Mechanical Properties of Human Fetal Talus

Roza Mahmoodian MS, Jeremi Leasure BS,
Hemanth Gadikota MS, Franco Capaldi PhD,
Sorin Siegler PhD

Published online: 14 January 2009
© The Association of Bone and Joint Surgeons 2009

Abstract Mechanical characterization of human cartilage anlagen is required to effectively model congenital musculoskeletal deformities. Such modeling can effectively explore the effect of treatment procedures and potentially suggest enhanced treatment methods. Using serial MRI, we have noted shape changes of the cartilaginous hindfoot anlagen in patients with clubfoot, suggesting they are soft and deformable. We therefore determined the stress relaxation behavior of cartilage plugs obtained from third-trimester stillborn fetuses in unconfined and confined compression geometries. The material parameters determined were the aggregate modulus $H_A = 0.15 \pm 0.07$ MPa, Poisson's ratio $\nu = 0.4 \pm 0.06$, Young's modulus $E_s = 0.06 \pm 0.03$ MPa, and permeability coefficients $k_0 = 2.01 \pm 0.8 \times 10^{-14} \text{ m}^4 \text{ N}^{-1} \text{ s}^{-1}$ and $M = 4.6 \pm 1.0$. As compared with adult articular cartilage, stiffness was an order of magnitude lower than the values reported in the literature, suggesting the relative softness of the tissue, and the permeability was an order of magnitude higher, indicating relative ease of flow in the tissue. Poisson's ratio also was close to the higher end of the range reported in previous

studies. Such material is expected to deform and relax to larger extents. These findings are consistent with the deformability of the cartilage anlagen during manipulation and casting for treatment of clubfoot.

Introduction

In the fetus, the skeleton initially consists of a cartilage “anlage” (plan) that gradually ossifies during fetal and postnatal development. Deficiency or retardation of this normal development causes congenital musculoskeletal anomalies such as various types of clubfoot deformity with delayed growth and ossification. Based on surface models from serial MRIs, we previously reported Ponseti clubfoot treatment deforms these cartilage anlagen [9]. Building a model to describe the development of cartilaginous anlagen may be used to create a general understanding of the response of the involved tissue to mechanical loads and explore new treatment options or infer the direct relationship between an existing treatment and the observed changes in growth pattern. Such studies can potentially provide guidance for the betterment of treatments.

During the maturation process, most of the tissue within the cartilage anlage ossifies. The articulation surfaces remain cartilaginous to provide for low-friction, wear-resistant, and load-bearing contact areas. The material properties of articular cartilage have been extensively studied in animals [4, 27, 30, 31, 38, 42, 44, 46, 52, 55–57] and, to a lesser degree, in humans [3, 5, 6, 10, 11, 24, 25, 28, 33, 50, 51, 53]. Sah et al. [56] reported substantially lower stiffness in bovine fetal compared with adult articular cartilage. They also found the hydraulic permeability increased from fetus to adult. Brown and Singerman [10] reported the average permeability and equilibrium modulus

One or more of the authors have received funding from National Institutes of Health Grant AR053255 (SS) and the International Society of Biomechanics dissertation award (RM).

Each author certifies that his or her institution either has waived or does not require approval for the human protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research.

R. Mahmoodian, J. Leasure, H. Gadikota, F. Capaldi,
S. Siegler (✉)
Department of Mechanical Engineering and Mechanics,
College of Engineering, Drexel University, Philadelphia,
PA 19104, USA
e-mail: ssiegler@coe.drexel.edu

they obtained from human fetal proximal femoral epiphyses were in the range of values accepted for human adult articular cartilage. A solid-phase Poisson ratio of zero was inferred for all their specimens as a result of poor curve fitting. They found an inverse relationship between permeability and equilibrium modulus similar to that reported for adult articular cartilage.

Such material characterization is an essential part of a finite element model to mimic the tissue's development and adaptation, and closer approximation of the tissue material properties would yield more realistic results. We therefore characterized the mechanical properties of human talar cartilage anlage in fetuses nearing full term. Because the biomechanical properties change with growth, such results should be used carefully bearing the limitations and approximations in mind when interpreting the analysis results.

The compressive mechanical properties of talus anlage in stress relaxation were determined: parameters pertaining to the equilibrium state (aggregate modulus H_A in a laterally confined geometry, elastic modulus E_s , and Poisson's ratio ν) and the tissue's strain-dependent hydraulic permeability.

Materials and Methods

Because of our particular interest in clubfoot and its treatment, we chose the talus for this study, because this anlage is the most affected in clubfoot [26, 47]. We studied the tali of two stillborn fetuses in the third trimester. The fetal age was determined by measurement of crown-rump lengths: 30 and 32 weeks. The fetus legs were frozen when obtained and were kept frozen at -80°C until the day of dissection. The feet were thawed and carefully dissected to separate the tali, which were partly ossified (Figs. 1, 2). The tissue was sagittally sliced using a manual tissue chopper with a stage whose advancement was controlled by a micrometer. As a result of the presence of both bone and cartilage within the tali, obtaining uniform slices was not possible using a cryostat or vibrating microtome. Tissue slices were 1.5 mm thick; however, as a result of deflection of the blade within the bony region, some non-uniformity in the thickness was observed throughout the slices. One to four cartilage plugs were gathered from each slice using a biopsy punch with a diameter of 3.00 mm. Plugs were taken from the unossified regions. The thickness of cartilage plugs was measured individually at the time of the experiment. The mean $\pm$ standard deviation plug thickness was 1.61 ± 0.37 mm. The plugs were submerged in phosphate-buffered saline (PBS) and frozen at -80°C until the day of testing. We gathered a total 16 samples from all slices of the two tali; seven were collected from the younger specimen and nine from the older.

Fig. 1 A photograph shows the dissected hind foot and tibia of a third-trimester stillborn fetus.

On the day of testing, samples were thawed at room temperature and allowed 1.5 hours in PBS to equilibrate before the experiments. Each sample was first tested in confined compression, allowed to recover for 1.5 hours at room temperature, and finally tested in unconfined compression. The samples were submerged in normal saline solution (0.15 mol/L NaCl) containing enzyme inhibitors (2 mmol/L EDTA, 5 mmol/L benzamide, 10 mmol/L N-ethylmaleimide, 1 mmol/L phenylmethylsulfonyl fluoride) during testing and recovery time between tests. A custom-designed testing apparatus (displacement resolution, 5 μm ; force resolution, 0.6 mN) with a smooth, stainless steel chamber was used for both test configurations. In the confined compression, the samples were placed inside a confining chamber with impervious walls and bottom (F 3) and pressed on with a porous indenter (pore size, 50 μm ; porosity, 40%). In the unconfined fixture, the plugs were placed on an impervious surface using a flat solid indenter with a smoothly polished tip. In the latter configuration, all contact surfaces were lubricated with silicone grease to reduce friction.

Before collecting the data, we applied a preconditioning load of 3.3 N (0.47 MPa) to the samples for 60 seconds, removed the load, and then applied a tare load of 0.06 N (8.5 kPa) for 900 seconds. Thickness of each sample was measured after the tare load was applied right before each actual stress relaxation test. By taking readings from the load cell, the stepper motor revolutions, which are convertible to length, were counted from when the indenter touched the top surface of the plugs (when the force started to rise just above zero) until the prespecified tare load value

was reached. This value was subtracted from the pretare-load thickness, which was measured in the same manner, to get the posttaring thickness that was used for analysis. The prescribed displacement history consisted of four ramps (5% strain each) at a displacement rate of 1 $\mu\text{m}/\text{second}$ followed by a stress relaxation period of 500 seconds. The stress relaxation period was chosen such that the change in the stress value at the end of the period was smaller than 100 Pa/minute. The loading protocol was the same for both configurations. Reproducibility of the measurements was previously examined for both biologic (bovine ankle articular cartilage) and nonbiologic (rubber) reference samples. Repeated testing on the same sample in both confined and unconfined compression produced material parameters that varied by less than 10%.

Dry weight of the tissue was calculated using a lyophilizer.

Before deformation, photographs of the cartilage plugs were taken using a high-resolution camera (Canon EOS Rebel XTi; Canon USA, Inc, Lake Success, NY) with a macro lens fixed on a stage. The diameters were calculated by fitting a circle to the plug cross-section with a custom-written code using MATLAB[®] (R2006a) image processing toolbox (The Mathworks Inc, Natick, MA). The average sample diameter was 3.29 ± 0.14 mm.

The compressive mechanical properties of talus anlage in stress relaxation were determined: parameters pertaining to the equilibrium state (aggregate modulus H_A in a laterally confined geometry, Poisson's ratio ν , and elastic modulus E_s) and the tissue's strain-dependent hydraulic permeability. Various models have been used to describe the biomechanical behavior of soft connective tissue under uniaxial compressive loading, including elastic [23], biphasic [44], triphasic [34], poroviscoelastic [39, 40], fibril-reinforced [37, 57], and transversely isotropic models [14, 19]. For a detailed listing of various models, refer to Garcia et al. [20]. We used the linear biphasic theory

Fig. 2 A photograph shows a medial view of a sagittal slice of talus displaying the cartilaginous and ossified regions. Ossification proceeds outward with growth.

developed by Mow et al. [44], which is well applicable to any biphasic tissue in the body (mature cartilage, meniscus, ligaments, and so on) where the tissue is assumed to be a soft, porous, isotropic, homogeneous, and permeable elastic solid filled with interstitial fluid with both phases assumed to be intrinsically incompressible. The friction between the tissue, indenter, and test fixture is neglected in this theory. As a result of the small strains used in the experiments, infinitesimal strain assumptions were used to simplify the model. However, the strain dependence of the permeability was included within the model. Using the following function for intrinsic permeability [35]:

$$k = k_0 \exp(Me) \quad (1)$$

where k_0 and M are intrinsic material parameters and e is the dilation of the solid matrix (true strain), the linear biphasic theory reduces to the so-called nonlinear diffusion equation for uniaxial confined compression tests [43]:

$$H_A \frac{\partial^2 u}{\partial z^2} = \frac{1}{k_0} \exp\left(-M \frac{\partial u}{\partial z}\right) \frac{\partial u}{\partial t}, \quad 0 < z < h \quad (2)$$

where $u(z, t)$ is the axial displacement of the solid phase, H_A is the aggregate elastic modulus, and h is the tissue thickness. An equivalent of this equation for the unconfined compression tests was not needed because only the equilibrium results of those experiments were analyzed.

The compressive mechanical properties of talus anlage were extracted: the elastic modulus (E_s) and aggregate modulus (H_A) were determined by fitting a line to the equilibrium stress-strain curves of unconfined and confined compression tests, respectively (Fig. 3), and the permeability parameters (k_0 and M) were determined by finding

Fig. 3 A graph shows the equilibrium stress-strain data. Under the infinitesimal strain conditions, the slopes of the curves remain nearly constant.

Fig. 4 A graph shows the transient response of the tissue tested in the confined compression configuration with the best curve fit for finding permeability coefficients.

the best fit to the stress-time curve from the confined compression tests using Equations 1 and 2 in a custom-written code in MATLAB[®] (Fig. 4). The coefficient of determination used to assess the quality of curve fits was

$$r^2 = 1 - \frac{\sum (y - y_{\text{est}})^2}{\sum (y - \bar{y})^2} \quad (3)$$

where y represents the experimental variable, y_{est} is the theoretical variable, and $\bar{y}$ is the mean value of y [32].

The Poisson's ratio was determined indirectly from solving the following equation for ν :

$$2H_A \nu^2 + (H_A - E_s)\nu - (H_A - E_s) = 0 \quad (4)$$

Results

The material parameters obtained using the transient and equilibrium experimental data (Figs. 3–5) and averaged over all the samples were the equilibrium properties: $H_A = 0.15 \pm 0.07$ MPa, $E_s = 0.06 \pm 0.03$ MPa, and $\nu = 0.4 \pm 0.06$; and the initial hydraulic permeability and its strain-dependence coefficient $k_0 = 2.01 \pm 0.8 \times 10^{-14} \text{ m}^4 \text{ N}^{-1} \text{ s}^{-1}$ and $M = 4.6 \pm 1.0$. There was no significant difference between the two test groups (p values were 0.85 for H_A , 0.13 for E_s , 0.22 for ν , 0.71 for k_0 , and 0.44 for M) (Table 1). The coefficient of determination for the

Fig. 5 A graph shows confined and unconfined compression test data. It is evident the stress values are higher in confined compression as a result of lateral confinement.

nonlinear curve fitting of permeability parameters was $r^2 = 0.910 \pm 0.041$ (Fig. 4). Linear fits to the equilibrium stress-stretch data (Fig. 3) yielded $r^2 = 0.979 \pm 0.026$ and $r^2 = 0.963 \pm 0.029$ for confined and unconfined compression, respectively. The specimens recovered $97.1\% \pm 2.6\%$ of their initial thickness as measured just before the confined and unconfined experiments.

The dry weight of the samples was $13.1\% \pm 1.8\%$.

Discussion

Clubfoot may be treated nonoperatively [29, 47, 48] or operatively [15]. The Ponseti technique consists of serial manipulation and plaster casting of the infant foot [48], which has gained popularity since introduced by Ponseti in the 1940s. Brand et al. [9] reported immediate shape changes in the anlagen after manipulation and casting in a study of this technique. Effectiveness of such treatments resulting from the application of manipulative forces could be attributed to these anlagen being predominantly cartilaginous and thus “soft” and deformable. Therefore, building a detailed mechanical model can be a reliable means to explore the relationship between the manipulation

Table 1. Mechanical parameters listed separately for the two cadavers (mean $\pm$ standard deviation): aggregate modulus (H_A), permeability coefficients (k_0 and M), Young's modulus (E_s), and Poisson's ratio (ν)

Plug groups	H_A (MPa)	k_0 ($10^{-14} \text{ m}^4 \text{ N}^{-1} \text{ s}^{-1}$)	M	E_s (MPa)	ν
Subject 1 (n = 9)	0.15 ± 0.09	2.4 ± 1.0	4.2 ± 1.0	0.05 ± 0.03	0.42 ± 0.05
Subject 2 (n = 7)	0.15 ± 0.05	1.9 ± 0.5	4.7 ± 1.1	0.07 ± 0.03	0.38 ± 0.07

forces and the observed changes in growth pattern. Once a clear understanding of the nature of these relationships has been established, it can be used to both improve current treatments and suggest new treatment techniques. Such models require careful characterization and understanding of the material properties of the developing cartilage anlage. We therefore studied the stress relaxation behavior of human talar anlage in this study by applying the linear biphasic theory in the confined and unconfined compression configurations. The parameters determined were the aggregate modulus H_A , Poisson's ratio ν , elastic modulus E_s and the tissue's strain-dependent hydraulic permeability.

It should be noted, as a result of the presence of vasculature in cartilage anlage with the ongoing ossification process, the assumption of biphasic mixture was a heuristic approach and a more detailed model encapsulating such details seems useful. Additionally, the linear biphasic theory by itself has inherent limitations resulting from the homogeneity, isotropy, zero friction, and infinitesimal strain condition assumptions for experiments, the latter of which remained valid under the strictly controlled conditions of our study. However, the reader should bear in mind while using these results the infinitesimal strain assumption may no longer hold, depending on the corresponding simulated physiological conditions. To account for larger deformations, the biphasic theory in finite deformation or other theories accommodating such nonlinearities must be applied to the experiments and to extract the material parameters. Also, although samples were taken from areas away from the ossification center, the homogeneity assumption may still not be accurate as the tissue composition and structure may differ in the vicinity of the ossific nucleus from regions farther out. Studies designed to address this issue can provide insight into the presence and degree of this variation.

As a result of difficulty in obtaining the cadaveric material, we only tested plugs harvested from two fetuses. Testing more samples is likely to provide more accurate estimations of the mechanical behavior of the material and lower the standard deviations caused by variations in different sources. Considering the relatively small number of samples tested, the standard deviations in this study are comparable to those obtained by others (Table 2). The age dependency of the mechanical properties of the tissue must also be considered when implementing the results.

We found low stiffness of the anlagen cartilage consistent with the deformability of the anlagen during clubfoot treatment. The literature suggests large variability among the mechanical properties of articular cartilage reported in the literature, which may be attributable in part to different testing geometries, varying test sites, and different species (Table 2). Similarities and differences between the biology and biochemistry of various species cartilages, regardless

of the anatomic similarity between their sources, are important factors in explaining the differences in mechanical properties.

Cartilage consists of two phases: the interstitial fluid and the solid matrix, which is composed primarily of collagen fibers and proteoglycans (PGs). Tissue biochemical composition and the ultrastructure of the extracellular matrix together determine the resulting biomechanical properties of cartilage [53]. Although a considerable amount of work describes the structure-function relationships [3, 22, 27, 30, 38, 50, 51, 53, 55, 56], the exact relationships are not well understood. The biomechanical properties of cartilage have been mainly associated with the collagen network and glycosaminoglycan constituents (negatively charged chains attached to the core protein of PGs) of the extracellular matrix [22, 45]. The differences between our results for fetal developing cartilage and the literature values for adult or fetal articular cartilage of various species may be attributed to the same source.

The average aggregate modulus and equilibrium Young's modulus (0.15 ± 0.07 and 0.06 ± 0.03 MPa) obtained in our study were about an order of magnitude smaller than the literature values for adult articular cartilage (Table 2). The slope of the stress-stretch curve proved to remain constant at the strain levels used in the experiments in both confined and unconfined configurations (lines fitted to the equilibrium stress-stretch data yielded $r^2 = 0.979 \pm 0.026$ and $r^2 = 0.963 \pm 0.029$, respectively), justifying the use of infinitesimal strain theory and the linear elastic material model for the solid matrix.

The site- and depth-dependent studies by Treppo et al. [53] suggested a negative correlation between the water content and the equilibrium aggregate modulus (H_A) in the adult human knee and ankle. They also reported the equilibrium aggregate modulus increased for all joint surfaces with increasing sulfated glycosaminoglycan/wet weight. Similar relationships have also been reported by others [45, 56]. The Young's modulus is strongly related to the PG content [30, 49].

The Poisson's ratio we observed in this study was fairly large (0.40 ± 0.06) compared with the literature range for compression tests (approximately 0.0–0.48) [5, 6, 24, 25, 28, 42, 45, 58]. Few studies have been performed on the fetal tissue [55, 56, 58], especially on humans [10]. Wong et al. [58] directly measured Poisson's ratio of fetal bovine samples using an image-based technique in unconfined compression and reported the equilibrium Poisson's ratio increased with age (from 0.09 ± 0.02 in fetal to 0.26 ± 0.11 in adult tissue) in bovine articular cartilage. Disregarding the issue of difference in species studied, our results contrast with those findings. Indeed, combined with the Poisson's ratio, Athanasiou et al. [5] determined for the proximal side of adult human talus ($\nu = 0.00$ – 0.06), the

Table 2. Some results from previous studies along with the test sites and testing configurations

Source article	H_A (MPa)	k ($10^{-14} \text{ m}^4 \text{ N}^{-1} \text{ s}^{-1}$)	ν	Test	Location
Athanasiou et al. [5]	0.92–1.25	0.080–0.164	0.00–0.06	Creep indentation	Proximal talus of human ankle
Athanasiou et al. [6]				Creep indentation	Lateral condyle
	0.701 $\pm$ 0.228	0.1182 $\pm$ 0.0207	0.098 $\pm$ 0.069		Human
	0.894 $\pm$ 0.293	0.0426 $\pm$ 0.0197	0.396 $\pm$ 0.023		Bovine
	0.603 $\pm$ 0.237	0.0774 $\pm$ 0.563	0.300 $\pm$ 0.057		Dog
	0.778 $\pm$ 0.176	0.4187 $\pm$ 0.1545	0.236 $\pm$ 0.044		Monkey
	0.537 $\pm$ 0.258	0.1806 $\pm$ 0.1049	0.337 $\pm$ 0.092		Rabbit
	0.530 $\pm$ 0.094	0.2173 $\pm$ 0.0730	0.000 $\pm$ 0.000		Human patellar groove (all adult)
Boschetti et al. [8]	0.397 $\pm$ 0.095	0.0875 $\pm$ 0.0412	—	Permeability test/Confined compression creep	Human adult tibial plateau
Hayes and Mockros [24]	—	—	0.42/0.37 ^e	Uniaxial/torsion creep	Human adult tibial plateau
Brown and Singerman [10]	E_s 0.699 $\pm$ 0.346	0.251 $\pm$ 0.159	0.00	Stress relaxation	Human fetal proximal femoral chondroepiphysis
Williamson et al. [56]	H_{A0}	$\log_{10}(k_{p0})^*$	—	Unconfined compression	Bovine
	0.11 $\pm$ 0.03	–15.8	—	Confined compression	Fetal distal femur
	0.27 $\pm$ 0.02	–16.1	—		Infant calf
	0.31 $\pm$ 0.03	–16.5	—		Adult knee
Current study	0.15 $\pm$ 0.07	2.01 $\pm$ 0.8	0.4 $\pm$ 0.06	Stress relaxation Confined and unconfined compression	Human fetal ankle talus

* Results varied with location and degree of offset compression; the presented results correspond to 45% offset compression; these are approximate values read from the graphs in [56]; i = instantaneous; e = equilibrium.

observed trend between age and Poisson's ratio is quite the opposite. Kiviranta et al. [30] did not address the relationship with age in their study of Poisson's ratio; however, harvesting samples from sites bearing various collagen compositions (bovine knee and shoulder), they observed the collagen network primarily controlled the Poisson's ratio. Using Fourier transform infrared imaging, they reported a negative correlation between the collagen content and Poisson's ratio. They also implemented a finite element model applying the fibril reinforced biphasic theory to confirm the mechanical properties of the collagen network influenced the Poisson's ratio. Other work on fetal tissue [56] studied the function-composition relationships of developing bovine articular cartilage in compression. These authors did not report on Poisson's ratio but found a marked increase in collagen content during development and associated the developmental changes in biomechanical properties to this phenomenon. More specifically, they suggested the load bearing-related mechanical properties of cartilage (increased modulus, decreased permeability) improved with age. Based on the combination of age, function, and composition studies on similar tissues, the low collagen content seems mainly responsible for the large Poisson's ratio we have ascertained for human fetal tissue as well. Poisson's ratio and Young's modulus are inversely related [30]. A high value of Poisson's ratio also suggests relatively low apparent compressibility, which tends to act as a barrier to fluid transport and counteract the effect of permeability on the ease of it.

The strain-dependent exponential representation of permeability yielded good model fits ($r^2 = 0.910 \pm 0.041$) to the experimental data. Our results indicate there is an order of magnitude difference between the fetal and adult talus permeability in the free-swelling state (k_0), with the fetal talus being more permeable. This is greater than the difference reported by Williamson et al. [56] between the femoral condyle and patellofemoral groove samples of fetal, calf, and adult bovine. According to Maroudas et al. [41], no major changes affecting material transfer occur in the cartilage matrix after death, provided the cartilage is stored at a suitably low temperature; therefore, we do not expect the double freeze-thaw cycles to have substantially influenced the permeability of the tissue [21, 36, 54, 59].

More permeable matrix facilitates the fluid flow in the tissue. One function to which permeability contributes is the transport of nutrients throughout the tissue. Cartilage canals containing blood vessels, cells, and extracellular matrix are found in epiphysis of long bones and in cartilaginous anlagen of small and irregular bones such as talus [1, 2, 7, 12, 13, 16, 17]. The cartilage model of fetal talus is well vascularized throughout by cartilage canals [1, 12] to nourish the cartilage and provide osteogenic tissue, with four arteries contributing to the blood supply of its

ossification nucleus [18]. The fluid flow in the developing anlagen is of a bicompartamental nature owing to the matrix porosity and the cartilage canals. The permeability obtained in the present work is the overall tissue-level permeability. In the investigation of Williamson et al. [56] on fetal articular cartilage, the hydraulic permeability negatively correlated with the concentration of both glycosaminoglycans and collagen as well as dry weight per unit volume. Because these concentrations are in line with the other mechanical properties we obtained, we presume they are also responsible for the part of the order-of-magnitude-higher permeability caused by the composition of cartilage matrix. The other contributing factor besides the matrix composition can be the presence of cartilage canals.

The dry weight measurements ($13.1\% \pm 1.8\%$) indicated the tissue is highly hydrated. The low dry weight is consistent with the high permeability and low stiffness results, considering the cartilage matrix gets softer and more permeable as the water content increases [3, 56].

In-depth studies are required to understand the reasons for differing properties between mature and developing human cartilage. In brief, the relatively high Poisson's ratio, high permeability, low dry weight, and low stiffness moduli we found are consistent with and are confirmed by previous work on developing cartilage. At the organ level, our observation of deformation of the anlagen by the manipulation and casting of clubfeet [9] is a clear manifestation of the properties we report.

Acknowledgments We thank Dr Richard Brand for help in the design and conduct of the study and Dr Schneck of the Department of Anatomy and Cell Biology at Temple University, Philadelphia, PA, for providing the cadaveric material.

References

1. Agrawal P, Atre PR, Kulkarni DS. The role of cartilage canals in the ossification of talus. *Acta Anat (Basel)*. 1984;119:238–240.
2. Agrawal P, Kulkarni DS, Atre PR. The participation of cartilage canals in the ossification of the human fetal calcaneum. *J Anat*. 1986;147:135–142.
3. Armstrong CG, Mow VC. Variations in the intrinsic mechanical properties of human articular cartilage with age, degeneration, and water content. *J Bone Joint Surg Am*. 1982;64:88–94.
4. Ateshian GA, Warden WH, Kim JJ, Grelsamer RP, Mow VC. Finite deformation biphasic material properties of bovine articular cartilage from confined compression experiments. *J Biomech*. 1997;30:1157–1164.
5. Athanasiou KA, Niederauer GG, Schenck RC Jr. Biomechanical topography of human ankle cartilage. *Ann Biomed Eng*. 1995; 23:697–704.
6. Athanasiou KA, Rosenwasser MP, Buckwalter JA, Malinin TI, Mow VC. Interspecies comparisons of in situ intrinsic mechanical properties of distal femoral cartilage. *J Orthop Res*. 1991; 9:330–340.
7. Blumer MJF, Longato S, Richter E, Pérez M, Konakci K, Fritsch H. The role of cartilage canals in endochondral and perichondral

- bone formation: are there similarities between these two processes? *J Anat.* 2005;206:359–372.
8. Boschetti F, Miotti C, Massi F, Colombo M. An experimental study on human articular cartilage permeability. *Proceedings of the Second Joint EMBS/BMES Conference.* 2002, pp. 2581–2582.
 9. Brand RA, Siegler S, Pirani S, Morrison WB, Udupa JK. Cartilage anlagen adapt in response to static deformation. *Med Hypotheses.* 2006;66:653–659.
 10. Brown TD, Singerman RJ. Experimental determination of the linear biphasic constitutive coefficients of human fetal proximal femoral chondroepiphysis. *J Biomech.* 1986;19:597–605.
 11. Chen SS, Falcovitz YH, Schneiderman R, Maroudas A, Sah RL. Depth-dependent compressive properties of normal aged human femoral head articular cartilage: relationship to fixed charge density. *Osteoarthritis Cartilage.* 2001;9:561–569.
 12. Cheng X, Wang Y, Qu H. Intrachondral microvasculature in the human fetal talus. *Foot Ankle Int.* 1997;18:335–338.
 13. Cheng X, Wang Y, Qu H, Jiang Y. Ossification processes and perichondral ossification groove of Ranvier: a morphological study in developing human calcaneus and talus. *Foot Ankle Int.* 1995;16:7–10.
 14. Cohen B, Lai WM, Mow VC. A transversely isotropic biphasic model for unconfined compression of growth plate and chondroepiphysis. *J Biomech Eng.* 1998;120:491–496.
 15. Cummings RJ, Lovell WW. Operative treatment of congenital idiopathic club foot. *J Bone Joint Surg Am.* 1988;70:1108–1112.
 16. Delgado-Baeza E, Santos-Alvarez I, Epeidegui-Torre TE, Miralles-Flores C. Cartilage canals in the tarsal navicular of the human fetus and infant. *Int Orthop.* 1993;17:30–33.
 17. Fritsch H, Brenner E, Debbage P. Ossification in the human calcaneus: a model for spatial bone development and ossification. *J Anat.* 2001;199:609–616.
 18. Fritsch H, Schmitt O, Eggers R. The ossification centre of the talus. *Ann Anat.* 1996;178:455–459.
 19. Garcia JJ, Altiero NJ, Haut RC. An approach for stress analysis of transversely isotropic cartilage under impact load. *J Biomech Eng.* 1998;120:608–613.
 20. Garcia JJ, Cortes DH. A nonlinear biphasic viscohyperelastic model for articular cartilage. *J Biomech.* 2006;39:2991–2998.
 21. Gleizes V, Viguier E, Féron JM, Canivet S, Lavaste F. Effects of freezing on the biomechanics of the intervertebral disc. *Surg Radiol Anat.* 1998;20:403–407.
 22. Grodzinsky AJ. Electromechanical and physicochemical properties of connective tissue. *Crit Rev Biomed Eng.* 1983;9:133–199.
 23. Hayes WC, Keer LM, Herrmann G, Mockros LF. A mathematical analysis for indentation tests of articular cartilage. *J Biomech.* 1972; 5:541–551.
 24. Hayes WC, Mockros LF. Viscoelastic properties of human articular cartilage. *J Appl Physiol.* 1971;31:562–568.
 25. Hori RY, Mockros LF. Indentation tests of human articular cartilage. *J Biomech.* 1976;9:259–268.
 26. Irani RN, Sherman MS. The pathological anatomy of idiopathic clubfoot. *Clin Orthop Relat Res.* 1972;84:14–20.
 27. Jurvelin JS, Arokoski JPA, Hunziker EB, Helminen HJ. Topographical variation of the elastic properties of articular cartilage in the canine knee. *J Biomech.* 2000;33:669–675.
 28. Kempson G, Spivey C, Swanson S, Freeman M. Patterns of cartilage stiffness on normal and degenerate human femoral heads. *J Biomech.* 1971;4:597–609.
 29. Kite JH. Non-operative treatment of congenital clubfeet: a review of one hundred cases. *South Med J.* 1930;23:337.
 30. Kiviranta P, Rieppo J, Korhonen RK, Julkunen P, Töyräs J, Jurvelin JS. Collagen network primarily controls Poisson's ratio of bovine articular cartilage in compression. *J Orthop Res.* 2006;24:690–699.
 31. Korhonen RK, Laasanen MS, Töyräs J, Rieppo J, Hirvonen J, Helminen HJ, Jurvelin JS. Comparison of the equilibrium response of articular cartilage in unconfined compression, confined compression and indentation. *J Biomech.* 2002;35:903–909.
 32. Kvalseth TO. Cautionary note about R2. *Am Statist.* 1985;39: 279–285.
 33. Kwan MK, Lai WM, Mow VC. A finite deformation theory for cartilage and other soft hydrated connective tissues—I. Equilibrium results. *J Biomech.* 1990;23:145–155.
 34. Lai WM, Hou JS, Mow WC. A triphasic theory for the swelling and deformation behaviors of articular cartilage. *J Biomech Eng.* 1991;113:245–258.
 35. Lai WM, Mow VC. Drag-induced compression of articular cartilage during a permeation experiment. *Biorheology.* 1980;17: 111–123.
 36. Lee KE, Pelker RR. Effect of freezing on histologic and biomechanical failure patterns in the rabbit capital femoral growth plate. *J Orthop Res.* 1985;3:514–515.
 37. Li LP, Soulhat J, Buschmann MD, Shirazi-Adl A. Nonlinear analysis of cartilage in unconfined ramp compression using a fibril reinforced poroelastic model. *Clin Biomech.* 1999;14:673–682.
 38. Lu XL, Sun DDN, Guo XE, Chen FH, Lai WM, Mow VC. Indentation determined mechano-electrochemical properties and fixed charge density of articular cartilage. *Ann Biomed Eng.* 2004;32:370–379.
 39. Mak AF. Unconfined compression of hydrated viscoelastic tissues: a biphasic poroviscoelastic analysis. *Biorheology.* 1986;23: 371–383.
 40. Mak AF. The apparent viscoelastic behavior of articular cartilage—the contributions from the intrinsic matrix viscoelasticity and interstitial fluid flows. *J Biomech Eng.* 1986;108:123–130.
 41. Maroudas A, London, Bullough P, Oxford, Swanson SAV, Freeman MAR. The permeability of articular cartilage. *J Bone Joint Surg Br.* 1968;50:166–177.
 42. Mow VC, Gibbs MC, Lai WM, Zhu WB, Athanasiou KA. Biphasic indentation of articular cartilage—II. A numerical algorithm and an experimental study. *J Biomech.* 1989;22: 853–861.
 43. Mow VC, Holmes MH, Lai WM. Fluid transport and mechanical properties of articular cartilage: a review. *J Biomech.* 1984;17: 377–394.
 44. Mow VC, Kuei SC, Lai WM, Armstrong CG. Biphasic creep and stress relaxation of articular cartilage in compression: theory and experiments. *J Biomech Eng.* 1980;102:73–84.
 45. Mow VC, Ratcliffe A, Poole AR. Cartilage and diarthrodial joints as paradigms for hierarchical materials and structures. *Biomaterials.* 1992;13:67–97.
 46. Parsons JR, Black J. The viscoelastic shear behavior of normal rabbit articular cartilage. *J Biomech.* 1977;10:21–29.
 47. Ponseti IV. *Congenital Clubfoot: Fundamentals of Treatment.* Oxford, UK: Oxford University Press; 1996.
 48. Ponseti IV, Smoley EN. Congenital club foot: the results of treatment. *J Bone Joint Surg Am.* 1963;45:261–344.
 49. Rieppo J, Töyräs J, Nieminen MT, Kovanen V, Hyttinen MM, Korhonen RK, Jurvelin JS, Helminen HJ. Structure-function relationships in enzymatically modified articular cartilage. *Cells Tissues Organs.* 2003;175:121–132.
 50. Rivers PA, Rosenwasser MP, Mow VC, Pawluk RJ, Strauch RJ, Sugalski MT, Ateshian GA. Osteoarthritic changes in the biochemical composition of thumb carpometacarpal joint cartilage and correlation with biomechanical properties. *J Hand Surg [Am].* 2000;25:889–898.
 51. Roberts S, Weightman B, Urban J, Chappell D. Mechanical and biochemical properties of human articular cartilage in

- osteoarthritic femoral heads and in autopsy specimens. *J Bone Joint Surg Br.* 1986;68:278–288.
52. Soulhat J, Buschmann MD, Shirazi-Adl A. A fibril-network-reinforced biphasic model of cartilage in unconfined compression. *J Biomech Eng.* 1999;121:340–347.
 53. Treppo S, Koepp H, Quan EC, Cole AA, Kuettner KE, Gordinsky AJ. Comparison of biomechanical and biochemical properties of cartilage from human knee and ankle pairs. *J Orthop Res.* 2000;18:739–748.
 54. vanOsCh GJVM, Blankevoort L, Kraan PMvd, Janssen B, Hekman E, Huiskes R, Berg WBvd. Laxity characteristics of normal and pathological murine knee joints in vitro. *J Orthop Res.* 1995;13:783–791.
 55. Williamson AK, Chen AC, Masuda K, Thonar EJ-MA, Sah RL. Tensile mechanical properties of bovine articular cartilage: variations with growth and relationships to collagen network components. *J Orthop Res.* 2003;21:872–880.
 56. Williamson AK, Chen AC, Sah RL. Compressive properties and function-composition relationships of developing bovine articular cartilage. *J Orthop Res.* 2001;19:1113–1121.
 57. Wilson W, Donkellar CCv, Rietgergen Bv, Ito K, Huiskes R. Stresses in the local collagen network of articular cartilage: a poroviscoelastic fibril-reinforced finite element study. *J Biomech.* 2004;37:357–366.
 58. Wong M, Ponticiello M, Kovanen V, Jurvelin JS. Volumetric changes of articular cartilage during stress relaxation in unconditioned compression. *J Biomech.* 2000;33:1049–1054.
 59. Woo SL, Orlando CA, Camp JF, Akeson WH. Effects of post-mortem storage by freezing on ligament tensile behavior. *J Biomech.* 1986;19:399–404.