J. Anat. (1979), **129**, 4, pp. 823–831
With 16 figures
Printed in Great Britain

High resolution scanning electron microscopy of elastic cartilage

ELLEN HOLM NIELSEN AND PETER BYTZER

Winslow Institute of Human Anatomy, University of Odense, Campusvej 55, 5230 Odense M, Denmark

(*Accepted* 2 *January* 1979)

INTRODUCTION

Since the advent of the scanning electron microscope (SEM) many papers dealing with the structure of cartilage as seen in the SEM have appeared, but most of them have been concerned with the surface structure of articular cartilage. Only a few (Clarke, 1971, 1974; Zimny & Redler, 1972; Ornoy & Langer, 1978) have described the inner structure of either articular or epiphyseal cartilage.

Elastic cartilage has never been studied with the SEM. With the advent of high resolution techniques it is now possible to obtain a more detailed understanding of the structures seen in the transmission electron microscope (TEM) (Holm Nielsen, 1976). We have therefore studied the chondrocytes, their relations to the lacunae, and the arrangement and morphology of the fibrils of the territorial and interterritorial matrices, in the elastic cartilage of the rat epiglottis, using a high resolution SEM technique.

MATERIALS AND METHODS

Eight adult rats were anaesthetized with ether or with an intraperitoneal injection of 5 % Nembutal sodium, 0·7 ml/100 g body weight. The rats were perfused for 10 minutes through the left ventricle of the heart with 2·5 % glutaraldehyde in 0·1 M sodium cacodylate buffer, pH 7·3, or with 1·5 % glutaraldehyde and 1 % paraformaldehyde, at a pressure of 90–110 mm Hg. The epiglottis was removed and cut into small pieces, which were fixed in the same fixative from 1–7 days at 4 °C. The specimens were washed several times in 0·1 M cacodylate buffer, pH 7·3, post-fixed in 1 % OsO_4 for 60 minutes at room temperature and dehydrated in increasing concentrations of acetone (25 %, 40 %, 60 %, 75 %, 90 %, 95 %, 100 %) for 2½ hours at 4 °C. Some of the specimens were frozen in liquid Freon 22 and fractured before the critical point drying procedure. The specimens were mounted with silver-paint, coated with gold [50 nm] in a sputter coater, and examined in a Jeol 100 CX electron microscope, with an EM-ASID-4D scanning attachment, operated at 20 and 40 kV.

OBSERVATIONS

The elastic cartilage of the rat epiglottis, like cartilage elsewhere, is made up of chondrocytes placed singly or in isogenic cell groups in lacunae in an intercellular substance (Fig. 1). Each lacuna is surrounded by a rim of condensed intercellular substance called the territorial matrix or ring, the rest of the intercellular substance being referred to as the interterritorial matrix. The cartilage is covered with perichondrium.

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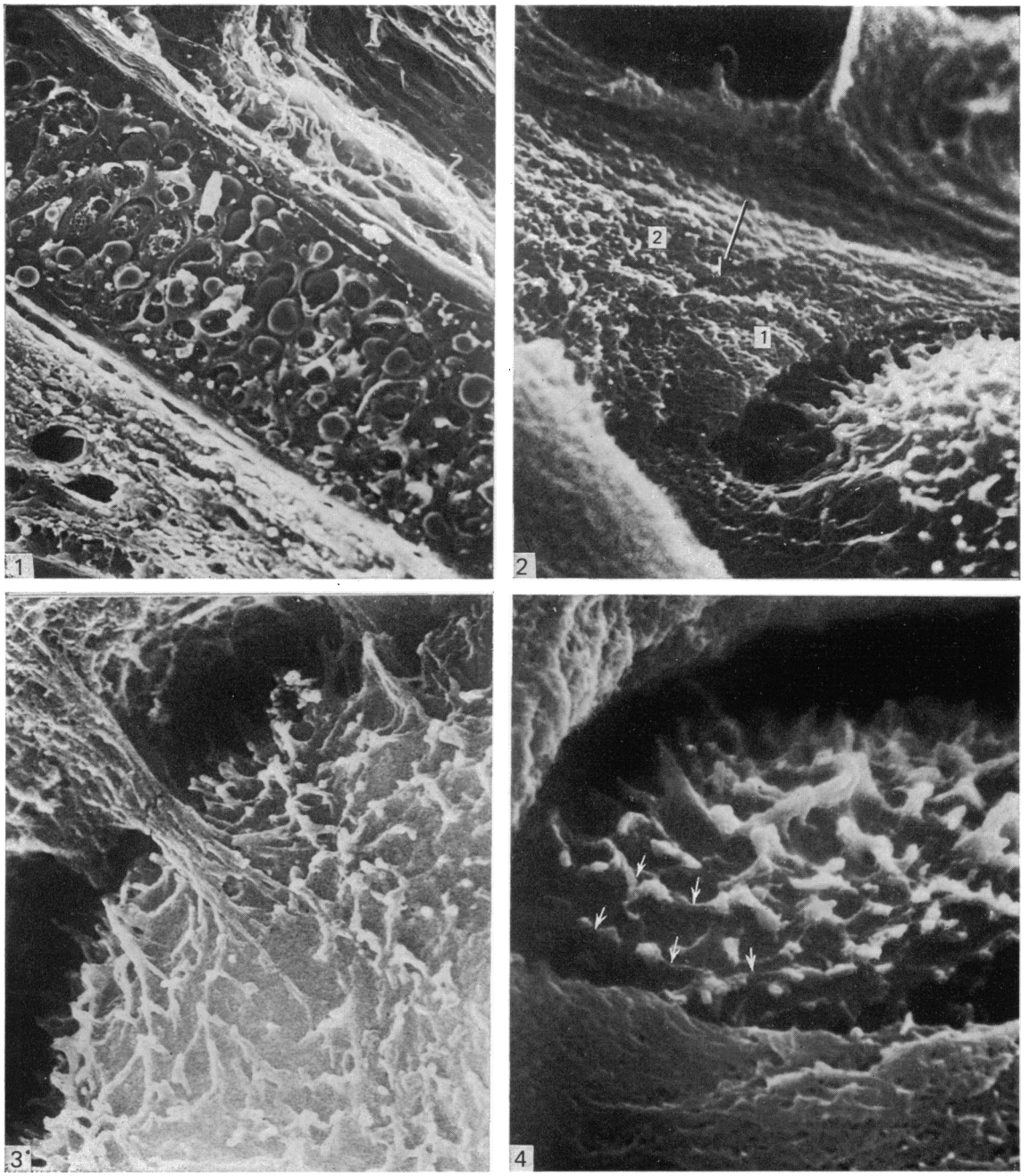


Fig. 1. Transverse section of elastic cartilage of rat epiglottis, showing chondrocytes and the sparse amount of intercellular substance. × 300.

Fig. 2. Chondrocyte in lacuna surrounded by territorial matrix (1). The border between the territorial and the interterritorial matrices (2) is marked by the arrow. × 8000.

Fig. 3. A bundle of territorial fibrils attached to the microvillous processes and to the chondrocyte surface. × 10000.

Fig. 4. Chondrocyte with rows of microvillous processes twined around the cell and joined by narrow ridges (arrows). × 15000.

All chondrocytes were situated in lacunae surrounded by a territorial ring (Fig. 2). Cells in the centre of the cartilage were round, while cells near the periphery were oblong with their long axes parallel to the perichondrium. Some lacunae contained two chondrocytes with flat abutting surfaces.

In all lacunae there was a 0·5 to 2 μm gap between the cell and the lacunar wall (Fig. 2). In this gap cellular processes and long fibrils originating from the lacunar wall were seen, but as a rule neither the processes nor the fibrils traversed the full width of the gap. In a few places, however, we observed a region of fusion between the cell and the lacunar wall consisting of fibrils interdigitating with and connected to microvillous processes and the cell surface (Fig. 3). These connexions had obviously been affected by the traction induced by cellular shrinkage during preparation. Fibrils were sometimes seen to project from the cells. As these fibrils looked like those found in the territorial matrix, we are convinced that they had remained stuck to the cells as these shrank away from the lacunar wall during the preparation of the specimen.

The cellular surfaces were studded with irregularly arranged microvillous processes. In a few cells, however, a certain pattern was discernible in the form of long rows of processes spirally twined around the cell (Fig. 4). The microvillous processes were from 0·3 to 1·0 μm long with a mean diameter of 0·2 to 0·3 μm. Many of the processes ramified from low crests, which were now and then joined by narrow ridges. Some of the processes had swollen or bifurcated tips.

In empty lacunae the territorial matrix was fully exposed as a cup-shaped structure often partly detached from the surrounding interterritorial matrix (Fig. 5). In the detached areas no morphological connexions were visible between the two.

The thickness of the territorial ring varied from 0·6 to 1·2 μm, with a typical value around 0·75 μm. It consisted of a finely woven mat (Fig. 6) of fibrils crossing at different angles with small thickenings at the points of intersection (Fig. 7). The fibrils ramified or bifurcated frequently, making it impossible to measure their lengths. All fibrils were arranged tangentially to the lacunae. In some areas sheets made up of paired fused fibrils were seen (Fig. 8).

The diameters of the fibrils varied from 40 to 100 nm with a numerical predominance of fibrils with diameters around 65 nm. The thick and thin fibrils were woven randomly.

In most cases the territorial ring was sharply demarcated from the interterritorial matrix by a zone of fibrils arranged in parallel with only very few crossing fibrils (Fig. 9). Figure 10 also shows this as well as the more complex layer of territorial matrix just visible underneath the parallel zone.

The interterritorial matrix consisted of a finely woven mat of fibrils with diameters from 50 to 120 nm (Fig. 11). In contrast to the arrangement in the lacunar wall, where all fibrils lay in the same plane, the structure in the interterritorial matrix was more three-dimensional but still the majority of the fibrils were arranged in parallel with the lacunar wall. This could be the reason the interterritorial matrix was generally exposed in sheets. In such sheets, fibrils arranged in parallel were interconnected by thinner fibrils. At regular intervals a pair of fibrils joined to form a common fibril which then seemed to alter its original course to form part of a neighbouring sheet (Fig. 12). Small globules with diameters of 110 to 180 nm were now and then observed in the interterritorial matrix (Fig. 13). We did not observe any specific ultrastructure in these globules.

At ultra-high-resolution the fibrils of both territorial and interterritorial matrices

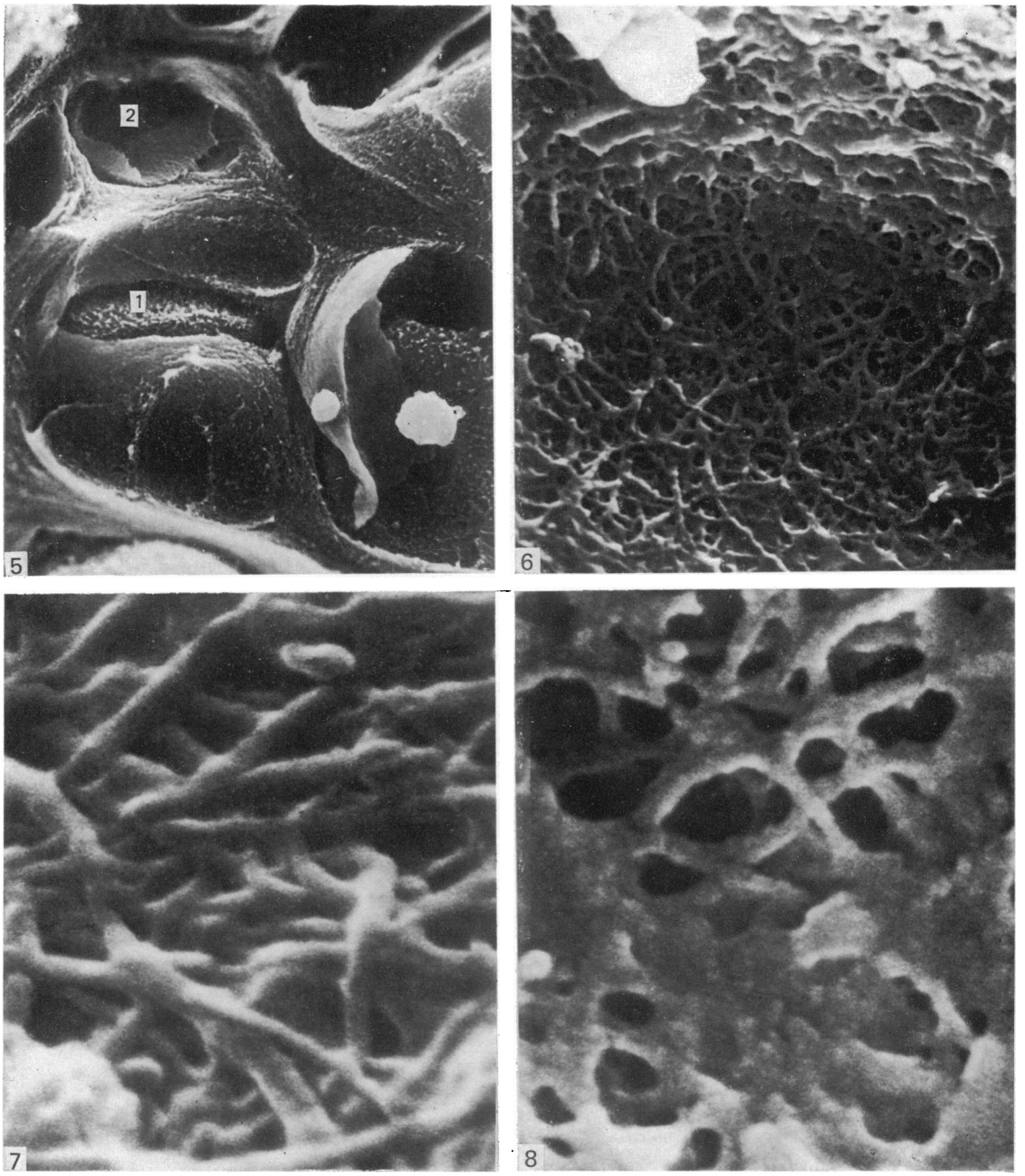
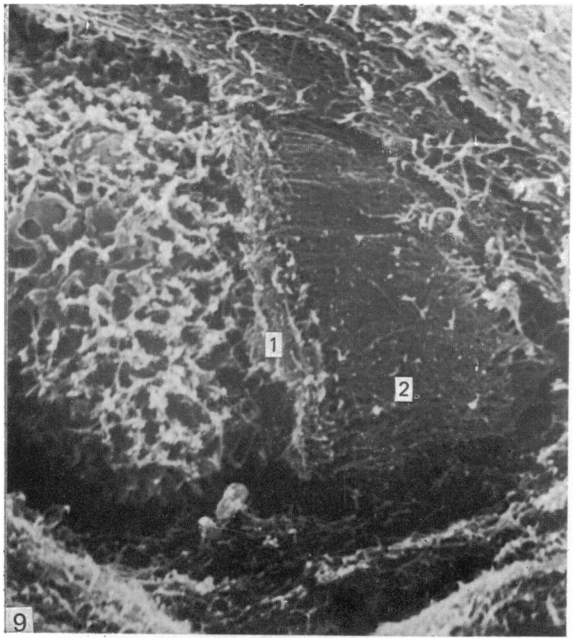


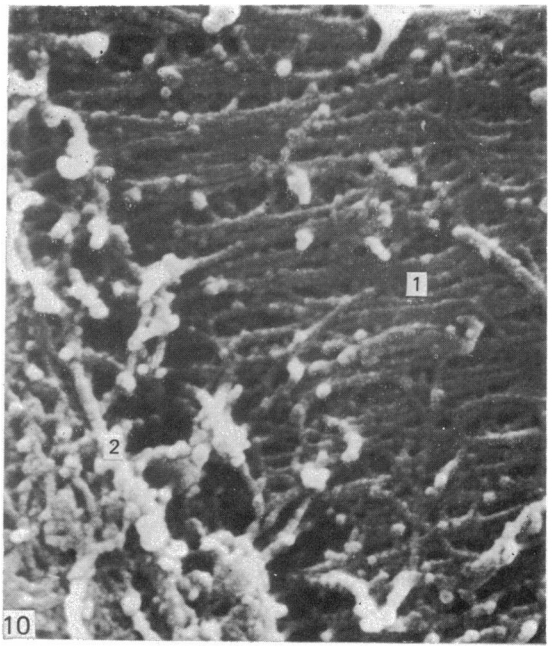
Fig. 5. Section through elastic cartilage showing a chondrocyte in its lacuna (1) and an empty lacuna with partly detached territorial matrix (2). ×2000.

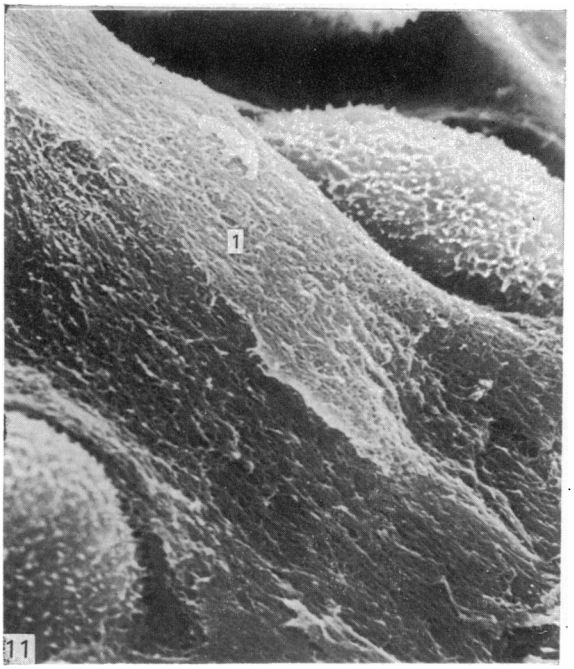
Fig. 6. The woven mat of territorial matrix in the lacunar wall. ×15000.

Fig. 7. Detail from Fig. 6 showing the crossing of fibrils. ×80000.

Fig. 8. Detail from Fig. 6 showing sheets of fused fibrils. ×60000.







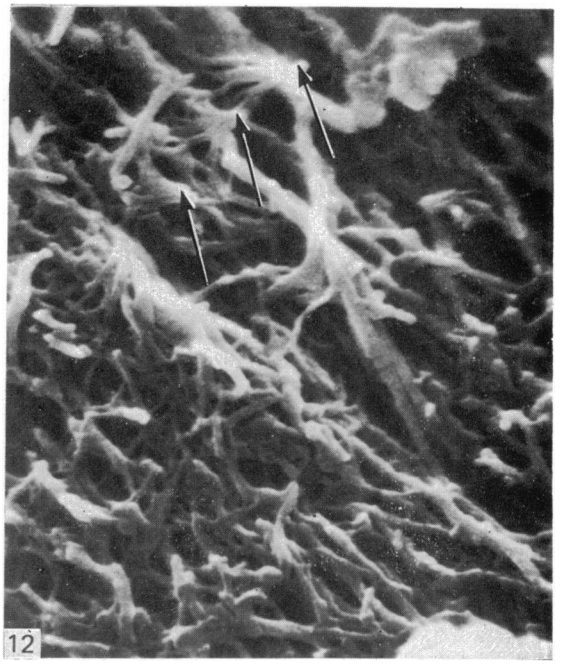
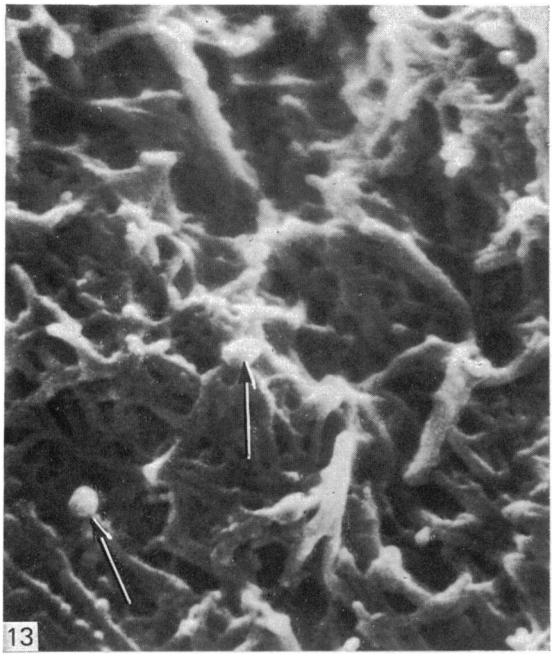


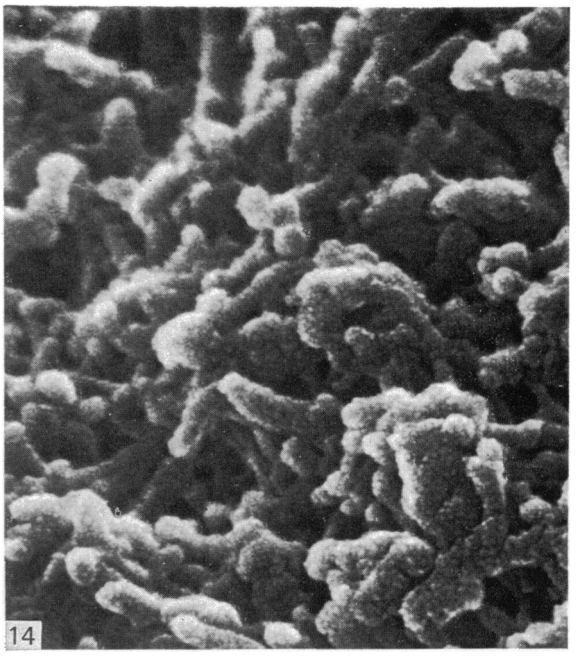
Fig. 9. Chondrocyte partly covered by the territorial matrix (1) and the zone of parallel fibrils (2). × 6000.

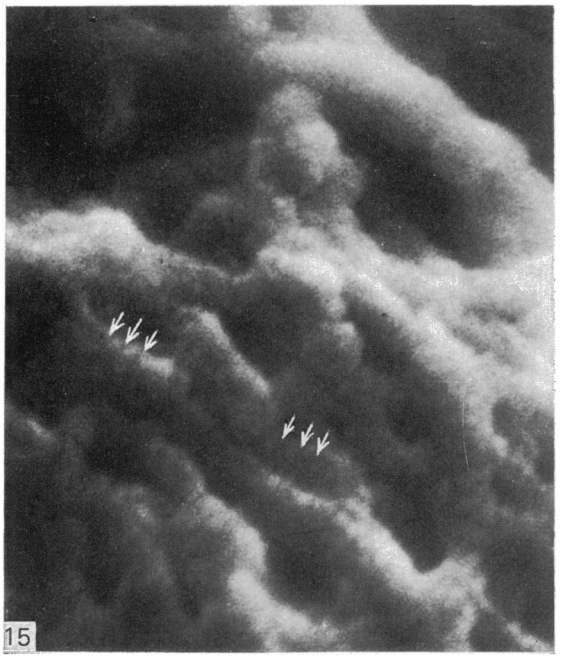
Fig. 10. Detail from Fig. 9 showing the parallel fibrils (1) and the territorial matrix (2). × 30000.

Fig. 11. Transverse section through the elastic cartilage, with chondrocytes in their lacunae and the interterritorial matrix (1) with longitudinally arranged fibrils. × 3000.

Fig. 12. Interterritorial matrix with parallelly arranged fibrils some of which are joining each other and leaving the plane of section (arrows). × 20000.







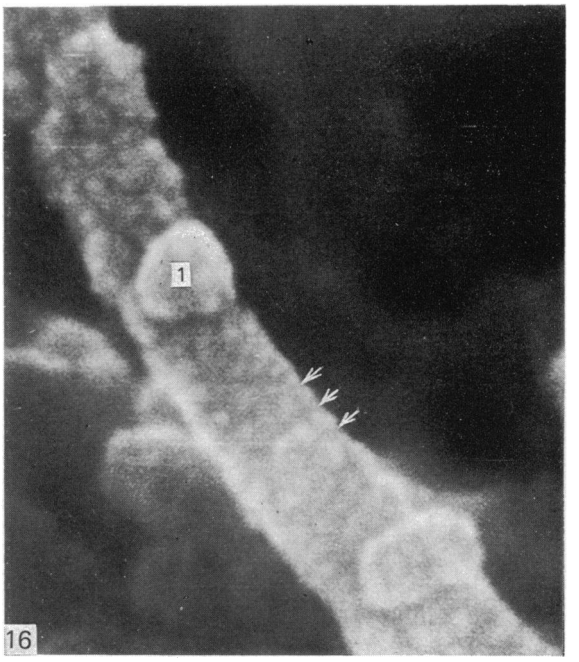


Fig. 13. Interterritorial matrix with a few globules between the fibrils (arrows). × 30000.

Fig. 14. Interterritorial matrix showing the fibrils finely spotted by small circular protuberances. × 50000.

Fig. 15. Fibrils from interterritorial matrix, with regularly spaced protuberances arranged circumferentially around the fibril in the form of ridges (arrows). × 150000.

Fig. 16. A single fibril from the interterritorial matrix showing an attached globular structure (1), and the regularly spaced protuberances arranged in the form of ridges (arrows). × 200000.

showed a regular pattern of small circular protuberances so that they appeared finely spotted (Figs. 14, 15). These protuberances measured 15 nm in width, and they were always spaced 120 to 190 nm apart. In some areas the protuberances assumed the form of regularly spaced ridges perpendicular to the long axis of the fibril at intervals of 12 to 19 nm. Along the fibrils were irregularly placed globular structures, some of which seemed more or less incorporated into the fibrils, while others were attached to the fibrillar surface (Fig. 16). The globular structures were spotted in the same manner as the fibrils, and their diameters matched those of the fibrils.

DISCUSSION

According to transmission electron microscopic investigations the ultrastructure of hyaline and elastic cartilage is very much the same, with the exception of the elastic component of the latter (Serafini-Fracassini & Smith, 1974; Holm Nielsen, 1976). The results in previous investigations with the SEM describing the inner structure of hyaline cartilage differ from the results presented in this study, probably because of differences in the methods of specimen preparation. The present preparation methods, together with the high-resolution SEM technique, have produced results not achieved before, e.g. the appearance of the chondrocyte surface, the anchoring of the chondrocytes in the lacunae, and the arrangements of the fibrils of the matrices.

The surface of a chondrocyte which had only retracted a little from the lacunar wall was described as smooth by Zimny & Redler (1972), but as covered with protrusions by Clarke (1974). In the present investigation it was obvious that the chondrocytes were studded with microvillous processes, many of which ramified from low crests, and had swollen or bifurcated tips.

In accordance with the observations of elastic cartilage in the TEM (Holm Nielsen, 1976) we found that the matrix consisted of a fine fibrillar network.

The lacunar wall, made up of the territorial matrix, was a finely woven mat of tangentially arranged fibrils crossing each other, leaving small openings into which the tips of the microvillous processes could penetrate. This is unlike articular cartilage where a specialized territorial region of fibrils was rarely seen (Clarke, 1974).

In our investigation it was possible for the first time to establish that chondrocytes are anchored to the lacunar wall by fibrils protruding from the territorial matrix, which connect either to the surface of the microvillous processes or to the cell surface in between the processes.

The zone of straight fibrils arranged in parallel which demarcated the lacunar wall from the interterritorial matrix, and the three dimensional network of the interterritorial matrix consisting of sheets with joining fibrils, was not observed by Clarke (1971) in articular cartilage.

It is highly remarkable that we failed to observe the 1–2 μm thick elastic fibres seen in the TEM which are situated in the middle of the septa between the lacunae in the form of coherent bands or separated clumps of elastin (Serafini-Fracassini & Smith, 1974; Holm Nielsen, 1976). We are at present totally unable to explain why elastin could not be identified in the SEM.

The traditional interpretation of cartilage matrix, based on TEM investigations, is of a network of collagen fibrils, and of proteoglycans (chondroitin sulphate) in the form of polygonal non-membrane-bounded granules (matrix granules) embedded in fine filaments.

In the TEM the diameter of the collagen fibrils has been found to vary from

20–90 nm (Smith, Peters & Serafini-Fracassini, 1967; Thyberg, Lohmander & Friberg, 1973; Sanzone & Reith, 1976; Holm Nielsen, 1976). The diameter of the fibrils measured in our investigation (40–120 nm) accords with the thickest of the fibrils seen in the TEM. The fact that neither Clarke (1971), nor ourselves using a high-resolution SEM technique, could make out the finest collagen fibrils (below 40 nm) probably reflects differences in the methods of preparation of material for TEM and SEM.

Because of the results of previous TEM investigations, we consider that the fibrils seen by us with SEM are collagen, in spite of the fact that no typical cross-band was seen. Clarke (1971), who also failed to observe the expected banding, suggests that the fibrils are coated with some substance. In our specimens we were able to distinguish a substructure in the fibrils, which has not been described before, namely a system of circular protuberances arranged around the fibrils in a regular pattern which in some places assumed the appearance of ridges running perpendicular to the long axis of the fibril. These protuberances may well be the groups of spots (3 nm) found alongside the collagen fibrils and representing two proteoglycan molecules tangentially attached to the a and b_1 bands of a collagen period (Serafini-Fracassini & Smith, 1976). The different form, and the different size and distance between the spots, as observed in TEM and SEM, is again probably explicable in terms of the different methods of preparation of TEM and SEM specimens.

The finely spotted globules attached to the surface of the fibrils we suppose to be the 'matrix granules', consisting of aggregates of proteoglycans (Thyberg, 1977).

The 110–180 nm globules seen between the fibrils in the interterritorial matrix are probably membrane-bounded matrix vesicles like those seen in epiphyseal cartilage with SEM by Ornoy & Langer (1978), for our globules likewise do not show any surface substructure. The lesser number of such globules in elastic cartilage is in agreement with the results obtained with TEM (Holm Nielsen, 1976).

SUMMARY

The elastic cartilage of the rat epiglottis was studied with a high-resolution SEM technique. The chondrocytes were found to be anchored in their lacunae by fibrils running in from the territorial matrix. This matrix exhibited a dense network of fibrils arranged tangentially around the lacunar cavity. The fibrils of the interterritorial matrix however, formed a three dimensional network of sheets with interconnecting fibrils. The SEM has shown up for the first time a substructure in the fibrils in the form of circular protuberances arranged circumferentially around the fibrils and forming ridges 12–19 nm apart. We suggest that the fibrils are collagen, and the protuberances are the proteoglycans attached to the collagen fibrils. Globules seen attached to the fibrils are most probably 'matrix granules' as observed in other kinds of cartilage. The total inability to visualize elastin with the high resolution SEM is puzzling.

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