

## Submicroscopic structure of canine articular cartilage

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**ABSTRACT:** Canine articular cartilage was studied in male dogs at age 1, 4, 5 and 8 years. Samples collected from four hip joints and two humeral joints in each age category were processed by standard methods to be examined by scanning and transmission electron microscopy. The cartilage of both joints was similar in structure. In the superficial cartilage layer of one-year-old animals, individual spindle-shaped chondrocytes in the extracellular matrix were, together with associated collagen fibrils, located parallel to the surface. When viewed by scanning electron microscopy, they were distinctly prominent above the surrounding surface. Changes in the thickness and arrangement of both the chondrosynovial membrane and intercellular matrix were apparent in the 4-, 5- and 8-year-old animals, indicating the onset or progression of an osteoarthritic process. The middle cartilage layer in young animals showed elliptical chondrocytes occurring in pairs. The voluminous cytoplasm contained a great amount of granular endoplasmic reticulum, a large Golgi complex and numerous transport vesicles. The pericellular matrix, up to 1 µm thick, was composed of aperiodic fibrils. In the old animals the pericellular matrix was absent and was replaced by thick collagen fibrils with a marked periodicity. The deep cartilage layer in young dogs included groups of three to four chondrocytes situated in a common territory. The cytoplasm contained distinct bundles of intermediary filaments. The pericellular matrix occasionally formed septa between adjoining cells. The intracellular matrix included bundles of collagen fibrils arranged in a matted structure. In the old animals aggregation of chondrocytes into groups almost disappeared. The cytoplasm showed only short cisternae of granular endoplasmic reticulum, small numbers of mitochondria and transport vesicles, frequent lipid droplets and small glycogen deposits. The intercellular matrix consisted of only short collagen fibrils with no distinct periodicity.

**Keywords:** chondrocytes; pericellular matrix; intercellular matrix; arthritis; dog

Zelander was the first to describe the submicroscopic structure of articular cartilage in 1959. Since then this tissue has been studied and described in most of the mammalian species (for review see Horky and Tichy, 1995).

The hyaline articular cartilage arises, during skeleton development, from mesenchyma as a part of the cartilage blastema of the bone anlage that becomes the bone during ossification. However, the ossification process, by which the preformed bone anlage is gradually degraded, does not involve the articular cartilage. This remains preserved but its part facing the articular cavity undergoes several changes during the following ontogenetic develop-

ment. The cartilage morphology is predominantly affected by the pulls exerted by developing muscles and ligaments in the vicinity of a joint. The first fetal movements and later the pressure produced by the moving organism may also be involved. The stage of these morphological changes is related to foetal age (for review see Horky, 1991a).

The articular cartilage is mainly involved in the joint's mechanical functions; in order to function properly, it must meet two essential requirements: resist pressure and facilitate gliding of two adjacent surfaces. Pressure resistance is achieved by the structure and arrangement of the two components of the intercellular matrix (Bloebaum and Wilson,

1980; Horkey, 1980; Clark, 1990; Clark et al., 2003). The involvement of chondrocytes in motion mechanics is minimal but they play a key role in the synthesis of the extracellular matrix responsible for mechanical properties. Much attention has also been paid to the cartilage surface layer that is important for smooth movement of the adjoining articular surfaces (Wolf, 1975; Horkey, 1980; Giles, 1992; Teshima et al., 1995; Sandell and Aigner, 2001).

The ultrastructure of canine articular cartilage was first described by Wiltberger and Lust (1975). Further data on this canine structure was contributed by the work of Ghadially (1983) and by his more general, earlier study that dealt with the surface development of articular cartilage under physiological conditions (Ghadially et al., 1977).

In the following period, a spate of data has appeared on degenerative or inflammatory lesions in the articular cavity that affected the structure or surface development of articular cartilage (e.g., Greisen et al., 1982; Orford et al., 1983; Teshima et al., 1995). In addition, several studies concerning histochemical, immunochemical, surgical (Van Tienen et al., 2003), orthopaedic (Wyland et al., 2002) and rheumatologic (Tirgari and Vaughan, 1975) features associated with cartilage have been published. Much information has been provided on changes in articular cartilage width (Kiviranta et al., 1992) and on manifestation of apoptosis (Kim et al., 2000; for review see Sandell and Aigner, 2001).

Our review of literature on the submicroscopic structure of canine articular cartilage revealed that there is very little information available on the structure of this tissue and that, for instance, scanning electron microscopy (SEM) has rarely been used. The aim of this study then was to revise and extend the existing data.

## MATERIAL AND METHODS

Samples of articular cartilage from four hip and two humeral joints were collected from dogs aged 1, 4, 5 and 8 years. They were taken from regions subjected to mechanical stress. For transmission electron microscopy (TEM), strips of 1 × 1 × 3 mm were cut out from the samples and immediately fixed in 400 mmol/l glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. The tissue was subsequently decalcified using two solutions of 0.1 M EDTA containing 400 mmol/l glutaraldehyde at pH 7.2, for 60 min each. It was then allowed to stand in the decalcifica-

tion solution overnight and was eventually rinsed for 30 min in two baths, each containing 0.1 M phosphate buffer at pH 7.4. Fixation was carried out in two baths of 40 mmol/l  $O_5O_4$  solution in phosphate buffer at pH 7.4. Dehydration, immersion and embedding in Durcupan ACM were carried out using standard procedures. Ultrathin sections were made on an LKB Nova ultramicrotome and stained with lead citrate or with uranyl acetate and lead citrate. The sections were viewed and photographed in a Tesla BS 500 electron microscope.

Samples for scanning electron microscopy were fixed in 5% and 10% formaldehyde. Dehydration was terminated using the method of desiccation at the critical point and the samples were gold-coated in a Balzers SCD 040 sputtering apparatus. They were examined and photographed in a Tesla BS 300 scanning electron microscope.

## RESULTS

In the specimens of articular cartilage investigated, three layers, i.e., superficial, middle and deep, were clearly distinguishable. They are described in detail below. All cell types in these layers were described with the exception of the transition types of chondrocytes at the tide-mark area; these were not studied because of their considerable uniformity.

### Submicroscopic structure of the superficial layer

In one-year-old animals, the superficial layer contained only a small number of chondrocytes. These were situated parallel to the surface. Their nuclei were elongated and hyperchromatic, and the zonula nucleum limitans varied in appearance. The nucleus usually included a reticular-type nucleolus. The cytoplasm contained infrequent cisternae of the granular endoplasmic reticulum (GER) and occasional lysosomes. The cell membrane on both poles extended to form long projections, which gave the cell a fibrocyte-like appearance. In these projections and in the perinuclear region, bundles of intermediate filaments were observed (Figure 1).

The extracellular matrix was clearly distinguished into pericellular and intercellular matrix. The pericellular matrix enclosed chondrocytes into lacunae that were slightly wider towards the surface facing the articular cavity. In young animals, surface layer

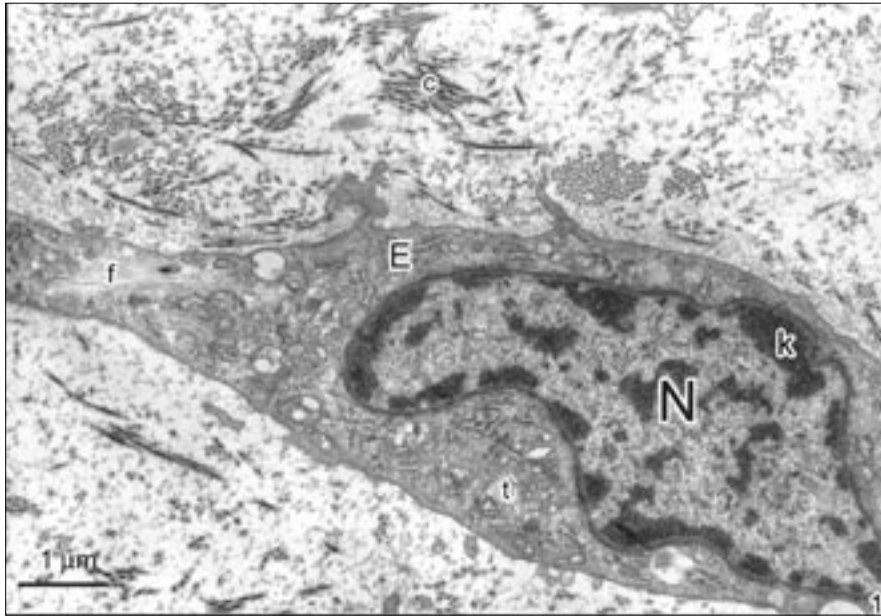


Figure 1. Chondrocyte of the superficial layer. Nucleus (N), karyosomes (k), granular endoplasmic reticulum (E), transport vesicles (t), collagen fibrils (c); × 14 000

chondrocytes, elongated and spindle-like in shape, protruded above the level of the surrounding extracellular matrix. Generally, they occurred singly and only occasionally in pairs. The chondrosynovial membrane was smooth, with only a mild corruga-

cavity was covered with a layer, 0.8 to 1 μm thick, consisting of aperiodic fibrils and short collagen fibrils (Figure 3).

The superficial layer of articular cartilage in the 4-, 5- and 8-year-old groups had a different appearance. There were changes in both the pericellular and intercellular matrix. The bundles of collagen fibrils were thinner, with no regular arrangement, making the whole layer thinner. The greatest changes were observed on the surface of this layer. In some areas, the chondrosynovial membrane was shaped into pyramid-like formations (Figure 4) that developed into the ridges shown by SEM (Figure 5). Some chondrocytes were surrounded by a furrow caused by collapse of the pericellular matrix. Owing to a reduction in intercellular matrix, chondrocytes came closer to the surface (Figure 6). The cytoplasm in some of these chondrocytes contained thick bundles of intermediate filaments (Figure 6). Because of articular cartilage exposure, aperiodic and collagen fibrils were in direct contact with the synovial fluid. Some collagen fibrils were of double thickness, which is typical of arthritic changes in articular cartilage. Bundles of aperiodic fibrils were also seen deeper under the surface of the partly disintegrated cartilage (Figure 3).

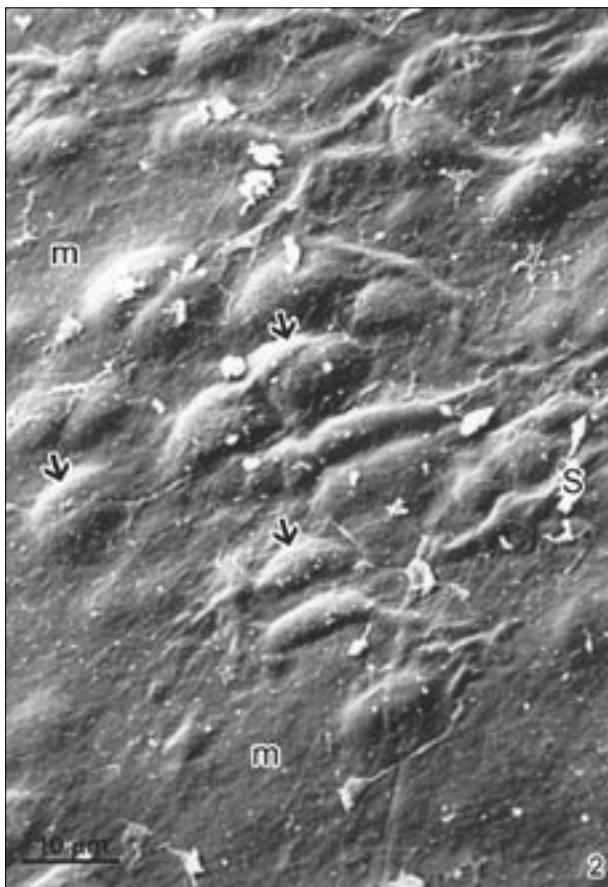


Figure 2. Articular cartilage surface. Chondrocytes penetrating into the articular cavity (→). Fine corrugated chondrosynovial membrane (m). Remnants of the synovial fluid (S); SEM, × 1 300

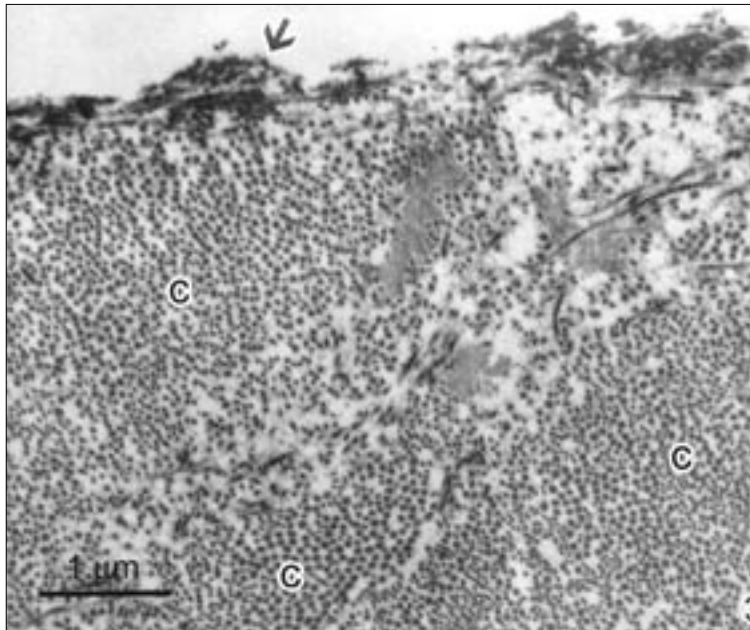


Figure 3. Articular cartilage surface in a young dog. Chondrosynovial membrane (→) with fine aperiodic fibrils. Bundles of collagen fibres (c) matted together; × 14 000

### Submicroscopic structure of the middle layer

In the one-year-old animals, the middle layer chondrocytes were located singly or in pairs in lacunae. They were usually oval in shape and from 15 to 18 μm in size. They contained a nucleus with a thin layer of diffuse chromatin at the nuclear envelope. The zonula nucleus limitans was always

developed. The nucleus usually contained one nucleolus.

The cytoplasm of these chondrocytes was large and contained numerous GER cisternae. These consisted of fine granular medium electron-dense matter. Small mitochondria were present in large numbers and the Golgi complex was spread over several regions. Among the cisternae, there were many large transport vesicles with contents varying

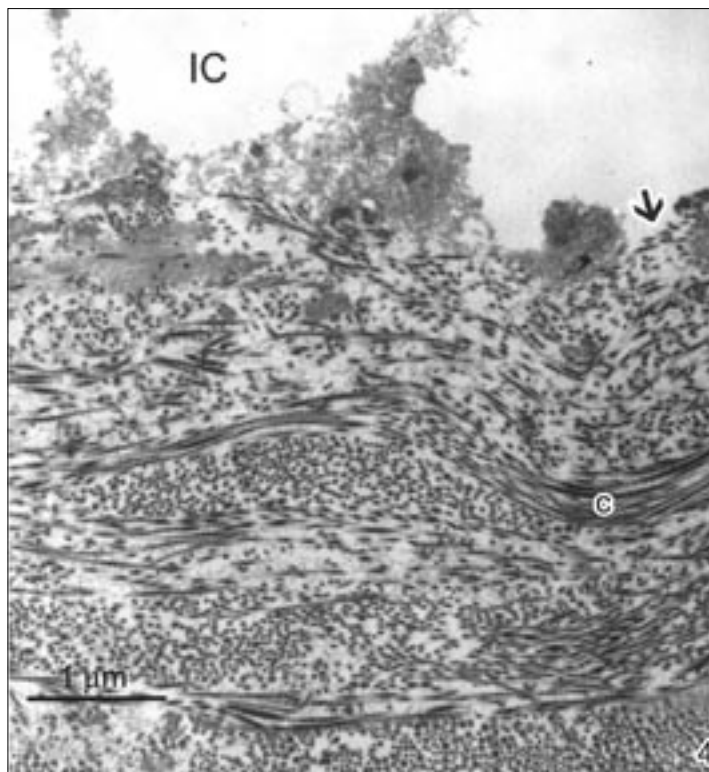


Figure 4. Articular cartilage surface in an old dog. Corrugated chondrosynovial membrane is absent in some regions (→) and collagen fibrils are in contact with the articular cavity (IC). Disarranged bundles of collagen fibrils (c); × 14 000

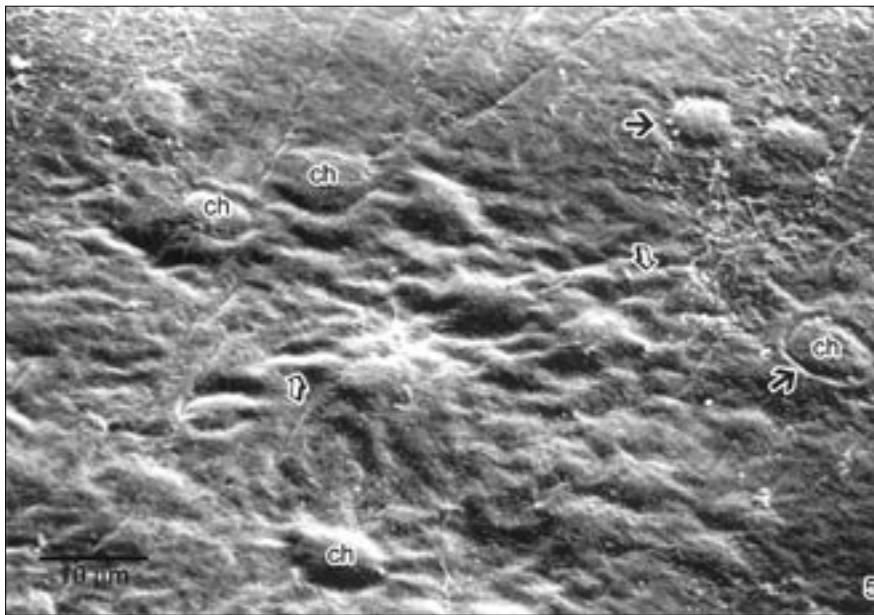


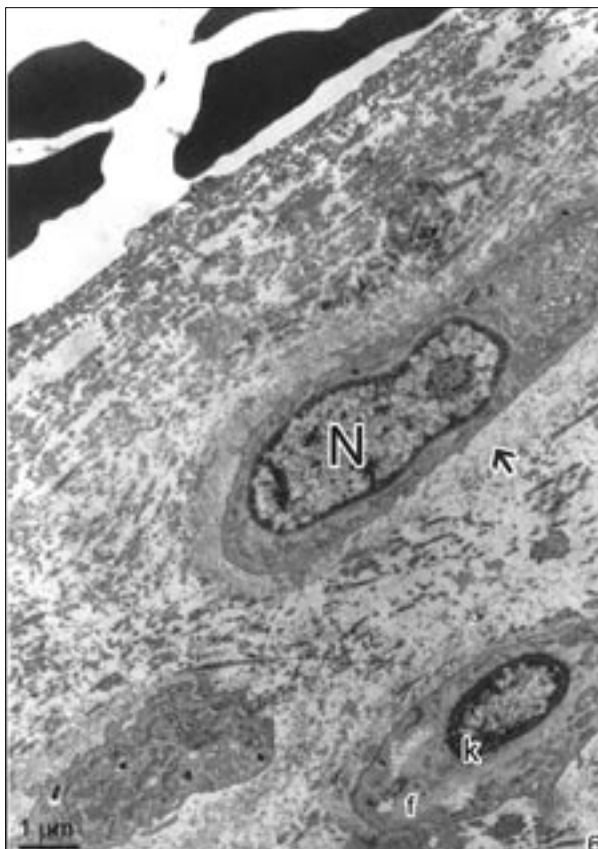
Figure 5. Small number of chondrocytes (ch) on the surface of articular cartilage in an old dog; in some chondrocytes the pericellular matrix has collapsed (→). Chondrosynovial membrane is deeply corrugated (⇒), SEM, × 1 500

in density. In the majority of cells the cytoplasm contained large vacuoles and glycogen deposits. The cell membrane extended, as short projections, into the pericellular matrix.

The pericellular matrix created a wide space (0.8 to 1 μm) around each chondrocyte; this space was occasionally penetrated by collagen fibrils extend-

ing from the neighbouring intercellular matrix (Figure 7).

In the old animals, the chondrocytes of this layer did not differ much from those in young animals. However, marked changes were observed in both the pericellular and intercellular matrix. The former was either partly or completely missing in most of the chondrocytes. The space thus created was penetrated by thick collagen fibrils that showed distinct periodicity; as a result, the differences between the pericellular and intercellular matrix disappeared. In many cases the intercellular matrix was adjoined directly to the chondrocytes (Figure 8).



### Submicroscopic structure of the deep layer

In the one-year-old animals, this layer was characterized by groups of three to four chondrocytes restricted to one region (Figure 9). The cells were either separated by septa composed of fine collagen fibrils or were tightly attached to each other. Nuclei usually did not differ from those of middle layer chondrocytes, but occasionally, in some nuclei, chromatin was aggregated in large karyosomes near to the nuclear envelope. GER cisternae in the cytoplasm were infrequent. The Golgi complex had the usual structure. Lysosomes, vacuoles and

Figure 6. Chondrocytes of articular cartilage in an old dog. Nucleus (N) with numerous karyosomes (k), reduced pericellular matrix (→); bundles of intermediate filaments (f) situated close to the nucleus; × 8 000

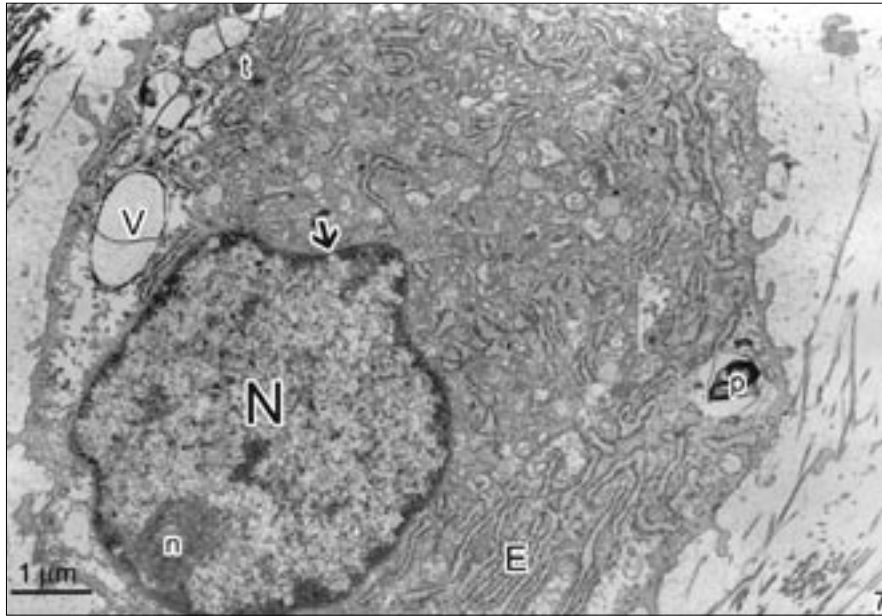


Figure 7. Chondrocyte of the middle layer in a young dog. Nucleus (N) zonula nucleolimitans (→), nucleolus (n), numerous cisternae of granular endoplasmic reticulum (E), vacuoles (V), transport vesicles (t), pseudomyelin structure (p);  $\times 10\,000$

bundles of intermediate filaments were regular findings. The cell membrane was smooth, with occasional projections. The pericellular matrix was about  $1\ \mu\text{m}$  in width. The intercellular matrix contained bundles of collagen fibrils that encircled the groups of chondrocytes. The bundles themselves were matted together.

In the old animals, the chondrocytes in the deep layer occurred individually, were oval in shape and  $10$  to  $12\ \mu\text{m}$  in size. The nucleus had an irregular shape and chromatin was distributed in the form of small karyosomes. The zonula nucleolimitans was usually absent. The cytoplasm contained a small number of short GER cisternae, mitochon-

dria and transport vesicles. Large lipid droplets and small glycogen deposits were frequently found. The pericellular matrix, about  $1\ \mu\text{m}$  in width, was electron-dense. The intercellular matrix was composed of short collagen fibrils, with no distinct periodicity, matted together (Figure 10).

## DISCUSSION

The amorphous ground substance with its fibrillar component, i.e., the extracellular matrix, plays an important role in the functioning of articular cartilage in synovial joints. Its presence in the super-

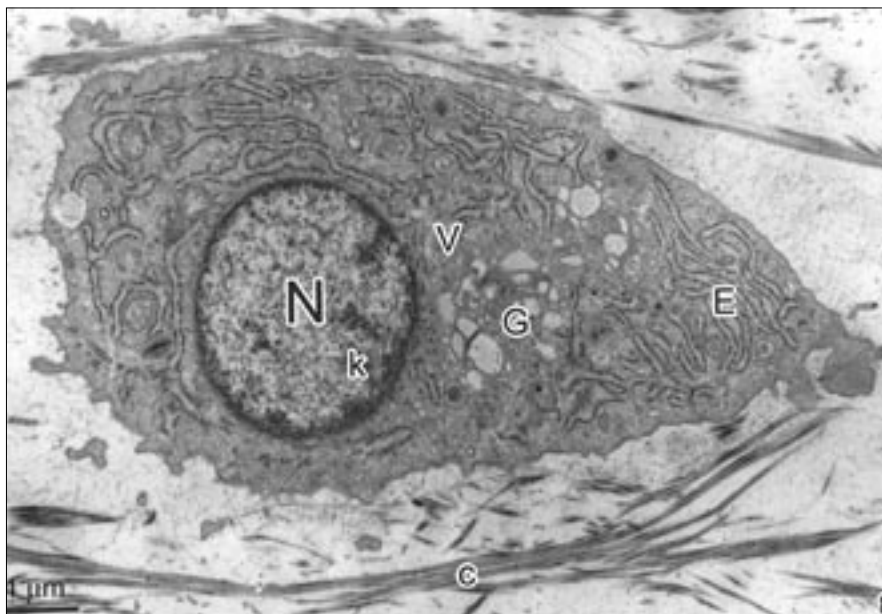


Figure 8. Chondrocyte of the middle layer in an old dog. Nucleus (N) with karyosomes (k), granular endoplasmic reticulum (E), dilated vesicles of the Golgi complex (G), transport vesicles (V). In the pericellular matrix thick collagen fibres (c) with distinct periodicity;  $\times 10\,000$

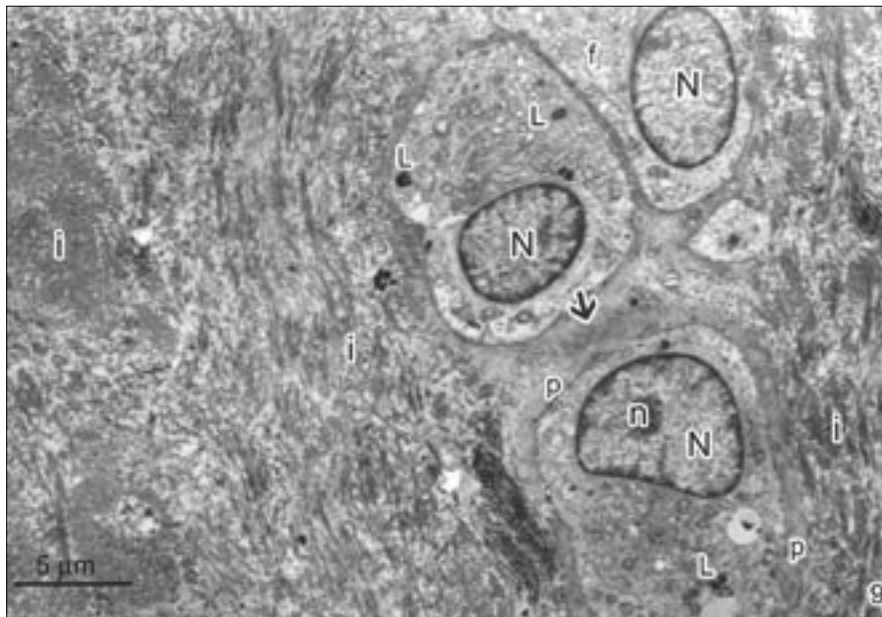


Figure 9. Deep layer of articular cartilage in a young animal. Nuclei (N) of chondrocytes, nucleolus (n), lysosomes (L), bundles of intermediate filaments (f). Pericellular matrix (p) fills lacunae and creates septa between chondrocytes (→). Intercellular matrix encircles the territory (i); × 3 500.

ficial layer facilitates the sliding and lubrication of adjoining surfaces whereas, in the middle and deep layers, it is responsible for resistance to mechanical stress and its distribution over the subchondral bone. The cellular component of articular cartilage is involved in cartilage synthesis, a fact reported many years ago by Sheldon and Kimbal (1962) and Freeman and Kempson (1973). The equine articular cartilage includes three layers, a finding that was also made in other mammals (Palfrey and Davies, 1966; Horky, 1991a,b, 1994a,b and others). The observations reported here are in agreement with the results obtained, with the use of magnetic resonance imaging, by Modl et al. (1991) and also

with our previous findings made in the articular cartilage of several mammalian species studied by TEM (Horky, 1980; Ghadially, 1983; Bozdech et al., 1990; Horky, 1994a,b).

Our previous observations on the chondrosynovial membrane in the horse and other mammals are in accordance with the findings of Meachim et al. (1965), Wolf (1975), Stockwell and Meachim (1979), Giles (1992) and Kamalanathan and Broom (1993). All these findings are in contradiction to the views of McCutchen (1966) and Maroudas (1973) who stated that the chondrosynovial membrane is produced by ultrafiltration of synovial fluid through the surface cartilage layer. In our opinion

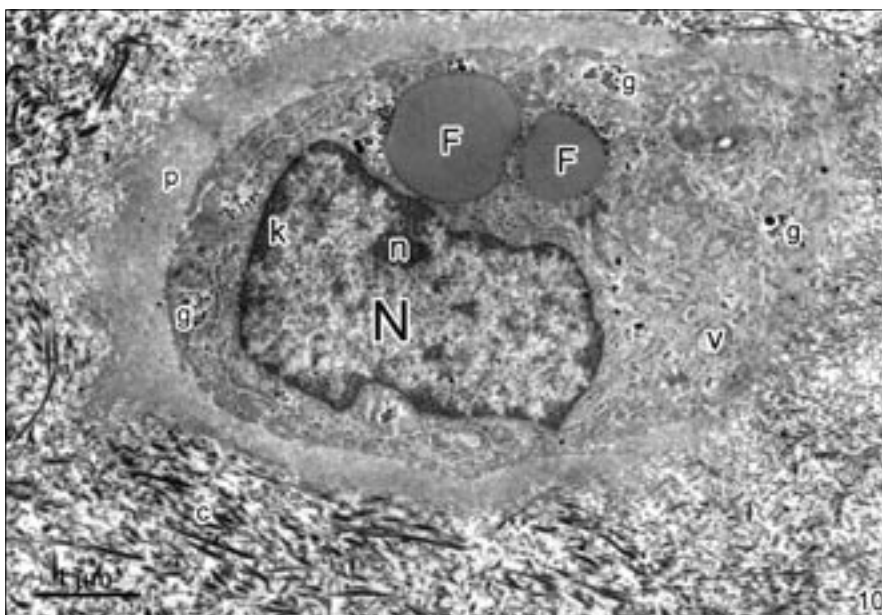


Figure 10. In the deep layer of articular cartilage of old animals, chondrocytes are localized individually. Nucleus (N) with numerous karyosomes (k) and a nucleolus (n). Short cisternae of granular endoplasmic reticulum in the cytoplasm, occasional transport vesicles (v), large lipid droplets (F), numerous small glycogen deposits (g). Dense pericellular matrix (p), short collagen fibres without periodicity (c) in the intercellular matrix; × 12 000

supported by previous repeated observations (see references cited above), this structure is synthesized by bundles of aperiodic filaments located either on, or immediately below, the articular cartilage surface, without their polymerisation into typical collagen fibrils. This process gives rise to a membrane, very thick in the prenatal period and becoming thinner with increasing age. This membrane is capable of making the adjoining surfaces, which are often under extreme pressure, move over each other smoothly (Chappuis et al., 1983; Swann et al., 1985; Hills, 1989). These surfaces are lubricated by synovial fluid, a product of synovialocytes, in which the most effective component is lubricin. In an intact articular cartilage, the presence of synovial fluid is able to reduce the friction coefficient to 0.01.

The chondrosynovial membrane becomes thinner with ageing but the number of collagen fibrils in the intercellular matrix increases. The fibrils are responsible for cartilage elasticity, while proteoglycans in the amorphous ground substance are responsible for resistance to mechanical stress. The fibrillar arrangement found in this study corresponded well with the observations by Bloebaum and Wilson (1980) and Clark (1990) as well as with the results of our previous studies carried out on several mammalian species, involving both prenatal and postnatal periods (Horky, 1986, 1987, 1989, 1991a,b, 1993, 1994a,b). The superficial layer has been found to contain bundles of aperiodic fibrils varying in width and length. In the horse, the bundles are not as large as, for instance, those in the goat (Horky, 1994b), which is obviously due to their loosening. Other authors have also paid attention to this superficial layer that plays an important role in the synthesis of the fibrillar component of articular cartilage (Broom, 1986; Clark, 1990; Jeffery et al., 1991; Hedlung et al., 1993).

The superficial layer of canine articular cartilage had a different appearance in different regions, a sign typical of arthritis (Horky, 1980; Ghadially, 1983; Bozdech et al., 1990). Wiltberger and Lust (1975) who compared normal and degenerative hip joints in dogs and Grondalen (1974a,b), who studied osteochondrosis and arthritis in pigs, made similar findings. These are also supported by histological studies on articular cartilage repair after artificial damage in the marmoset (Bibb and Robinson, 1993).

The structure of the middle layer of articular cartilage in dogs was similar to that described in other mammalian species (Gilmore a Palfrey, 1987; Horky, 1989, 1991a,b, 1994a,b). However, the ar-

rangement of fibrils in the intercellular matrix was different. The pericellular matrix was poorly developed so that collagen fibrils came into direct contact with chondrocyte cell membranes; this agreed with our previous observations made in the feline articular cartilage (Horky and Tichy, 1995). In the canine cartilage, collagen fibrils did not encircle chondrocytes as they did in the articular cartilage of cats (Horky, 1993).

The deep layer of articular cartilage did not differ in structure from those studied in other mammals (Hedlung et al., 1993; Horky, 1991c, 1993, 1994b).

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