

Effects of enzymatic degradation on the frictional response of articular cartilage in stress relaxation

Ines M. Basalo^a, David Raj^b, Ramaswamy Krishnan^a, Faye H. Chen^c, Clark T. Hung^b, Gerard A. Ateshian^{a,b,*}

^aDepartment of Mechanical Engineering, Columbia University, SW Mudd, Mail Code 4703, 500 West 120th Street, New York, NY 10027, USA

^bDepartment of Biomedical Engineering, Columbia University, SW Mudd, Mail Code 4703, 500 West 120th Street, New York, NY 10027, USA

^cDepartment of Orthopedic Surgery, Columbia University, 622 West 168 Street, PH11-Center, New York, NY 10032, USA

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Abstract

It was recently shown experimentally that the friction coefficient of articular cartilage correlates with the interstitial fluid pressurization, supporting the hypothesis that interstitial water pressurization plays a fundamental role in the frictional response by supporting most of the load during the early time response. A recent study showed that enzymatic treatment with chondroitinase ABC causes a decrease in the maximum fluid load support of bovine articular cartilage in unconfined compression. The hypothesis of this study is that treatment with chondroitinase ABC will increase the friction coefficient of articular cartilage in stress relaxation. Articular cartilage samples ($n=34$) harvested from the femoral condyles of five bovine knee joints (1–3 months old) were tested in unconfined compression with simultaneous continuous sliding (± 1.5 mm at 1 mm/s) under stress relaxation. Results showed a significantly higher minimum friction coefficient in specimens treated with 0.1 μ /ml of chondroitinase ABC for 24 h ($\mu_{\min} = 0.082 \pm 0.024$) compared to control specimens ($\mu_{\min} = 0.047 \pm 0.014$). Treated samples also exhibited higher equilibrium friction coefficient ($\mu_{\text{eq}} = 0.232 \pm 0.049$) than control samples ($\mu_{\text{eq}} = 0.184 \pm 0.036$), which suggest that the frictional response is greatly influenced by the degree of tissue degradation. The fluid load support was predicted from theory, and the maximum value (as a percentage of the total applied load) was lower in treated specimens ($77 \pm 12\%$) than in control specimens ($85 \pm 6\%$). Based on earlier findings, the increase in the ratio $\mu_{\min}/\mu_{\text{eq}}$ may be attributed to the decrease in fluid load support.

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1. Introduction

Articular cartilage functions as the primary bearing material of synovial joints, providing very low friction and wear. Many experimental studies have established that the cartilage friction coefficient increases with time following load application (McCutchen, 1959, 1962; Walker et al., 1968; Longfield et al., 1969; Malcom, 1976; Forster and Fisher, 1996; Ateshian et al., 1998;

Forster and Fisher, 1999; Krishnan et al., 2004), starting very low upon loading (~ 0.002 – 0.02) and reaching much higher values after sustained periods of time (~ 0.1 – 0.4). Different modes of lubrication have been proposed to explain the low friction coefficient of cartilage (MacConaill, 1932; Charnley, 1959; Walker et al., 1968; Dowson et al., 1969; Longfield et al., 1969; Swann et al., 1984; Dowson and Jin, 1986; Hills, 1989), but its time-dependent behavior has been attributed most convincingly to the time-varying interstitial fluid pressure within the loaded cartilage layers, as proposed by McCutchen (1959, 1962) and supported by others (Malcom, 1976; Macirowski et al., 1994; Forster and Fisher, 1996; Ateshian, 1997; Ateshian et al., 1998).

*Corresponding author. Department of Mechanical Engineering, Columbia University, SW Mudd, Mail Code 4703, 500 West 120th Street, New York, NY 10027, USA. Tel.: +1-212-854-8602; fax: +1-212-854-3304.

E-mail address: ateshian@columbia.edu (G.A. Ateshian).

In this hypothesized mechanism, the interstitial water of cartilage pressurizes upon loading, supporting most of the applied load transmitted across the contact interface. Consequently, when the interstitial pressure is high, the frictional load transmitted by the collagen-proteoglycan solid matrix is considerably reduced, yielding a very small friction coefficient. As the pressure subsides with time, the frictional force on the solid matrix increases, along with the friction coefficient. In our recent study (Krishnan et al., 2004), we provided direct experimental evidence in support of this mechanism by simultaneously measuring the friction coefficient and interstitial fluid load support in a cartilage-against-glass experiment. A strong negative linear correlation was observed between these variables, confirming the hypothesized mechanism.

The implication of these findings is that the frictional response of cartilage is not limited to a surface phenomenon, since the magnitude and duration of interstitial fluid pressurization is dependent on the mechanical properties of the cartilage layers. Enzymatic treatment of articular cartilage has been widely used to simulate the matrix degradation that occurs with osteoarthritis and to investigate the role of each component in the mechanical response of the tissue. Chondroitinase ABC can be used to selectively degrade the proteoglycans without affecting the collagen matrix. A decrease in proteoglycans following treatment with chondroitinase ABC and other proteolytic enzymes has been associated with a decrease in the shear (Zhu et al., 1993) and Young's modulus (Lyyra et al., 1999), changes in the tensile response (Schmidt et al., 1990), an increase in the hydraulic permeability (Lotke and Granda, 1972), a decrease in the compressive stiffness (Bonassar et al., 1995) and alterations in the frictional response (Pickard et al., 1998; Jin et al., 2000; Kumar et al., 2001).

In our recent study (Basalo et al., 2003a, b), it was confirmed that the peak interstitial fluid load support in bovine cartilage decreases significantly following enzymatic degradation with collagenase or chondroitinase ABC. Therefore, the objective of this study was to investigate the effect of chondroitinase ABC treatment on the frictional response of articular cartilage in stress relaxation. Based on our earlier findings, the hypothesis was that chondroitinase ABC treatment would increase the minimum friction coefficient of bovine articular cartilage in this testing configuration.

2. Methods

2.1. Specimen preparation

Forty-five cylindrical cartilage plugs (\varnothing 8 mm) were harvested from the femoral condyles of five healthy 1–3

months-old bovine joints obtained from a local abattoir and stored at -20°C in phosphate buffered saline solution (PBS). On the day of testing, the specimens were first thawed at room temperature. The subchondral bone and part of the deep zone of each plug were removed using a sledge microtome (2400; Leica Microsystems, Inc., Bannockburn, IL) to produce a uniform thickness (final average thickness: 1.39 ± 0.19 mm), leaving the articular surface intact. \varnothing 4.78 mm plugs were further cored out from these samples for subsequent testing. Specimens were divided into four groups: control ($n=12$), chondroitinase treatment ($n=11$), PBS treatment ($n=11$), and unloaded chondroitinase treatment ($n=11$). Specimens in the chondroitinase treatment and unloaded chondroitinase treatment groups were digested with 0.1 u/ml chondroitinase ABC (Sigma, St. Louis, MO) in a buffer solution containing 50 mM Tris-HCl, 60 mM sodium acetate and 0.02% bovine serum albumin (pH 8.0) at 37°C for 24 h under gentle agitation. The unloaded chondroitinase group was incorporated in order to determine whether frictional testing contributes to further proteoglycan loss. Specimens in the PBS treatment group were incubated in PBS under the same conditions. Those in the control group received no treatment prior to frictional testing.

2.2. Friction measurements

The friction apparatus (Fig. 1) consists of a sliding stage (MTC-100; Nutec, Deer Park, NY) with a motion control module (SB 1391; ACS Tech 80, Maple Grove, MN) to provide reciprocal translational motion; a stepper micrometer to provide normal load application (18503; Oriol Instruments, Stratford, CT), connected to a linear variable differential transformer to measure specimen deformation (HR100, Shaevitz Sensors, Fairfield, NJ). Normal and frictional loads were measured with a multiaxial load cell (20E12A-M25B, JR3 Inc., Woodland, CA., normal load 0–10 lbs, tangential load 0–3 lbs, accuracy 1% of maximum load). Cartilage specimens were placed within a small recess at the center of the test chamber, with the articular side facing up. Friction measurements (cartilage against a 1 mm thick glass slide) were performed in unconfined compression stress-relaxation, at room temperature, with the specimen and glass surface immersed in PBS during the entire duration of the experiment.

A low magnitude creep tare load was applied in order to ensure proper contact between the platen and the specimen. A constant load of 1.8 ± 0.4 N with continuous reciprocal sliding (± 1.5 mm at 1 mm/s) was applied until creep equilibrium was achieved (~ 2500 s).

The application of the tare load was followed by a stress-relaxation test where a 10% compressive strain was applied at a constant rate of 0.1%/s, also with

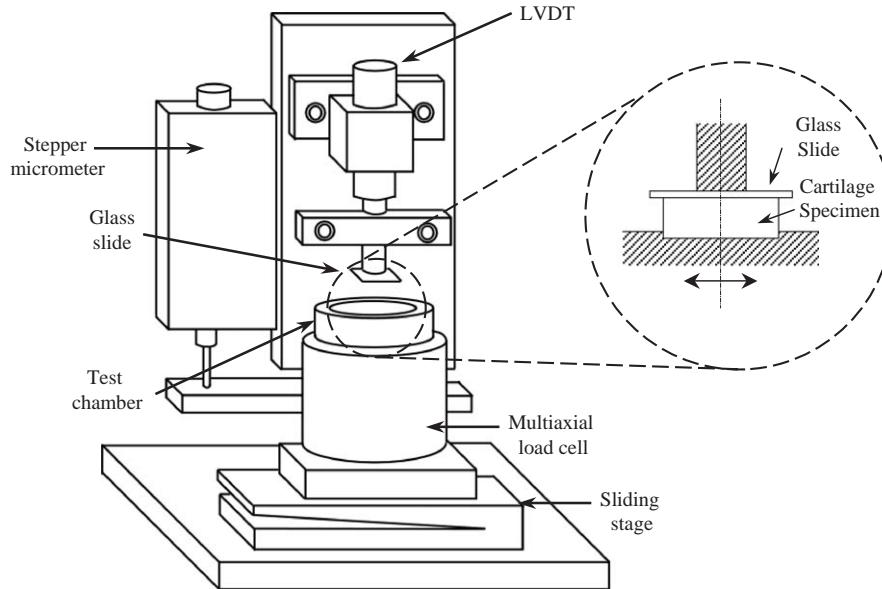


Fig. 1. Diagram of the friction testing device. The detail shows the orientation of the specimen relative to the glass slide and the sliding direction.

continuous reciprocal sliding. Stress-relaxation was used because it allows controlling the applied strain and maintaining it within the acceptable range for the linear biphasic theory (Mow et al., 1980).

The time-varying frictional force (F), the normal force (W) and specimen axial deformation were monitored during the test. The time-dependent friction coefficient was calculated from the ratio of the friction force and normal force ($\mu_{\text{eff}} = F/W$), and its minimum (μ_{min}) and equilibrium (μ_{eq}) values were tabulated for each specimen.

2.3. Determination of interstitial fluid load support

The fluid load support W^P/W is defined as the fraction of the total applied normal load W that is supported by the interstitial fluid when it pressurizes. W^P/W is not measured directly in this study, but its time-dependent behavior can be inferred from the measured load response $W(t)$ and axial displacement $u(t)$, based on our recent formulation (Soltz and Ateshian, 2000):

$$\frac{W^P}{W} = \underbrace{\frac{H_{+A} + \lambda_2}{H_{+A} - \lambda_2}}_{\alpha} \underbrace{\left[1 - \frac{W_0}{W} - \left(\frac{W_{\text{eq}} - W_0}{W} \right) \left(\frac{u - u_0}{u_{\text{eq}} - u_0} \right) \right]}_{f(W,u)}. \quad (1)$$

In this expression, H_{+A} is the tensile aggregate modulus, λ_2 is the “off-diagonal” modulus, $W_0 = W(t=0)$ is the tare load magnitude, $W_{\text{eq}} = W(t \rightarrow \infty)$ is the equilibrium normal load, $u_0 = u(0)$ is the deformation under the tare load and $u_{\text{eq}} = u(t \rightarrow \infty)$ is the equilibrium deformation. The function $f(W, u)$ is completely deter-

mined from experimental measurements, however without further characterization of the mechanical properties of each sample, the ratio $\alpha = (H_{+A} + \lambda_2)/(H_{+A} - \lambda_2)$ is a priori unknown.

Our previously proposed friction model (Ateshian et al., 1998) formulated the following dependence between the transient friction coefficient and interstitial fluid load support,

$$\frac{\mu_{\text{eff}}}{\mu_{\text{eq}}} = 1 - (1 - \varphi) \frac{W^P}{W}, \quad (2)$$

where φ is the fraction of the contact area over which the solid-to-solid contact occurs. Substituting Eq. (1) into Eq. (2) predicts a linear relationship between $\mu_{\text{eff}}/\mu_{\text{eq}}$ and $f(W, u)$. From a linear regression performed on this response, the slope $(1 - \varphi)\alpha$ can be determined. As a first approximation, $1 - \varphi$ is taken as the water content of cartilage at the articular surface (Ateshian et al., 1998), which averages to 0.91 for both normal and proteoglycan-depleted immature bovine cartilage (Torzilli et al., 1990). α can then be estimated from the slope of the linear regression, along with W^P/W from Eq. (1).

2.4. Biochemical analyses

Following frictional tests, specimens were equilibrated in PBS for 1 h. After measuring their wet weights (M220, Denver Instruments, Denver, CO), specimens were subsequently lyophilized overnight and reweighed dry to obtain the water content. Following 16-h papain (Sigma, St. Louis, MO) digestion, the glycosaminoglycan (GAG) content was determined using a 1,9-dimethyl-methylene blue assay with chondroitin-6-sulfate (Sigma,

St. Louis, MO) as the standard (Farndale et al., 1982). The total hydroxyproline content was determined using a colorimetric method (Stegeman and Stalder, 1967), and converted into total collagen content using a mass ratio of collagen to hydroxyproline of 7.25 (Williamson et al., 2001). GAG and collagen contents were normalized by the wet weight.

2.5. Statistical analyses

Statistical analyses were performed using the SAS v.8 software package (SAS Institute Inc., Cary, NC). One-way ANOVA ($\alpha = 0.05$) and Bonferroni post hoc testing were used to detect statistical differences in the minimum (μ_{min}) and equilibrium (μ_{eq}) friction coefficients, the ratio μ_{min}/μ_{eq} , the predicted fluid load support and the biochemical composition between control and treated groups.

3. Results

The average responses of the time-dependent friction coefficient μ_{eff} for all specimens in the control and chondroitinase treatment groups are shown in Fig. 2, normalized by their respective equilibrium values, μ_{eq} . At the start of the stress-relaxation test, μ_{eff}/μ_{eq} is equal to unity; during the ramp compression it decreases down to a minimum value, μ_{min}/μ_{eq} , achieved at the end of the ramp, and then relaxes back to unity. The time constant for the relaxation phase (the time required for μ_{eff}/μ_{eq} to rise from μ_{min}/μ_{eq} by $(1 - e^{-1})(1 - \mu_{min}/\mu_{eq})$) was 297 ± 168 s for the chondroitinase treatment group, compared to 405 ± 107 s for the control and 362 ± 145 for the PBS treatment groups ($p = 0.29$ and 0.31 , respectively).

Average values of μ_{min} and μ_{eq} are reported in Fig. 3. There was a significant increase in μ_{min} and μ_{min}/μ_{eq}

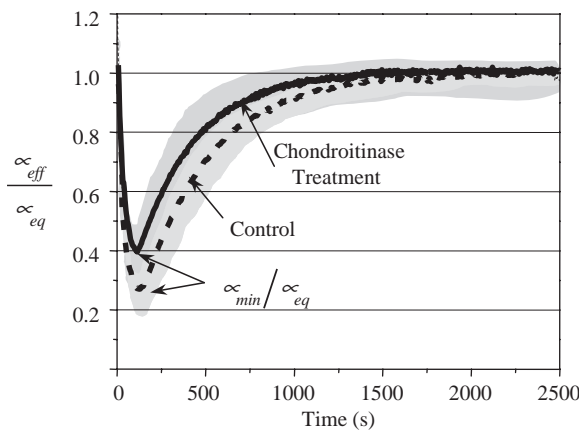


Fig. 2. Average response and standard deviation of the friction coefficient for the chondroitinase treatment and control groups.

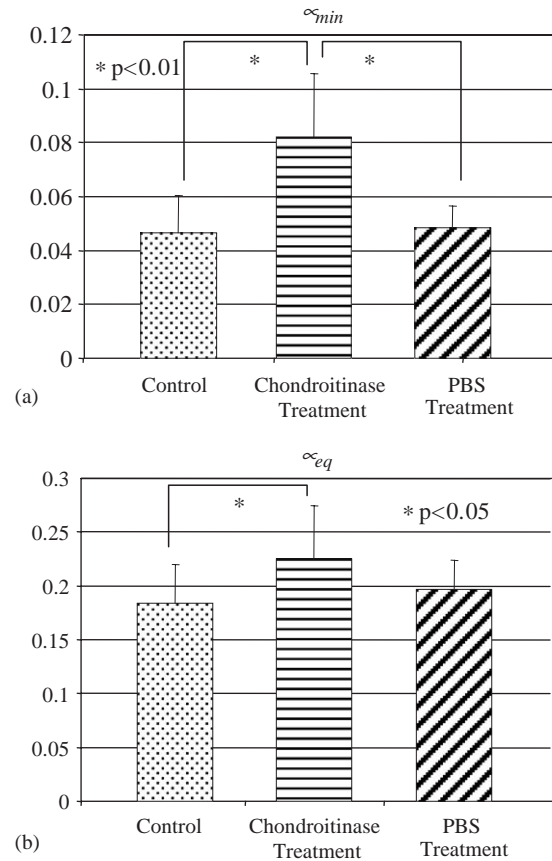


Fig. 3. Average (a) minimum and (b) equilibrium friction coefficient for all groups.

after treatment with chondroitinase ABC in comparison to control and PBS treatment groups ($p < 0.05$) (Fig. 2a). No significant differences were found in μ_{min} or μ_{min}/μ_{eq} between the control and PBS treatment groups ($p = 1.0$). A significant increase was also observed in μ_{eq} between chondroitinase and control treatment groups ($p < 0.05$), but not between chondroitinase and PBS treatment groups ($p = 0.08$) (Fig. 2b).

Linear regression of μ_{eff}/μ_{eq} versus $f(W, u)$ yielded coefficients of determination near unity ($R^2 = 0.992 \pm 0.008$ for the control group and $R^2 = 0.985 \pm 0.009$ for the chondroitinase treatment group), as shown for representative samples in Fig. 4. The slope of this response yielded coefficients α as summarized in Table 1, which also presents the corresponding maximum values of the function $f(W, u)$. The interstitial fluid load support, $W^P/W = \alpha f(W, u)$ (Eq. 1), was then predicted, with the average response over all specimens in the control and treated groups presented in Fig. 5. The peak fluid load support, $W^P/W|_{max} = \alpha f(W, u)|_{max}$, which occurred at the end of the ramp compression, was smaller in the chondroitinase treatment group ($77 \pm 12\%$) than in the control group ($85 \pm 6\%$) and PBS treatment group ($85 \pm 5\%$) ($p < 0.05$).

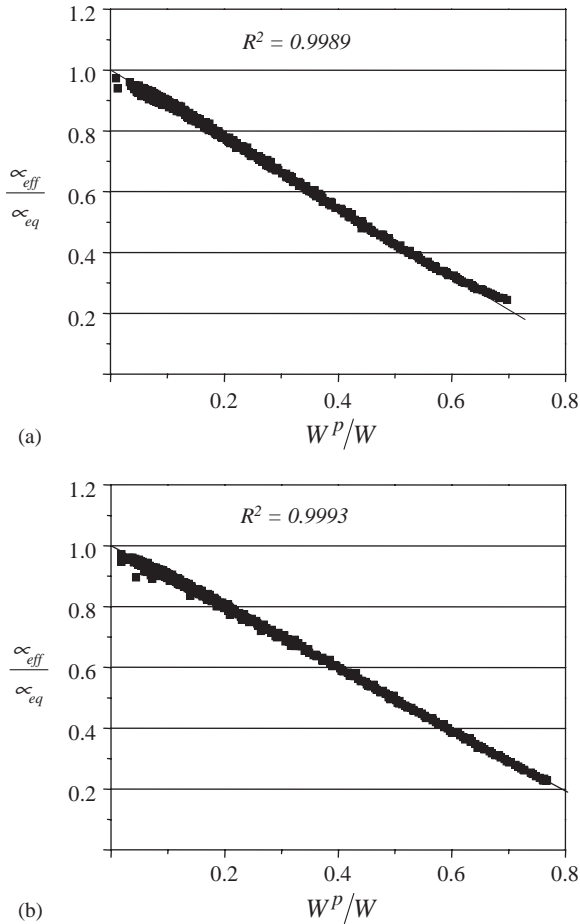


Fig. 4. Fluid load support versus friction coefficient for representative samples in (a) control group and (b) chondroitinase treatment group.

Table 1

Maximum value of the function $f(W, u)$, evaluated from load and displacement data using Eq. (1), and coefficient α obtained from the linear regression of $\mu_{\text{eff}}/\mu_{\text{eq}}$ using Eqs. (1) and (2), with $\phi = 0.91$

	Max $f(W, u)$	α
Control	0.7338 ± 0.0627	1.1573 ± 0.0767
Chondroitinase treatment	$0.6546 \pm 0.0828^*$	1.1747 ± 0.1143
PBS treatment	0.7430 ± 0.0537	1.1514 ± 0.0525

Statistically significant difference with respect to the control group is denoted by (* $p < 0.01$).

Results from the biochemical analyses are summarized in Table 2. The water content was not significantly affected by treatment with 0.1 u/ml of chondroitinase ABC or by incubation in PBS for 24 h. Treatment with chondroitinase ABC caused a significant decrease in GAG content in the chondroitinase treatment groups compared to the control and PBS treatment groups ($p < 0.05$). No statistical differences were found in the collagen content between any of the groups ($p = 1.0$),

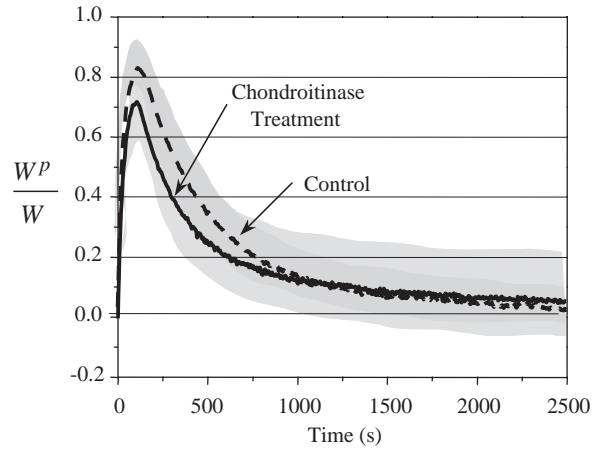


Fig. 5. Average response and standard deviation of the predicted fluid load support for the chondroitinase treatment and control groups.

indicating that treatment with chondroitinase ABC did not have any enzymatic effect on the collagen matrix.

No differences were found in the biochemical composition between the chondroitinase treatment and unloaded chondroitinase treatment groups, confirming that the tissue degradation resulted from the enzymatic treatment with chondroitinase ABC and not from the frictional testing or incubation in PBS.

4. Discussion

The main objective of this study was to investigate the effects of enzymatic treatment on the frictional response of articular cartilage. The predicted peak fluid load support was found to decrease after treatment with chondroitinase ABC, and was accompanied by a significant increase in both μ_{min} and $\mu_{\text{min}}/\mu_{\text{eq}}$ with respect to the control group. These results strongly support the hypothesis that loss of interstitial fluid load support following enzymatic digestion increases the minimum friction coefficient. The drop in interstitial fluid load support is consistent with our recent study (Basalo et al., 2003a, b), where treatment of immature bovine cartilage samples with chondroitinase ABC significantly reduced the measured peak interstitial fluid load support from $83 \pm 12\%$ to $48 \pm 16\%$. The lower GAG content ($0.8 \pm 0.4\%$) in the treated specimens of this recent study might be responsible for the more pronounced drop in fluid load support after the enzymatic treatment.

The time-dependent response of the friction coefficient (Fig. 2) was characteristic of stress-relaxation tests (Ateshian et al., 1998). Because of equilibrium tare conditions prior to the initiation of the stress-relaxation test ($W^P/W = 0$, Fig. 5), μ_{eff} was initially equal to μ_{eq} ; it

Table 2
Biochemical composition of all treated and control specimens

	Water (%)	GAG (% wet weight)	Collagen (% wet weight)
Control	82.0±2.2	3.3±0.8	9.5±2.1
Chondroitinase treatment	81.4±4.9	1.7±0.8*	10.8±1.2
PBS treatment	83.9±2.0	2.5±0.8	10.1±1.0
Unloaded chondroitinase treatment	83.6±3.1	1.4±0.9*	10.4±1.1

Statistically significant difference with respect to the control group is denoted by (* $p < 0.01$).

then decreased with increasing compression, reaching its minimum value at the end of the ramp-loading phase, coinciding with the occurrence of the peak fluid load support. It subsequently increased back toward its equilibrium value during the relaxation phase, as W^P/W returned to zero. The negative linear correlation between the friction coefficient and the predicted fluid load support, which is evident in the results of Fig. 4, confirms our recent study where μ_{eff} and W^P/W were measured directly and similarly found to correlate (Krishnan et al., 2004). This result gives further evidence to the hypothesis that interstitial fluid pressurization plays a primary role in governing the frictional response of articular cartilage.

In addition, the equilibrium friction coefficient μ_{eq} also increased by treatment with chondroitinase ABC. At equilibrium conditions, the interstitial fluid pressurization has reduced to zero (McCutchen, 1959; Forster and Fisher, 1996; Ateshian, 1997) and all the contribution to the frictional response comes from the solid matrix of the tissue. This suggests that the composition of the extracellular matrix and the interaction between the proteoglycans and the collagen matrix may also have an influence on the frictional behavior.

The fact that the friction coefficient of the treated specimens reached equilibrium conditions somewhat (though not statistically) faster than specimens in the control group might be attributed to an increase in hydraulic permeability due to a loss of proteoglycans (Lotke and Granda, 1972; Maroudas, 1979). A higher permeability implies a higher rate of fluid flow within the tissue, which will make the specimen reach equilibrium conditions faster.

Previous studies have investigated the effect of enzymatic digestion in the frictional response of articular cartilage. Kumar et al. (2001) also found an increase in the friction coefficient of porcine articular cartilage that was previously treated with chondroitinase ABC. They did not find any difference in the frictional response between specimens incubated in PBS and in a solution containing 3.5% of albumin, 0.5% of globulin and 0.375% HA. Based on these results, the use of PBS as an incubation control medium in the current study, instead of one also containing 0.02% BSA, was deemed appropriate.

On the other hand, Pickard et al. (1998) tested articular cartilage after chondroitinase AC treatment and found no significant difference in the frictional response in creep at different load magnitudes. They found a small, though statistically insignificant decrease in the equilibrium friction coefficient after the enzymatic treatment and they observed that the treated specimens reached their equilibrium displacement more rapidly. The difference between chondroitinase ABC and AC is not likely to explain the contradictory outcome with the current study or Kumar's, because chondroitinase ABC additionally degrades chondroitin sulfate B, found in dermatan sulfate, which is present only in negligible amounts in articular cartilage. A potential explanation is that the large contact stresses applied in Pickard's study may generate elevated interstitial fluid pressurization even in enzymatically degraded samples, because high strains may produce nonlinear stiffening of the solid matrix. Since interstitial fluid pressurization was neither measured nor estimated theoretically in their study, this potential explanation remains to be verified in future studies.

This study provides experimental evidence that if the mechanism of interstitial fluid pressurization is in some way compromised, the frictional properties will be correspondingly affected. The findings suggest that the frictional response of cartilage is not simply a surface phenomenon but significantly depends on the mechanical integrity of the tissue matrix and the interaction of the extracellular matrix with the interstitial fluid. It is well known that osteoarthritis is characterized by tissue degradation that includes loss of proteoglycans, collagen network fibrillation and increased tissue hydration (Freeman and Meachim, 1979). The increase in friction coefficient after enzymatic treatment shown in this study suggests that the frictional properties and hence the normal functioning of the tissue would be affected by the disease and may also promote further tissue degeneration.

Acknowledgements

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