Scanning Electron Microscopy of the Normal and Rheumatoid Synovial Membranes

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The inner surface of the synovial membrane has gained increasing interest with the progress of study on the pathohistological changes in this tissue. The previous methods for the study of the normal and pathological synovial surface have been represented either by arthroscopy which enables the observation, though macroscopic, in situ or by binocular microscopy of biopsy specimens and of tissues taken in surgery or autopsy. These methods have been, however, insufficient for the elucidation of minute relief in the synovial surface. As for the submicroscopic surface structure, it has been only imagined on the basis of the knowledge obtained from the transmission electron microscopy of sectioned specimens that the synovial surface should be markedly rough because of the presence of cellular and subcellular projections (see the three-dimensional illustration by Barland, Novikoff and Hamerman, 1962). The surface of the articular cartilage in normal and rheumatoid joints was observed under the electron microscope by Takeda (1968) by means of the replica method. This method, however, has not been applied to the study of the surface of the synovial membrane as far as the authors know.

The light and electron microscopic changes in the rheumatoid synovial membrane are characterized by a more or less conspicuous proliferation of the lining cells and of the cellular, fibrilar and vascular elements of the underlying connective tissue. Fibrous and/or amorphous debris is known to be deposited on the lining cell surface (Norton and Ziff, 1966; Ghadially and Roy, 1967). It seems of high interest to see what changes in the surface view might occur in correspondence to these changes which have hitherto been observed only in tissue sections.

In the present study, normal and rheumatoid synovial membranes were observed with a scanning electron microscope. This microscope, which has recently been proved by Barber and Boyde (1968) to be applicable for the examination of the surface structure of the ciliated epithelia, is used, as far as the authors know, for the first time in the study of the joint wall.

Materials and Methods

Pieces of the normal synovial membrane were taken from the left knee joint of an 18-year-old male during mid-thigh amputation because of a sarcoma in the tibia. Rheumatoid synovial membranes were obtained in surgical operations: 1) from the
Fig. 1. Normal synovial surface of the knee joint. 18-year-old male.

A: A survey in a low magnification (×360).

B: A view in a higher magnification (×3,600) showing the projections of the second order (corresponding to the individual lining cells) provided with those of the third order (cytoplasmic processes).
left knee joint of a 30-year-old female of rheumatoid arthritis judged as “classical”
type according to the criteria of the American Rheumatism Association (ROPES et al.,
1959); 2) from the right wrist joint of a 27-year-old female with “definite” type; and
3) from the left elbow joint of a 57-year-old female with “definite” type rheumatoid
arthritis.

The specimens, about 1 × 1 cm large and 0.5 cm thick, were gently washed by
a pipette with physiological saline and then fixed for 24 hrs in a 10% neutral for-
malin. After being rinsed in distilled water, they were transferred through a series
of ascending concentrations of aceton, from 50 through 70, 90 to 100%. The spe-
cimens were then allowed to air dry (BARBER and BOYDE, 1968).

The synovial surface of these specimens was coated with gold in a vacuum-
evaporator. The specimen was rotated around the vertical axis while the evapora-
tion was made with the angle of 45° to the specimen. The thickness of the coated
gold was checked by preliminary scanning electron microscopy and the coating
was repeated if too thin. Usually the specimens showing a slight gold glance of the
surface gave the best result.

Observation and photography were carried out with a JSM-2 scanning electron
microscope (Japan Electron Optics Laboratory Co., Ltd.) with 16 or 25 KV accele-
rating voltage.

**Observations**

**Normal synovial membrane**

Under the scanning electron microscope the surface of the synovial membrane
showed projections and indenations of three different dimensions.

The projections of the first order were the synovial folds and villi which are
familiar in macroscopic anatomy. Those of the second order were the numberless
projections of oval, cony and irregular shapes which evenly covered most part of
the surface. These protrusions varied considerably in size (2–5 µ in thickness) and
stood loosely to each other, i.e., keeping a distance of a few micra (Fig. 1, 2).

The projections of the third order were lump-like or papillary in shape and

Fig. 2. Normal synovial surface in a high magnification
(×3,600). 18-year-old male. Note the strawberry-like projec-
tions (of the second order) which correspond to the lining
cells and their small processes.
Fig. 3. A part of the normal synovial surface of an 18-year-old male.

A: Note that, in place of the protrusions of the second order, slightly depressed areas probably corresponding to the individual lining cells are recognized. A few erythrocytes (contamination) are seen. $\times 3,600$

B: The area marked in Figure A in a higher magnification ($\times 10,000$). Note the protrusions of the third order (cytoplasmic processes) of a fairly constant size and distribution.
fairly constant in thickness (0.1—0.2 μ) and were protruded from those of the second order, giving the latter a knarry appearance (Fig. 1-B, 2).

In a small part of the synovial membrane the projections of the second order failed and, instead, slightly depressed areas of 5—10 μ diameter were recognized which probably corresponded to flattened individual cells. In this case the papillary projections of the third order covering the whole surface were observed more clearly than otherwise (Fig. 3).

**Rheumatoid synovial membrane**

The scanning electron microscopy clearly demonstrated the unusually large and complex synovial villi whose occurrence is well known in rheumatoid joints. Some were represented by finger-like forms (Fig. 4), whereas others by cauliflower-like ones (Fig. 5). Also in the parts lacking in such special projections, the rheumatoid synovial surface was characterized by its very irregular elevations and depressions. Complex grooves formed here and there resembled stalactite caves (Fig. 6, 7).
Projections of the second order were distributed irregularly on the base of the undulations of the first order. They occurred, especially in the specimens from the rheumatoid knee joint, rather sporadically (Fig. 6, 7, 8). They were mostly polyp-like or nipple-like in shape and varied markedly in size, measuring mostly 3–6 μ in thickness. Their round heads as well as the other parts of the synovial surface were covered with protrusions of the third order which appeared thicker (0.2–0.3 μ) and lower than in the normal case. In the specimens from the knee joint of the 30-year-old patient these protrusions were especially low and appeared as dull and inconspicuous lumps attached to the round head (Fig. 6-B, 7-B). In this rheumatoid knee joint, the synovial surface showed larger and smaller areas of a homogeneous and smooth aspect suggesting a deposition of a muddy substance (Fig. 6, 7).

**Fig. 5.** Cauliflower-like villi in the elbow joint of a 57-year-old female with “definite type” rheumatoid arthritis. The protrusions of the second order (individual lining cells) are recognized already in this medium magnification (×1,200).

**Fig. 6.** Synovial surface in the knee joint of a 30-year-old female with “classical type” rheumatoid arthritis.

A: The whole surface is irregularly undulated and polyp-like structures are protruded here and there. ×3,600

B: The marked area of Figure A in a higher magnification (×10,000). This round protrusion probably corresponds to a swollen lining cell. Note that the protrusions of the third order (cytoplasmic processes) are low and inconspicuous.
Fig. 6
Fig. 7. Synovial surface of the same joint as in Figure 6.

A: Protrusions of the second order are represented by the sporadic polyp-like structures. A large part of the surface appears smooth and "muddy". \( \times 3,600 \)

B: The marked area of Figure A in a higher magnification (\( \times 10,000 \)). The inconspicuous appearance of the cytoplasmic processes may be ascribed to the deposition of the "muddy" substance.
Fig. 8. Synovial surface in the elbow joint of a 57-year-old female with "definite type" rheumatoid arthritis. In this case the "muddy" substance is not recognized. However, the very irregular size and disposition of the lining cell protrusions (of the second order) and their roundly swollen appearance seem characteristic of rheumatoid change. A: ×3,600, B: ×10,000
Discussion

Normal synovial membrane

It is especially impressive in the scanning electron microphotographs of the joint wall, that the surface area of the synovial membrane is conspicuously enlarged by the protrusions of the different three orders described above. This undoubtedly reflects the well known fact that the transport of substances is very active through the synovial surface (Adkins and Davies, 1940; Langer and Huth, 1960; Cochran, Davies and Palfrey, 1965).

The protrusions of the first order may need no explanation as they simply correspond to the synovial plicae in macroscopic anatomy which fill the dead spaces of the joint cavity. The protrusions of the second order, measuring 2—5 μ in thickness are believed to be the projected tops of individual lining cells. To confirm this assumption, a rough measurement of the normal human lining cells in ultrathin sections was made using the electron microphotographs of previous authors (Barland, Novikoff and Hamerman, 1962) and of one of the present authors (Inoue, unpublished data). The thickness of the protruded part of the lining cells thus measured corresponded well with the size of the projections in question under the scanning electron microscope.

It may now be natural to identify the protrusions of the third order with the “tentakelähnliche cytoplasmatische Fortsätze” observed in animals (Langer and Huth, 1960) and with the “finger-like cell processes” described in human synovial membrane (Barland, Novikoff and Hamerman, 1962) under the transmission electron microscope. According to some authors (Barland, Novikoff and Hamerman, 1962; Hirohata and Kobayashi, 1964; Ghadially and Roy, 1967; Barland, Smith and Hamerman, 1968), the lining layer of the normal human synovial membrane consists of cells of two different types; the Type A cells characterized by rich vacuolar and lysosomal elements are provided with more numerous cytoplasmic processes than the ergastoplasm-rich B cells. The present scanning electron microscopic observation could lead to no conclusion on a possible difference in the cell surface of the lining layer with respect to the different cell types.

Rheumatoid synovial membrane

The proliferation of the lining cells and the underlying cellular and fibrous tissue elements which is known as one of the characteristic changes in rheumatoid arthritis seems to be well manifested in the irregularly elevated and swollen aspects in the surface view of the synovial membrane shown in the present study.

The protrusions of the first order correspond to that unusual growth of villi which is familiar in the arthroscopy of the rheumatoid joints. Light microscopic studies in sectioned preparations indicate that these villi may often contain larger and smaller lymph follicles.

The protrusions of the second order which appear in polyp-like forms under the scanning electron microscope probably correspond to the elevation of individual lining cells. The protrusions agree in thickness (3—6 μ) with those of the sectioned lining cells shown in the transmission electron microphotographs by Norton and Ziff (1966) and by one of the present authors (Inoue, unpublished data). Figure 3 in the paper of Norton and Ziff shows a section of a large and round lining cell
swelling up into the joint cavity which is very much like the polyp-like projections in the present study.

The possibility may not be excluded that a part of the polyp-like bodies would be represented by leucocytes, though these cells which are contained in the joint fluid of rheumatoid patients more or less numerously are believed to have been washed away at least for the most part by physiological saline.

The protrusions of the third order, or the cytoplasmic processes, in the rheumatoid synovial surface may be characteristically low and dull as compared with those in the normal joint. The cytoplasmic processes seem as if smoothed out by the swelling of the cell. The inconspicuousness of the processes may be, however, at least partly ascribed to a slight deposition of the muddy debris of the rheumatoid synovia on the lining cell surface.

The surface view as shown in Figures 6 and especially 7 which suggests a deposition of a larger amount of this "mud" was recognized only in the specimens from the knee joint. This seems to correspond to the clinical experience that the "mud" is often conspicuous in this joint which may contain an especially large amount of fluid in rheumatoid inflammation. In the transmission electron micrographs of sections the "mud" appears as either granular or fibrous substance more or less intimately attached to the synovial surface (Norton and Ziff, 1966; Ghadially and Roy, 1967) and may be difficult to be completely washed by physiological saline. Because of the reduction of the cytoplasmic processes of the lining cells, the surface area of the rheumatoid synovial membrane seems, in spite of its conspicuous villus formation, to be smaller than the normal one. Moreover, if one takes it into account that the lining cells may be covered by the muddy debris, the effective surface of the synovial membrane for the transport of substances is believed to be diminished in rheumatoid arthritis more or less markedly.

The description on the surface structure and its rheumatoid changes in the present paper is based on the findings obtained from a small number of cases and need confirmations or revisions by further investigations in a larger number of normal and pathological cases. It is especially intended in this paper to propose that the scanning electron microscope is a very effective tool for studying and recording the surface view and its changes of the joint walls.

Summary

Normal and rheumatoid synovial membranes obtained in surgical operations were fixed in 10% formalin, dehydrated in aceton, dried in air, coated with gold and examined under a JMS-2 type scanning electron microscope.

1. In the normal synovial surface, individual lining cells were protruded fairly uniformly into the joint cavity. These protrusions were covered with small papillary cytoplasmic processes.

2. In the rheumatoid synovial membrane which was characterized, already on the macroscopic level, by a conspicuous unevenness of the surface and by a formation of unusual villi, the lining cells were protruded into the cavity more irregularly and sporadically than in the normal case. Some cells swelled up roundly high into the synovial cavity. The lining cells may carry lower and less conspicuous cyto-
plasmic processes than the normal ones. Some areas of the synovial surface looked smooth and amorphous suggesting a possible deposition of the muddy debris of the rheumatoid synovia.

3. The present study proves that scanning electron microscopy provides an excellent method for studying and recording the surface aspect and pathological changes of the synovial membrane.

References

Barber, V. C. and A. Boyde: Scanning electron microscopic studies of cilia. Z. Zellforsch. 84: 269-284 (1968).