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THREE-DIMENSIONAL OBSERVATION OF COLLAGEN FRAMEWORK OF LUMBAR INTERVERTEBRAL DISCS

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The anatomy and pathology of the intervertebral discs have seldom been described. The arrangement of the collagen fibers has recently been examined using light microscopy (Saunders & Inman 1940, Coventory et al. 1945, Hirsch & Schajowitz 1952, DePalma & Rothman 1970), X-ray crystallographic techniques (Horton 1958) and electron microscopy (Sylvén et al. 1951, Happey et al. 1964, Gomibuchi 1964). However, no complete demonstration is found of the three-dimensional architecture of the intervertebral discs at the ultrastructural level. In the present study, scanning electron microscopy (SEM) was used to examine the fibrous framework of the discs.

MATERIALS AND METHODS

The lumbar intervertebral discs with the connected vertebral bodies were obtained from Wistar rats, adult hybrid dogs and from humans of various ages. The freshly removed discs were washed with physiological saline and divided into two parts on the vertical and the horizontal planes using a sharp razor. Specimens for SEM were prepared by two methods; half of the specimen was immersed in 2.5 per cent glutaraldehyde and postfixed in 1 per cent osmium tetroxide, and the other half of the specimen was immersed for 2–3 hours in chymotrypsin solution, washed with phosphate buffer and fixed similarly. The samples were dried by the critical point method, evaporated with goldpalladium and examined by U-3 SEM (JEOL Ltd.) and field emission type SEM (Hitachi Ltd.). Samples from the same source as used in SEM investigation were examined by polarized light microscopy.

RESULTS

Intervertebral discs of rats

The horizontally cut surfaces of the rat lumbar discs showed the concentric structure of the lamellae of annulus fibrosus. The lamellae in

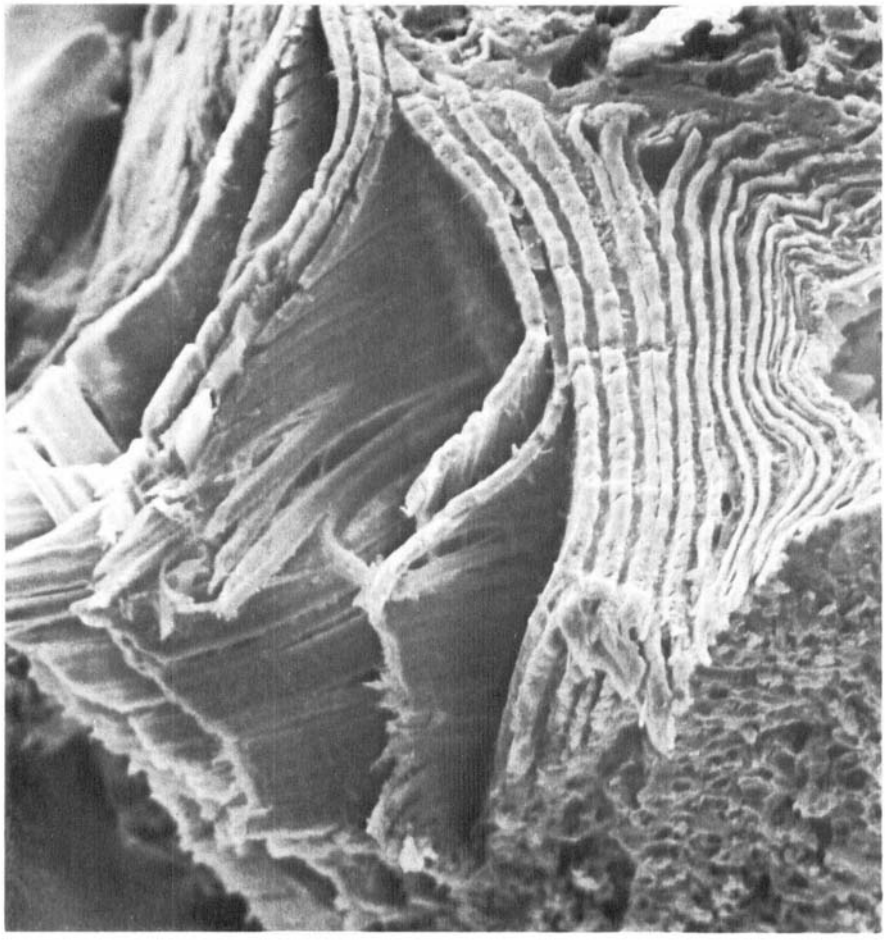

Figure 1. Three-dimensional framework of the fibrous bundles forming the annulus fibrosus of a rat. The bundles forming each lamella successively cross and run obliquely to the vertebral bodies above and below. × *120.*

the anterior areas measured approximately 20 μ in thickness, while the lamellae were thinner in the posterior areas. Each lamella contained numerous fine fibrils, 0.1–0.2 μ in diameter which were lined in an uniform direction. Observations of specimens whose cut surfaces were peeled and separated allowed a clear view of the architecture. The concentric lamellae appeared in neat layers. Each lamella was split into regularly arranged fibrillar bundles of about 10 μ in diameter. The fibers of the lamellae ran obliquely from one vertebra to the next. The

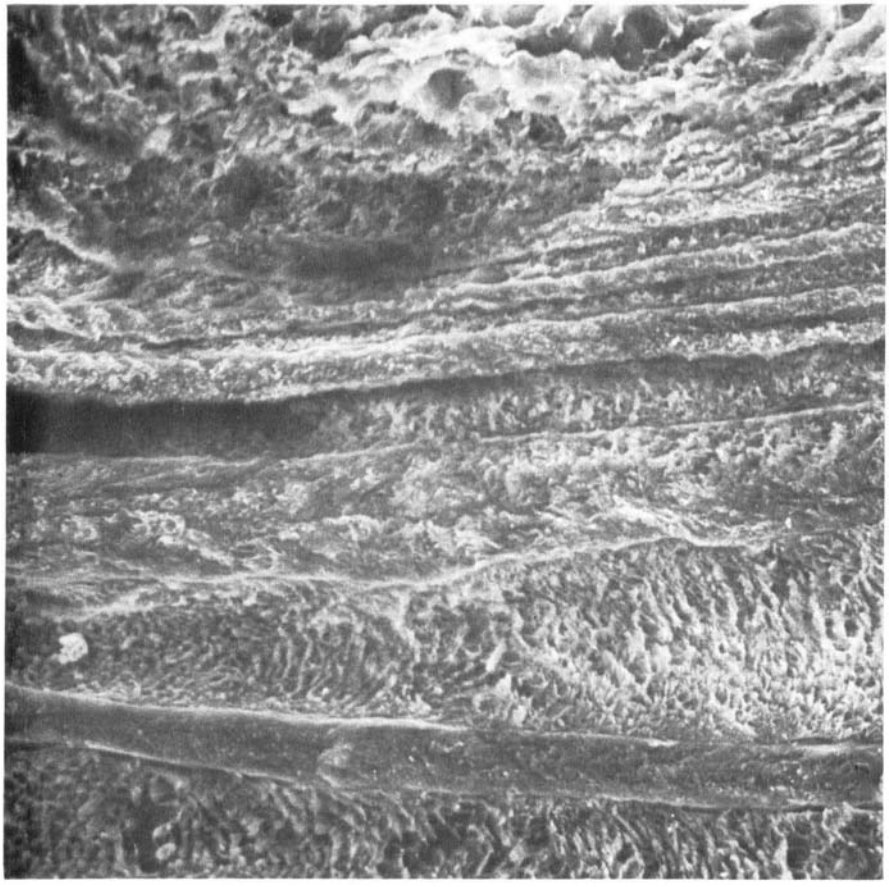

Figure 2. Horizontally cut surface of a dog disc showing transitional area of the nucleus pulposus and the annulus fibrosis. × *120.*

fibers in successive layers were interwoven with alternate lamellae (Figure 1).

Intervertebral discs of dogs

In horizontally cut surfaces of discs from dogs, irregular nets of fine fibrils of the nucleus usually overlapped one another forming a thin membraneous structure. These collagen fibrils in the nucleus pulposus gradually entered into the lamellar structure of the annulus fibrosus, and the thickness of the lamellae increased from the inner to the outer layers (Figure 2). In the middle layer of the annulus, the individual

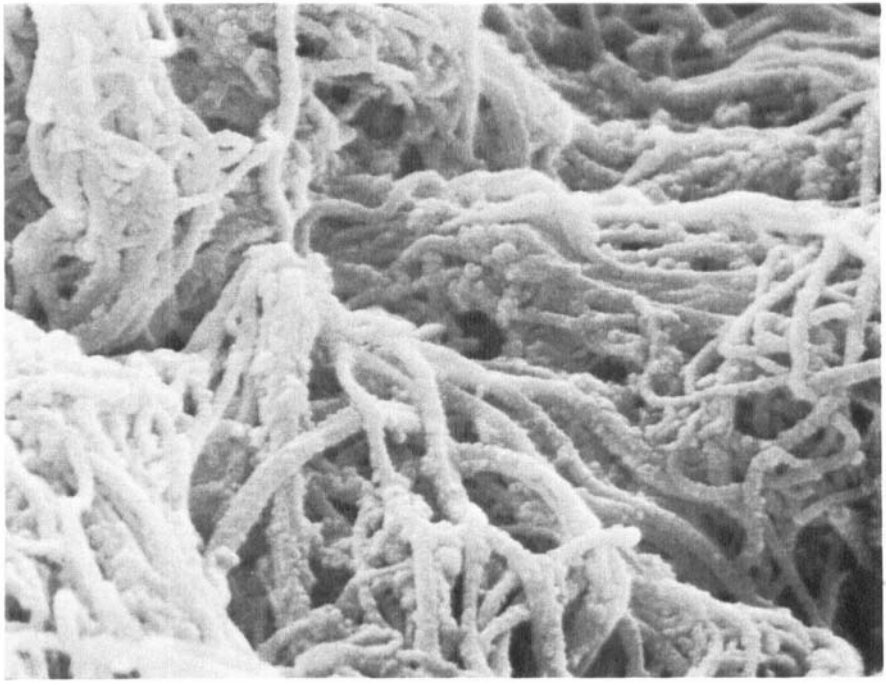

Figure 3. High power view of an adult human nucleus demonstrating the collagen framework, irregularly aligned. × 12,000.

lamellae measured about 50 μ in thickness and were composed of fibrillar bundles.

Intervertebral discs of humans

The fibrillar arrangement of the nucleus in adults showed an irregular network. Most of the fibrils measured 0.1–0.15 μ in diameter, and granular particles of 0.05–0.08 μ in size were attached to the fibrillar surfaces (Figure 3). The characteristic periodicity of the banding structure was found on the fibrils. The granular particles probably correspond to the mucopolysaccharide-protein (MPSP) complex.

A regular orientation of the annular lamellae was found in the adult human discs with a slight increase in thickness from the inner to the outer lamellae. However, lamellae of the posterior areas of the disc were generally thinner than those of the anterior areas and were partly interwoven with each other. The lamellae of the annulus easily peeled

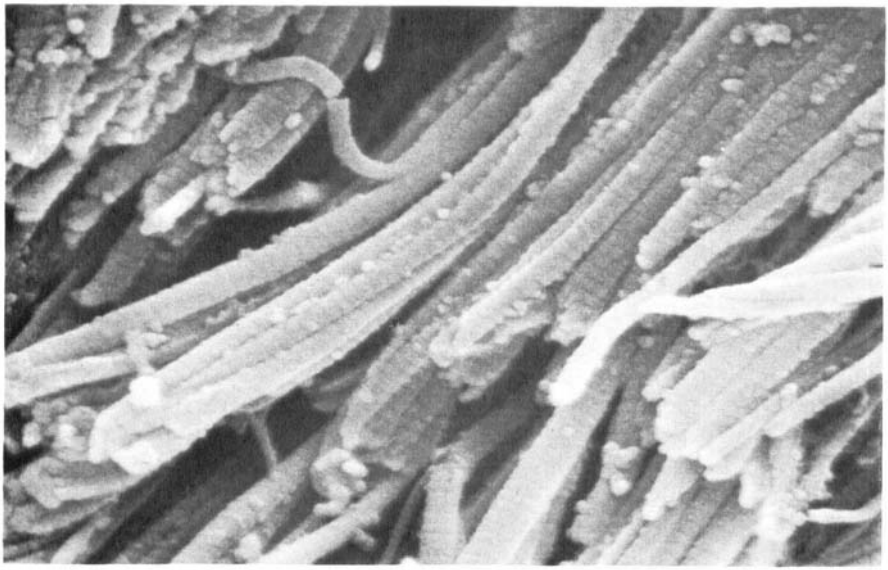

Figure 4. Field emission SEM picture clearly showing the regular orientation of collagen fibrils in the annulus which have been mostly denuded by chymotrypsin treatment. × *31,000.*

off after treatment with chymotrypsin. The collagen fibrils were 0.1–0.2 μ in diameter with a regular periodicity of about 600 Å in width. Granular particles of the remaining MPSP were seen on the separated fibrils (Figure 4). Stereoscopic views of vertically cut surfaces indicated that the slant fibrillar lamellae of the annulus adjacent to the nucleus entered into the cartilage plate. However, no connection of fibrils was found between the nucleus and the cartilage plate. The cartilage plate was made of a close network of fibrils running parallel to the vertebral bodies.

DISCUSSION

Light microscopic studies of the disc (Saunders & Inman 1940, Coventory et al. 1945, Hirsch & Schajowitz 1952, DePalma & Rothman 1970) have drawn attention to its functional properties. These studies have suggested that the collagen fibers of one lamella crossing the fibers of the next lamella contribute to the strength and elasticity of the disc. However, the precise arrangements of the fibrous components of the intervertebral discs were not adequately demonstrated because of methodological limitations. Our SEM images of the cut and teased

surfaces of the disc allowed examinations of the fibrous architecture of the three different parts of the disc (Inoue 1973, Takeda 1975). Sylvén et al. (1951) demonstrated by transmission electron microscopy (TEM) that the collagen fibrils of the nucleus measure 170–1260 Å in diameter and that the periodicity is 560–712 Å in width. The fine fibrils found in the present study showed a larger diameter than the collagen fibrils previously described by TEM (Sylvén et al. 1951, Happey et al. 1964, Gomibuchi 1964). This discrepancy in fibrillar diameter may be due to MPSP that are known to cover the fibrillar surfaces in TEM.

The nucleus from young human subjects is gel-like and contains much MPSP and less collagen fibrils (Hirsch et al. 1953). Happey et al. (1964) reported that networks in the nucleus are thinner than those in the annulus. In the present study, it was difficult to find differences of fibrillar thickness between the nucleus and the annulus.

The present SEM examinations also demonstrated that the lamellae in the anterior areas were slightly wider than those in the posterior areas where interlacing arrangements of lamellae were present. It can be inferred that the architecture of the intervertebral disc is fairly different in the anterior and posterior areas of the lamellae but that the fibrillar size of the lamellae does not differ.

Our observations indicate that the fine fibrillar network of the cartilage plate resembles that of the articular cartilages previously investigated by SEM (Clarke 1971, Inoue 1975). This network probably facilitates the flow of metabolities and water from the vertebral bodies to the nucleus by compression and decompression of tissues (Nachemson et al. 1970).

On the other hand, Hirsch & Sonnerup (1968) reported that the shock-absorbing function was based upon hydrodynamic action which probably depends upon hydroscopicity of the MPSP in the nucleus (Macnab 1969). Furthermore, the present study suggests that shock-absorption relates not only to the nucleus but also to the annulus, as the discs are composed of markedly tough fibrillar lamellae that enclose the nucleus (Hirsch & Sonnerup 1968, Macnab 1969). The intervertebral discs are also well-developed in adjusting to shock. Aging and pathological processes may cause degeneration of this three-dimensional architecture.

In the present study, it was found that chymotrypsin easily digests the extra-fibrillar substances and denudes the collagen fibrils. This indicates that the disc can be affected by chemical agents produced by the degenerating discs.

SUMMARY

Lumbar intervertebral discs obtained from rats, dogs and humans were examined by scanning electron microscopy. The nucleus pulposus was constructed of a loose network of fine fibrils and formed lamellated membranes in the peripheral areas. The annulus fibrosus was composed of concentric lamellae of fibrous bundles that ran uniformly in each lamella and crossed over to the bundles of adjoining lamellae. The lamellae were made of fine fibrils measuring 0.1–0.2 μ in diameter, corresponding to matured collagen fibrils. The cartilage plate consisted of a close meshwork of collagen fibrils which interconnected with the annular fibrils. From these results, it was concluded that the intervertebral disc was well developed for shock absorption at the light microscopic and ultrastructural levels. In specimens treated with chymotrypsin, the extra-fibrillar substances were easily digested in the nucleus, as well as in the annulus. The intervertebral disc may thus be easily affected by chemical agents.

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