The fine structure of the surface and superficial layer of the articular cartilage is of special interest for the analysis of the functions and pathological changes of the joint. The arrangement of the collagen fibrils in the surface layer of the articular cartilage has been investigated by the Spaltlinie method (BENNINGHOFF, 1925), by phase contrast microscopy (MACCONAILL, 1951) and by polarization microscopy (MACCONAILL, 1951; TAKEDA, 1968). The transmission electron microscope study by TAKEDA (1968) using replicas seems to be the only work which treats with the fine surface structure of the articular cartilage including rheumatoid changes.

WALKER and his co-workers (1969) recently demonstrated the surface view of the articular cartilage by scanning electron microscopy. Although they described the occurrence of bundles of collagen fibrils and of spherical particles which they presumed to represent an “acid-protein complex of spherical configuration,” their electron micrographs seem not very adequate for a detailed analysis of the surface structures of the cartilage presumably because of an inappropriate method of specimen preparation. The scanning electron micrographs of the articular surface included in the preliminary report by MacCall (1968) do not seem to clarify the detailed structure either.

The present authors who recently reported on the normal aspect and the rheumatoid changes in the surface of the synovial membranes under the scanning electron microscope (FUJITA, INOUE and KODAMA, 1968), attempt in this paper to extend their observation to the surface structure of the articular cartilage which is known to be greatly damaged during the early stage of rheumatoid arthritis.

Materials and Methods

Samples of normal articular cartilage were obtained from the lateral condyle of an 18-year-old male during mid-thigh amputation because of a sarcoma in the tibia. The cartilage and the synovial membranes of the knee joint were judged to be normal macroscopically and light microscopically.

Rheumatoid articular cartilages were taken from the head of the radius of a 24-year-old female during the arthroplasty of the elbow joint and from the lateral condyle of the femur of a 57-year-old female during the débridment of the knee joint. These cases were classical rheumatoid arthritis according to the criteria of the American Rheumatism Association (ROPES et al., 1959). The synovial membranes from these patients showed a marked hypertrophy and unusual growth of villi, and the latter
The specimens, after being gently washed with physiological saline, were fixed for 24 hrs in 10% neutral formalin. They were dehydrated in a series of ascending grades of acetone, i.e. 50, 75, 90% and two changes of absolute acetone, and were then allowed to dry in air. The surface of the specimens was then coated with carbon and gold in a vacuum-evaporator. The evaporation was made with an angle of about 45°, specimens being rotated around the vertical axis. The specimens were observed and photographed with JSM-2 type scanning electron microscope (Japan Electron Optics Laboratory Co., Ltd.) using a beam accelerating voltage of 16 or 25 kV.

**Observations**

**Normal cartilage surface**

The surface of the normal cartilage showed a striking unevenness under the scanning electron microscope in contrast to the smoothness seen with the naked eyes. The unevenness was caused by fiber-like elevations of two different dimensions. Those of larger dimension recognizable already at a low magnification were parallel ridges of 5-7 μ thickness, presumably corresponding to the bundles of collagen fibrils. The elevations of smaller dimension were fibrils of 0.1-0.3 μ thickness and densely interwoven into a network covering the large bundles, though their main course corresponded to the direction of the latter. Small pits, about 0.1-1.0 μ in diameter, were sometimes seen in the meshes of the network. Occasionally fibers and fibrils were partly detached from the surface. In Figure 1-A one recognizes some of these detached fibers which are anchored by fibrils netting them.

**Rheumatoid cartilage surface**

The scanning electron microscope showed marked changes in the cartilage surface of both the radius and the lateral condyle of the femur from the patients with rheumatoid arthritis.

The free surface in the center of the former cartilage showed no parallel ridges and bundles. A low magnification view demonstrated a remarkably large sheet of fibrilar net partly desquamated from the surface of the cartilage base (Fig. 2-A). The fibrils were conglomerated into a thick elevation in the center of the sheet and extended like web to the periphery. Numerous granular particles with a diameter of 0.3-0.5 μ were recognized on the fibrilar mass and they tended to be attached to individual fibrils. (Fig. 2-B).

Undermining the fibrilar net there were cavities which had more or less deeply eroded the cartilage substance. Free cells of the synovial fluid often remained in the cavities (Fig. 3-A, B).

In the circumferential face of the head of the radius the changes above described were even more conspicuous (Fig. 4-A). Especially impressive was the accumulation of abundant spherical and ovoid granules on the surface of the fibrilar masses. Some granules appeared attached to the side of individual fibrils, whereas some others as if incorporated by the fibrils thus causing a beaded appearance of the latter (Fig. 4-B, 5). A few granules appeared to be attached to the end of the fibrils (Fig. 5). In Figure 5 granules of relatively large size (about 0.5 μ) are grouped like a bunch of grapes.
Fig. 1. Surface view of the normal femoral condyle of an 18-year-old male.
A. Large bundles of collagen fibrils show parallel undulations. A few are raised from the base and connected by foot-like fibers. ×1,200
B. The marked part of Figure A in a higher magnification. Note the dense network of fibrils and some small pits in its meshes. ×3,000
Fig. 2. The rheumatoid articular surface of the head of radius. 24-year-old female.
A. A mass of fibrils partly desquamated from the surface. ×1,200.
B. A closer view of the marked area of Figure A showing granular particles attached to the fibrils. ×3,600
Fig. 3. A part of the articular cartilage in a rheumatoid elbow joint.
A. A dirty mass of fibrils and an undermining cavity are shown. ×1,200
B. A closer view showing fibrils attached to small granular particles. A red cell and a synovial free cell of unknown nature are seen. ×3,000
Fig. 4. The circumferential face of the same rheumatoid cartilage as in Figure 3.
A. Fibril sheets and undermining cavities are seen.  $\times 3,600$
B. The marked area of Figure A in a higher magnification showing fibrils and granular substance attached to them. Some fibrils appear beaded.  $\times 10,000$
In the rheumatoid knee joint, the cartilage surface macroscopically presented pannus formation from the surrounding synovial tissues and ulcerations. Scanning electron micrographs from the articular area of the lateral condyle showed more numerous and more variously formed cavities, but less frequent detachment of fibrilar nets and bundles than in the above described elbow joint (Fig. 6). Deep cavities with skelet-like rests of fibril nets and granular particles attached to them were shown (Fig. 6-B).

Discussion

Normal cartilage surface

The present study indicates the occurrence of fibrous structures of two different dimensional orders in the normal cartilage surface. The parallel ridges of 5–7 μ thickness are most likely bundles of collagen fibrils. Although the appearance of the undulation in the surface may possibly have been accentuated by the shrinkage of the tissue during the drying procedure, the presence of parallel bundles is doubtless because some

Fig. 5. Granular particles accumulated in a grape-like bunch. Circumferential face of a rheumatoid elbow joint. ×10,000
Fig. 6. Surface view of the articular cartilage of the lateral condyle of the femur from a rheumatoid knee joint. 57-year-old female.
A: showing round excavations of various sizes. Few fibrilar components are shedding. ×1,200
B: Cavities deeply undermining into the cartilage matrix and web-like fibrilar sheets remaining in the cavities. ×3,000
fibers of corresponding dimension and direction have been found raised from the cartilage surface, as shown in Figure 1. Raised bundles fixed by the "roots" of fibrils to the underlying cartilage were described also by Walker and his associates (1969). The thin bands of 0.1–0.3 μ thickness are without doubt the collagen fibrils in the sense of word used by light microscopists. Each of them may correspond to a bundle of ten or more collagen filaments which are known to transmission electron microscopists to be 500–1,000 Å thick. It thus seems probable that the surface layer of the articular cartilage is composed of collagen fibrils which are interwoven to form thick bundles on one hand, and on the other, combine the individual bundles and fix them to the base.

This view is compatible with the early description by Benninghoff (1925) that the collagen fibriles of the articular cartilage form arcades which stand on the deep calcified layer of the cartilage, the apical fibrils running tangentially to the surface. The electron microscopic observations of replicas by Takeda (1968) indicate that the superficial collagen fibrils are arranged in a constant direction in mono-axial joints, whereas irregularly in multi-axial ones. The present result obtained in a mono-axial joint seems to confirm partly the description by Takeda (1968). Observation under the scanning electron microscope of the cartilage in various types of joints seems to be a profitable theme of study.

It should be emphasized that the fibrous system above described is naked in the surface of cartilage. It can hardly be believed that the highly viscous ground substance of the cartilage has been washed away by physiological saline. That the collagen fibrils are naked in the surface of articular cartilage has been shown by Takeda (1968) by the use of electron-microscopy of replicas, though MacConaill (1951), using a phase contrast microscope, insisted on the presence of a thin superficial layer consisting of pure hyaline substance without fibrous element. As for the small pits found in the meshes of the fibrils, it is not known whether or not they correspond to the "cartilage canals" described in human condylar cartilage by Haines (1963) and Blackwood (1965).

Rheumatoid cartilage surface

The present study reveals desquamation of fibrous sheet and formation of deep cavities in the rheumatoid articular cartilage. It has been known that in chronic arthritis a pannus growing over the cartilage surface erodes the cartilaginous substance and replaces it with dense fibrous tissue. Some authors ascribed the erosion of the cartilage to a change in enzymatic activity in the rheumatoid synovial fluid (Thomas, 1965; Fell and Thomas, 1960). Ziff and his co-workers (1960) reported that the homogenates of rheumatoid synovial membrane lowered the viscosity of cartilage mucoproteins. Dingle (1962) proposed that arthritic changes may activate lysosomal enzymes which are involved in the destruction of cartilaginous tissue. In fact, some studies proved an elevated lysosomal enzyme level in synovial membranes obtained from rheumatoid patients (Luscombe, 1963; Barland et al., 1964).

The observation by Takeda (1968) of replicas taken from a knee joint of a rheumatoid patient shows irregular unevenness covered by a muddy substance without any visible fibrous element. The discrepancy of this finding from ours may possibly be attributed to different types of rheumatoid lesions.

The nature of the granular particles attached to the surface of the rheumatoid cartilage is problematic. Walker and his associates (1969) found similar granules...
under the scanning electron microscope and suggested that they correspond to acid protein complexes. The description of these authors that the spherical granules congregate preferentially on undamaged rather than on damaged cartilage surface is incompatible with our observation because, in the present study, the normal articular cartilage was free of those granules. The occurrence of beaded fibrils and the apparent attachment of a granule at the end of a fibril as recognized in the present study suggests a possibility that the granules might correspond to a product of destroyed fibrils.

Following the preceding paper of these authors on the surface changes in rheumatoid synovial membranes (FUJITA, INOUE and KODAMA, 1968), the present paper gives evidence that also the pathological changes in the surface of articular cartilage can be examined simply and effectively with the scanning electron microscope. The present observations themselves will be extended and, perhaps, revised by the use of further materials of study obtained from various joints and various patients.

Summary

Specimens of articular cartilage were obtained during surgical operations from the lateral condyle of a normal femur and from the head of the radius and the femoral condyle of rheumatoid patients. The specimens were fixed in 10% formalin, dehydrated in acetone, dried in air and coated with carbon and gold to be observed in a scanning electron microscope.

1. The surface of the normal cartilage showed parallel ridges of 5-7 µ width densely netted by fibrils of 0.1-0.3 µ thickness. It was emphasized that these probable collagen fibrils were naked in the cartilage surface.

2. In the rheumatoid cartilage surfaces sheets of fibril nets were partly desquamated and the cartilage substance was irregularly excavated presumably by an elevated action of lytic enzymes. Granules of unknown nature abundantly occurred on the fibrils.

3. This study proves the applicability of scanning electron microscopy to the ultrastructure research of the cartilage surface in normal and pathological conditions.

手術中に得られた正常な大腿骨髄部およびリウマチ患者の膝骨頭部、大腿骨髄部の軟骨片を、それぞれ 10% ポルマリン固定、アセトン脱水、空気中で乾燥、カーボンおよび金の蒸着を施し、走査電子顕微鏡で観察した。

1. 正常膝関節の大腿骨髄部表面は、5〜7 µの太さの線維束が規則正しく並び、高倍で見ると、その上を 0.1〜0.3 µ 大の繊細な線維の網目がおおっており、軟骨表面にはこれらの線維成分が露出している。

2. リウマチ関節の軟骨表面では、線維網の剝離、ある種の融解酵素の活性化を示唆する陥凹や空洞の形成を見る。またその本体は分からないが、無数の小顆粒物質が軟骨表面に付着している。

3. 走査電子顕微鏡は、正常あるいは病的関節の軟骨表面における微細構造の観察にも優れた方法であることがわかった。
References

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