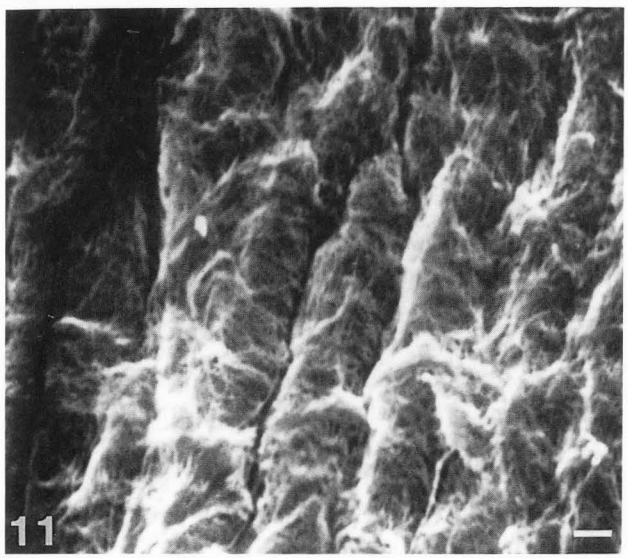
XRD investigation We analyzed a small part of the samples because there were only a few well-defined mineralized zones. The XRD pattern showed a small number of peaks corresponding to calcite, $CaCO_3$ (Fig. 12). It was not possible to carry out an EDS analysis because these samples mainly consisted of organic matter.

Conclusions

Given the necessity to explain the etiology and pathogenesis of discal calcifications and endoarticular loose bodies, we were led to perform comparative examinations using different analysis techniques. These techniques allowed us to clarify the nature of the structure examined from both a morphological and a chemical-compositional point of view. SEM observations were essential in order to describe the morphology of







Figures 9-11. Scanning electron micrographs of an endoarticular loose body.

Figure 9. At low magnification a concave region on the surface of the loose body. Bar = 0.2 mm.

Figure 10. Loose bundles of fibers are present in the central area of the hollow. Bar = 2 μm.

Figure 11. Compact bundles of fibers at the border of the hollow. Bar = 2 μm.

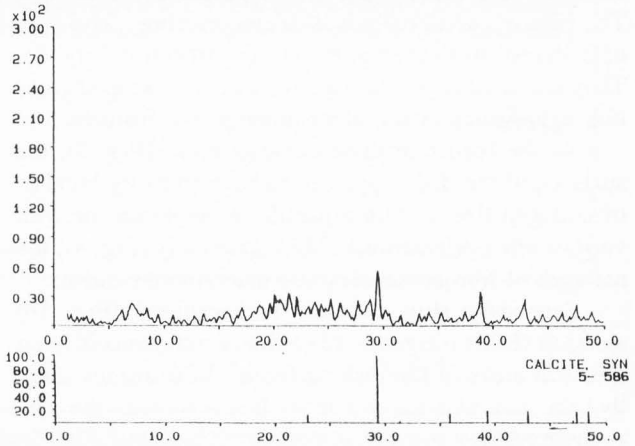


Figure 12. Chemical-compositional analysis of an endoarticular loose body. XRD pattern of the loose body.

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the disks, the presence of collagen fiber bundles in the border areas of the disks, and the presence of areas in which a well-defined structural arrangement was no longer recognizable. With BSE examinations, we were able to highlight the structurally different areas. The EDS analysis allowed us to characterize the basic chemical composition of such areas, whereas with the XRD analysis, we were able to define the crystalline phases.

Using these different analysis techniques, we were able to observe that endodiscal calcifications are composed of hydroxyapatite, whereas the endoarticular loose bodies are composed of calcite ($CaCO_3$).

In light of these findings and of the patients' histories, we can draw some interesting and useful etiopathogenic conclusions. It is possible, despite the limited number of cases, to assume the existence of considerable differences between the two types of lesions examined, although they both seem to result from non-reactive processes. Discal calcifications were present in three subjects affected by articular pathologies due to functional overloading. During arthrotomy, we could observe that the morphology and the position of the discal

deformity were in agreement with the occlusal characteristics of the subjects examined, as if, such a deformity were the result of an intrinsic incapability of the disk to tolerate functional stresses and requests.

Therefore, in these cases, we can assume that the discal lesions may result from a chronic inflammatory process which determines TMJ osteoarthrosis with a dysplastic phenomena affecting the articular disk.

The endoarticular loose bodies were present in a male subject and were, without any morphological remodeling or functional limitations, classifiable as Wilkes stage 2 (Wilkes, 1978). In this case, we can assume that the lesion is the result of an acute inflammatory process which, by modification of the mechanisms of the synovial fluid turnover cycle, caused the accumulation of catabolytes in the articular chamber, the deposition of calcium salt, and the formation of collagen conglomerations around such precipitates.

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Discussion with Reviewers

Reviewer I: Is there any reason why the specimens were not coated with carbon first to do the elemental analysis and then covered with gold for SEM morphological studies?
H. Mishima: The samples were sputtered with gold on SEM-EDS. Could you explain the reason you have used gold instead of carbon?
Authors: We agree that the spattering of samples with carbon yields better working conditions for observation with EDS and BSE, but our first aim was to make only a morphological study and we worked according to the standard methods. Only later, the characteristics of the observed samples induced us to extend the investigation with BSE, EDS and XRD analysis.

Reviewer I: Given the stated conditions of the microprobe analysis, would one not have expected much greater counts?
Authors: Our working conditions were devised to ensure that the organic parts of the samples were not damaged. In any case, these conditions gave qualitatively reliable results.

Reviewer I: Can any more information be given about how the calcite is distributed in the endoarticular loose bodies? Is it appropriate to built hypotheses on such limited material? Could it be related to time, and that if analysed after a longer period, hydroxyapatite might be present. Could calcite have been present somewhere in the discal calcifications?
Authors: The calcite seems to be distributed on a network of collagen fibers. Given that the mineral composition of calcified disk differ from that of endoarticular loose bodies, a pathogenetic hypothesis seems reasonable. Clearly, the confirmation of this hypothesis will require a much larger case series than the present one.

H. Mishima: Were other elements aside from Ca and P detected in calcified disks by EDS investigation?
Authors: We did not detect other elements.

H. Mishima: I think that collagen fibers may have some function in the initial calcification of the endodiscal calcification. Please comment.
Authors: We agree, but feel that the this function would be associated with modifications in the matrix which, with the data currently available, we are unable to confirm.

M.M. Sharawy: Can the hydroxyapatite crystals be detected in the synovial fluid of some of these cases?
Authors: We have not examined the synovial fluid because of difficulties in the surgical procedure.

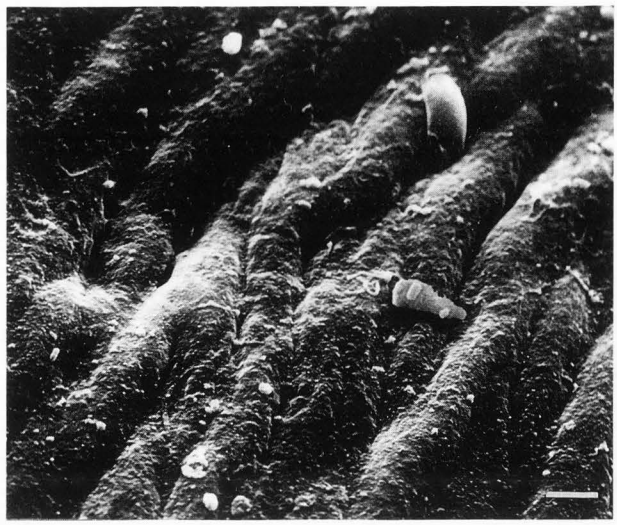

Figure 13. Control disk. Bar = 5 μm.

Reviewer I: A reader with no background to the topic would have difficulty appreciating any morphological change without a view of the control disk.
R.P. Scapino: Can you please provide some information related to observations on normal discs taken from sites comparable to those of the pathological specimens.
Authors: Figure 13 presents a scanning electron micrograph of a control disk. The surface of the disk has an undulated configuration due to the regular arrangement of the compact bundles of collagen fibres.