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Material properties of articular cartilage in the rabbit tibial plateau

Maria L. Roemhildt^{a,*}, Kathryn M. Coughlin^a, Glenn D. Peura^a, Braden C. Fleming^b, and Bruce D. Beynon^a

^aMcClure Musculoskeletal Research Center, Department of Orthopaedics and Rehabilitation, University of Vermont, 95 Carrigan Drive, 412 Stafford Hall, Burlington, VT 05405, USA

^bBrown Medical School, Rhode Island Hospital, Biomechanics Laboratory, CORO West, Suite 404, 1 Hoppin Street, Providence, RI 02903, USA

Abstract

The material properties of articular cartilage in the rabbit tibial plateau were determined using biphasic indentation creep tests. Cartilage specimens from matched-pair hind limbs of rabbits approximately 4 months of age and greater than 12 months of age were tested on two locations within each compartment using a custom built materials testing apparatus. A three-way ANOVA was used to determine the effect of leg, compartment, and test location on the material properties (aggregate modulus, permeability, and Poisson's ratio) and thickness of the cartilage for each set of specimens.

While no differences were observed in cartilage properties between the left and right legs, differences between compartments were found in each set of specimens. For cartilage from the adolescent group, values for aggregate modulus were 40% less in the medial compartment compared to the lateral compartment, while values for permeability and thickness were greater in the medial compartment compared to the lateral compartment (57% and 30%, respectively). Values for Poisson's ratio were 19% less in the medial compartment compared to the lateral compartment. There was also a strong trend for thickness to differ between test locations.

Similar findings were observed for cartilage from the mature group with values for permeability and thickness being greater in the medial compartment compared to the lateral compartment (66% and 34%, respectively). Values for Poisson's ratio were 22% less in the medial compartment compared to the lateral compartment.

Keywords

Cartilage; Rabbit; Mechanical testing

1. Introduction

Measurements of the material properties of articular cartilage have been frequently used to document the progression of osteoarthritis in animal models (Hasler et al., 1999). Several techniques such as confined compression, unconfined compression and indentation testing have been employed (Mow et al., 2005). An advantage of the biphasic indentation creep test (Mak et al., 1987; Mow et al., 1989), in which a flat, porous, and indenter tip is pressed into the articular surface with a known force while the resulting displacements are recorded, is that it can be performed in situ on osteochondral specimens. These data can then be used to calculate the aggregate modulus, permeability, and Poisson's ratio of the articular cartilage (Mak et al., 1987; Mow et al., 1989). The test is based on the biphasic model, which assumes cartilage

* Corresponding author. Tel.: +1 802 656 3823; fax: +1 802 6564247. maria.roemhildt@med.uvm.edu (M.L. Roemhildt).

consists of an isotropic, porous solid phase within which a fluid phase is imbibed. The rate of fluid flow is determined by the permeability. It is responsible for the creep rate that is thought to reflect the proteoglycan content of the tissue (Mak et al., 1987; Mow et al., 1989). The aggregate modulus is based on the equilibrium displacement and is thought to reflect the integrity of the type II collagen matrix and proteoglycan content (Mak et al., 1987; Mow et al., 1989).

Although the rabbit is frequently used as an animal model for cartilage research, there is little consensus in the literature with regard to the normal variations in the material properties of articular cartilage about the rabbit knee. Differences in the sex and age of specimens used, testing methods, or test location prevent direct comparison of results (Parsons and Black, 1977; Athanasiou et al., 1991; Lane et al., 1979; Naumann et al., 2002; Hoch et al., 1983; Sah et al., 1997; Wei et al., 1998; Rasanen and Messner, 1996, 1999). The primary objective of this study was to determine if there are differences in tibial articular cartilage properties with respect to leg (left vs. right), joint compartment (medial vs. lateral), and location of test site (a central region vs. a posterior region) in the normal rabbit knee. A secondary objective was to determine if any factor-dependent effects were consistent across different populations of rabbits.

2. Materials and methods

2.1. Preparation of test specimens

Matched hind limbs of New Zealand white rabbits were harvested within 4 h of euthanasia. Specimens from adolescent rabbits (male, greater than 4 months of age, $n = 12$) or mature rabbits (female, approximately 12 months of age, $n = 7$) were used. Tibial plateaus were excised and sectioned to separate the medial and lateral compartments. Specimens were wrapped in gauze saturated with lactated Ringer's solution and frozen to -80°C until testing. Care was taken to protect the articular surfaces while the specimens were harvested.

2.2. Mechanical testing setup

Biphasic indentation creep tests (Mak et al., 1987; Mow et al., 1989) were performed on a custom materials testing system. The system consists of a servomotor-driven linear stage (M-510; Physik Instruments) to which a 50 g load cell (FTD-G-50; Lucas) is attached (Fig. 1A). A servo-controller (DMC-1710; Galil Motor Control) drives the stage in either load or displacement control. A laser interferometer (Excel Precision) measures the displacement between a fixed reference point and the live side of the loadcell to provide a direct measurement of specimen deflection. The interferometer and load cell accuracies are $0.10\text{ }\mu\text{m}$ and 0.05 g , respectively. The system has a minimal incremental motion of $0.05\text{ }\mu\text{m}$. The testing device was positioned on a vibration-damping platform (#2208-44-12; Kinetic Systems). A plane-ended, porous, 1.0 mm diameter indenter tip (Athanasiou et al., 1991) was machined from sintered stainless steel with a $20\text{ }\mu\text{m}$ filtration rating (Martin Kurz & Co., Inc., Mineola, NY) and utilized. The indenter tip was readily interchanged with a guide for specimen positioning and a needle probe to measure the thickness of the articular cartilage.

2.3. Mechanical testing protocol

Osteochondral specimens were thawed in a bath of Ringer's solution for approximately 45 min and were then mounted to an aluminum block using cyanoacrylate adhesive. The specimen was secured on the material testing system within a spherical clamp that allowed five-degree of freedom of motion to help align the specimen with the indenter. A thin mirror, 1 mm in diameter, was placed on the surface of the cartilage at the selected test location. The specimen within the clamping system was positioned such that the primary laser beam and the reflected beam were coincident; indicating that the mirror and cartilage surface were aligned normal to

the indenter tip with the help of a positioning guide (Fig. 1B). The clamping system was locked into place using a vacuum clamp and the positioning guide was replaced with the indenter tip. The specimen reservoir was filled with Ringer's solution until the specimen was submerged. After positioning, the specimen was allowed to rehydrate for 10–15 min before the indentation test proceeded.

Indentation tests were performed on two sites within each compartment: (1) at the center of the load bearing area (which corresponded to the tibiofemoral contact location when the knee was at approximately 60° of flexion), and (2) slightly posterior and towards the central eminence relative to the first location (corresponding to a load-bearing location when the knee was in a more flexed position) (Fig. 2).

During the creep indentation test, a tare load of –3.50g was applied to the cartilage surface for 900 s. Subsequently, a test load of –10.00 g was applied until the creep response reached equilibrium as the temporal load-displacement response was recorded at 1 Hz. The criterion for equilibrium was less than a 1 μm change in displacement over a 900-s period.

Following completion of the creep test and a recovery period equal to the test duration, the cartilage thickness was determined at the precise test site while maintaining specimen position and orientation by interchanging the indenter tip and 50 g load cell with a blunt-needle probe (~0.12mm in diameter) attached to a 2.3 kg load cell (sensitivity = 0.07 g, not accounting for systemic noise; LGP 310; Cooper Instruments). The rate of displacement was 0.5mm/s. The load cell and displacement outputs were recorded at 128 Hz as the needle penetrated into the cartilage tissue; a change from zero slope on the load-displacement chart identified contact with the cartilage surface and a sudden rise in force indicated contact with the deep calcified layer (Fig. 3; Athanasiou et al., 1991). Next, the specimen was repositioned and the indentation and thickness protocols were repeated to test the second location within each compartment. The total testing time for each compartment was typically 3–4 h. Following indentation testing, cartilage surfaces were stained with India ink and graded for surface smoothness and presence of splits using the categories of Jurvelin et al. (1983).

2.4. Analysis

The intrinsic material properties of the articular cartilage were calculated by curve-fitting the load–displacement response with the biphasic indentation creep solution via a nonlinear regression procedure (Mow et al., 1989). Due to differences between the theoretical model and experimental results in the early initial time response, the early portion of the creep response was not used in the determination of material properties (Mow et al., 1989; Setton et al., 1994). Conditions assumed for the solution of the biphasic indentation problem, such as a frictionless shear interface between the indenter tip and cartilage as well as perfect step loading, are difficult to verify or achieve experimentally. These discrepancies are thought to cause divergence of the theoretical and experimental data during the initial portion of the creep response (Mow et al., 1989) therefore the initial portion of the data containing 70% of the total equilibrium deformation was excluded from the curve-fit analysis, as is routine when using this technique (Mow et al., 1989; Setton et al., 1994). The collected creep-indentation data were filtered prior to determination of the material properties to adjust for difference in sampling rate that was used (Appendix 1).

To evaluate the primary aim, three-factor analyses of variance (ANOVA) with repeated measures were used to determine if there was an effect produced by leg (left vs. right), compartment (medial vs. lateral), and/or location (central vs. posterior) on the tibial cartilage properties: aggregate modulus (H_A), permeability coefficient (k), Poisson's ratio (ν_s), and measured thickness (h). Pooled means and standard errors were used to compare levels of each factor. This analysis was performed independently for each material property in the adolescent

and mature data sets. Additionally, a *t*-test was used to compare compartment specific data between adolescent and mature groups for each material property.

3. Results

The applied load typically resulted in nominal strain in the cartilage of less than 10% (mean strain for all samples = $8.1 \pm 74.5\%$).

3.1. Adolescent rabbit cartilage

Aggregate modulus, permeability, Poisson's ratio, and thickness were found to be similar between left and right legs, *p*-values = 0.98, 0.59, 0.73, and 0.62, respectively (Table 1).

In contrast, the material properties differed between medial and lateral compartments of the tibia (Table 1). Values for the aggregate modulus were 40% less in the medial compartment (pooled mean $\pm$ pooled standard error = 0.95 ± 0.14 MPa) compared to the lateral compartment (1.32 ± 0.16 MPa, *p* = 0.004). Values for permeability were 57% greater in the medial compartment ($12.1 \times 10^{-16} \pm 2.2 \times 10^{-16}$ m⁴/Ns) compared to the lateral compartment ($5.2 \times 10^{-16} \pm 1.1 \times 10^{-16}$ m⁴/Ns) (*p* < 0.001). Poisson's ratio values were 19% less in the medial compartment (0.32 ± 0.02) compared to the lateral compartment (0.38 ± 0.01) (*p* < 0.001). The thickness values were 30% greater in the medial compartment (0.71 ± 0.05 mm) compared to the lateral compartment (0.50 ± 0.03 mm) (*p* < 0.001).

There was a strong trend for cartilage thickness to vary between test locations ($h_{\text{Central}} = 0.63 \pm 0.05$ mm vs. $h_{\text{Posterior}} = 0.58 \pm 0.05$ mm) (*p* = 0.06) (Table 1). Evaluation of the medial compartment revealed a trend for aggregate modulus to differ between test locations ($H_{\text{A Med central}} = 1.11 \pm 0.12$ MPa vs. $H_{\text{A Med posterior}} = 0.78 \pm 0.15$ MPa) (*p* = 0.08). There was no effect of test location in the lateral compartment. No other two or three-way interactions were observed.

3.2. Mature rabbit cartilage

Similar testing and analysis were also performed on articular cartilage from mature rabbits (Table 2).

There was no effect of leg or location for aggregate modulus, permeability, Poisson's ratio, and thickness. Values for permeability were 66% greater in the medial compartment ($19.0 \pm 12.0 \times 10^{-16}$ m⁴/Ns) compared to the lateral ($6.4 \pm 3.1 \times 10^{-16}$ m⁴/Ns) (*p* = 0.002). Poisson's ratio values were 22% less in the medial compartment ($0.25 \pm .11$) compared to the lateral compartment ($0.31 \pm .06$) (*p* = 0.04). Thickness values were 34% greater in the medial compartment (0.80 ± 0.10 mm) compared to the lateral compartment (0.53 ± 0.11 mm) (*p* < 0.001).

Further evaluation revealed a trend for aggregate modulus to differ between compartment for the posterior location ($H_{\text{A Med posterior}} = 1.37 \pm 0.58$ MPa vs. $H_{\text{A Lat posterior}} = 1.83 \pm 0.57$ MPa) (*p* = 0.035).

There was no effect of compartment for the central location. No other two or three-way interactions were observed.

3.3. Comparison of adolescent and mature groups

Comparison of outcome measures, grouped by compartment, using the student's *t*-test revealed significant differences between the adolescent and mature data sets in the medial and lateral compartments (Table 3). Values for aggregate modulus, permeability and thickness_{Medial} were

greater in the Mature group compared to the Adolescent group. Values for Poisson's ratio were less in the Mature group compared to the Adolescent group.

4. Discussion

Previous studies have observed a greater thickness and elastic modulus in the medial relative to the lateral compartment in the rabbit tibial plateau (Hoch et al., 1983; Rasanen and Messner, 1996). In the present study, there was a similar finding for thickness, however, the aggregate modulus was greater in the lateral compartment relative to the medial. In addition, there was a strong trend for test location to have an effect on material properties. This may indicate that different regions of cartilage behave distinctly. This is of interest in studies of osteoarthritis which use material properties to track disease progression as baseline values may vary between test region locations.

Variation in cartilage material properties over the tibial plateau surface has been studied in other species with specific interest in comparing regions covered and not covered by the meniscus (Appleyard et al., 1999; Setton et al., 1994). Setton et al. found that permeability and thickness values for articular cartilage from areas not covered by the menisci were greater than those for areas covered by the menisci in canines (Setton et al., 1994). A similar trend was observed in the present study for thickness and aggregate modulus (medial compartment), with values of each being greater for the central location compared to the posterior location. The rabbit menisci are relatively small and may migrate during rollback of the femur on the tibia plateau during normal range of motion in the rabbit knee. The central test site used in this study was uncovered by the meniscus while the posterior site is thought to have been partially covered by the meniscus.

The present study focused on cartilage specimens from rabbits approximately 4–5 months and greater than 12 months of age. In the rabbit, growth plates close and cartilage is considered histologically mature between 6 and 8 months of age (Kavanagh and Ashhurst, 1999). The literature suggests that the material properties of adolescent cartilage may differ from mature rabbit cartilage. Wei and colleagues have observed a decrease in cartilage stiffness in rabbits with maturation when evaluated at approximately 3, 5, and 8 months (Wei et al., 1997, 1998). In contrast, others have reported no significant difference in Young's modulus between late adolescent (6 months) rabbit cartilage compared to adult (19 months) (Hoch et al., 1983; Rasanen and Messner, 1996; Wei et al., 1998). In the present study aggregate modulus, permeability, and thickness were greater in the mature group compared to the adolescent group. Although the absolute values of cartilage material properties varied between specimens of the different groups used in this study, the statistical analysis revealed consistency in factors affecting each population. The effect of compartment was observed in both the adolescent and mature data sets. The potential for age related effects should be considered if experimental studies are conducted over long periods of time and indicate that a tightly controlled population of animals should be used.

No differences were detected between right and left legs for each set of specimens studied. This finding is important since the contralateral leg is frequently used as a control during experimental treatments.

The sampling rate of 1 Hz used to monitor the creep response in this study differed from the sampling criterion used by others (Athanasίου et al., 1994). This difference increased the weight of the tail portion of the experimental data curve and was found to increase error between the experimental data and the predicted theoretical solution which influenced the calculated material properties. Therefore, to ensure minimal error between experimental data and the

predicted theoretical solution the collected creep-indentation data were filtered to optimize the curve fitting procedure (Appendix 1).

In this study a needle probe was used to determine the thickness of the articular cartilage. The needle probe has been a common technique to measure the cartilage thickness (Athanasίου et al., 1991; Hoch et al., 1983; Wei et al., 1998; Rasanen and Messner, 1996, 1999; Setton et al., 1994). In a study comparing thickness measurement techniques, the thickness values of canine articular cartilage determined using a needle probe technique showed high, linear correlation with values determined through optical techniques. The optically determined values were approximately 3% higher than values determined by needle probe (Jurvelin et al., 1995). Since the thickness measurement is used in the determination of material properties, it is estimated that values for aggregate modulus and permeability that are presented here would differ less than 0.62% and -4.4%, respectively, from values calculated from optical measurements of thickness.

The structural properties obtained for normal cartilage were highly variable, particularly the permeability coefficient. Some of the variation may be explained by the initial condition of the cartilage. Grading of the cartilage following India ink staining revealed that the lateral compartments had mostly smooth, intact surfaces with the occasional presence of superficial split lines while medial compartments had slightly rougher surfaces and/or the presence of superficial or deep splits. However, no significant correlation was noted between any of the cartilage properties and cartilage scores. All specimens used in the present study were from normal rabbits that had not received any experimental intervention to their knees, suggesting that some degree of variation in cartilage properties exists at baseline.

The rabbit model is frequently used in the study of the progression of osteoarthritis and in the evaluation of different types of intervention using material properties as outcome measures. Given that cartilage material properties vary not only compartmentally, but may also vary over the surface of a given compartment, care must be exercised in the selection of consistent test locations throughout an experiment. Testing additional regions within a given compartment would further the understanding of how cartilage properties vary over the surface of the tibial plateau in the rabbit.

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Appendix 1. Description of filtering procedure for creep indentation data

Due to a difference in the sampling rate at which the creep-indentation data were collected compared to those used by others, the collected creep-indentation data were filtered prior to determination of the material properties. This was performed to optimize the curve fitting procedure as determined by a minimum, average, and normalized RMS error between the experimental and theoretical curves for the data set. For these rabbit cartilage specimens, the optimal filtering algorithm involved computing a factor, f , which resulted in a total of 70 data points. These points were spaced along the time axis such that the first filtered data point (t_n, y') corresponded to $f\%$ of the distance along the time axis between the time corresponding to 70% deformation ($t_{70\%}$) and the time at equilibrium (t_{eq}). In a similar manner, the next point (t_{n-1}, y'') corresponded to $f\%$ of the distance along the time axis between the time corresponding to 70% deformation, and the time corresponding to the preceding data point (t_n, y'). These steps were repeated until 70 points had been selected. This technique weighted initial points heavier on a linear time scale and was found to minimize the RMS error between the predicted and

measured creep curves as compared to unfiltered data and other filtering techniques. Fig. A1 shows the filtered and unfiltered data for one specimen.

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Fig. 1. Materials testing system: (A) schematic of creep test setup, (B) Interchangeable positioning guide consisting of a translucent, collecting platform with a pinhole.

Fig. 2.
Locations of testing sites on the tibial plateau.

Fig. 3.

Force vs. displacement graph for needle probe test. The measured thickness was determined from the point of contact between the needle probe and cartilage surface (deviation from zero slope) until reaching the tidemark (inflection point before a rapid increase in load).

Fig. A1.

The filtered data (+) is weighted towards the initiation of the test load. The unfiltered data is shown as a solid line (—).

Table 1

Cartilage properties of the Adolescent tibial plateau determined from indentation test

ADOLESCENT		Aggregate modulus (MPa)		Permeability ($\times 10^{-16} \text{m}^4/\text{Ns}$)		Poisson's ratio		Thickness (mm)	
Side	Position	Medial	Lateral	Medial	Lateral	Medial	Lateral	Medial	Lateral
Right	Central	1.18 (0.43)	1.33 (0.58)	12.5 (6.0)	5.8 (7.2)	0.31 (0.06)	0.39 (0.03)	0.74 (0.10)	0.51 (0.11)
	Posterior	0.79 (0.22)	1.23 (0.65)	12.8 (9.9)	4.7 (1.7)	0.31 (0.05)	0.37 (0.04)	0.71 (0.19)	0.49 (0.11)
Left	Central	1.05 (0.45)	1.29 (0.20)	11.0 (6.6)	5.4 (2.2)	0.34 (0.05)	0.37 (0.03)	0.78 (0.15)	0.50 (0.12)
	Posterior	0.76 (0.71)	1.44 (0.69)	12.1 (8.0)	4.8 (2.5)	0.31 (0.10)	0.38 (0.05)	0.62 (0.22)	0.51 (0.10)
R-L Diff	Central	0.13 (0.42)	0.05 (0.46)	1.5 (6.0)	0.4 (6.4)	-0.03 (0.04)	0.02 (0.04)	-0.04 (0.12)	0.02 (0.08)
	Posterior	0.03 (0.77)	-0.22 (0.55)	0.8 (11.5)	0.2 (2.8)	0.00 (0.11)	-0.01 (0.05)	0.09 (0.05)	-0.03 (0.12)

Values are given as the mean, with the SD in parentheses; R-L Diff = the paired Right-Left leg differences ($n = 12$).

Table 2
Cartilage properties of the Mature tibial plateau determined from indentation test

MATURE	Aggregate modulus (MPa)				Permeability ($\times 10^{-16} \text{ m}^4/\text{Ns}$)				Poisson's Ratio				Thickness (mm)			
	Side	Position	Medial	Lateral	Medial	Lateral	Medial	Lateral	Medial	Lateral	Medial	Lateral	Medial	Lateral		
Right		Central	1.79 (0.96)	1.68 (0.38)	15.8 (5.8)	6.1 (4.1)	0.27 (0.10)	0.29 (0.06)	0.82 (0.10)	0.55 (0.10)						
		Posterior	1.24 (0.43)	1.80 (0.42)	18.6 (6.1)	5.4 (2.1)	0.25 (0.10)	0.34 (0.06)	0.84 (0.12)	0.49 (0.10)						
Left		Central	1.57 (0.34)	1.60 (0.74)	20.9 (19.6)	7.3 (3.5)	0.23 (0.13)	0.31 (0.06)	0.78 (0.15)	0.53 (0.12)						
		Posterior	1.50 (0.74)	1.87 (0.72)	20.5 (13.3)	6.9 (2.9)	0.26 (0.11)	0.30 (0.07)	0.78 (0.15)	0.54 (0.14)						
R-L Diff		Central	-0.19 (0.38)	0.26 (0.53)	-5.2 (21.6)	-1.2 (5.4)	-0.04 (0.19)	-0.02 (0.09)	-0.03 (0.13)	0.02 (0.08)						
		Posterior	-0.48 (0.94)	-0.03 (0.91)	-1.9 (17.3)	-1.5 (2.1)	0.01 (0.17)	0.04 (0.08)	-0.01 (0.16)	-0.05 (0.16)						

Values are given as the mean, with the SD in parentheses; R-L Diff = the paired right-left leg differences ($n = 7$).

Table 3

Comparison of cartilage properties between Adolescent and mature groups

	Aggregate modulus (Mpa)			Permeability ($\times 10^{-16} \text{ m}^4/\text{Ns}$)			Poisson's ratio			Thickness (mm)		
	Medial	Lateral	M-L Diff	Medial	Lateral	M-L Diff	Medial	Lateral	M-L Diff	Medial	Lateral	M-L Diff
Adolescent	0.95 (0.50)	1.32 (0.55)	-0.38 (0.64)	12.1 (7.6)	5.2 (3.9)	6.9 (7.6)	0.32 (0.07)	0.38 (0.04)	-0.06 0.07	0.71 (0.18)	0.50 (0.11)	0.21 (0.18)
Mature	1.53 (0.65)	1.74 (0.57)	-0.21 (0.89)	19.0 (12.0)	6.4 (3.1)	12.5 (12.9)	0.25 (0.10)	0.31 (0.06)	-0.06 0.13	0.80 (0.10)	0.53 (0.11)	0.27 (0.16)
<i>p</i> -Value	<0.001	0.003	<i>0.349</i>	0.004	0.031	<i>0.128</i>	0.002	<0.001	0.818	0.017	<i>0.296</i>	<i>0.129</i>

Values are given as the mean, with the SD in parentheses; M-L Diff = the paired medial-lateral compartment differences.