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Collagen fibre arrangement in the tibial plateau articular cartilage of man and other mammalian species

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ABSTRACT

Experimental animal models are frequently used to study articular cartilage, but the relevance to man remains problematic. In this study animal models were compared by examination of the collagen fibre arrangement in the medial tibial plateau of human, cow, pig, dog, sheep, rabbit and rat specimens. 24 cartilage samples from each species were prepared and maximum cartilage thickness in the central tibial plateau measured. Samples were fixed, dehydrated, freeze-fractured and imaged by scanning electron microscopy (SEM). At low magnification, 2 different arrangements of collagen fibres were observed: leaf-like (human, pig, dog) and columnar (cow, sheep, rabbit, rat). The porcine collagen structure was the most similar to that of man. This arrangement was consistent from the radial to the upper zones. Under higher magnification at the surface of the leaves, the collagen was more randomly oriented, whereas the columns consisted of parallel collagen fibrils. The maximum thickness of cartilage did not correlate with the type of collagen arrangement but was correlated with the body weight of the species ($r = 0.785$). When using animal models for investigating human articular cartilage function or pathology, the differences in arrangement of collagen fibres in tibial plateau cartilage between laboratory animals should be considered especially if morphological evaluation is planned.

Key words: Collagen; knee joint.

INTRODUCTION

The basic biochemical, biomechanical and morphological characteristics of articular cartilage are frequently studied in relation to pathological changes and the effectiveness of different treatment methods. The experimental and descriptive findings derive from a wide variety of animal species. For example, human and bovine cartilage is used for biomechanical studies, the dog is a popular subject to study deficiency of the anterior cruciate ligament and the rabbit is frequently used to study the effects of meniscectomy or to evaluate the treatment of cartilage defects (Hoch et al. 1983; Torzilli et al. 1983; Korkala et al. 1984; Mow et al. 1984; Wakitani et al. 1989; Moran et al. 1992; Setton et al. 1994; Marshall & Chan, 1996). Nevertheless, species-specific differences in morphological and biomechanical characteristics must be considered in the choice of laboratory animals for the study of articular cartilage.

The morphological and biomechanical properties of articular cartilages in different laboratory animals have been compared. For example a difference in cell density in femoral condyle cartilage of 8 different mammals has been demonstrated (Stockwell, 1971). Simon (1971*a*) calculated differences in compressive stresses between cow, sheep, dog, rat and mouse articular cartilage. Significant differences in intrinsic material coefficients from in situ biphasic creep experiments of bovine, canine, human, monkey and rabbit distal femoral cartilage were demonstrated by Athanasiou et al. (1991). The morphology of articular cartilage from different animals has been compared with respect to the collagen structure. Benninghoff (1925) introduced the partition of articular cartilage into 4 different layers or zones. He used polarised light

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microscopy to examine the orientation of collagen fibres in human, dog, cat and rabbit articular cartilage. Collagen fibres were described as arising in a radial manner from the subchondral bone before arching over to run tangential to the surface and finally returning to the subchondral bone. Clark (1985, 1990) demonstrated by SEM a columnar arrangement of collagen fibres in rabbit patellar and distal femoral cartilage. Comparisons of human, dog and rabbit tibial plateau cartilage showed similar general patterns of collagen fibre arrangement in all 3 species (Speer & Dahners, 1979; Clark, 1991). In a polarised light and transmission electron microscopic study of articular cartilage of different mammals (rat, guinea pig, rabbit, dog, pig, human) Zambrano et al. (1982) confirmed the classical description of the collagen arrangement as the Gothic arches of Benninghoff (1925). However, none of these investigators has directly addressed the question of species related variations in the 3-dimensional arrangement of collagen structure.

Collagen is the structure-providing element of articular cartilage and contributes to the main function of this tissue which is to transmit loads. Motivated by the lack of information on differences in collagen arrangements between different laboratory animals and in particular by the lack of comparisons with human cartilage, we investigated the arrangement of collagen fibres within tibial plateau articular cartilage of the most frequently used laboratory animals and compared them with that in man. Additionally, in order to examine whether a certain species-specific cartilage thickness is associated with a specific arrangement of collagen fibres, maximum cartilage thickness in the central medial tibial plateau was measured and related to the species body weight.

MATERIALS AND METHODS

Preparation of samples

Intact knee joints from humans, cow, pig, dog, sheep, rabbit and rat were obtained either immediately or within a maximum of 20 h after death. Four different animals from each species were studied and 6 samples from each animal were harvested. Only samples from skeletally mature and middle-aged individuals were used (for man: 27–49 y). After opening the joint, the cartilage surface was examined to exclude cartilage exhibiting fibrillation of the surface. During preparation, the samples were kept wet by rinsing with pH 7.4 buffered Ringer's solution. Cubic samples (maximum size $4 \times 4 \times 5$ mm) were harvested from the medial tibial plateau, retaining a thin layer of subchondral bone. Samples were taken from the central weight bearing, meniscus free region. Prior to chemical fixation the maximum thickness of the articular cartilage was measured for each sample as described below.

Fixation for SEM

In order to control for fixation artifacts, half of the samples were preserved by microwave enhanced fixation and the other half by conventional fixation (Richards & Kääb, 1996). They were rinsed for 10 min in 0.1 mol l^{-1} PIPES (piperazine-N,N′-bis-2-ethane sulphonic acid) buffer pH 7.4 at 20 °C. Samples were immersed in the fixing solutions and then placed in a conventional microwave oven (Miele Electronic M696, Gütersloh, Germany). Microwave irradiation was administered at a constant 450 W until a temperature of 40 °C was reached, as measured by a probe placed alongside the specimen. Primary fixation was in 2.5% glutaraldehyde with 4% paraformaldehyde. The cubes were postfixed in 0.2% osmium tetroxide and stained in 2% uranyl acetate for periods of 60 min each at 20 °C. After each fixation step, the sample was immediately placed in PIPES buffer (pH 7.4) at 4 °C, with one change for a total time of 20 min. Conventional (aqueous) immersion fixation was used for the other half of the samples. In this case primary fixation was in 2.5% glutaraldehyde with 4% paraformaldehyde for 4 h. Secondary fixation and staining were identical to the microwave group.

Fixed samples were dehydrated using a series of graded ethanol solutions of 50%, 60%, 70%, 80%, 90%, 100% and 100% for 15 min each respectively. After dehydration, freeze-fracturing was performed on specimen containing 100% ethanol, after cooling in liquid nitrogen. The cartilage was fractured with a broad sharp chisel in a direction perpendicular from the subchondral bone to the surface. Additionally, half of the fractured specimens were fractured again vertically at a right angle to the first fracture, in order to reveal the collagen arrangement in 2 orthogonal planes (Clark, 1990).

The samples were dried with a Polaron E3000 critical point drier (Agar Scientific Ltd, Essex, UK), using CO_2 as a transitional fluid. The specimens were coated with 8 nm of gold in a Baltec MED 020 unit (Baltec AG, Balzers, Liechtenstein) and examined with a Hitachi S-4100 field emission SEM (Hitachi Ltd, Tokyo, Japan). The SEM was operated in secondary electron detection mode at an accelerating voltage of 1–2 kV and an emission current of 10 μA.

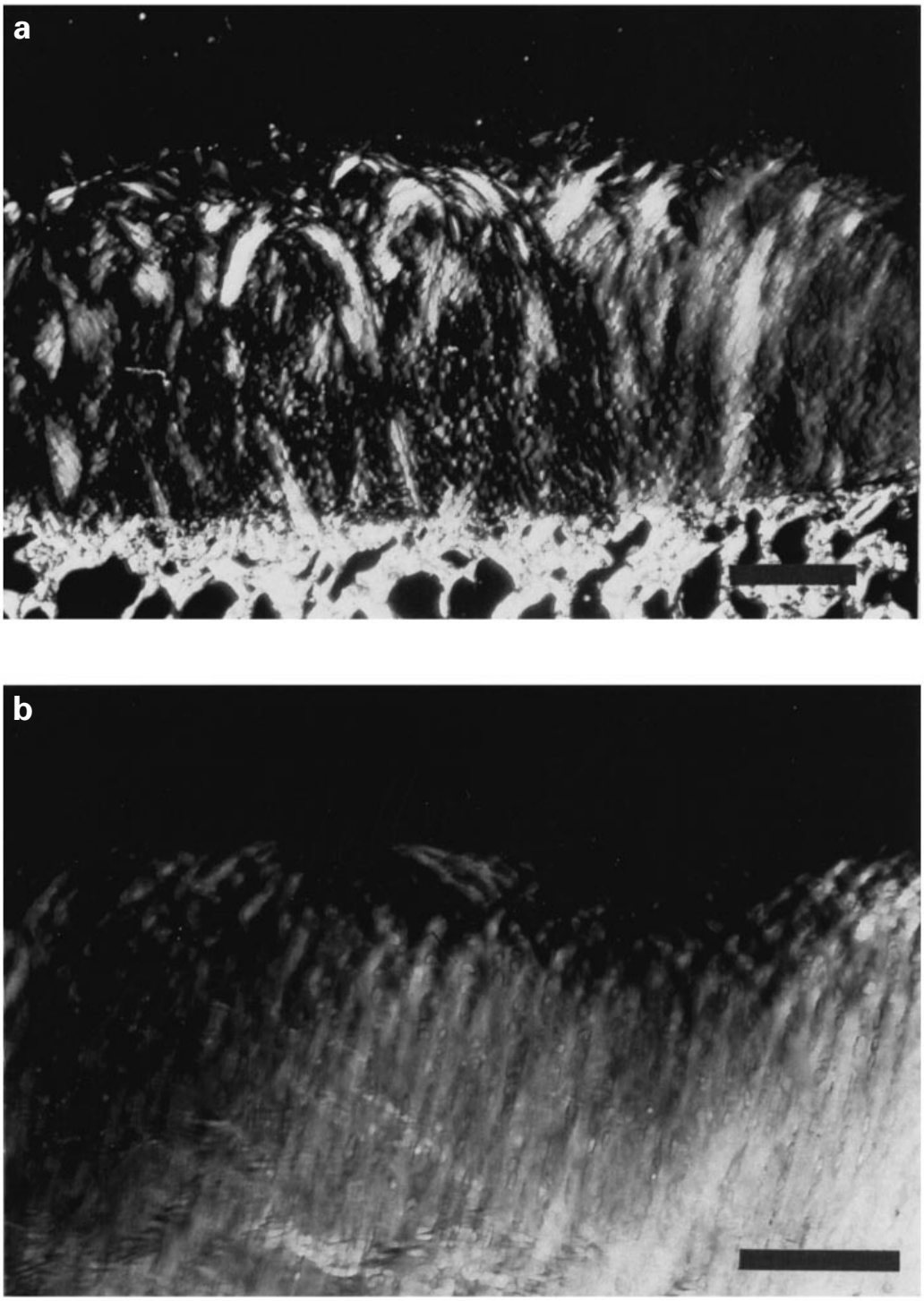


Fig. 1. Polarised light photomicrographs of section of specimen through the medial tibial plateau. (*a*) Human: parallel giant bundles of collagen fibres, curving in the transitional zone to run parallel to the surface. Bar, 1 mm. (*b*) Thinner collagen fibre bundles in the rabbit. Bar, 0.1 mm.

The terminology used to indicate the cartilage layers or zones follows that of others (Benninghoff, 1925; Clarke, 1971; Lane & Weiss, 1975; Clark, 1985). The zone beneath the surface, the tangential zone, consists of collagen fibres lying parallel to the surface. The radial or deep zone begins at the calcified zone and runs towards the tangential zone. The zone connecting the radial and tangential zone is termed the transitional zone.

Light microscopy

From each species 6 specimens were prepared for histological examination. The samples were fixed in

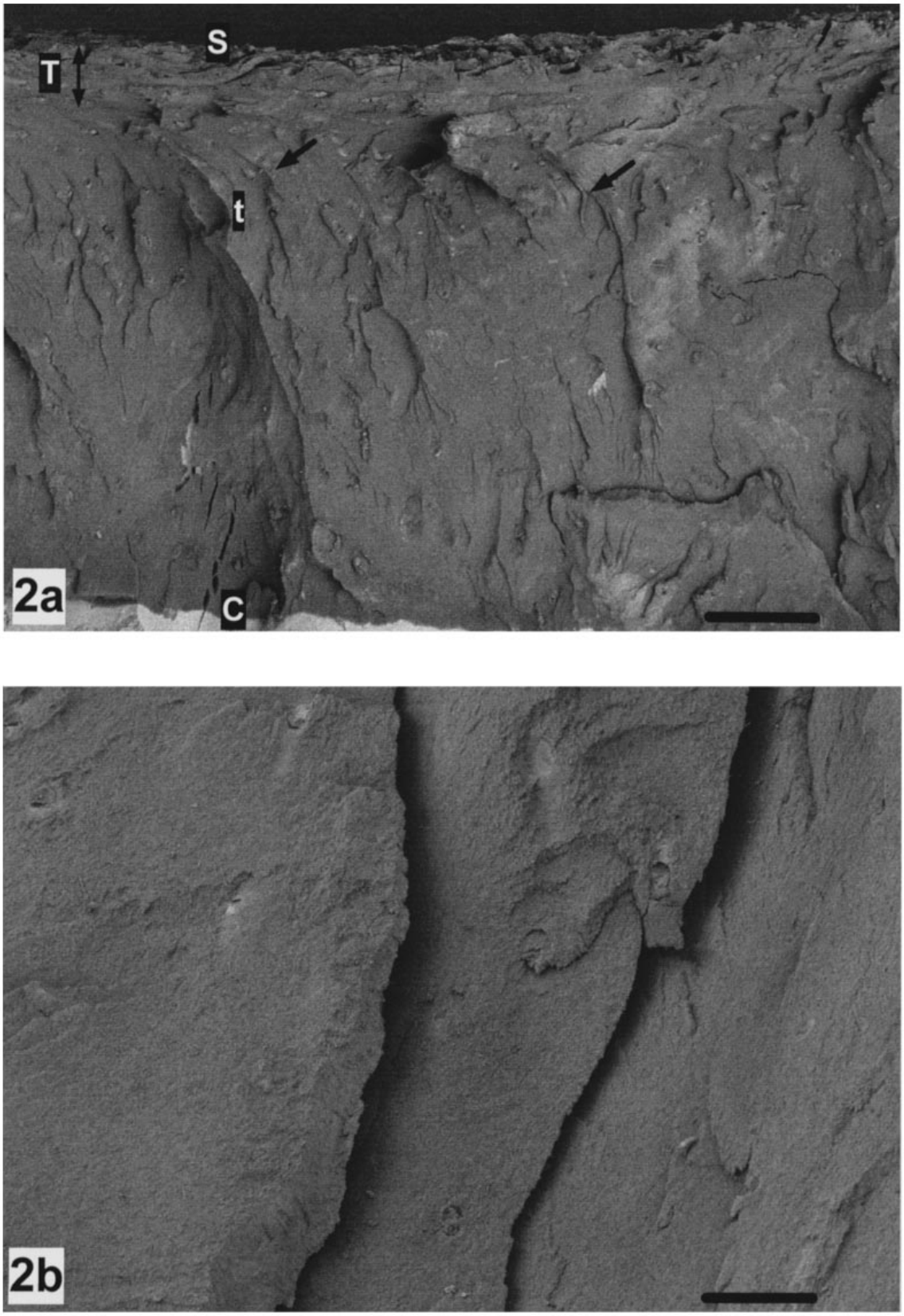
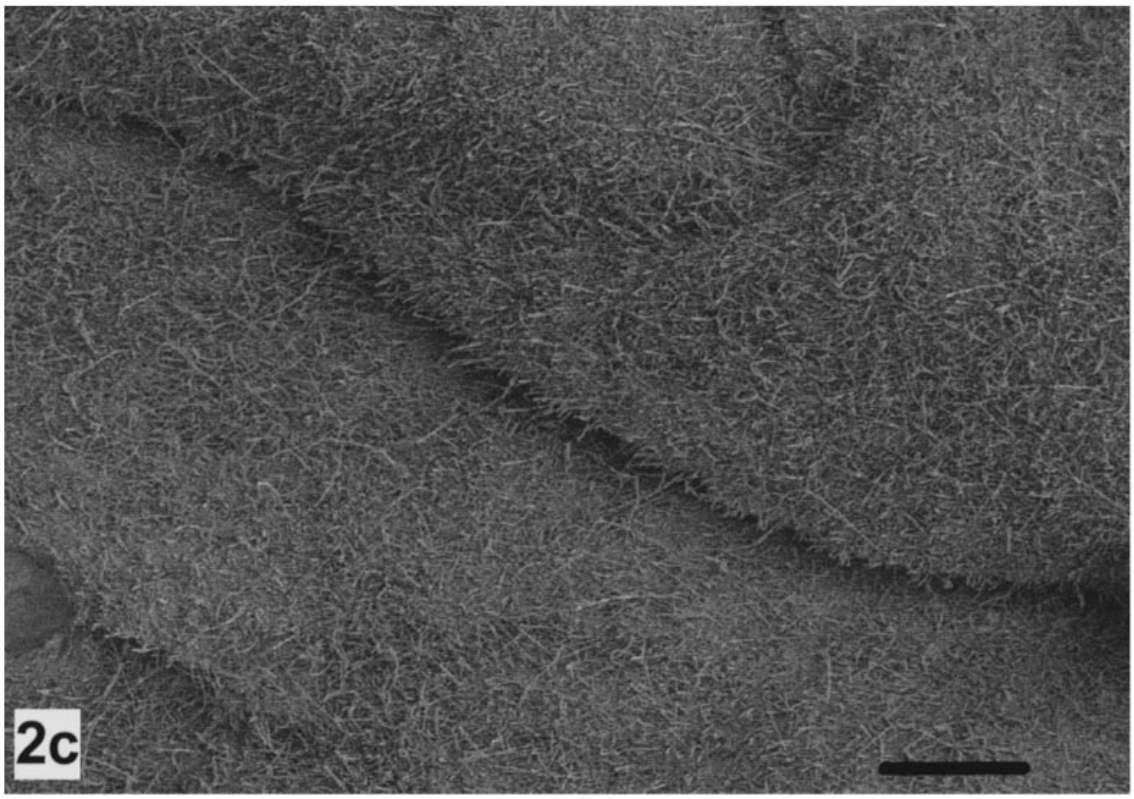


Fig. 2. Freeze-fracture face of human cartilage. (*a*) Leaves of collagen fibres arising from the calcified cartilage (C) and running to the surface (S). In the transitional zone (t) the leaves are curved (arrow) tangentially (T, tangential zone, arrow). Bar, 500 μm. (*b*) Medium magnification of leaves with gaps between in the radial zone. Bar, 50 μm. (*c*) Higher magnification, showing a more random network within the leaves (radial zone). Bar, 15 μm.



Figs 2*a*, *b*. For legend see opposite.

buffered 4% formaldehyde, dehydrated, transferred into 100% xylol and infiltrated and embedded in methyl methacrylate. Polymerisation was completed after 48 h at 60 °C. Sections were cut with a microtome (Sawing microtome 1601, Wetlzar, Germany) around 200 μm thick and then ground and polished (Polishmachine Stähli-Lapp, Stähli AG, Biel, Switzerland) to 80 μm in thickness. These cuts were used either unstained for polarised light microscopic examination or stained with haematoxilin-eosin, resofucsin or azan for light microscopic evaluation (Romeis, 1989).

Thickness measurement

Maximum cartilage thickness of all samples that were used for SEM preservation was measured while the sample was immersed in Ringer's solution. Only samples which were cut perpendicular to the bone were used. The cartilage front face was viewed under a stereomicroscope at a magnification of ×32. The thickness from the calcified cartilage to the surface was traced with a cursor on a calibrated digitising table (Kontron Electronics, Munich, Germany). A mirror allowed the projection of the cursor onto the sample in the field of view. The maximum thickness in the medial tibia plateau from the different animals of each species was taken. The maximum but not mean thickness from the tibial plateau was taken in order to avoid high deviation of values due to intraspecies local variation. Statistical analysis of the relation of maximum thickness and body weight of each species was performed using a linear regression analysis.

RESULTS

Polarised light microscopy and light microscopy

Polarised light microscopy revealed the presence of collagen fibres in all species. These collagen fibre bundles ran perpendicularly from the calcified cartilage towards the surface. In the transitional zone, the fibres formed intersecting, arch-like structures. In the human the collagen fibres were broad and appeared as giant bundles (Fig. 1*a*). In all other species, the collagen fibres appeared as thinner bundles or columns (Fig. 1*b*). Bovine and porcine cartilage, which were most similar to man on SEM, showed no aggregation of collagen fibres; instead, the bundles were thinner. By light microscopy no overall collagen fibre organisation was visible since fibres could not be traced from the calcified cartilage to the surface and no significant differences among species were detected.

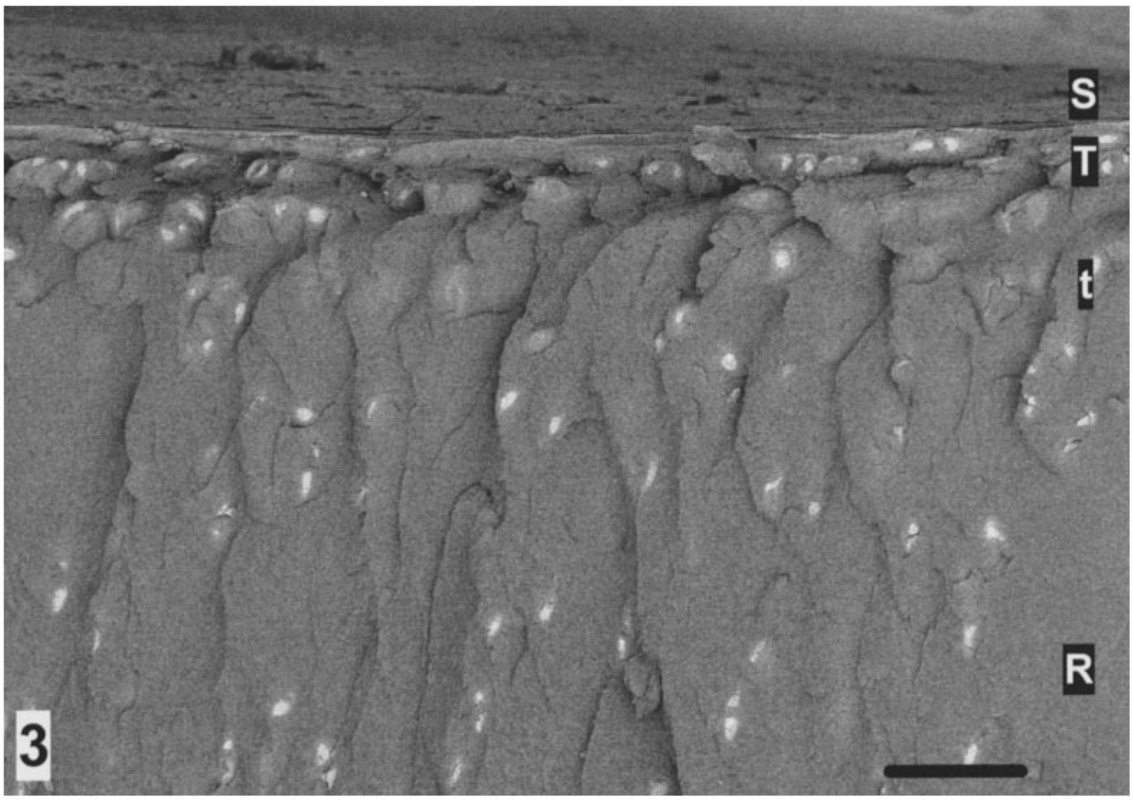


Fig. 3. Porcine cartilage: leaf structure comparable to that of man. Surface (S), tangential (T), transitional (t) and upper radial (R) zone. Bar, 60 μm.

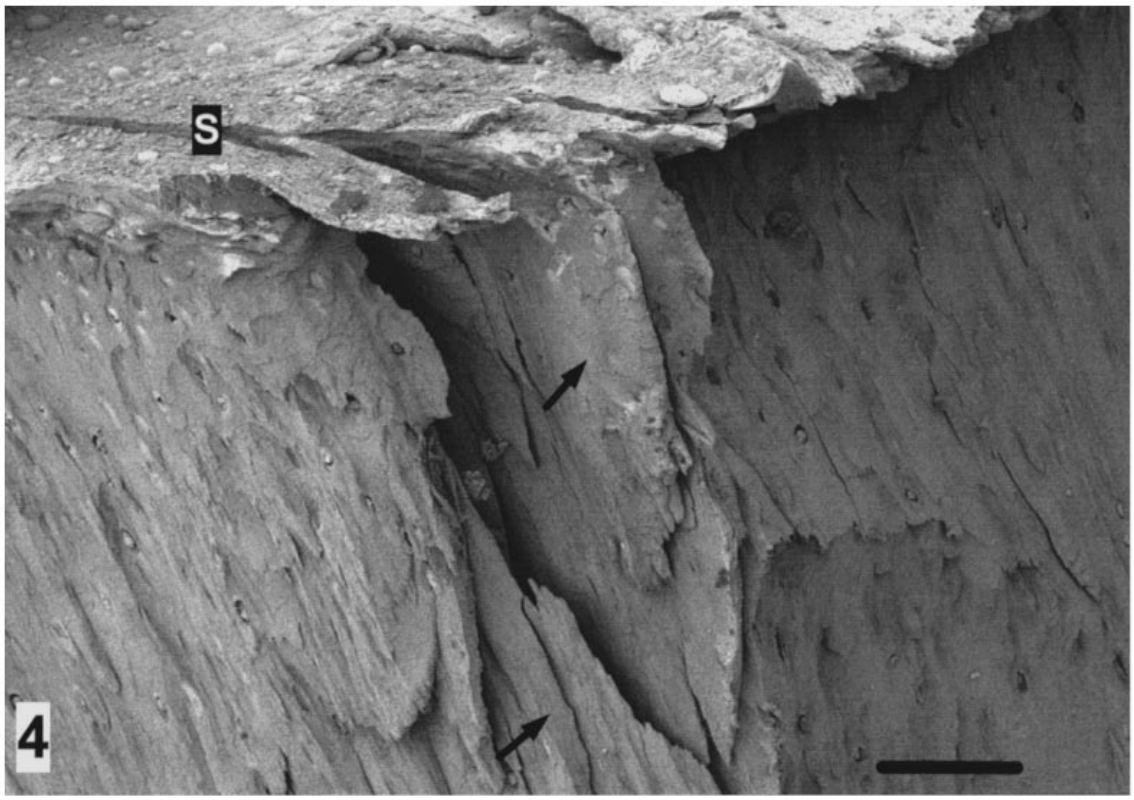


Fig. 4. Dog cartilage: 2 vertical fractures meet at a right angle. There is a more leaf-like arrangement of the collagen network (arrows). The leaves are oriented vertical to the surface (S). Bar, 90 μm.

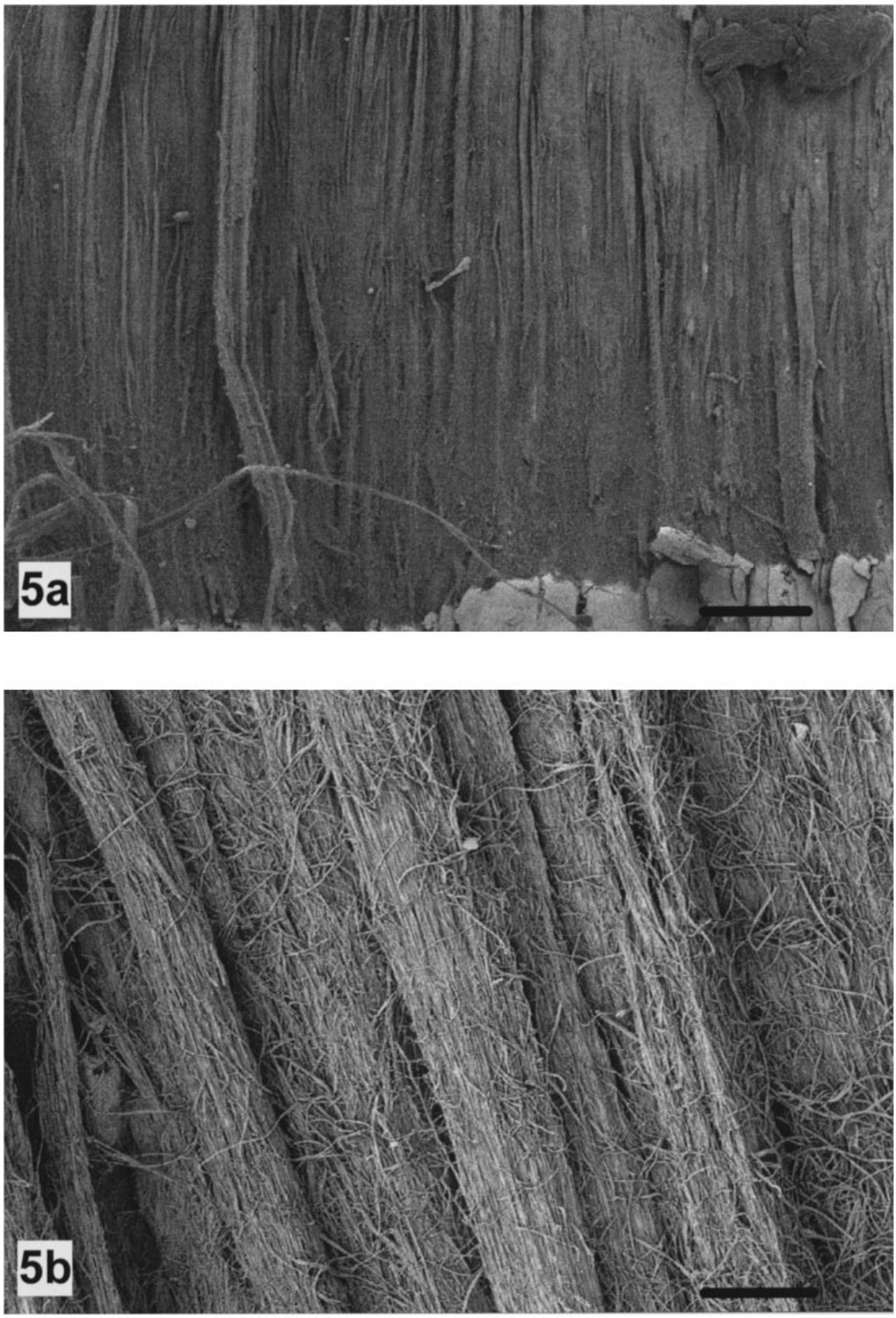
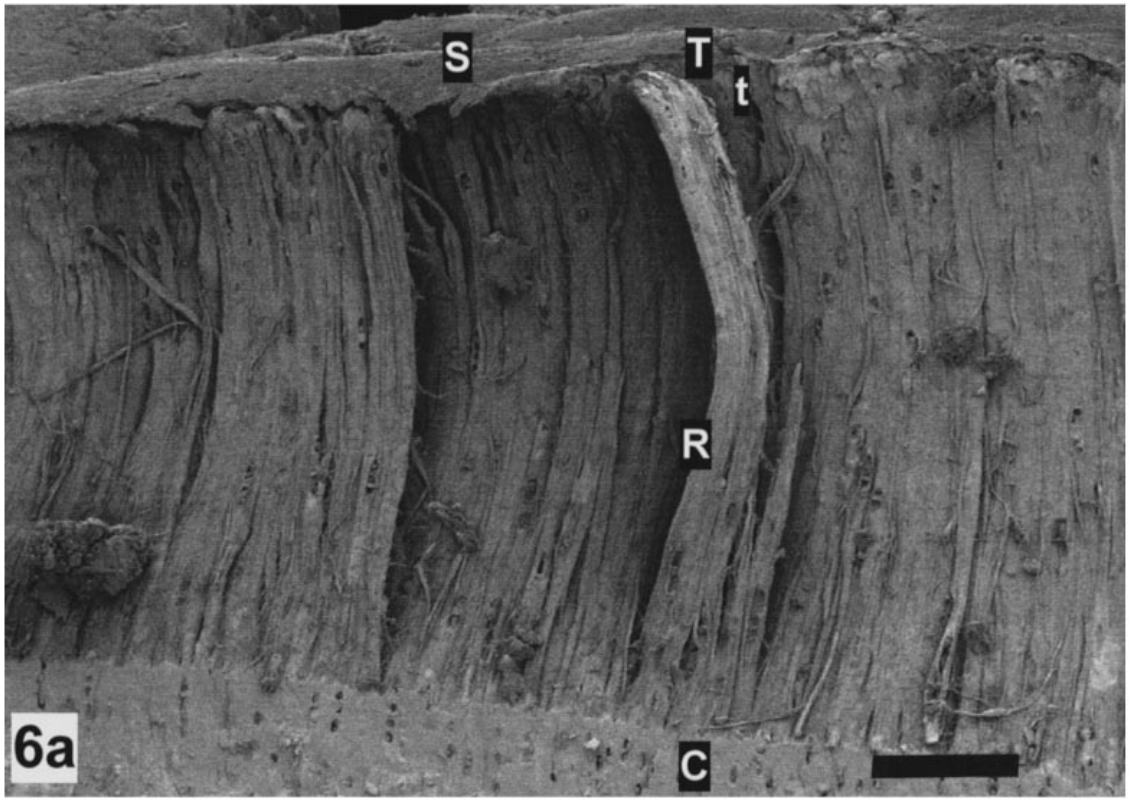


Fig. 5. Bovine cartilage: collagen is arranged in a columnar manner in the radial and transitional zones. (*a*) Columns run perpendicular to the surface. Bar, 150 μm. (*b*) A column consists of parallel collagen, perpendicular to the surface in the radial zone. Some fibres run obliquely. Bar, 10 μm.



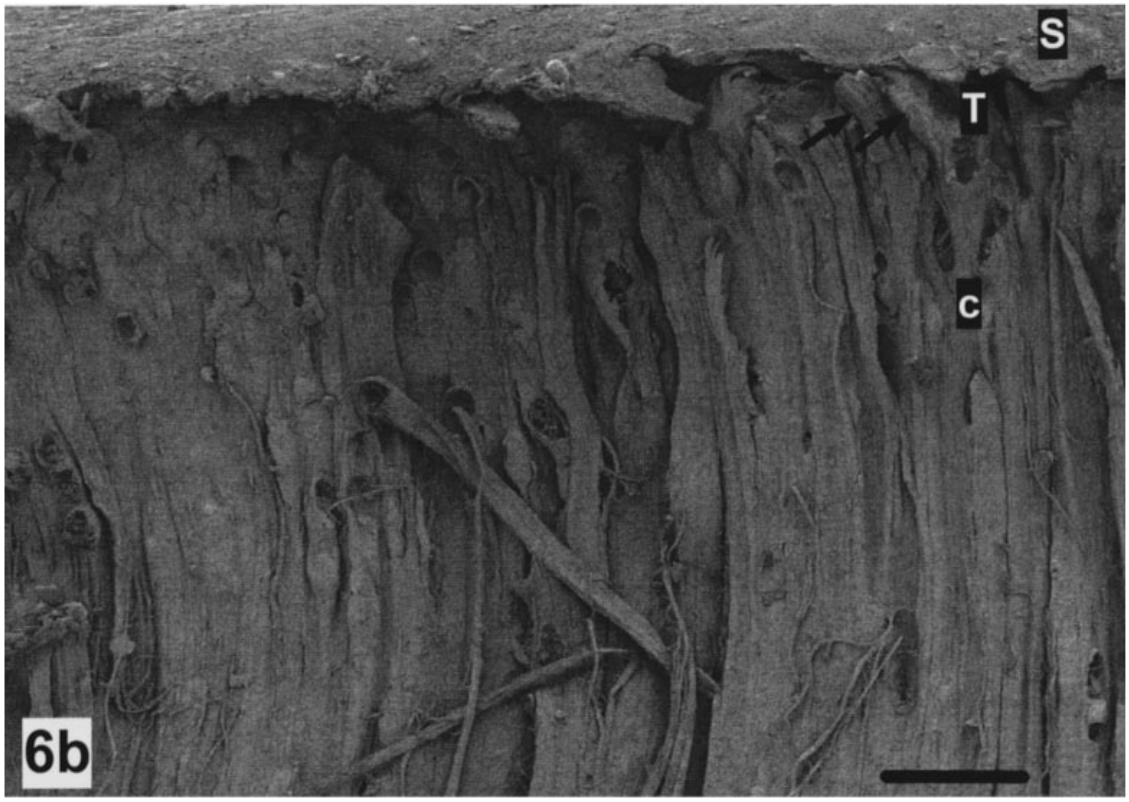


Fig. 6. Rabbit cartilage: columnar arrangement of collagen fibres. (*a*) Overview: surface (S), tangential (T), transitional (t), radial (R) and calcified (C) zones. Bar, 150 μm. (*b*) Superficial one third of the cartilage with columnar arrangement of the collagen fibres showing a slight bending of the fibres under the surface (arrow) to the tangential zone (T). s, surface; c, chondrocytes in their lacunae. Bar, 50 μm.

Fig. 7. Rat cartilage: columnar arrangement of collagen fibres, running parallel and perpendicular to the surface in the radial zone. Bar, 5 μm.

Electron microscopy

No qualitative difference was observed between cartilage fixed either by microwave enhanced or conventional fixation. With both fixation techniques, no signs of major shrinkage were observed. The cartilage surface was almost smooth or showed minor ridges and humps in all species (Fig. 6*b*).

The structure of the collagen fibres as exposed on freeze-fracture faces showed a species-specific fracture pattern. At low magnification, the exposed collagen fibres were arranged as leaves or columns. The human cartilage exhibited a clear leaf-like arrangement where the collagen was organised in broad flat leaves or sheets which merged one with another (Fig. 2). Porcine cartilage showed the most similar arrangement of collagen fibres to the human (Fig. 3). The dog also showed a more leaf-like arrangement (Fig. 4). On the other side, cow, sheep, rabbit and rat had a more columnar arrangement of the collagen matrix (Figs 5*a*, 6*a*, 7). This arrangement was also observed in samples which were fractured at right angles. Columns were visible on both fracture surfaces, while leaves showed a partly columnar appearance only in one fracture plane (Fig. 4). The diameter range of individual columns varied somewhat among species. It ranged between 8–30 μm in the cow, 5–15 μm in the sheep, 5–15 μm in the rabbit and 4–8 μm in the rat.

This general arrangement of collagen fibres as leaves or columns was consistent throughout the deep or radial and the transitional zone. While it was not possible to trace a single leaf from the calcified cartilage to the surface, it was feasible to trace columns in all species exhibiting the columnar pattern (Figs 2*a*, 6*a*). While the different zones were visible in each specimen, the zones were of different relative thickness (percentage thickness with respect to full thickness) in different species. The deep or radial zone was between 60 % and 80 % and the transitional zone approximately 10 %–15 % of total thickness in all species. The tangential zone, where the collagen fibres or leaves were oriented almost parallel to the surface, was relatively thick in man (approximately 10 %, 250 μm) and thinner in other species, e.g. in the rabbit (approximately 3–4 %, 10 μm).

At higher magnifications, no preferred collagen fibril organisation within the individual leaves in human, pig or dog cartilage could be detected (Fig. 2*c*). Within the fibre columns, however, the fibrils were essentially parallel and perpendicular to the surface (Figs 5*b*, 7). This parallel organisation was present throughout the course of the fibres. Individual

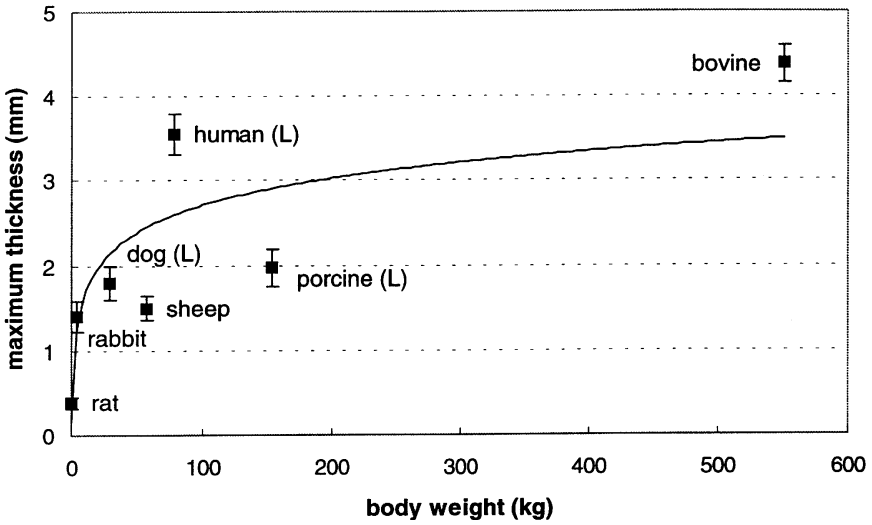


Fig. 8. Maximum thickness of articular cartilage in the central medial tibial plateau. There is a correlation between body weight and maximum cartilage thickness ($r = 0.785$, $P < 0.001$). Species with a leaf-like arrangement of collagen fibers are marked (L).

collagen fibrils were observed crossing between leaves or columns (Figs 2*c*, 5*b*, 7). Within the matrix, chondrocytes were visible in their lacunae. In all species there was a higher number of chondrocytes in the upper cartilage layers when compared with the deeper zones (Figs 3, 6*b*).

Maximum cartilage thickness

Maximum cartilage thickness in the medial tibia plateau was ($r = 0.785$, $P < 0.001$) associated with species body weight (Fig. 8). Bovine cartilage was the thickest with 4.4 mm (± 0.22) cartilage thickness and a body weight of 550 kg (± 24). Human cartilage was the second thickest with 3.6 mm (± 0.34), and a body weight of only 78 kg (± 4.2). In all the other species the cartilage was less than 2 mm thick. There was no correlation between cartilage thickness or body weight and the general collagen arrangements as leaves or columns. The cartilage was relatively thick in man and rabbit in relation to body weight, when compared with the other species.

DISCUSSION

The 3-dimensional arrangement of articular cartilage collagen fibres in various mammals used in cartilage research including human articular cartilage was compared. We found that there were 2 general types of collagen arrangement, a more leaf-like arrangement in man, pig and dog and a more columnar arrangement in cow, sheep, rabbit and rat.

The medial tibial plateau cartilage was examined in this study since it is a common site of cartilage degeneration in man (White et al. 1991). As a consequence, numerous experimental studies have focused on the tibial plateau (Aston & Bentley, 1986; Kobayashi et al. 1995).

SEM is an appropriate method to show the collagen arrangement of fibres since not only can individual fibres be imaged but also relatively large specimens can be evaluated for the 3-dimensional arrangement of collagen fibres. This structure cannot be analysed if the sample has been cut (Zambrano et al. 1982; Boyde & Jones, 1983; Debont et al. 1986). However, all methods used for the preparation of articular cartilage samples for SEM probably lead to the formation of morphological artifacts. The best documented types of artifact include dimensional changes and disruption of the matrix when the sample is cut or fractured to expose a viewing surface. The overall shrinkage due to fixation, dehydration and drying can be as much as 30% (Boyde & Jones, 1983; Richards & Kääb, 1996). This shrinkage can produce artifacts. However, the general organisation of collagen fibres should not change with general shrinkage of the specimen. Using cryoscanning electron microscopy, Kobayashi et al. (1995, 1996) reported a leaf-like structure in the porcine tibial plateau. With this method artifacts resulting from fixation and drying would not be expected, although different artifacts from freezing might form. Freeze-fracturing of dehydrated tissue in ethanol produces relatively little plastic deformation of the resulting surfaces in comparison with cutting and will show the collagen arrangement since fracture planes tend to follow natural cleavage planes (Boyde & Jones, 1983; Clark, 1990). However, with the columnar pattern, fibres were also found to be running obliquely between parallel fibres. This may be a result of displacement of fibres during freeze-fracturing

(Clark, 1985). Also the more random oriented collagen fibril arrangement within the leaves may be due to fibril cross-links exposed by freeze-fracturing. Since the preparation was similar for all species, no differences in the interspecies collagen arrangement would be expected resulting from preparation artifacts. Enzymatic digestion was avoided, since it is an additional step in preserving cartilage which would not have offered any advantages in this study but could have introduced additional artifacts. It should be noted that the proteoglycans can obscure the surface of collagen fibrils, masking the banding pattern (Minns & Stevens, 1977; Speer & Dahners, 1979). Quacchi et al. (1992) showed on bovine nasal septum enzymatic digestion resulting in an increase in the frequency of thin fibrils, but only a minimal difference in the mean fibre diameter. For ultrastructural analysis and quantitative measurement on collagen fibril diameter, which was outside the scope of this study, the digestion is necessary in order to visualise individual fibrils clearly (Segawa & Takiguchi, 1992). However, the general arrangement of the collagen fibres will not be affected by dispensing with digestion (Clarke, 1971; Clark, 1985, 1991). This is especially the case, where all specimens were treated in the same manner for comparative analysis.

While there are numerous SEM studies analysing the arrangement of collagen fibres in human articular cartilage, only a few comparisons between species have been made. Comparison with these previous studies is difficult since specimens were taken from different joints and varying locations within a joint, or prepared for SEM imaging by a wide variety of methods (Clarke, 1971; Bullough et al. 1985; Jeffery et al. 1991). Within his classical arcade model, Benninghoff (1925) demonstrated that tangential fibres are an extension of the radial fibres which cross obliquely in the transitional zone. In our study, in the species which showed a more columnar arrangement, the fibres could be traced from the calcified cartilage to their oblique orientation in the tangential zone matching the findings of Benninghoff. For the species that showed a more leaf-like arrangement, this tracing of radial fibres was not possible. This might be due to leaf fracturing in the transitional zone (Clark, 1990). In an SEM study, Clark (1991) demonstrated a collagen arrangement in human, dog, and rabbit cartilage similar to the present study. The more leaf-like arrangement of collagen fibres is known for human cartilage (Clarke, 1971; Minns & Stevens, 1977; Boyde & Jones, 1983; Teshima et al. 1995). Jeffery et al. (1991) also showed a leaf-like arrangement of collagen fibres in bovine metacarpal cartilage.

With respect to the association between collagen arrangement, cartilage thickness and species body weight, the question on the significance of the collagen arrangement on the load bearing function of the tissue arises. It is known that cartilage thickness relates to body weight of rat, dog, sheep and cow tibial plateau (Simon, 1971*a*), or femoral condyle (Stockwell, 1971). The deformation of cartilage in general increased with cartilage thickness but no differences in deformation between species for similar cartilage thickness were found (Simon, 1971*b*). We also found a correlation between body weight and maximum cartilage thickness in the tibial plateau. Man and rabbit had a relatively thick cartilage when compared with their body weight than was found in other species. This may be explained by the fact that man is the only non-quadruped out of this group while all the others, except the rabbit, bear a greater proportion of the body on the forelimbs. Therefore, these 2 species may be exposed to higher static peak stresses (Simon, 1971*a*; Biewener, 1989). In our study we could not detect any relationship between cartilage thickness or body weight of different species and the general collagen arrangement as leaves or columns. No obvious reason of classification, habits or biomechanics can be discovered to explain why some species have the more leaf-like and others the more columnar arrangement of collagen fibres in the tibial plateau. The variation in function (e.g. different forces acting on the knee joint or varying contact areas) of the knee joint of different species may be a reason for the variation of the fibre arrangement. On the other hand, the collagen fibre arrangement could vary due to location; means differ from joint to joint within a species. However, this was not shown for the species examined in this study but might be the subject of further research.

In all mammals the articular cartilage serves the same main function, to transmit loads while providing for low friction. Therefore it is not surprising that cartilage exhibits the same structural components in all species, i.e. hydrated proteoglycans and a highly organised collagen fibre structure. However, the species-specific differences in general collagen arrangement in the medial tibial plateau should be considered in selecting animal models for the study of human cartilage pathology or treatment.

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