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CHARACTERIZATION OF COMPRESSIVE BEHAVIOR OF DEVELOPING HUMAN TALUS

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ABSTRACT

Mechanical characterization of human cartilage anlagen is required in order to effectively model congenital musculoskeletal deformities. Such modeling can effectively explore the effect of treatment procedures and potentially suggest enhanced treatment methods. We therefore determined the stress relaxation behavior of cartilage plugs obtained from third-trimester still-born fetuses in unconfined and confined compression geometries. The material parameters determined were the aggregate modulus H_A = 0.15 ± 0.07 MPa, permeability coefficients k_0 = 2.01 ± 0.8 × 10^{-14} $m^4 N^{-1} s^{-1}$ and M = 4.6 ± 1.0, Young's modulus E_s = 0.06 ± 0.03 MPa, and Poisson's ratio ν = 0.4 ± 0.06. As compared to adult articular cartilage, stiffness was an order of magnitude lower than the values reported in the literature, inferring 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 found in previous studies. Such material is expected to deform and relax to larger extents.

INTRODUCTION

In the fetus, the skeleton initially consists of cartilage "anlage" which gradually ossifies during fetal and postnatal development. Faulty or retarded development of this system causes congenital musculoskeletal anomalies. Understanding the mechanisms of retarded or deficient development is essential in improvement of treatment options. Additionally, a model describing the development of cartilaginous anlagen may be used to propose and test new treatment options or infer the direct relationship between an existing treatment and the observed changes in growth pattern under treatment or abnormal conditions. Such studies can potentially provide guidance for the betterment of treatment results.

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 biomechanics of *articular* cartilage has been extensively studied in animals [1-12], and to a lesser degree in humans [13-24]; however, to our knowledge such information is nonexistent for the developing cartilage *anlage*. Since the material definition is an important part of the finite element models where a closer approximation of the material properties yields more realistic results, the present work aims to study and characterize the mechanical properties of human talar cartilage anlage by utilizing established analytic models for soft tissue.

Congenital talipes equinovarus (clubfoot) is the most obvious of all childhood foot deformities as well as one of the most common, which can be easily diagnosed at birth by its characteristic appearance. Amongst the involved bones in this disorder is the talus (ankle bone) being the most affected [25, 26]. Therefore, this anlage was chosen for our first study bearing in mind that similarity between the tarsal anlagen tissues due to similarity in the developmental stage can allow for using the results as approximations for anlage other than talus for which the properties were determined.

The experimental results were analyzed using the biphasic theory developed by Mow et al. [7] where the

cartilage is assumed to be isotropic and homogenous while the friction between the tissue, indenter, and test fixture is neglected. Infinitesimal strain assumptions were used while the strain-dependence of the permeability was included within the model. 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, and elastic modulus E_s) and the tissue's strain-dependent hydraulic permeability.

MATERIALS AND METHODS

Various models have been used to describe the biomechanical behavior of soft connective tissue under uniaxial compressive loading, including elastic [27], biphasic [7], triphasic [28], poroviscoelastic [29, 30], fibril-reinforced [12, 31], and transversely isotropic models [32, 33]. For a detailed listing of various models refer to Garcia et al. [34]. In the current work the biphasic theory was applied [7] which is well applicable to any biphasic tissue in the body (mature cartilage, meniscus, ligaments, etc.).

The tali of two still-born fetuses in the third trimester were studied. The fetal age was determined by measurement of crown-rump (CR) lengths. The feet were carefully dissected to separate the tali which were partly ossified (Fig. 1, Fig. 2). The tissue was sliced using a manual tissue chopper with a stage whose advancement was controlled by a micrometer. Due to 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 due to deflection of the blade within the bony region,

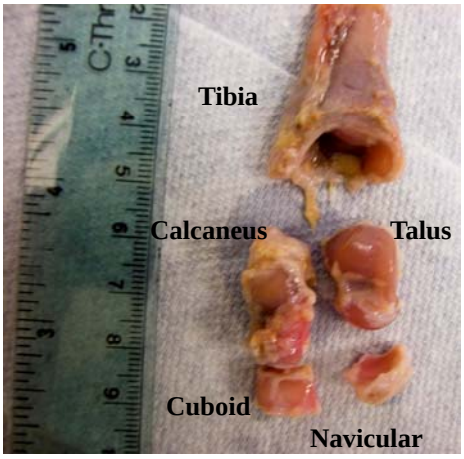

Fig. 1 Dissected hind foot and tibia of the third-trimester still-born fetus.

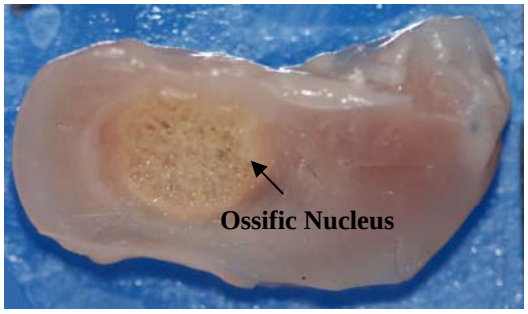

Fig. 2 Medial view of a sagittal slice of talus displaying the cartilaginous and ossified regions.

some nonuniformity in the thickness was observed throughout the slices. Cartilage plugs were gathered from each slice using a biopsy punch with a diameter of 3.00 mm. The thickness of cartilage plugs was measured individually at the time of the experiment. The average plug thickness was 1.61 ± 0.37 mm. The plugs were submerged in PBS (phosphate buffer solution) and frozen at -80°C until the day of testing. We gathered sixteen samples from the tali. Seven were collected from the younger specimen and nine from the older.

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 *M* NaCl) containing enzyme inhibitors (2 *mM* EDTA, 5 *mM* benzamadine, 10 *mM* N-ethylmaleimide, 1 *mM* PMSF) during testing. A custom-designed testing apparatus (displacement resolution 5μm, 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, and pressed upon with a porous indenter (pores size 50μm, 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 in order to reduce friction.

Prior to collecting the data, we applied a pre-conditioning load of 3.3N (0.47 MPa) to the samples for 60 s, removed the load, and then applied a tare load of 0.06N (8.5 kPa) for 900 s. Thickness of each sample was measured after the tare load was applied, and before each actual stress relaxation test. By taking readings from the load cell, the stepper motor revolutions were counted from when the indenter touched the top surface of the plugs (when the force started to rise just above zero) until the pre-specified tare load value was reached. The prescribed displacement history consisted of four

Table 1 Mechanical parameters listed separately for the two cadavers (mean ± SD): aggregate modulus (H_A), permeability coefficients (k_0 and M), Young's modulus (E_s), Poisson's ratio (ν)

Plug groups	H_A (MPa)	k_0 ($10^{-14} m^4 N^{-1} s^{-1}$)	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

ramps (5% strain each) at a displacement rate of $1\,\mu m/s$, followed by a stress relaxation period of $500\,s$. The stress relaxation period was chosen such that the change in the stress value at the end of the period was smaller than $100\,Pa/\min$. The loading protocol was the same for both configurations. Reproducibility of the measurements was previously examined for both biological and non-biological reference samples. Repeated testing on the same sample in both confined and unconfined compression produced material parameters that varied by less than 10%.

Prior to deformation, photographs of the cartilage plugs were taken using a high resolution camera (Canon EOS REBEL XTi) 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 average sample diameter was found to be 3.29±0.14*mm*.

The experimental results were analyzed using the biphasic theory developed by Mow et al. [7] which assumes the tissue is a soft, porous, isotropic, homogenous and permeable elastic solid filled with interstitial fluid, with both phases intrinsically incompressible [7]. The friction between the tissue, indenter, and test fixture is neglected in this theory. The strain-dependence of the permeability was included within the model using the following function for intrinsic permeability [35] :

$$k = k_0 \exp(Me) \tag{1}$$

where k_0 and M are intrinsic material parameters and e is the dilation of the solid matrix (true strain). With (1), the linear biphasic theory reduces to the so called *nonlinear diffusion equation* for uniaxial confined compression tests [36]:

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

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 the above equation for the unconfined compression tests was not needed since only the equilibrium results of those experiments were analyzed in order to extract the Poisson's ratio.

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 was determined by finding 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

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

where y represents the experimental variable, y_{est} is the theoretical variable, and $\bar{y}$ is the mean value of y [37].

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

$$2H_A\nu^2 + (H_A - E_s)\nu - (H_A - E_s) = 0 \tag{4}$$

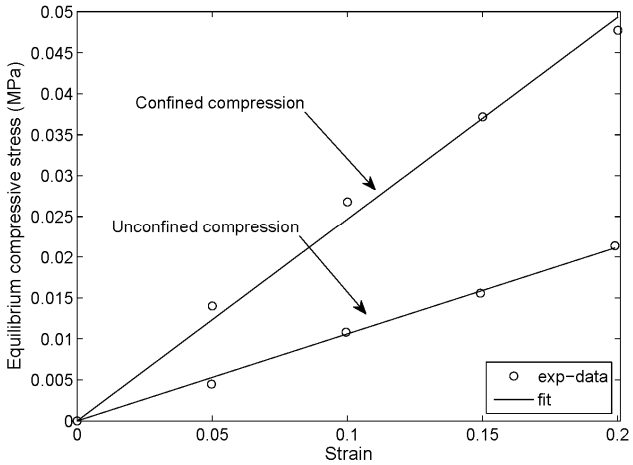

Fig. 3 The equilibrium stress-strain data. Under the infinitesimal strain conditions the slope of the curves remains nearly constant.

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

Source article	H_A (MPa)	k ($10^{-14} m^4 N^{-1} s^{-1}$)	ν	Test	Location
Athanasiou et al. [14]	0.92-1.25	0.080-0.164	0.00-0.06	Creep indentation	Proximal talus of human ankle
Athanasiou et al. [17]	0.701/0.588	0.118/0.113 $\times 10^{-14}$	0.098/0.074	Creep indentation	Femoral condyle Lateral/medial
Boschetti et al. [38]	0.397 ± 0.095	0.0875 ± 0.0412	-	Permeability test/Confined compression creep	Human adult tibial plateau
Hayes and Mockros [19]	-	-	0.42^i/0.37^e	Uniaxial/torsion creep	Adult tibial plateau
Brown and Singerman [24]	E_s = 0.6988 ± 0.3460	0.251 ± 0.159	0.00	Stress relaxation Unconfined compression	Fetal proximal femoral chondroepiphysis

i: instantaneous, e: equilibrium

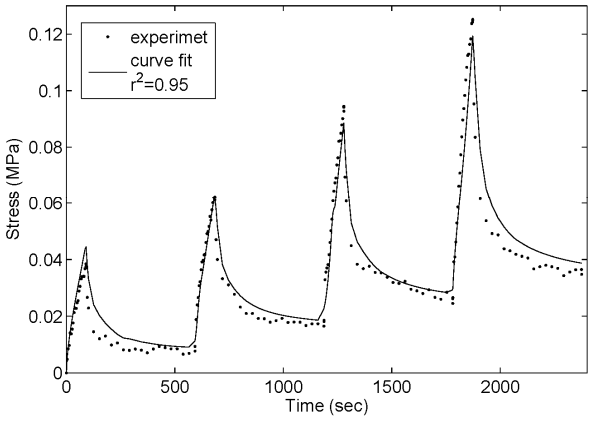

Fig. 4 Transient response of the tissue tested in the confined compression configuration with the best curve fit for finding permeability coefficients.

RESULTS

The material parameters averaged over all the samples were H_A = 0.15 ± 0.07 MPa, k_0 = 2.01 ± 0.8 × $10^{-14} m^4 N^{-1} s^{-1}$, M = 4.6 ± 1.0, E_s = 0.06 ± 0.03 MPa, and ν = 0.4 ± 0.06 (Table 1 lists the values of the two groups separately). The coefficient of determination for the nonlinear curve fitting of permeability parameters was r^2 = 0.910 ± 0.041 (Fig. 5). The specimens recovered 97.1 ± 2.6% of their initial thickness as measured just before the confined and unconfined experiments. Linear fits to the equilibrium stress-stretch data yielded r^2 = 0.979 ± 0.026 and r^2 = 0.963 ± 0.029 for confined and unconfined compression respectively (Fig. 3).

The dry weight of the samples was 13.1 ± 1.8 %.

DISCUSSION

Building a detailed mechanical model can be a reliable means to explore mechanisms behind normal and deficient growth prenatally and postnatally, and the changes in growth pattern due to treatments. Once a clear understanding of the nature of these mechanisms has been established, it can be used to both improve current treatments and suggest new treatment techniques. Such models of congenital foot disorders 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 biphasic theory in the confined and unconfined compression configurations.

The literature suggests large variability among the mechanical properties of articular cartilage reported in the literature, which may be due in part to different testing geometries, varying test sites, and different species. For comparison, Table 2 provides a few results from the previous studies on humans. Similarities and differences between the biology and biochemistry of various species cartilages, regardless of the anatomical 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 [16]. Considerable amount of work has been done in efforts to correlate the structure-function relationships [2, 3, 5, 10, 11, 15, 16, 18, 20, 39], however 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 [39, 40]. 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 $0.06 \pm 0.03\,MPa$) obtained in our study were about an order of magnitude smaller than the literature values for adult articular cartilage. 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 assumption and the linear elastic material model for the solid matrix.

The site and depth-dependent studies by Treppo et al. [16] suggested a negative correlation between the water content and the equilibrium aggregate modulus (H_A) in adult human knee and ankle. They also found the equilibrium aggregate modulus increased for all joint surfaces with increasing sulfated glycosaminoglycan/ wet weight. Similar relationships have also been reported by others [11, 40]. The Young's modulus also has been found to be strongly related to the PG content [3, 41].

The Poisson's ratio we obtained in this study was fairly large (0.40 ± 0.06) compared to the literature range for compression tests (~0.0-0.48) [6, 13, 14, 17, 19, 21, 40, 42]. Few studies have been performed on the fetal tissue [10, 11, 42] especially in humans [24]. Wong et al. [42] 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. [14] determined for the proximal side of adult human talus ($\nu_s = 0.00\text{-}0.06$), the observed trend between age and Poisson's ratio is quite the opposite. Kiviranta et al. [3] 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 FTIR imaging they found a significant 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 [11] studied the function-composition relationships of developing bovine articular cartilage in compression. These authors did not report on Poisson's ratio; however, they 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 to be mainly responsible for the large Poisson's ratio we have ascertained for human fetal tissue as well. Poisson's ratio and young's modulus were previously found to be inversely related [3]. 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), and the fetal talus being more permeable. This is greater than the difference reported by Williamson et al. [11] between the femoral condyle and patellofemoral groove samples of fetal, calf, and adult bovine. According to Maroudas et al. [43] 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.

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 as well as in cartilaginous anlagen of small and irregular bones such as talus [44-50]. The cartilage model of fetal talus is well vascularized throughout by cartilage canals [47, 48] in order to nourish the cartilage and provide osteogenic tissue, with four arteries contributing to the blood supply of its ossification nucleus [51]. The fluid flow in the developing anlagen is of a bicompartmental 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. on fetal articular cartilage [11], the hydraulic permeability negatively correlated with the concentration of both GAGs and collagen as well as dry weight per unit volume. Since 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 (ϕ_0 = 13.1 ± 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 [11, 18].

In-depth studies are required to understand the sources of the differentiation between mature and developing human cartilage. In brief, the relatively high Poisson's ratio, high permeability, low dry weight and low stiffness moduli found in this study were consistent with the previous works on what seems to be also the composition of the talar cartilage anlage at hand.

It is worth noting that due to 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. The reader should bear in mind also that the infinitesimal strain assumption may no longer hold depending on the corresponding simulated physiological conditions. In order to account for larger deformations, the biphasic theory in finite deformation or other theories accommodating such nonlinearities must be applied to the experiments and in order to extract the material parameters.

Due to difficulty in obtaining the cadaveric material, plugs harvested from two fetuses were tested. Testing more samples is likely to provide better estimations of the mechanical behavior of the material. The age dependency of the mechanical properties of the tissue must also be considered when implementing the results into other studies.

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