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Quantitative structural organization of normal adult human articular cartilage

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Summary

Objective: Data pertaining to the quantitative structural features and organization of normal articular cartilage are of great importance in understanding its biomechanical properties and in attempting to establish this tissue's counterpart by engineering *in vitro*. A comprehensive set of such baseline data is, however, not available for humans. It was the purpose of the present study to furnish the necessary information.

Design: The articular cartilage layer covering the medial femoral condyle of deceased persons aged between 23 and 49 years was chosen for the morphometric analysis of cell parameters using confocal microscopy in conjunction with unbiased stereological methods. The height of the hyaline articular cartilage layer, as well as that of the calcified cartilage layer and the subchondral bone plate, were also measured.

Results: The mean height of the hyaline articular cartilage layer was found to be 2.4 mm, the volume density of chondrocytes therein being 1.65%, the number of cells per mm^3 of tissue 9626 and the mean cell diameter 13 μm. Other estimators (including matrix mass per cell and cell profile density) were also determined.

Conclusions: A comparison of these normal human quantitative data with those published for experimental animals commonly used in orthopaedic research reveals substantial differences, consideration of which in tissue engineering strategies destined for human application are of paramount importance for successful repair.

Key words: Human, Articular, Cartilage, Morphometry.

Introduction

Adult human articular cartilage possesses a unique structural organization and composition which endow it with the highly specific physical attributes that are necessary for fulfilling its functions within synovial joints[1]. Especially in large joints, individual entities of articular cartilage represent the largest units (spanning several millimeters) of avascular tissue within the human body. Notwithstanding this circumstance, articular cartilage tissue is very active metabolically, such a state being necessary to maintain its structural integrity, its physical and mechanical competence and its reactivity to load transmission and absorption[2].

A quantitative description of this tissue's three-dimensional structure is indispensable for a thorough understanding of the complex diffusional and permeability conditions that underlie its physiological functioning. Such data are also necessary for a full appreciation of articular cartilage pathology and repair. That traumatized or diseased articular cartilage manifests but a limited capacity to repair spontaneously[3] is a renowned circumstance, which has served as an incentive to numerous investigators to overcome the intrinsic problems, using, for example, tissue engineering techniques[4], cell and tissue transplantation approaches[5] and growth-factor-based strategies[6]. Success in these endeavors depends upon inducing articular cartilage regeneration in the true sense of the word. But this goal can be achieved only if researchers' efforts are guided by an intimate knowledge of the quantitative structural organization of human articular cartilage tissue at the cellular level, and such baseline data are, unfortunately, not available. Although there exist several qualitative descriptions of human articular cartilage structure at the macroscopic, histological and ultrastructural levels[7–10], only a few gross parameters, such as total tissue volume and thickness, have been selected for quantitative appraisal (by magnetic resonance imaging)[11–13]. Systematic quantitative analyses of microscopic estimators have been undertaken only in experimental animals[14,15].

It is the purpose of the present study to systematically analyse and quantify adult human knee-joint articular cartilage in one representative area, namely, the weight-bearing region of the medial femoral condyle, which is a frequent site of traumatic injury and pathologic degeneration. Cell and matrix parameters are analysed in adult humans aged between 23 and 49 years using the modern, unbiased stereological techniques devised by Cruz-Orive[16] and Gundersen *et al*.[17,18]. Pertinent data (such as the volume density of cells, the number of cells per unit volume of tissue, the number of chondrons per unit volume of tissue and matrix mass per cell) are gleaned not only for the articular cartilage layer as a whole, but also for each of the zones of which it is comprised. Data are then compared with those already available for experimental animals.

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Materials and methods

Tissue samples were derived from the bodies of eight adult humans (six males and two females) aged between 23 and 49 years. Individuals were the victims of traffic accidents, suicide or drug overdose and had suffered from neither acute nor chronic diseases of any kind. Permission for the removal of biopsies was granted by the University of Bern's Medical Ethics Commission and the undertaking itself performed in the Department of Forensic Medicine. Tissue was removed only from knee joints that manifested a healthy, glossy and completely intact articular cartilage surface. 2–3 cylindrical cartilage–bone explants, 4 mm in diameter, were drilled perpendicular to the articular surface from the weight-bearing region of one medial femoral condyle per individual within 48 h of death. Specifically, the area was defined as the medial line through the medial femoral condyle—opposing the non-meniscus-covered region of the medial tibial plateau in the extended knee-joint position—at a posterior distance of approximately 1–1.5 cm from the trochlea. Tissue cylinders were immediately transferred to a 5% solution of glutaraldehyde (buffered with 0.1 M sodium cacodylate, pH 7.4), within which they were fixed for 1 week at ambient temperature[19]. They were then stored in 70% ethanol at 4°C to minimize swelling and/or shrinkage effects[20] until required for microscopic examination. In a preliminary experiment using cartilage–bone cylinders derived from fresh young adult bovine shoulders, we elicited by stereoscopic microscopy that the tissue underwent neither swelling nor shrinkage during either glutaraldehyde fixation for 1 week at ambient temperature or its storage in 70% ethanol for a like period at 4°C. Hence, it seems probable that human cartilage–bone cylinders likewise remained unaffected by these phenomena.

Tissue cylinders (still immersed in 70% ethanol) were cut vertically into two halves using a razor blade, and the subchondral bone tissue trimmed horizontally to leave only a narrow layer. The undisturbed attachment of the subchondral bone plate to the articular cartilage is, however, essential to prevent swelling of the latter. 100-μm-thick sections were then cut parallel to the vertical plane using a vibratome (EMS OTS 3000-03, Electron Microscopy Sciences, Fort Washington PA, U.S.A.). These sections (still bathed in 70% ethanol) were sandwiched between a glass microscope slide and a coverslip (sealed with nail polish) and used immediately for analysis in the confocal microscope. The initial immersion of explants in glutaraldehyde serves a dual purpose: it fixes the tissue and provides an autofluorescence which improves cell contrast during confocal microscopy[19].

A laser scanning confocal microscope (MRC 600 LSC imaging system; Bio-Rad, Hertfordshire, U.K.) was used in conjunction with ×40 and ×60 oil-immersion objectives (numerical apertures=1.3 and 1.4, respectively) and an argon-laser light source (λ=488 nm). The height of the hyaline articular cartilage layer was measured optically, the zoom of the microscope being adjusted so that the height of the confocal image was one-eighth of the height of the cartilage. Each of the eight fields was sectioned optically at 2- or 4-μm intervals to a depth of 40 μm. To minimize noise, a Kalman filtering procedure was used to average five to six sequentially acquired images. These were photographed using slide film and viewed using a back projector, which projects the film directly onto point-counting or cycloid grids at a final magnification of ×870.

For measuring the heights of the calcified cartilage layer and the subchondral bone plate, one glutaraldehyde-fixed tissue cylinder was embedded in methyl methacrylate as previously described[6]. 200-μm-thick vertical sections were cut and one of these selected by an unbiased sampling procedure. It was then glued onto a plexiglas slide, polished and surface-stained with McNeil's Tetrachrome, basic Fuchsine and Toluidine Blue O. On a light micrograph of this section (magnification ×170), three vertical lines were drawn at a fixed distance apart, with a random start from the left-hand margin. The height of each layer was measured along these three lines using a ruler[21]. The delimiting border between the hyaline cartilage layer and the zone of calcified cartilage was defined by the tidemark and that between the calcified cartilage and the subchondral bone plate on the basis of their different tinctural properties. With respect to the lower boundary of the subchondral bone plate, the following rule was respected: when one of the vertical lines hit the bone marrow, the height was clearly determined by the interface. However, when a vertical test line fell in a region where the subchondral bone plate merged with the spongiosa, then no measurement was made at this point. In such a case, a fourth line was drawn.

For the high-resolution light microscopic illustration of zone architecture, a few blocks of glutaraldehyde-fixed tissue were post-fixed in 1% osmium tetroxide solution (buffered with 1.0 M sodium cacodylate buffer, pH 7.4) and embedded in Epon. 1-μm-thick semi-thin sections were cut using a Leica Ultracut E microtome and photographed in a Nikon Eclipse microscope (see[9] for methodological details).

In order to have a reproducible and reliable definition scheme as well as to exclude subjective bias or deviations from true verticality in the sectioning plane, articular cartilage zones were delineated on a quantitative basis. The superficial/tangential zone was defined as the first 10% of the tissue thickness, the transitional zone as the next 10% and the radial zone as the remaining 80% of the tissue thickness down to the tidemark. The radial zone was further divided into four subzones of equal thickness. Hence, stereological estimators were determined for six tissue zones (Figs 1–3).

Stereological dissectors[22,23] were represented by the tissue volume between serial optical sections (two sections per dissector in the superficial and transitional zones and four sections per dissector in the radial zones), which were 5 μm apart and encompassed an area of 180 μm×240 μm. The imaging of individual areas was varied from block to block using systematic random sampling principles[24,25], beginning at the left-hand margin and embracing the entire zone height.

The optical dissectors were photographed in color at a magnification of ×400. Individual frames were then projected at a magnification of ×870 for the performance of morphometric measurements. For each of the basic stereological estimators, i.e., volume density of cells, surface area density of cells, number of cell profiles per unit area and number of chondron profiles per unit area, the dissector consisted of a single section. These measurements were made using point and intersection counting methodologies[17,18]. For intersection counting, cycloid test lines were utilized[24]; for profile counting, the counting rule defined by Gunderson[22] was respected.

The dissector methodology[23] was applied for estimating the number of chondrocytes per unit volume of tissue, the number of chondrons per unit zone volume and the number

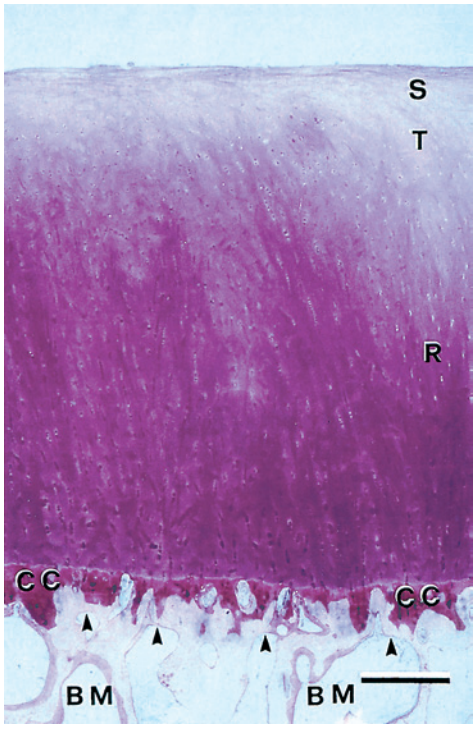

Fig. 1. Light microscopic overview of adult human articular cartilage taken from the medial femoral condyle. By definition, the superficial (S) and transitional (T) zones each constitute 10% of the height of the articular cartilage layer, the bulk of which (80%) is represented by the radial one (R). For analytical purposes, the latter is partitioned into an upper and a lower half, each of which is further subdivided into upper and lower portions. The layer of calcified cartilage (CC) and the subchondral bone plate (arrowheads) form relatively thin strata beneath the hyaline articular cartilage tissue. BM: bone marrow space. 100-μm-thick polished section of methacrylate-embedded tissue, surface-stained with McNeil's Tetrachrome, basic Fuchsine and Toluidine Blue O. Magnification bar=500 μm.

of cells per chondron within the radial zones (the superficial and tangential zones contain only single-cell chondrons).

The secondary estimators calculated were: mean cell volume ($\bar{v}$) [$\bar{v}=Vv/Nv$, where Vv represents the cell volume density and Nv the number of cells per unit tissue volume], mean cell surface area ($\bar{s}$) [$\bar{s}=Sv/Nv$, where Sv represents the surface density of cells within a unit tissue volume], mean horizontal (i.e., projected) cell diameter ($\bar{h}$) [$\bar{h}=Nv/N_A$, where N_A represents the number of cell profiles per unit tissue area] and mean matrix volume per cell ($\bar{v}$(m/c)) [$\bar{v}$(m/c)=Vv (matrix)/Nv (cells), where Vv (matrix) represents the volume density of the matrix within a unit tissue volume].

Data derived from the eight human samples were averaged, and the means, standard devations of the means, the coefficients of variation and the coefficients of error calculated.

Results

The human articular cartilage analysed in this study was derived from the medial femoral condyle of persons aged between 23 and 49 years at the time of their demise. The mean height of the hyaline layer in this region was 2.4 mm, variation between individuals being fairly high at 22%

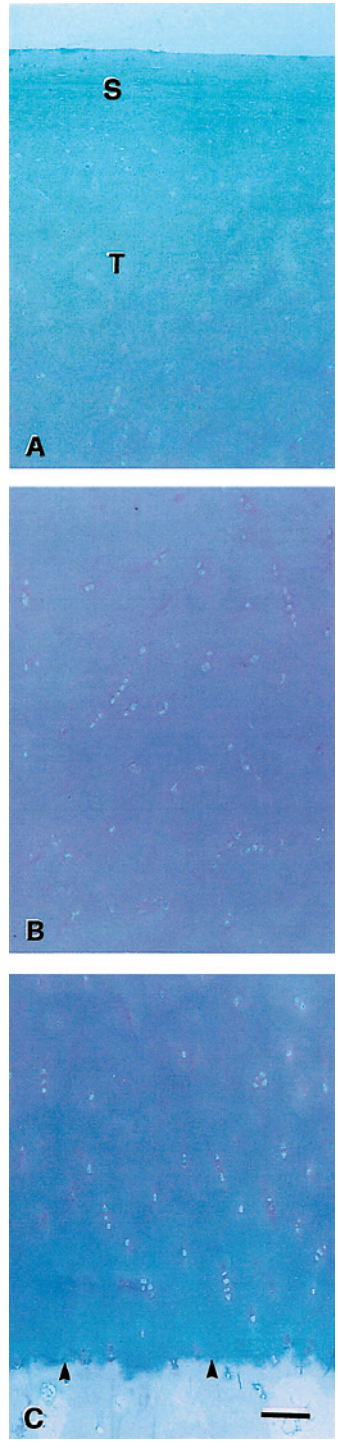

Fig. 2. High-magnification light micrographs of (A): the superficial (S) and transitional (T) zones, (B): the upper radial zone and (C): the lower radial zone. In the latter illustration, the tidemark is indicated by arrowheads. Chondrocytes within the superficial zone [upper portion of (A)] have a typically oval form, with the long axis orientated parallel to the articular cartilage surface, whereas those within the transitional one [lower portion of (A)] have a more rounded profile. Within the radial zone [(B) and (C)], chondrons are arranged preferentially in a vertical direction relative to the articular cartilage surface. Semi-thin (1-μm-thick) section of Epon-embedded tissue stained with Toluidine Blue 0. Magnification bar=100 μm.

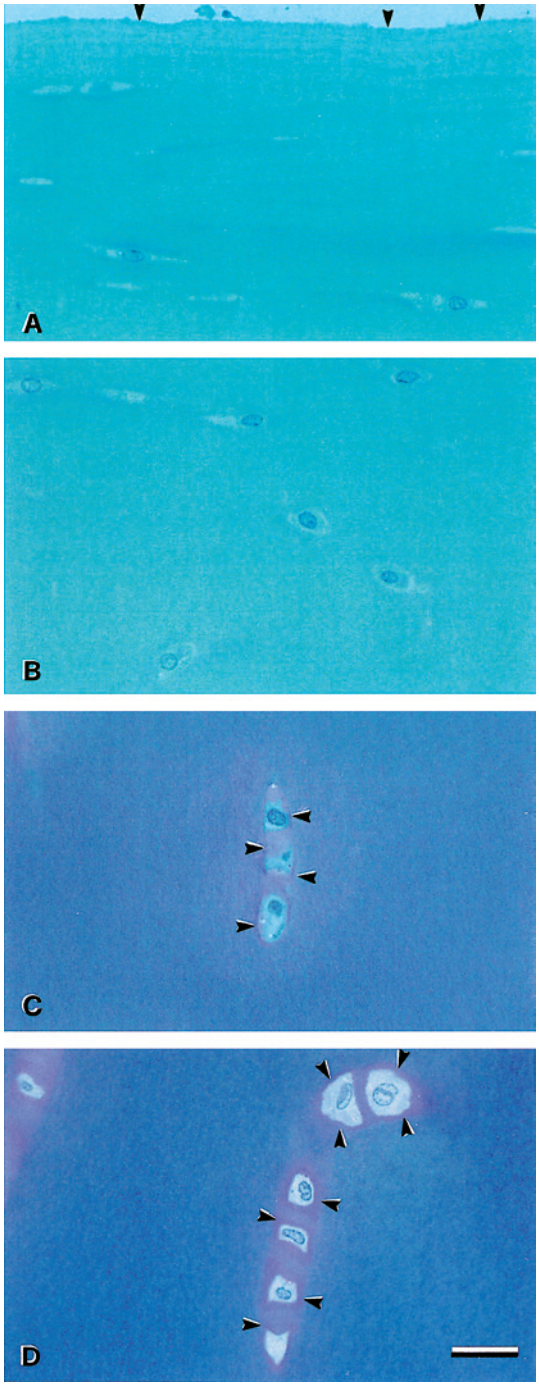

Fig. 3. High-resolution light micrographs of the four major zones into which normal human articular cartilage is partitioned. (A): Beneath the joint surface (arrowheads), cells within the superficial zone are flat and elliptic in form. (B): Within the transitional zone, chondrocytes have a more rounded profile and form single-cell chondrons, as indeed do those of the superficial one. (C) and (D) illustrate typical chondrocyte clusters (chondrons) within the upper (C) and lower (D) halves of the radial zone. As must be apparent from their scant distribution, these cell clusters control huge matrix domains. The arrowheads in (C) and (D) mark the boundary between the pericellular/territorial matrix compartment and the vast interterritorial one. Semi-thin (1-μm-thick) section of Epon-embedded tissue stained with Toluidine Blue 0. Magnification bar=20 μm.

Table I
Height measurements

Estimator	Mean height	Coefficient of variation
Height of hyaline articular cartilage layer	2.410 mm	22%
Height of calcified cartilage layer	0.134 mm	74%
Height of subchondral bone plate	0.190 mm	57%

(coefficient of variation); that of the calcified cartilage layer was 134 μm and that of the subchondral bone plate 190 μm (Table I).

Estimates of the primary, secondary and tertiary morphometric parameters are presented in Table II and Figs 4 and 5, respectively. These are expressed as a function both of individual zone volume—data pertaining to each of the six radial subzones being presented separately—and of the volume of the hyaline articular cartilage layer as a whole.

One of the most striking findings is how small a tissue volume is occupied by cells. Although the cell volume density varies considerably between zones—with the superficial one having the highest value and the lower radial zone the lowest (Figs 1 and 2)—the average for the whole articular cartilage layer is a mere 1.65%. Accordingly, the total tissue volume occupied by extracellular space is extremely large (100−1.65%=98.35%), the matrix domain controlled by a single chondrocyte being on average 104,040 μm^3 (Table II), but as high as 160,707 μm^3 in the lower half of the upper radial zone (see Table II and Fig. 4).

Calculations relating to chondrocyte geometry reveal the average horizontal diameter (13 μm), surface area (821 μm^2) and cell volume (1748 μm^3) to be fairly small (Table II and Fig. 4). The variation between individual cells within zones is likewise relatively small, which indicates that the zone-specific chondrocyte population is quite uniform in size. It should be borne in mind that these parameters relating to chondrocyte geometry are based upon observations made in the light microscope. At this level of magnification, small cytoplasmic processes extending from the cell surface are not resolved and therefore not included in the measurements. Nevertheless, the values presented afford some conception of just how small a surface area a single chondrocyte apposes to its matrix domain, whose synthesis, degradation and remodeling it controls.

Within the superficial and transitional zones, chondrons are exclusively single-cell units [Figs 1, 2(A) and 3(A), (B)], whereas throughout the entire radial one, they contain on average 5–8 chondrocytes [Figs 2(B),(C) and 3(C),(D), Table II and Fig. 5).

One estimator that is frequently quoted in the literature is the total number of cells contained within the volume of tissue underlying a 1-mm^2 area of the articular cartilage surface[26,27]. The present analysis reveals this to be 23,674 for humans (Table II and Fig. 5), which is surprisingly similar to the values quoted for several other mammalian species (see Discussion).

Discussion

In the present study, the hyaline articular cartilage layer covering the medial femoral condyle of young adult and middle-aged humans (23–49 years of age) with no known pathological affections or history of joint disease was subjected to a thorough stereological analysis.

Table II
Numerical values of basic stereological estimators

+	Estimator	S	T	U	V	L	K	A
A	Volume density of chondrocytes (Vv), %	2.59 (0.29)	2.13 (0.23)	1.94 (0.29)	1.25 (0.23)	1.50 (0.24)	1.21 (0.34)	1.65 (0.15)
B	Surface area density of chondrocytes (Sv), mm^2/mm^3	15 (1.09)	8 (0.65)	8 (0.81)	6 (0.76)	6 (0.97)	6 (0.97)	8 (0.55)
C	Number of chondrocytes per unit tissue volume (Nv), N/mm^3	24,018 (3188)	10,262 (466)	7302 (572)	6866 (849)	8879 (1062)	7941 (717)	9626 (511)
D	Number of chondrocytic profiles per unit area (N_A), N/mm^2	526 (48)	214 (18)	160 (16)	137 (15)	150 (19)	122 (17)	188 (13)
E	Mean chondrocyte volume [$v(c)$], μm^3	1237 (238)	2086 (219)	2866 (534)	1951 (397)	1732 (253)	1514 (410)	1748 (189)
F	Mean matrix volume per chondrocyte [$m(c)$], μm^3	45,901 (5917)	97,008 (5179)	141,561 (13,821)	160,707 (20,221)	127,002 (21,589)	132,713 (13,485)	104,040 (5053)
G	Mean horizontal cell diameter [$h(c)$], μm	11 (1.7)	14 (1.5)	14 (1.4)	12 (1.2)	14 (1.8)	12 (1.8)	13 (0.5)
H	Mean cell surface area [$s(c)$], μm^2	672 (55)	881 (56)	1203 (176)	948 (117)	782 (98)	721 (109)	821 (66)
I	Number of chondrons per unit tissue volume [Nv(chondrons)]	24,018 (3189)	10,262 (466)	1652 (277)	1348 (153)	1522 (227)	2464 (672)	4825 (480)
J	Mean matrix volume per chondron [$v(m$/chondron)], μm^3	45,901 (5917)	97,008 (5179)	857,628 (287,164)	809,968 (103,469)	899,740 (281,427)	563,426 (94,721)	217,669 (20,610)
K	Number of chondrocytes per chondron [N(cells/chondron)]	1 (0)	1 (0)	7 (2.7)	6 (1.3)	8 (2.3)	5 (1.0)	2 (0.2)
L	Number of chondrocytes beneath a 1-mm^2 area of the joint surface [N(cells/mm^2)]*	5697 (608)	2502 (137)	3596 (340)	3380 (480)	4481 (688)	4017 (575)	23,674 (1814)
M	Number of chondrons beneath a 1-mm^2 area of the joint surface [N(chondrons/mm^2)]**	5697 (608)	2502 (137)	811 (134)	655 (71)	771 (156)	1161 (301)	11,597 (972)

Values represent the means of determinations in 8 human knees, standard errors of the means being given in parentheses.

Abbreviations used: S=superficial zone; T=transitional zone; U=upper half of the upper radial zone; V=lower half of the upper radial zone; L=upper half of the lower radial zone; K=lower half of the lower radial zone; A=average for all zones of the entire hyaline articular cartilage layer.

*Number of chondrocytes and **number of chondrons contained within the volume of tissue underlying a 1-mm^2 area of the articular cartilage surface.

+Letters in this column refer to the graphical presentation of each set of data in Figs 4 (A–H) and 5 (I–M).

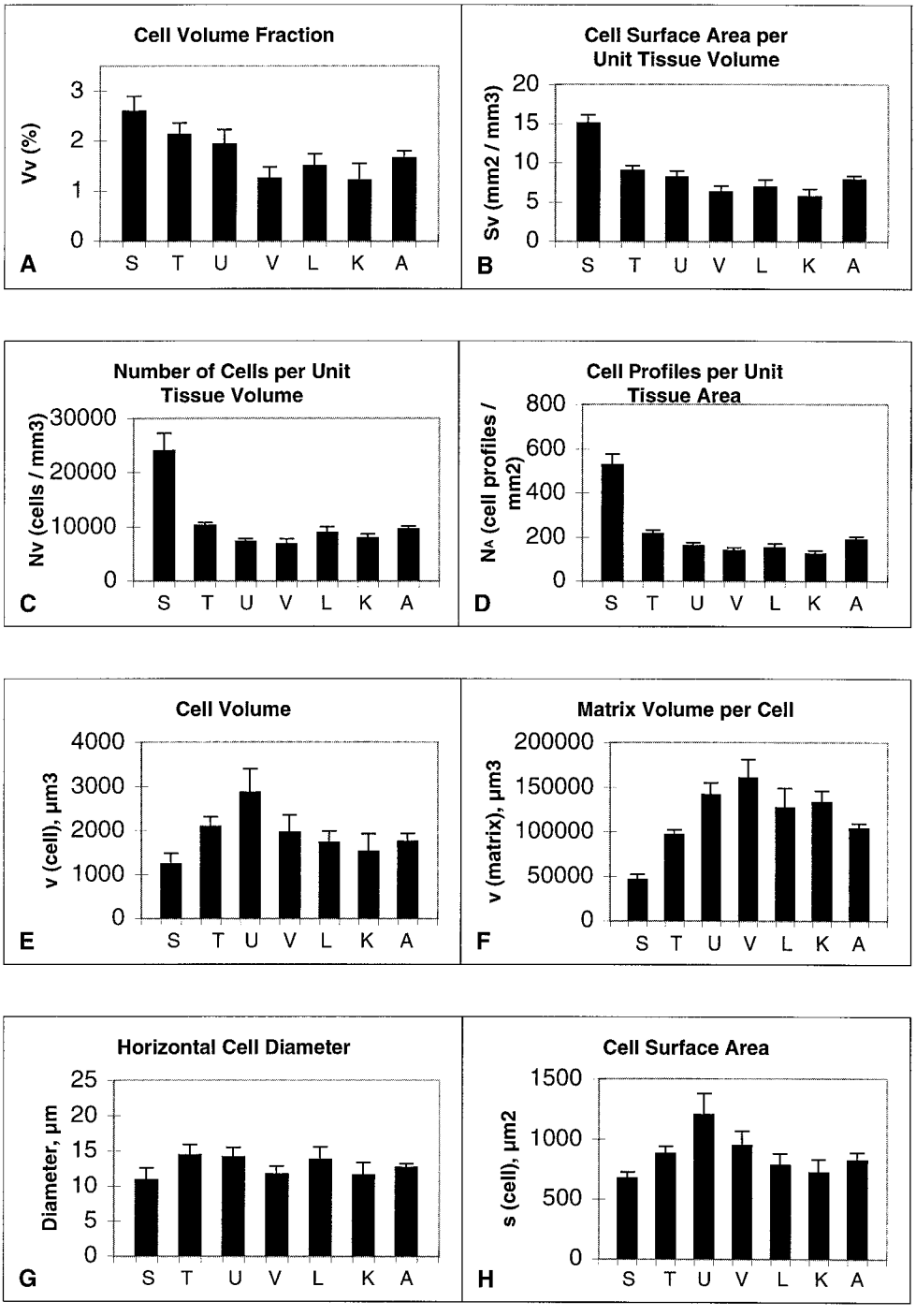

Fig. 4. Graphical presentation of stereological estimators.

The mean heights of the hyaline articular cartilage layer, the calcified cartilage layer (between the tidemark and the subchondral bone plate) and the subchondral bone plate (excluding the spongial trabeculae) were 2.4 mm (CE: 7.8%), 0.13 mm (CE: 26.2%) and 0.19 mm (CE: 20.1%), respectively. For a given joint, hyaline articular cartilage height depends upon the size of the animal species. In humans, therefore, this layer is much thicker than that in smaller mammals[14,26,27], such as rabbits[14] and sheep or goats[28,29], for which values of 0.4 mm and 0.7 mm, respectively, have been reported, but thinner than that in larger ones, such as the cow or elephant. On the other hand, both the calcified cartilage layer and the subchondral bone plate are much thinner in humans than they are in rabbits, goats or sheep[14,29]. The heights of the calcified cartilage layer and the subchondral bone plate are characterized by very high coefficients of error (approximately three times greater than that associated with the height of the hyaline articular cartilage layer). This circumstance is indicative of high natural biological variation in height, which reflects the pronounced interdigitation of these layers with underlying tissue. The physiological importance of this

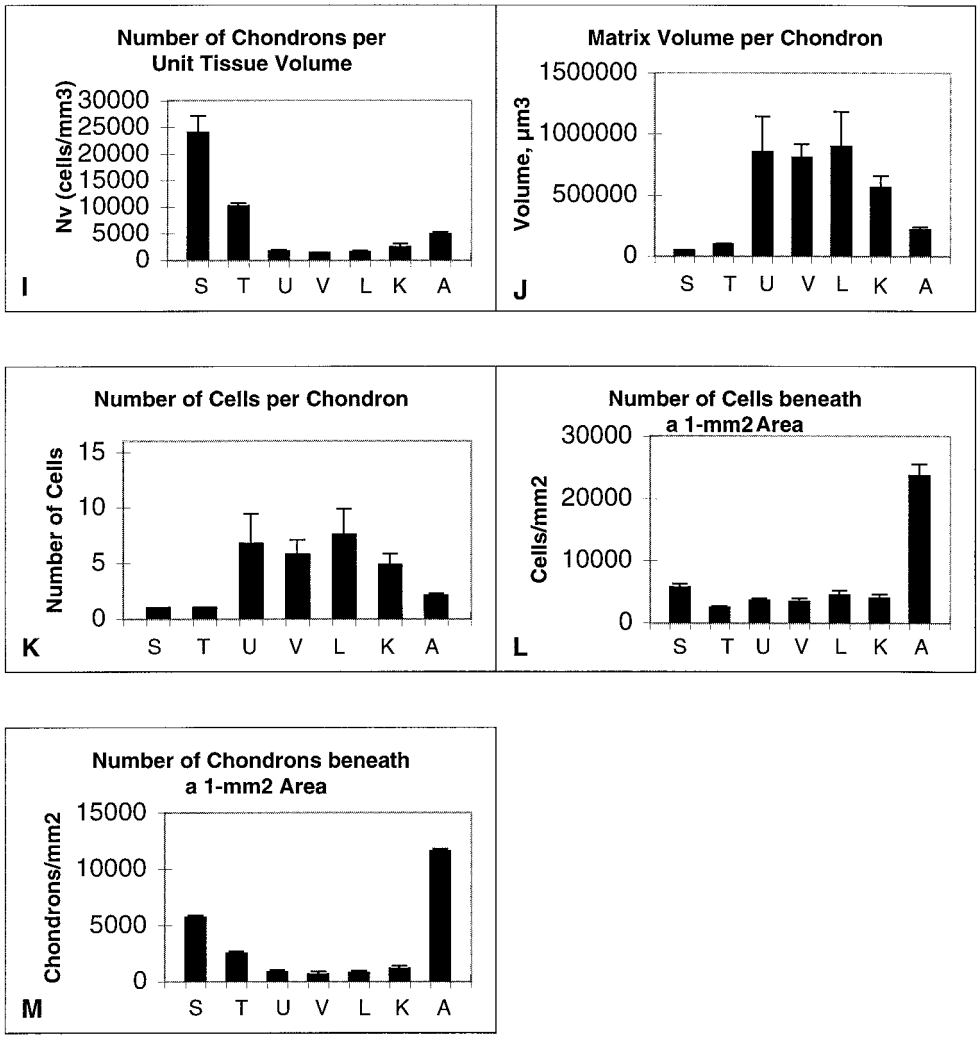

Fig. 5. Graphical presentation of chondron stereological data.

phenomenon is unknown and can only be speculated upon. On the one hand, it could improve the mechanical anchorage of these layers, enabling them to better withstand shear forces. On the other hand, interdigitation also increases the surface area of these layers, which could enhance the diffusional flow of nutrients into and through them to the lower hyaline articular cartilage zones. The values obtained for these two mineralized layers in the present study are somewhat lower than those estimated microscopically for other human joint areas[30,31] and those determined by magnetic resonance imaging for the human knee[12,32]. Since the said layers are each characterized by such an undulating contour, it is possible that their boundaries are thereby rendered somewhat hazy and broad rather than being sharp and narrow in magnetic resonance imaging, which cannot distinguish such nuances as individual entitities. This circumstance may lead to an overestimation of their heights.

The volume density (i.e., volume fraction) of chondrocytes within human articular cartilage tissue was not only very low, generally, with an average value of 1.65% (CE: 9%), but even decreased with increasing distance from the surface. Between the superficial zone and the lower portion of the lower radial zone, this parameter dropped by a factor of two (from 2.6% to 1.2%). Such low cell volume densities have not been encountered in any of the mammalian species commonly investigated in orthopaedic research. In rabbits and goats, for example, the average cell volume fraction for the entire articular cartilage layer is in the order of 12%[14,28]. Among human bodily tissues, articular cartilage is unique in having such a low volume density of cells. Given the great distance that separates chondrocytes from the nearest blood capillaries and their dependence upon diffusion for the delivery of oxygen and nutrients (from the synovium and the subchondral bone tissue), it is not surprising that their metabolic activities[33,34] are conducted chiefly along anaerobic pathways.

Consistent with data pertaining to the volume density of chondrocytes, the number of cells per unit volume of tissue (i.e., numerical volume density or cellularity) likewise decreased with increasing distance from the articular surface, down to the lower half of the upper radial zone. Between the superficial zone and the latter region, this parameter dropped three-and-a-half fold (from 24,018 to 6866 cells per mm^3). Such a profound decrease in cellularity has not been observed in any of the experimental animals thus far investigated[14].

For each zone, the number of cell profiles encountered within a unit area of a tissue section roughly paralleled the number of cells per unit volume, which indicates that the

zone-specific size distribution of chondrocytes did not vary markedly.

The horizontal diameter of chondrocytes was similar in each zone, the average value for the articular cartilage layer as a whole being 13 μm (range: 11–14 μm; CE: 4.2%). This value falls within the range documented for other mammalian species. On the other hand, both the surface area and volume of individual cells increased in parallel from the superficial zone to the upper portion of the upper radial one and then decreased towards the lower half of the lower radial zone. The largest cell volumes encountered (2866 μm^3) are similar to those measured in experimental animals such as the rabbit[14], but they are well below those documented for the rat growth plate (17,400 μm^3), wherein increases in cell shape and size serve as a means of boosting longitudinal bone growth[20].

The mean volume of matrix controlled by a single cell attained a value of 160,707 μm^3 in the lower portion of the upper radial zone (Table II and Fig. 4) and averaged 104,040 μm^3 (CE: 4.9%) for the hyaline articular cartilage layer as a whole. These volumes are about 10 times larger than those under the control of a single chondrocyte in adult rabbit articular cartilage. And in the growth plate, such huge matrix domains have not been encountered in any mammalian species investigated.[20] Their great compass in human articular cartilage may be one of the reasons (in addition to low oxygen tension) why remodeling activities within the farther reaches of the interterritorial matrix are generally so low.

In the superficial and transitional zones, chondrons are exclusively single-cell units [Figs 1, 2(A) and 3(A),(B)], whereas in the radial one they contain between five and eight chondrocytes [Figs 1, 2(B),(C) and 3(C),(D)]. These findings accord with data gleaned from other mammalian species[35,36]. The extracellular space intervening between two neighboring chondrons increases as a function of the number of chondrocytes it contains. But the matrix domain controlled by any one of the individual cells therein is not algebraically divisible by this number. The mean matrix volume controlled by an individual chondrocyte within five- or eight-cell chondrons of the radial zone ranges between 127,002 μm^3 and 160,707 μm^3, which exceeds that associated with the single-cell units of the superficial (45,901 μm^3) and transitional (97,008 μm^3) zones (Table II). Serial optical sectioning of chondrons within the radial zone revealed these to have the shape of very elongated oblate spheroids in a direction perpendicular to the articular cartilage surface, an impression of which may be gathered from Fig. 3(D).

When these findings for native tissue are compared with those relating to isolated chondrons maintained *in vitro*, it becomes clear how artifically small the matrix coat around each cell is under these latter conditions[35,36]. Hence, although cultured chondrons are used to study chondrocyte biology within such units, these set-ups are in fact of limited value in understanding extracellular matrix activity across the entire physiological domain.

One parameter that is frequently cited in the literature is the number of chondrocytes contained within the volume of tissue underlying a 1-mm^2 surface area of the articular surface in weight-bearing regions of the femoral condyle[14,15,27]. In humans, this value was found to be 23,674 chondrocytes per mm^2, which lies within the range documented for other mammalian species[14,27]. This similarity between species may seem surprising when one considers their very obvious differences in articular cartilage structure, organization and biomechanical needs. The clue to this enigma lies not in the number of cells *per se*, but in species-specific differences in the activity potential of a given population of chondrocytes and its capacity to elaborate a matrix of distinct composition and macromolecular organization[37,38].

The tremendous differences in structure and organization existing between the various zones of human articular cartilage tissue are striking. But, as yet, we can only speculate on their biomechanical implications. What is clear, however, is that the maintenance of this anisotropic structural organization is indispensable for the tissue's functional competence[19,39]. The complexity of this layer's architecture renders the task of simulating it *in vitro* an awesome one. How to surmount the difficulties involved represents a great challenge to scientists specializing in the field of tissue engineering. But the quantitative stereological data furnished by the present study should facilitate their undertaking.

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