Focal destruction and remodeling in guinea pig arthrosis

Edin de Bri[1], Kristina Jönsson[3], Finn P Reinholt[2] and Olle Svensson[1]

We investigated the proximal tibiae in adult, middle-aged and old (6, 12, and 30 months) guinea pigs with spontaneous arthrosis by quantitative morphometry. Since the cartilage destruction develops predominantly in the medial tibial plateau, the lateral side may serve as an internal control. In established lesions, cartilage destructions were focal, surrounded by a brim of thickened cartilage that was grossly intact, but showed cell clustering, hypertrophy and increased metachromasia. Peripherally, there was a distinct transition to normal-appearing cartilage. Subjacent to the cartilage ulcerations, subchondral bone was thickened. Compared to the lateral plateau the height of the calcified cartilage increased and the surface density of the osteocartilaginous interface decreased. Marrow depletion and cysts developed below the cartilage ulcerations. Cysts seem to develop through fibrous tissue partially undergoing cartilage metaplasia in tiny noduli that subsequently coalesce, liquefy centrally and expand. Superficially, similar fibrocartilaginous proliferations result in incomplete resurfacing and small areas of subchondral resorption; in the periphery of the joint, they form osteophytes. In the guinea pig, arthrosis tissue destruction is thus accompanied by local tissue proliferation.

Divisions of [1]Orthopedics, [2]Pathology and [3]Clinical Research Center, Karolinska Institute, K54, Huddinge Hospital, S-141 86 Huddinge, Sweden. Tel +46 8-7461000. Fax -7114292
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Clinically, arthrosis (OA) has been described as a nonspecific end-stage of organ failure (Radin and Rose 1986). Morphological studies have largely been performed on surgical specimens from patients with advanced disease and consequently little is known about initiating factors and pathogenetic mechanisms. To overcome these obstacles, various animal models have been used (Adams and Billingham 1982, Schwartz 1987). Most of these models, however, involve an intraarticular intervention, resulting in rapidly progressive changes, quite unlike primary arthrosis. However, we find naturally occurring OA-like conditions in animals, e.g., Dunkin Hartley guinea pigs (Bendele et al. 1989). A recent study (de Bri et al. 1995) presented quantitative data on articular cartilage and subchondral bone during development of OA in the guinea pig. In the present paper, we provide further details on the natural history of guinea pig OA, with special emphasis on interfaces and interactions between calcified and uncalcified tissues, using quantitative morphometry.

Animals and methods

Outbred male Dunkin Hartley guinea pigs (Møllegård, Copenhagen, Denmark) in 3 age groups (n 8 in each age group), 6-month-old (adult), 12-month-old (middle-aged) and 30-month-old (old), were killed by intraperitoneal injections of pentobarbital. The proximal tibiae were dissected out and soft tissue was removed. The specimens were fixed in neutral-buffered 4% formalin for 72 h, decalcified for 5–7 days in 40% formic acid, cut sagittally into two approximately equal portions, i.e., medial and lateral plateaus, and embedded in paraffin wax. At the random start, excluding the central portion involving the insertion of the cruciate ligaments, 10–12 histological sections were cut through each plateau at constant intervals, of 250 μm, and stained with hematoxylin and eosin. From these sections, volume densities (Vv) and surface densities (Sv) were measured by point- and intersection-counting (> 200 hits per item and plateau) in a projection light microscope (Reichert-Jung, Germany) at final magnifications of ×50 and ×125. In this microscope, the image is projected onto a screen, on which the transparent grid (lattice) used for counting points and line intersections is attached. The absolute volumes (V) of cysts, bone marrow and osteophytes can then be calculated. For measurements of height of calcified cartilage and Sv of the osteocartilaginous interface, 6–8 nondecalcified plastic-embedded step sections of the central compartment were stained with toluidine blue.

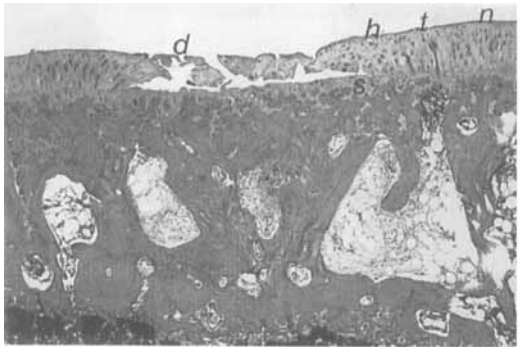

Figure 1. Micrographs of the medial tibial plateau of a 12-month-old Dunkin Hartley guinea pig showing osteoarthrotic (OA) changes, including cartilage destruction (d) and separation at the tidemark (s). The central cartilage lesion is surrounded by a rim of hypertrophic cartilage (h) with an increased height compared with the more peripheral normal cartilage (n). Note the rather abrupt transition (t) to normal cartilage. The bone below the lesion is sclerotic. Note the difference from the bone below normal cartilage. Also note that the metaphyseal bone has a different structure than epiphyseal bone. HE ×50.

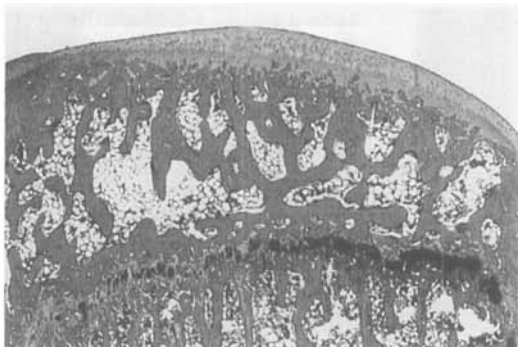

Figure 2. Micrograph from the lateral tibial condyle from a 12-month-old Dunkin Hartley guinea pig without OA changes, histologically normal-appearing articular cartilage without fibrillation or destruction. The subchondral bone density is not increased as in the medial plateau. No cysts or osteophytes were found. HE ×50.

Vv of the growth plate remnant was measured by point-counting at a magnification of ×50, using a conventional multipurpose test system (M42 grid) (Weibel 1979). Sv was measured by counting the intersections of the test grid lines with the lines formed by the sectioned surfaces, using the test grid designed by Baddeley et al. (1986). A coherent test system means that in a given grid the number of test points, the length of the test line and the test area have a certain fixed relationship to each other (Weibel 1979). As reference volume for Sv of vessels, we used the subchondral bone plate volume, defined as bone extending from the osteocartilaginous border to the first distal occurrence of non-osseous tissue. Cysts were defined as cavities larger than 100 μm, devoid of marrow cells, osteophytes as extra-articular osteocartilaginous tissue (extending beyond the original cortex). The reference volume for cysts and growth plate was the entire epiphysis.

The height of the calcified cartilage was measured on non-decalcified sections stained with toluidine blue. Surfaces of exposed calcified cartilage and cartilage subjected to horizontal splitting at the tidemark between calcified and uncalcified layers, were measured by intersection-counting with a cycloid grid for vertical sections (Baddeley et al. 1986), i.e., the number of intersections through the item in question, divided by the number of intersections with the smooth contour of the joint surface. The numbers of sections and measurements were determined in a pilot experiment, evaluated by cumulative mean plots. In the analyses of each histological section, the central (not meniscus-covered) compartment and the peripheral (meniscus-covered) compartment were measured separately, according to the gross anatomical estimates. The central compartment comprised about 30% of the joint surface. To control artefacts created by the tissue preparation procedure, type of fixative, fixation time, pH, osmolality, dehydration and embedding procedures were kept constant throughout the study. For statistical evaluation, Kruskall-Wallis non-parametric ANOVA and the Mann-Whitney U-test at a rejection level of $p = < 0.05$ and Spearman's test for correlation were used.

Results

All animals developed OA in the medial plateau (Figure 1), whereas the lateral plateau remained intact (Figure 2). Cartilage lesions were most pronounced in the central medial compartment, which showed gross areas of destruction in animals as young as 12 months of age (Table 1). Cartilage destruction was focal, with a "punched out" appearance, often extending down to the calcified layer. These lesions were surrounded by grossly intact but microscopically abnormal cartilage that usually extended about 1–2 mm into the peripheral compartment. The marginal zone of this cartilage ulceration was often thickened and characterized by cell clustering and proliferation. In this brim, increased metachromasia was observed in the radial zone, while cell necrosis, matrix destruction and shedding predominantly occurred at the cartilage surface. Peripherally, there was a rather abrupt transition to the normal appearing cartilage (Figure 1). Horizon-

Table 1. Exposure and horizontal splitting of calcified cartilage, percent (mean (SD) range) area of condylar articular surface (8 pigs in each age group

Age (months)	Exposed calcified cartilage (%)		Horizontal splitting (%)	
	Medial plateau	Lateral plateau	Medial plateau	Lateral plateau
6	0 (0) 0–0	0 (0) 0–0	1 (2) 0–3	0 (0) 0–0
12	16 (8) [a] 12–22	0 (1) 0–1	6 (5) [a] 3–11	0 (1) 0–1
30	17 (11) [a] 11–25	2 (4) 0–5	5 (5) [a] 3–10	0 (1) 0–1

[a] p = < 0.05 compared to values at 6 months

Table 2. Height of calcified cartilage (mean (SD) range) in guinea pigs of different ages (months; 8 pigs in each age group)

Age	Medial plateau (mm)	Lateral plateau (mm)
6	0.08 (0.01) 0.08–0.09	0.16 (0.01) 0.14–0.17
12	0.12 (0.01) [a] 0.11–0.14	0.18 (0.03) 0.13–0.22
30	0.11 (0.01) [a] 0.10–0.12	0.18 (0.01) 0.17–0.19

[a] p = < 0.05 compared to values at 6 months

Table 3. Surface density (mean (SD) range) of osteocartilaginous interface (mm^2/mm^3) of the central compartments (8 pigs in each age group)

Age	Medial plateau	Lateral plateau
6	45 (4.8) 40–51	28 (2.5) 26–32
12	31 (6.3) [a] 28–36	25 (4.9) 20–30
30	34 (4.8) [a] 30–36	23 (9.4) 18–31

[a] p = < 0.05 compared to values at 6 months

tal separation at the tidemark was virtually absent at the lateral side, but appeared in OA (Table 1). This phenomenon was commonly noted adjacent to areas with cartilage destruction, extending peripherally into the marginal zone.

In the central medial compartment, calcified cartilage height increased between 6 and 12 months of age (Table 2). Laterally, the calcified cartilage was initially higher, showing no change during the observation period. Paired measurements of calcified/uncalcified cartilage showed no correlation (r = 0.68).

The osteocartilaginous border was highly irregular, with deep extensions into the bone, giving it a coral-like appearance (Figure 3), with a surface about 5–6 times that of the articular surface. The Sv of the osteocartilaginous interface decreased in the medial plateau with increasing OA and remained unchanged in the lateral plateau (Table 3).

Bone proliferations were almost always focal and confined to areas below pathological articular cartilage; the sclerotic bone showing a sharp demarcation towards normal bone, roughly corresponding to the overlying cartilage changes. Moreover, the degree of bone sclerosis appeared to be roughly proportional to the severity of the adjacent cartilage lesions. Thus, in areas of cartilage destruction, the subchondral bone was dense, with thick bone trabeculae, often with numerous mosaic-like cement line lesions somewhat mimicking Paget's disease (Figure 3). Below the cartilage destruction, hematopoietic bone marrow cells were depleted, with only a limited number of stellate interstitial cells (Figure 4). In addition, the volume of bone marrow cavities decreased (data not shown). Superficial bone necroses were occasionally found, but only focally in small areas. Fractures with bone-forming callus were extremely rare.

Cysts, large fluid-filled spaces lined by a fibrous membrane (Figure 5), were a prominent feature in animals even as young as 12 months of age (Table 4). Substantial numbers of cysts were found only in the central compartment on the medial side. Some cysts

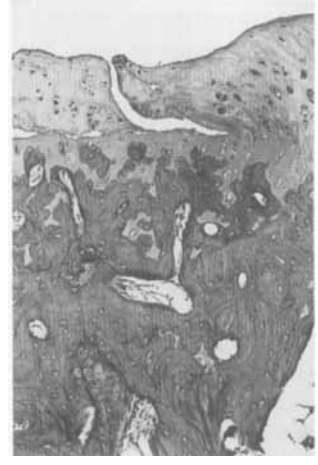

Figure 3. Micrograph from a 30-month-old Dunkin Hartley guinea pig showing that below the cartilage lesions, bone is thickened, showing extensive remodeling and there is also a depletion of hematopoietic bone marrow. Compare with normal hematopoietic marrow shown in Figure 2. In addition, the osteocartilaginous border has a coral-like, interdigitating configuration. HE ×125.

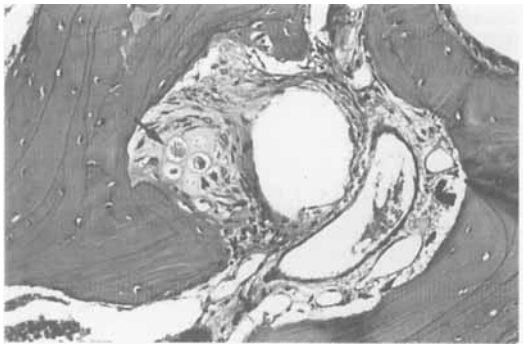

Figure 4. Larger magnification of the fibrocartilagninous lesion seen in Figure 3. Note that it contains mixed tissue, including cartilage (arrow). Subchondral proliferation of fibrous tissue, sometimes with cartilage metaplasia, formed osteoblast and osteoclast-scarce noduli in relation to resorption cavities. HE ×250.

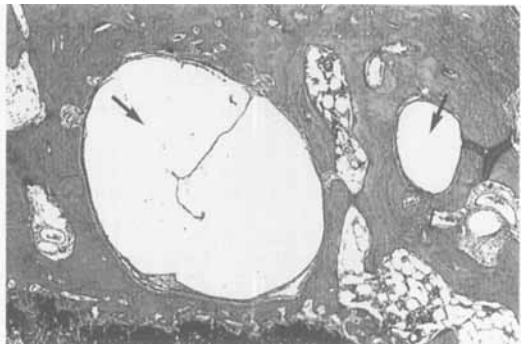

Figure 5. Micrograph from a 30-month-old Dunkin Hartley guinea pig showing two mature cysts of different size (arrows). The volume of the cysts increased with OA in the central medial compartment. To the right, a smaller cyst with fibrocartilaginous proliferation (f). HE ×125.

Table 4. Volume of cysts (mm^3) (mean (SD) range) in epiphyseal bone (8 pigs in each age group)

	Medial plateau (mm^3)		Lateral plateau (mm^3)	
Age (months)	Central compartment	Peripheral compartment	Central compartment	Peripheral compartment
6	0.10 (0.15) 0–0.29	0 (0) 0–0	0 (0) 0–0	0 (0) 0–0
12	1.36 (1.25) [a] 0–3.23	0.46 (1.02) 0–1.79	0.20 (0.55) 0–0.89	0.13 (0.37) 0–0.99
30	1.10 (1.04) [a] 0–2.85	0.23 (0.42) 0–0.78	0.49 (0.71) 0–1.59	0.08 (0.14) 0–0.41

[a] $p = < 0.05$ compared to values at 6 months

Table 5. Surface density (mm^2/mm^3) of capillaries (mean (SD) range) in subchondral bone (8 pigs in each age group)

	Medial plateau		Lateral plateau	
Age (months)	Central compartment	Peripheral compartment	Central compartment	Peripheral compartment
6	2.9 (0.4) 2.4–3.3	4.6 (1.0) 4.0–5.6	2.9 (0.3) 2.4–3.2	4.6 (0.7) 4.1–6.3
12	2.7 (0.6) 1.9–3.4	4.4 (1.0) 3.5–5.0	3.2 (0.9) 2.0–3.9	3.9 (0.5) 3.7–4.9
30	2.9 (0.7) 2.0–4.5	4.8 (1.8) 4.1–6.5	3.2 (0.7) 2.5–3.4	4.0 (0.8) 3.5–5.6

were also observed at the ligamentous insertions. Some smaller lesions were partly solid, containing fibrous tissue partially undergoing cartilage metaplasia (Figure 5). In general, the smaller the lesion, the larger its solid portion. In fact, there seemed to be a continuity in development from small solid noduli to mature cysts (Figures 4 and 5).

There was no increase of blood vessel Sv with age or disease. Vascularity was denser in the peripheral part of the joint (Table 5).

The osteophytes noted in the periphery of the medial plateau at 12 months of age increased further between 12 and 30 months of age (Table 6). Similarly to the development of cysts, there appeared to be a continuum from small areas of fibrous tissue to prominent exostoses, surfaced by cartilage with an intermediate layer of calcified cartilage and a subchondral bone plate.

As judged by the morphologic appearance, there was still an active bone formation at the physis of the youngest animals, but the Vv of the growth plate decreased with age (Table 7), remaining as a remnant in the oldest animals. The distal femur was not examined histologically, but it showed a similar gross arthropathy as in the tibia.

Table 6. Volume of osteophytes (mm^3) (mean (SD) range) (8 pigs in each age group)

Age	Medial plateau (mm^3)		Lateral plateau (mm^3)	
6	0 (0)	0–0	0 (0)	0–0
12	0.68 (0.48) [a]	0.10–3.28	0.09 (0.21)	0–1.43
30	1.46 (1.39) [a,b]	0–8.91	0.08 (0.22)	0–1.54

[a] p = < 0.05 compared to values at 6 months
[b] p = < 0.05 compared to values at 12 months

Table 7. Volume density of epiphyseal growth plate (mean (SD) range) (8 pigs in each age group)

Age	Medial plateau (mm^3)		Lateral plateau (mm^3)	
6	0.33 (0.02)	0.32–0.36	0.34 (0.02)	0.33–0.36
12	0.28 (0.02) [a]	0.26–0.29	0.28 (0.02) [a]	0.27–0.29
30	0.13 (0.01) [a,b]	0.12–0.14	0.14 (0.01) [a,b]	0.13–0.14

[a] p = < 0.05 compared to values at 6 months
[b] p = < 0.05 compared to values at 12 months

Discussion

We found a general arthrosis pattern: the cartilage lesions had a rather stereotype appearance, with a central cartilage destruction surrounded by proliferative cartilage. Excluding the superficial zone, the matrix of the marginal zone often appeared to be grossly intact and higher than the peripheral, non-OA cartilage. Striking features were the constant width of the proliferative rim and ulcerations that were paralleled by subjacent bone changes.

There was an increase in the height of the calcified cartilage that occurred parallel to OA on the medial side. An increase of calcified cartilage height in human OA has previously been demonstrated in resected femoral heads (Vignon et al. 1976). The calcified layer is probably important for restricting peak stresses to the resilient subchondral bone. In a computer model, Anderson et al. (1993) calculated that decreases in uncalcified cartilage and increases in the calcified layer, increase local shear stresses at the tidemark, supporting the notion that thickening of the calcified cartilage may be a pathogenetic factor in OA (Radin et al. 1991). On the other hand, even if the calcified layer on the lateral, non-OA side did not change, it was substantially thicker, which indicates that load is not the only factor affecting its height. In an investigation of the temporomandibular joint, Flygare et al. (1993) demonstrated a covariation between calcified and uncalcified cartilages. In contrast, we found no correlation between the paired measurements of calcified/uncalcified heights.

The importance of the stress concentration at the tidemark has been highlighted by the reported high clinical incidence of horizontal splitting in this area (Meachim and Bentley 1978). We found horizontal splitting mostly in conjunction with cartilage destruction in the medial plateau and it was not particularly common. However, the rather uniform appearance and co-localization with the other pathological changes suggest that it is an inherent part of the pathological process and not merely an artefact of preparation.

The intermediate forms between cysts and noduli may favor a continuous line of development (Figures 4 and 5). Noduli usually originated at bone trabeculae below the subchondral bone plate, the deep ones lacking continuity with the joint. The origin of the cells in the noduli is not clear, but the facts that they contained mixed tissues, and that there were intermediate forms between early fibrous lesions and mature cysts, may indicate that they developed from undifferentiated connective tissue cells. Another possibility is that they evolved from remnants of calcified cartilage scattered in bone trabeculae, but such islands contain only a very limited number of cells. Milgram (1983) concluded that cysts are formed through proliferation of myxomatous bone marrow cells. Irrespective of the cellular mechanisms underlying cyst formation, local overload is presumably a triggering factor, since cysts were mainly encountered below areas of severe cartilage destruction.

The noduli were surrounded by intact, viable bone that did not look like fracture callus. Similar observations have previously been reported by Milgram (1983), arguing against the suggestion that bone necrosis causes cysts (Rhaney and Lamb 1955). Alternatively, it has been claimed that cysts are formed by inflow of synovial fluid through a vent mechanism (Landells 1953). However, most investigators have failed to demonstrate communication (Milgram 1983). We found communication with the joint in only a small number of cysts, particularly at ligament insertions. These lesions were always small, and showed no herniation of a synovial membrane, nor did they contain debris. Cartilage metaplasia associated with remodeling in OA has been described previously (Collins 1949, Jaffe 1972, Havdrup et al. 1976), and Collins (1949) also briefly mentioned that cysts appear to be incidental to remodeling.

Subchondral bone is both structurally and functionally inseparable from cartilage. Although we found no fibrils crossing the cartilage/bone junction, it appears to be much stronger than the border at the calcification front, since natural or artefactual separation between bone and cartilage in histological preparations

is hardly ever seen. The strength of the osteocartilaginous border is probably largely explained by its coral-like, interdigitating configuration (Figure 3), distributing forces over a large surface. Its irregular and serrated border is obviously the result of ongoing remodeling, although the scarcity of piercing vessels and chondroclasts indicates low turnover. Yet, the decreased Sv of the osteocartilaginous interface in OA shows that cartilage extensions into the subchondral bone are partly resorbed along with the remodeling. However, the initial differences in the interface and height of calcified cartilage between the medial and lateral sides indicate differences in joint physiology, probably in terms of load.

Our observations illustrate the different bone structure of epiphysis and metaphysis: bone formation associated with OA was largely confined to the epiphysis, the physeal remnant presenting as a sharp border to the unaffected metaphyseal bone. In contrast to man, rodents do not completely close their physes. Although increased bone density was limited to the medial side, we do not think that the structural differences primarily is caused by a load distributing effect of the physeal remnant, since there were no differences between the lateral and medial parts of the physis. It seems more likely that the structural differences reflect the different histogenesis. Similar changes have been described in a rabbit overload model of OA (Dekel and Weissman 1978). Although bone sclerosis in human knee OA often is sharply localized (Havdrup et al. 1976), the sclerosis is not as pronounced as in guinea pigs or rodents.

Fractures were very rare in our material, in agreement with Cameron and Fornasier (1975) who observed that, although trabecular stress fractures occur normally, their incidence does not increase in OA. Neither do our results favor the hypothesis that gross bone necrosis is an important factor for the joint deformation in OA. In contrast, Harrison et al. (1953) claimed that serial infarctions cause bone destruction and deformation in OA.

The vascular supply does not appear to be a primary pathogenetic factor in this disease model, since vascular density did not change in subchondral OA bone. Moreover, the vascular density was highest in the periphery of the joint, where the OA changes were least pronounced. The hypothesis of a vascular pathogenesis in OA stems primarily from observations of venous engorgement in severe hip OA (Hulth 1959, Arnoldi et al. 1972). Another argument against the vascular theory is that increased vascularization does not generally precede tissue proliferation. In a study on a rabbit meniscectomy OA model, Moskowitz and Goldberg (1987) observed that most animals developed peripheral osteophytes and that the changes in vessels, soft tissue, and bone, evolved simultaneously. In their study the ostophytes, however, grew in weeks, in contrast to the slowly progressive growth in the present study. For anatomical and mechanical reasons, osteophytes grow only at the periphery of the joint. Facilitating factors may be the abundant vascularity; even under normal conditions cellular activity is highest at the periphery of the joint (Moskowitz and Goldberg 1987). In this area, proliferative chondrocytes and other mesenchymal cells with an immature appearance are located. In addition to metabolic factors, it has been suggested that mechanical traction by soft tissues at their insertion at the perichondrium, may play a role in the initiation of metaplasia (Bennet and Bauer 1937). The possible role of mechanical factors is supported by a study on meniscectomized sheep, in which Ghosh et al. (1990) reported that osteophytosis was proportional to the amount of postoperative weight-bearing exercise, and not correlated to regressive cartilage changes. Moreover, clinical studies indicate that osteophytosis is not directly associated with OA (Nilsson et al. 1982). In our study, osteophytes increased significantly only in the medial plateau, indicating that local rather than regional stimuli cause the proliferation. In the light microscope, the cartilage covering osteophytes sometimes had a quite normal articular cartilage appearance, but a recent investigation using in situ hybridization has shown expression of collagen I in osteophytic cartilage, indicating that the newly formed matrix is abnormal. (Aigner et al. 1993). Osteophytic cartilage is also reported to contain decreased amounts of keratan sulfate (Williams et al. 1988).

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