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Effect of nominal stress on the long term friction, deformation and wear of native and glycosaminoglycan deficient articular cartilage

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Summary

Objectives: Earlier *in vitro* studies have shown that the coefficient of friction (COF) of cartilage decreases with increasing load at the lower end of the physiological loading spectrum. At these lower load levels, depletion of glycosaminoglycans (GAGs) from native cartilage has been shown to elevate the COF levels. The current study evaluated the long-term friction, deformation and wear of native and GAG deficient cartilage at a wide range of physiological stress levels *in vitro*.

Methods: A pin-on-plate machine (sliding velocity: 4 mm/s and stroke length: 4 mm) was used to measure the COF of native and GAG deficient cartilage at applied contact stress levels of 0.5 MPa, 2 MPa, and 3.15 MPa in 7 h long friction tests with phosphate buffered saline (PBS) lubrication. The resultant deformation and wear of the cartilage samples due to the friction tests were measured using a height vernier apparatus and lubricant analysis respectively.

Results and conclusions: An increase in contact stress from 0.5 MPa to 3.15 MPa resulted in an increase in the COF and wear of native cartilage samples, due to cartilage tissue's inability to rehydrate itself completely and maintain a high fluid load support at the 4 mm stroke length under high contact stress levels. There was no effect of increasing contact stress levels on the COF and wear of GAG deficient cartilage samples due to the very high deformations observed in these samples and the smoothening of their surfaces under the higher loads, leading to the development of conforming surfaces during articulation.

Key words: Friction, Wear, Deformation, Articular cartilage, Glycosaminoglycans, Chondroitinase ABC.

Introduction

Synovial joints experience a range of loading conditions during activities such as standing, walking, running etc and the contact pressures in the human hip and knee joints can vary from 0.5 MPa to as high as 18 MPa while performing strenuous activities[1,2]. Articular cartilage tissue lines the ends of the long bones forming these synovial joints, and provides a low friction bearing surface while undergoing minimal wear. Loss or degeneration of cartilage tissue due to trauma or/and pathologies can severely restrict the joint function. Consequently, it is essential to study the tribological properties of articular cartilage under different loading conditions in order to understand cartilage behaviour in native and diseased states and to develop appropriate therapies.

The biphasic nature of articular cartilage is well documented in literature and contributes to its load bearing capacity[3–6]. When a load is applied on the cartilage surface, the almost instantaneous interstitial fluid pressurization leads to a very high fluid phase load support resulting in very low friction until the fluid load support reduces gradually to a small value while the applied load is transferred to the solid phase of cartilage. The fluid load support can be sustained at a high value as long as the contact migrates on the cartilage surface and the previously loaded tissue sufficiently rehydrates[6–9]. Glycosaminoglycans (GAGs) of cartilage, with their large number of negative charges (sulphate and carboxyl groups) and hydrophilic nature control the permeability of cartilage and hence play an important role in the biphasic lubrication of cartilage. Depletion of GAGs from cartilage tissue has been shown to result in increased coefficient of friction (COF) levels[10–14]. However, most of these *in vitro* studies have been performed at contact stress levels that fall at the lower end of the physiological loading spectrum.

In an earlier study, it was shown that an increase in nominal stress (up to 0.4 MPa) caused a reduction in the COF of native cartilage under both dynamic and static loading conditions[15], confirming the findings of other *in vitro* studies in the literature on the effect of varying nominal stress levels on cartilage friction[6,16–19]. While the reason for this behaviour is not clear, with the exception of the study by Pickard *et al.*[16], these studies were performed at the lower end of the physiological load spectrum (<0.5 MPa), and were short-term studies that lasted less than an hour. Pickard *et al.* showed that the COF of cartilage articulating against a polished metal plate under boundary lubrication conditions at 4 MPa was smaller than at a contact pressure of 0.5 MPa[16]. The effect of increasing the nominal stress to more physiological levels for extended periods of time, on the friction and wear of cartilage tissue under biphasic lubrication conditions is not known. It is implausible that the COF of cartilage will continue to reduce with increasing

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nominal stress levels since, physiologically, mechanical overloading has been shown to lead to joint injury and osteoarthritis[20]. Additionally, it may be important to consider the tangential friction force as well as the COF at different nominal stress levels.

In the current study, a pin-on-plate machine was utilized in a cartilage articulating against cartilage configuration to investigate the (1) effect of physiological nominal stress levels on the COF of native cartilage and any resulting degradation of the tissue, and (2) examine the hypothesis that, the increase in friction levels due to GAG loss when compared to native tissue, will be pronounced at physiological load levels and may even lead to extracellular matrix (ECM) damage during articulation. Specifically, the objectives included investigating the effect of nominal stress on the COF, deformation and wear of native and GAG deficient articular cartilage at three different nominal stress levels of 0.5 MPa, 2 MPa and 3.15 MPa.

Materials and methods

MATERIALS

Native and GAG deficient cartilage samples

Osteochondral plugs (9 mm diameter) and plates (ca. 2 cm × 2.5 cm × 1 cm) were acquired from the patellofemoral grooves of skeletally mature 18-month-old bovine knee joints (24 h after slaughter) following methods detailed in earlier studies[14,15,21]. Samples were harvested from only those knee joints that looked healthy without any signs of surface fibrillation or discoloration. A total of 36 pairs were harvested from at least 10 different animals and the specimens stored frozen in phosphate buffered saline (PBS) at −20°C until further use. Cartilage specimens were thawed in a water bath at 37°C for an hour before their actual use in any prescribed test, and all the specimens were used within a month of their extraction. Earlier studies comparing the tribological properties of cartilage tissue have shown no significant difference between fresh tissue and cartilage tissue that had undergone just one cycle of freeze–thawing[8,22,23].

GAG deficient cartilage samples (18 pairs) were obtained by treating native cartilage samples with 0.1 U/ml Chondroitinase ABC (CaseABC) enzyme (Sigma) for 24 h in an aseptically prepared buffer solution (pH 8) containing 50 mM Tris–HCl, 60 mM sodium acetate, 0.02% (w/v) bovine serum albumin and an antibiotic solution[14]. CaseABC enzyme at this strength and protocol has been shown to deplete more than 50% of the GAGs from the native cartilage samples[14].

METHODS

Friction tests

A pin-on-plate machine described in detail elsewhere[22,24] was used for all the friction tests. The cartilage pin was loaded and held static against the cartilage plate, which was fixed in a reservoir that reciprocated continuously for a set distance at a set speed. The frictional force between the pin and the plate was transmitted to a piezoelectric force sensor and a data acquisition system continuously logged the voltage output from the force sensor. The force sensor was calibrated with known weights in advance to enable the calculation of frictional force between the pin and plate. COF was calculated as the ratio of this measured frictional force to the applied normal load on the cartilage pin.

Friction tests were conducted at room temperature (20°C ± 2°C) in the Dynamic model (sliding velocity: 4 mm/s; stroke length: 4 mm) at three different nominal stress levels of 0.5 MPa, 2 MPa and 3.15 MPa on both native and GAG deficient cartilage pin and plate couples ($n = 6$). PBS was used as the lubricant with an initial volume of 25 ml at the start of each test. Previous studies have shown that the use of biological lubricants such as hyaluronic acid[21,25], 25% bovine serum[24] do not present any advantage in terms of lowering the COF compared to other neutral lubricants such as PBS and Ringer's in the Dynamic model. The friction data was collected at regular intervals until the end of each test (7 h), after which, the lubricant remaining in the reservoir (~21–23 ml) was collected carefully and stored frozen for further analysis.

Deformation and recovery measurements

The thickness of the cartilage tissue on the cartilage pin specimens, before (T_b) and immediately after (T_a) the friction tests was measured using a Nikon V-16D Profile Projector (1 μm resolution) at 10× magnification [Fig. 1(d)]. Eight measurements were taken around the circumference of each pin to calculate the average thickness of the cartilage tissue. Once T_a was recorded, the recovery of the deformed cartilage tissue was measured to quantify any permanent deformation and creep. Recovery with time was measured using a TRIMOS height vernier scale (resolution: 1 μm) with an attached dial test indicator (DTI) (resolution: 10 μm) that had a spring loaded lever arm as shown in Fig. 1. The combination of these instruments had a resolution of 1 μm and a repeatability of 10 μm. After T_a measurement, the samples were submerged in PBS to measure the recovery of the tissue until it reached equilibrium (around 2 h). The magnitude of recovery at equilibrium was termed T_{eq} [Fig. 1(d)].

The variables Reduction, Recovery and Permanent deformation were defined as shown below, calculated, and plotted based on the thickness and recovery measurements.

$$\%\ \text{Reduction} = \frac{T_b - T_a}{T_b} 100 \tag{1}$$

$$\%\ \text{Recovery} = \frac{T_{eq}}{T_b} 100 \tag{2}$$

$$\%\ \text{Permanent deformation} = \frac{T_b - (T_a + T_{eq})}{T_b} 100 \tag{3}$$

Reduction denoted the loss in thickness of the cartilage tissue at the end of 7 h friction tests due to the deformation under load and sliding. Recovery denoted the reversible creep, which could be recovered by soaking the cartilage samples in PBS after the friction tests until they reached equilibrium without any further increase in thickness. Permanent deformation represented the permanent loss of thickness in cartilage pin samples due to the 7 h friction tests.

Surface roughness measurements

The surface roughness of the cartilage plate samples was measured using a Form Talysurf Series 6 stylus profilometer (Rank Taylor Hobson; sensitivity − 20 nm). Ra was measured as an average of data collected over two 8 mm traces drawn 2 mm apart on the sample surface, both in the direction of translation and perpendicular to it (Gaussian Filter; 0.8 mm cut-off). The cartilage sample surfaces were kept moist by soaking them with PBS while the roughness data was collected.

Lubricant analysis

The lubricant collected at the end of each friction test and stored frozen was thawed and analyzed for its hydroxyproline and GAG contents. Out of the available volume of the lubricant from each test, 15 ml was separated and centrifuged at 40,000 RCF for 30 min at 4°C and subsequently frozen at −70°C overnight. The frozen lubricant samples were then freeze-dried at −40°C and 1 mbar pressure for 48 h. The freeze-dried residue was re-suspended in 1 ml distilled water, and either a hydroxyproline assay[26] indicative of collagen or a dimethylmethylene blue (DMB) assay for sulphated sugars[27] indicative of GAGs, was carried out on this 1 ml concentrate. The estimated hydroxyproline and sulphated sugar concentrations were adjusted back to the original 15 ml starting volumes. The sample size for each test condition was limited to three and three replicates were used for each sample in the assays. Soak controls were developed by soaking native cartilage pin and plate couples without any load or motion applied between them for 7 h in 25 ml PBS ($n = 6$). The PBS collected after the 7 h was analyzed in the same way as described above.

All the results were analyzed using two-factor Analysis of Variance (ANOVA) and pair-wise comparisons performed using Tukey's posthoc analysis.

Results

FRICTION TESTS

The COF increased significantly ($P < 0.01$) with increasing nominal stress for native cartilage (Fig. 2). There was no statistical difference between the friction levels at any of the three stress levels tested for GAG depleted cartilage samples. At 0.5 MPa nominal stress, GAG deficient cartilage samples had a significantly ($P < 0.04$ at 1 h loading time; $P < 0.006$ at 7 h loading time) higher COF when compared to native cartilage samples. However, at 2 MPa

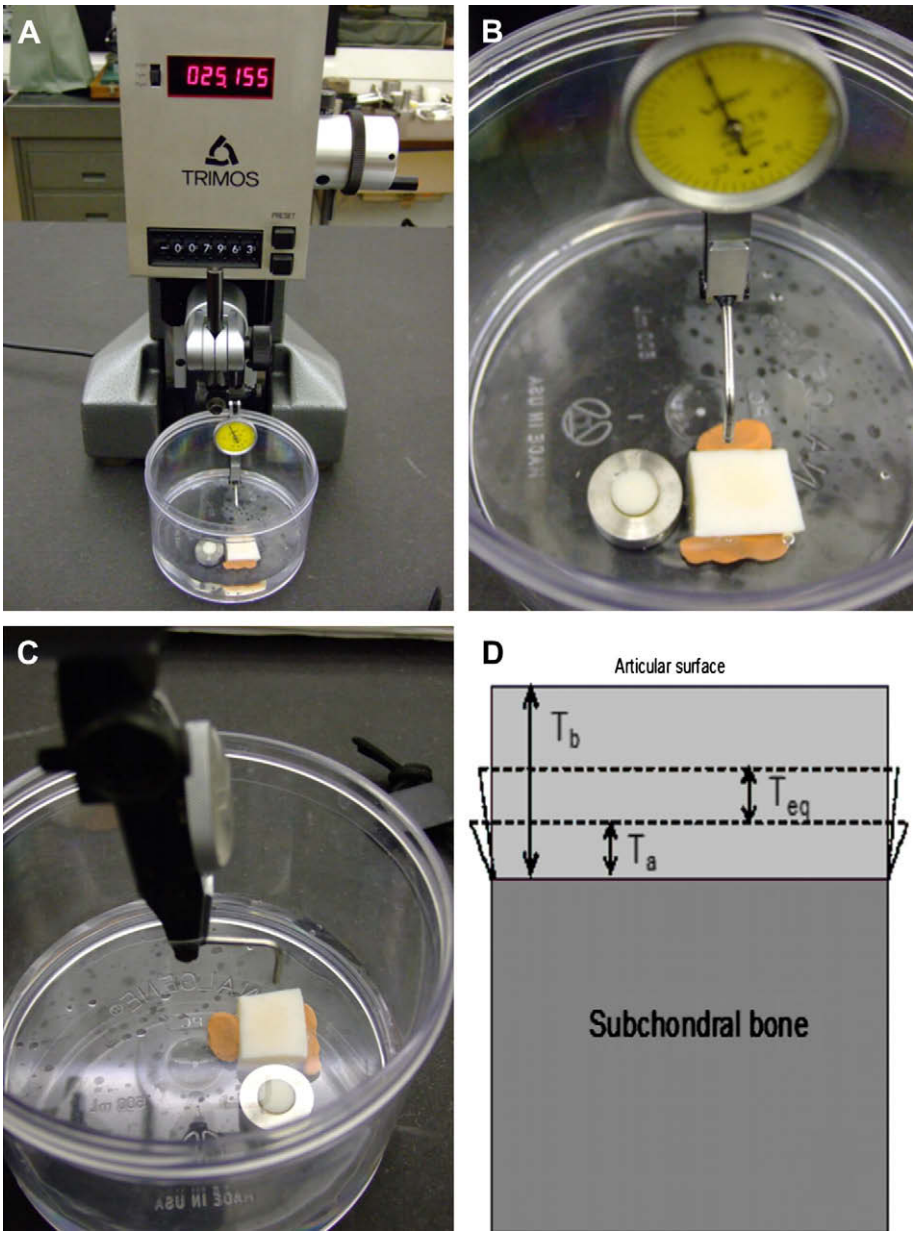

Fig. 1. Height vernier arrangement (1a, 1b, 1c) to measure the deformation and recovery of cartilage pins after friction tests. Exaggerated schematic of the cartilage pin (1d) denoting the variables T_b, T_a and T_{eq} measured during recovery analysis.

($P < 0.04$) and 3.15 MPa ($P < 0.01$), GAG deficient cartilage samples had significantly lower COF when compared to native cartilage samples at 7 h loading time (Fig. 2).

DEFORMATION AND RECOVERY MEASUREMENTS

Nominal stress had a significant effect ($P < 0.05$) on the thickness of cartilage pin tissue observed immediately after the friction tests, and also the permanent deformation in the tissue for both native and GAG deficient articular cartilage (Fig. 3). At 2 MPa, the variables 'Reduction' and 'Permanent deformation' were significantly ($P < 0.05$) lower for native cartilage when compared to the GAG deficient cartilage samples. The highest reduction in cartilage pin thickness immediately after the friction tests, and the highest permanent deformation of about 70% and 25% respectively, were observed at the highest stress level of 3.15 MPa for GAG deficient cartilage samples (Fig. 3). There was no significant difference in the recovery trends for cartilage tissue on the pins under any of the test conditions, except for native cartilage pins tested at 0.5 MPa, which recovered and reached equilibrium much sooner than pins tested under other conditions (Fig. 4).

Cartilage plate samples, especially GAG deficient samples and native samples tested at 3.15 MPa nominal stress, had prominent articulation track impressions in the form of depressions on them after the 7 h friction tests. The depth of these tracks from the surface of cartilage samples was measured using the same vernier height scale apparatus by measuring the average difference between the heights

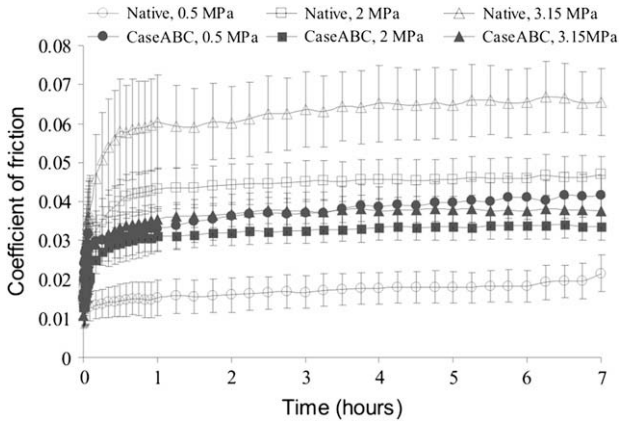

Fig. 2. Effect of contact stress on the COF of native and GAG deficient articular cartilage [error bars: SE ($n = 6$)].

at four points in the articulation track and four points adjacent to the track on each side. GAG deficient cartilage plate samples had much deeper articulation tracks on them when compared to their native counterparts (Table I).

LUBRICANT ANALYSIS

The lubricant (PBS) collected after each friction test appeared clear without any visible wear debris. The soak controls showed no detectable traces of either hydroxyproline or GAGs. Both the nominal stress ($P < 0.04$ for hydroxyproline, $P < 0.01$ for GAGs), and the state of the tissue (native or GAG deficient; $P < 0.05$ for hydroxyproline, $P < 0.01$ for GAGs) had a significant effect on the amounts of hydroxyproline and GAGs lost into the lubricant. For native cartilage tissue, increasing the nominal stress from 0.5 MPa to 3.15 MPa approximately doubled both the hydroxyproline and GAGs lost into the lubricant, indicating wear of the cartilage specimens (Fig. 5). There was a strong positive correlation between the amount of sulphated sugars lost into the lubricant and the permanent deformation observed at the end of friction tests for native cartilage pin specimens ($r^2 = 0.99$).

In the case of GAG deficient cartilage specimens, there was no significant difference between the amounts of

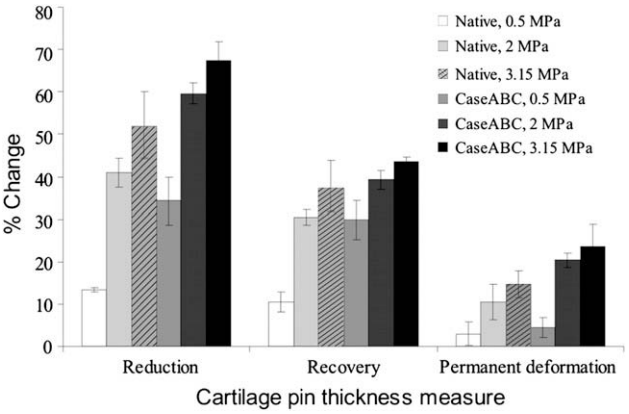

Fig. 3. Reduction, recovery and permanent deformation observed in cartilage pin samples after 7 h friction tests [error bars: SE ($n = 6$)].

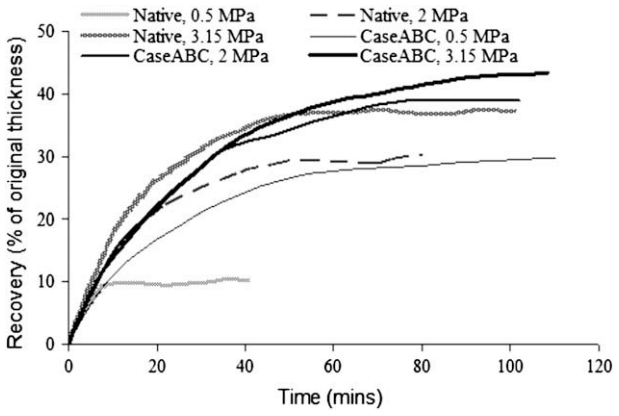

Fig. 4. Average ($n = 6$) recovery trends for cartilage tissue on the pins while soaked in PBS postfriction tests.

hydroxyproline or GAGs lost into the lubricant at any applied nominal stress level. However, in the case of GAGs lost, since all the GAG deficient cartilage specimens were treated with the same strength of CaseABC enzyme prior to testing, only a limited concentration of GAGs would be available in the tissue for exudation under any load. GAG deficient specimens lost significantly lower amounts of hydroxyproline ($P < 0.02$) and GAGs ($P < 0.0005$) into the lubricant when compared to native cartilage specimens at 3.15 MPa, while there was no significant difference between these quantities lost at 0.5 MPa and 2 MPa nominal stress levels between the two types of tissue.

SURFACE ROUGHNESS MEASUREMENTS

Roughness data collected from cartilage plates showed that nominal stress did not have any significant effect on the Ra of the samples. GAG deficient cartilage plates had significantly ($P < 0.001$) lower Ra values than that of native cartilage plates indicating smoothened surfaces for the former (Fig. 6). For cartilage plates, under each test condition, the Ra values measured in a direction perpendicular to the direction of translation in the articulation zone tended to be higher than the corresponding Ra values measured in the direction of translation. This difference was not statistically significant except for GAG deficient cartilage samples tested at the 3.15 MPa nominal stress level.

Discussion

Earlier studies have shown that the COF of native cartilage tissue decreases with increasing nominal stress

Table I
Depth of articulation track on the cartilage plate specimens immediately at the end of the 7 h friction tests

Friction test conditions		Depth of the articulation track (mm) on the cartilage plate samples [mean ± SE ($n = 5$)]
Native cartilage samples	0.5 MPa	No discernible articulation track
	2 MPa	0.087 ± 0.015
	3.15 MPa	0.152 ± 0.015
GAG deficient cartilage samples	0.5 MPa	0.216 ± 0.035
	2 MPa	0.224 ± 0.031
	3.15 MPa	0.324 ± 0.029

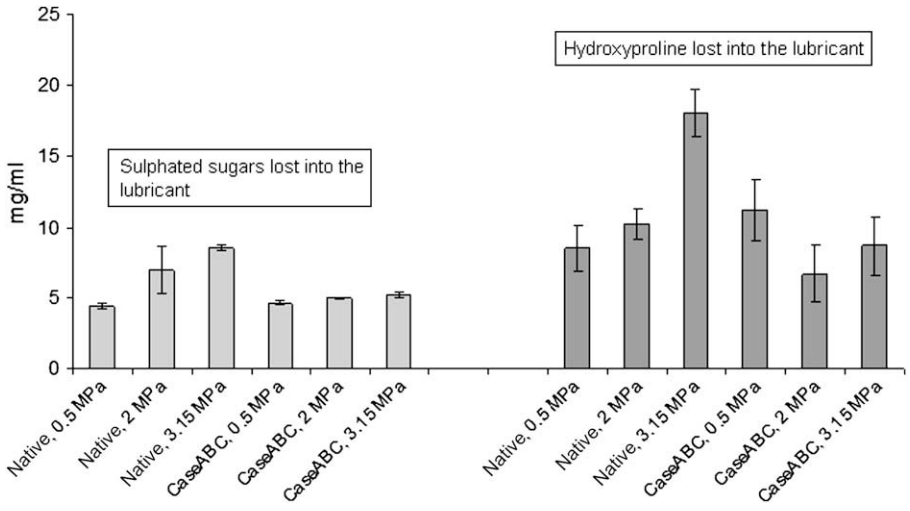

Fig. 5. The amounts [mean($n = 3$) $\pm$ SE] of sulphated sugars (GAGs) and hydroxyproline lost into the lubricant during the 7 h friction tests and estimated using lubricant analysis.

levels[15,16,19] and that the depletion of GAGs from the native cartilage tissue increases its COF[10–14]. However, most of these studies were performed at the lower end of physiological load spectrum and for short durations (<1 h). The current study explored the effect of nominal pressure at three levels – 0.5 MPa, 2 MPa, and 3.15 MPa on the friction and any resultant wear of native and GAG deficient articular cartilage. The duration of each friction test was extended to 7 h to investigate the efficiency of cartilage tissue in maintaining a low COF over extended periods of time at higher loads and the role of GAGs under such conditions.

EFFECT OF NOMINAL STRESS ON THE FRICTION AND WEAR OF NATIVE CARTILAGE

In an earlier study, we reported that under Dynamic model, similar to the one used in the current study, the COF decreased with increasing nominal pressure levels up to a value of 0.4 MPa[15]. Comparison of the COF at 1 h loading time point, showed that the same trend continued and the COF at 0.5 MPa in the current study was lower than that observed at 0.4 MPa in the previous study. However, further increase in the nominal stress levels up to a value of 3.15 MPa led to an increase in the COF. Although different friction rigs were used in these two studies, they were standardized and verified to be producing similar friction levels with both a standard couple (GUR 1120 Polyethylene pin articulating against a 314 stainless steel plate, Ra: 0.005 μm) and a biological couple (bovine cartilage pin articulating against a cartilage plate) under similar test conditions.

A plot of COF against nominal stress at a loading time of 1 h for native cartilage derived from collated data (previous study[15] and the current study) is shown in Fig. 7. The COF was found to initially decrease with increasing nominal stress, until it reached a critical point between 0.5 MPa and 2 MPa, after which it increased rapidly. Even more interesting was the trend observed in the measured tangential frictional force with increasing nominal stress (Fig. 7). The frictional force did not change much at nominal stress levels below 0.5 MPa, but increased non-linearly at higher stress levels. At nominal stress levels less than 0.5 MPa, the fluid load support in the cartilage tissue may have remained unchanged at a very high level, with shear effects between the

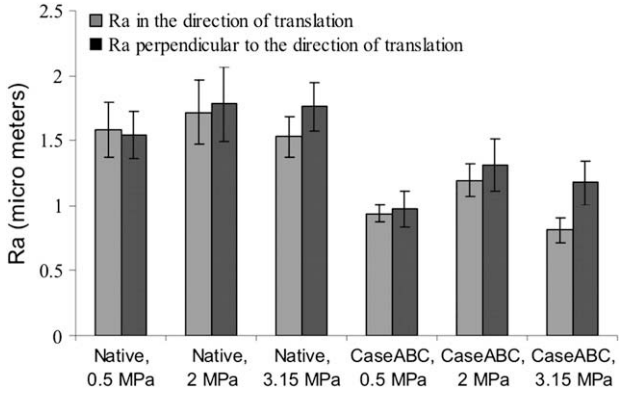

Fig. 6. Surface roughness measures of native and GAG deficient (CaseABC treated) cartilage plate samples after 7 h friction tests under three different contact stress levels [error bars: SE ($n = 6$)].

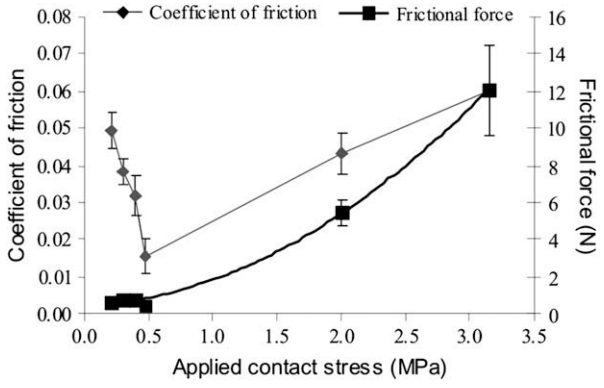

Fig. 7. Variation of friction force and the COF with applied contact stress for native cartilage in a pin-on-plate configuration with a 4 mm stroke length at 1 h loading time [error bars: SE ($n = 6$)].

cartilage surfaces contributing to the small changes in the frictional force. However, at higher nominal stress levels, a reduction in the fluid load support may have compounded with shear effects at the surface to result in an increased frictional force as well as the COF.

The reduction in the fluid load support of native cartilage at higher nominal stress levels was postulated to be due to the failure of the tissue to rehydrate as effectively as it does at the lower nominal stress levels. To test this hypothesis, a preliminary retrospective investigation was carried out by repeating the friction tests with native cartilage at 3.15 MPa nominal stress with a 10 mm stroke length instead of the original 4 mm, and keeping the other parameters unchanged. Increasing the stroke length from 4 mm to 10 mm at 3.15 MPa, decreased the COF levels to those observed at 0.5 MPa nominal stress and 4 mm stroke length (Fig. 8). This finding suggested that while a 4 mm stroke length may provide sufficient time for unloaded cartilage tissue to rehydrate itself and maintain a very high fluid load support at lower stress levels ($<$0.5 MPa), it may not be enough for cartilage tissue rehydration at higher nominal stress levels ($>$2 MPa), leading to a lower level of fluid phase load support and higher frictional forces. Hence, the stress level at which the COF started to increase in the current study may not be unique to cartilage tissue itself, but may depend on factors that determine the rate and amount of cartilage tissue rehydration under any specific set of operating conditions.

The inability of cartilage tissue to rehydrate effectively at short stroke lengths and high nominal stress levels, also explained the raised but steady COF at higher stress levels. Under these conditions, the magnitude of fluid phase load support would have reduced to a lower optimum value that was sustained for the duration of the test. This would increase the load borne by the solid phase of cartilage as manifested by the higher friction levels. An increase in the hydroxyproline and GAGs lost into the lubricant indicated concomitant biochemical deterioration in the tissue and displacement of ECM components into the lubricant. While an increase in nominal stress did not have any effect on the Ra of the cartilage specimens, it did result in higher deformation levels in the cartilage tissue. A permanent deformation of 15% at 3.15 MPa compared to 4% at 0.5 MPa, on native cartilage pins suggested that the ability of cartilage tissue to swell back to its natural state after unloading was diminished under these conditions.

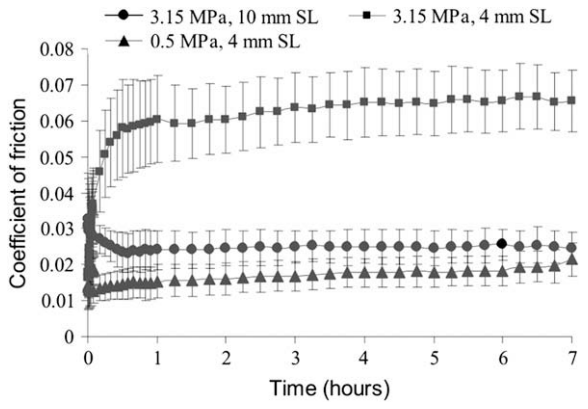

Fig. 8. Effect of stroke length used in the friction tests on the COF levels of native cartilage at 3.15 MPa contact stress [error bars: SE ($n = 6$); SL − stroke length].

EFFECT OF NOMINAL STRESS ON THE FRICTION AND WEAR OF GAG DEFICIENT CARTILAGE

There was no significant difference between the COF levels for GAG deficient cartilage samples at any of the stress levels tested. At 0.5 MPa, GAG deficient cartilage samples had a significantly higher COF than those of native cartilage samples. This was in good agreement with other studies in the literature that have reported an increase in friction with GAG depletion from cartilage[10–14]. The higher COF has been shown to be due to the (partial) loss of biphasic lubrication due to GAG depletion, or the loss of GAGs in the superficial layer on the cartilage surface or a combination of both. At stress levels of 2 MPa and higher, GAG depleted cartilage samples had significantly lower COF and wear compared to their native cartilage counterparts and a closer look at the deformation levels and Ra measures of these samples may reveal the reason behind the findings.

At nominal stress levels of 2 MPa and higher, GAG deficient cartilage pin specimens had lost more than 60% of their initial thickness by the end of the friction tests and failed to recover completely in PBS with a residual deformation of more than 20%. Similarly, the GAG deficient cartilage plate specimens had much deeper articulation track imprints at the end of the friction tests compared to the native cartilage specimens. These high deformation levels observed for GAG depleted cartilage tissue supported our earlier findings that GAG depletion results in a loss of compressive stiffness[14]. Additionally, GAG deficient cartilage plates had significantly lower Ra values than the native cartilage plate specimens (Fig. 6) indicating smoothening of the cartilage surfaces during the test. Based on the deformation and roughness data, it was postulated that the gross morphological changes occurring during the friction tests with GAG deficient cartilage samples may have led to the formation of conforming/congruent surfaces during articulation and an unexpected contribution from some form of hydrodynamic lubrication resulting in low COF levels. This may also have masked any increase in the COF due to the loss of biphasic lubrication. Such a condition may also explain the lower amounts of hydroxyproline lost into the lubricant during the friction tests for GAG deficient cartilage samples when compared to the native cartilage samples. The hypothesis of the study that at higher stress levels, the difference between the friction levels of native and GAG deficient cartilage samples would be pronounced, was rejected based on these findings.

Caution should be exercised while extending/translating these results to an *in vivo* situation. The cartilage specimen acquisition process can sometimes alter the tissue structure and when opposed in a pin-on-plate configuration, these two factors may create loading conditions which do not completely replicate what happens in the joint. A few studies in the literature associate early stage GAG loss in the etiology of osteoarthritis, and one of the broader goals of this study was to examine if GAG loss can lead to surface disruption and degradation of the ECM. No such indications were found, due to the very high deformation levels of GAG deficient cartilage that may have influenced the friction and wear properties. The authors contribute these very high deformation levels partly to the unidirectional translation, constant loading configuration used in this study, and hope to examine this issue further in a more physiologically pertinent experimental configuration involving whole/partial joint condyles under multidirectional motion and dynamic loads simulating gait.

Lubricant analysis provided a simple and reliable albeit indirect, route to characterize the biochemical degradation of cartilage specimens. However, the method in its current form, did not provide any information regarding the spatial distribution of this biochemical degradation on the cartilage specimens, and failed to differentiate between the wear of individual components of a cartilage pin and plate couple. Although no visible surface damage was observed in any of the tests, microscopic evaluation may have gleaned any micro-structural changes at the cartilage surface. In the future, lubricant analysis will be coupled with the recent advances in imaging cartilage tissue using MRI methods to provide comprehensive information about wear of articular cartilage under different *in vitro* test conditions.

Conflict of interest

The authors have no conflict of interest.

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