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Chondroitin sulphate: an effective joint lubricant?

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Summary

Objective: The effect of chondroitin sulphate (CS) treatment on the friction and deformation characteristics of native and glycosaminoglycan (GAG) deficient articular cartilage was investigated.

Methods: Friction tests were conducted at 0.4 MPa load, in Static and Dynamic models, to determine the startup coefficient of friction (COF) and dynamic COF, respectively. *Native cartilage*: For each cartilage pin and plate couple, the COF was determined under three consecutive tests – (1) baseline COF in PBS (2) COF in CS lubricant and (3) COF again in PBS, after 24 h CS treatment. *GAG deficient cartilage*: For each cartilage pin and plate couple, the baseline COF was determined in PBS initially and again following enzymatic treatment to deplete GAGs. The specimens were then soaked in CS solution for 24 h and the COF determined again in PBS. In a similar manner, friction tests were replaced with indentation tests to study the deformation of the tissue.

Results: CS at 50 mg/ml significantly lowered the startup COF of native cartilage both as a lubricant and a treatment solution. In the dynamic model, where the fluid load support is sustained at a high level, CS failed to have any effect on the COF of native cartilage. GAG depletion raised the friction and deformation levels of cartilage, and subsequent CS treatment failed to lower them to their native levels.

Conclusion: CS proved to be an effective lubricant for cartilage under mixed-mode lubrication conditions. However, supplemental CS that diffused into the specimens had no influence on the fluid load support of cartilage.

Key words: Friction, Deformation, Articular cartilage, Chondroitin sulphate, Chondroitinase.

Introduction

Osteoarthritis is a debilitating disease that involves the loss of cartilage tissue in the articulating joints. One of the common approaches for curbing the progression of osteoarthritis is the use of oral supplements of chondroitin sulphate (CS) and glucosamine or variants of these, although their utility is still debatable[1,2]. An alternative approach, that has not been considered might be to directly supplement the affected joints with CS that may contribute significantly to the biomechanical function of cartilage tissue.

Glycosaminoglycans (GAGs) play an important role in the friction and deformation properties of articular cartilage[3]. GAG molecules contain sulphate and carboxyl groups, which due to their highly charged nature, trap water and other small molecules between their chains, creating a Donnan osmotic equilibrium partially responsible for the overall compressive stiffness of the cartilage tissue[4]. The charged nature of these GAG chains, also offers strong resistance to interstitial fluid flow leading to the very low permeability values for articular cartilage. Hence, when a load is applied, the interstitial fluid flow is accompanied by large drag forces that generate pressure gradients which are capable of supporting the applied load until the fluid gets completely exuded away into the joint space or other unloaded regions. As the interstitial fluid pressurization subsides, the applied load is gradually transferred to the solid phase of articular cartilage and at equilibrium conditions, all the load is borne by the solid phase. Forster and Fisher[5] coined the term 'biphasic lubrication' to describe this phenomenon as it was based on the biphasic theory proposed by Mow *et al.*[6] The fluid load support can be maintained at a high level as long as the rehydration of the previously loaded areas of the tissue is allowed. At equilibrium conditions, when the fluid load support is minimal, boundary lubrication comes into effect.

Clinically, a decrease in the rate of GAG synthesis is indicated in the later stages of osteoarthritis[7]. Under *in vitro* experimental setups, GAG loss has been shown to result in raised coefficient of friction (COF) levels[8–12] and deformation[10,13,14] in the cartilage tissue. In such cases, supplementing these joints with CS, the most abundant GAG in articular cartilage, may prove beneficial. In an *in vitro* study, Basalo *et al.*[15] have shown that CS can reduce the friction coefficient of native articular cartilage tissue while articulating against polished glass. The effect of supplemental CS on GAG deficient articular cartilage is yet to be investigated.

The current study examined the hypothesis that CS lubricant/treatment can be an effective contributor to biphasic and mixed-mode lubrication of articular cartilage. An *in vitro* pin-on-disk setup was used to study the effect of CS on the startup and dynamic friction properties of native and GAG deficient articular cartilage in a cartilage articulating against cartilage configuration. Indentation tests were used to study

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the effect of CS treatment on the deformation of cartilage tissue. A qualitative fluorescence microscopy study was undertaken to investigate the localization of supplemental CS into cartilage tissue.

Materials and methods

MATERIALS

Cartilage samples

Articular cartilage pin (9 mm diameter) and plate (*ca* 20 mm × 25 mm × 10 mm) samples were acquired from patello-femoral grooves of 18 month old bovine knee joints with the help of specially designed drill-aided corers and a hand-saw. The subchondral bone (~10 mm thick) was retained along with the cartilage tissue for ease of handling the specimens and proper fixation during the friction and indentation tests. More than 36 pairs of pin and plate specimens were harvested from 12 different joints obtained from 12 different animals. Cartilage plate and pin specimens were stored frozen (−20°C) in PBS until further use. Prior to any testing, specimens were defrosted in a water bath at 37°C. Cartilage specimens were used within a month of acquisition. 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[16–18].

Chondroitinase ABC (CaseABC) enzyme solution

CaseABC enzyme (from Proteus Vulgaris, Sigma) was utilized to deplete GAGs from cartilage. Cartilage specimens were treated for 24 h at 37°C with the enzyme solution at a concentration of 0.1 U/ml in an aseptic buffer solution (pH 8) containing 50 mM Tris−HCl, 60 mM sodium acetate, 0.02%(w/v) bovine serum albumin and an antibiotic solution[10]. CaseABC enzyme at this strength and protocol has been shown to deplete more than 50% of the GAGs from native cartilage samples[10].

CS lubricant

CS was tested at two different concentrations − 10 mg/ml and 50 mg/ml dissolved in PBS. CS 10 mg/ml represented the physiological concentrations of CS isomers found in bovine cartilage tissue[19,20], and CS 50 mg/ml was chosen to represent a supersaturated CS solution that can be used for joint supplementation. Preliminary dimethylmethylene blue (DMB) assays showed that 50 mg/ml CS increased the CS concentration of the tissue significantly. CS was made by mixing CS A (Biochemika, 27042), CS B (Sigma, C3788), and CS C (Sigma, C4384) in the ratio of 1:0.1:1 at both the concentrations. This ratio was derived from the literature which estimated the concentrations of different types of CS in the articular cartilage of 1.5−2 years old heifers[20]. Cartilage samples were treated with CS solution for 24 h at 4°C under mild agitation using a shaker at 300 rpm.

METHODS

All the friction tests were conducted on a reciprocating motion pin-on-plate machine[21] at a load of 25 N (0.4 MPa nominal contact stress). The cartilage pin was loaded and held static against the cartilage plate, which was fixed in a reservoir that could reciprocate 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 that was pre-calibrated with known weights, 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. Two friction models were used − Dynamic (4 mm/s sliding velocity; 4 mm stroke length; 1 h long) that measured the COF under biphasic lubrication conditions, and Static model (4 mm/s startup velocity) that recorded the startup COF after a constant period of pre-loading (5 s, 0.5, 2, 5, 10 and 20 min) under mixed-lubrication conditions. Previous studies have shown that in the Dynamic model COF remains low for long duration[10,21,22] due to effective biphasic lubrication associated with cyclic unloading of the cartilage plate. In contrast, in the Static case, the friction rises with increasing pre-loading time indicating reduced fluid load support, increased solid phase contact and a high dependency on lubrication mechanisms such as boundary[5,21]. A low sliding speed of 4 mm/s was adopted to ensure the contacts remained in a mixed-mode lubrication regime during sliding and hence eliminating any fluid film formation between the articulating surfaces[5,16].

Effect of CS treatment on native cartilage

For each native cartilage pin and plate couple, the baseline COF was determined initially in PBS lubricant. PBS was then replaced with CS lubricant, and the friction test was repeated on the specimens with a recovery period of at least 2 h (in PBS) between the tests. Immediately after the second friction test, the same cartilage specimens were soaked in freshly prepared CS solution for 24 h and the friction test repeated again in fresh PBS lubricant. The process was repeated for both CS solution concentrations, 10 mg/ml and 50 mg/ml, with a set of new cartilage specimens and under both Static and Dynamic friction models. Additionally, the effect of soaking cartilage specimens in plain PBS for 24 h was studied using a control group consisting of six pairs of native cartilage pins and plates. The same steps outlined above were followed except that these specimens were treated with PBS for 24 h instead of CS solution before determining the repeat COF.

Effect of CS treatment on GAG deficient articular cartilage

Initially, for each cartilage pin and plate couple, the baseline COF was determined in PBS in the Dynamic friction model. The cartilage pin and plate specimens were then treated with the CaseABC enzyme as described above and the COF measured again in PBS. The same cartilage pin and plate specimens were then treated for 24 h with CS solution (50 mg/ml) at 4°C and the friction test repeated in PBS lubricant to measure the COF. Static friction model was not used with GAG deficient cartilage since it has already been shown in an earlier study[10] that loss of GAGs did not result in an increased COF under such conditions.

In addition to the friction characteristics, the effect of CS treatment on the deformation of GAG deficient cartilage was studied in a similar fashion to that described above, except that the friction tests were replaced by indentation tests[10,21]. The indentation tests were carried out with a solid flat-ended stainless steel 3 mm diameter indenter against cartilage plate specimens in a PBS bath. The deformation of the tissue with time was recorded under a creep load of 1 N (0.14 MPa contact stress) for 50 min. At the end of the indentation tests, the thickness of the cartilage tissue on the plate specimens was estimated using a needle indenter.

The results from the friction and indentation tests were analyzed using Analysis of Variance (ANOVA) within-subject design, and pair-wise comparisons were performed using Bonferroni *post-hoc* analysis[23].

DMB assay

The 1, 9-dimethylmethylene blue (DMB) assay[24] was used to estimate the amount of sulphated sugars (representative of GAGs) in native cartilage, and samples treated with CS 10 mg/ml and 50 mg/ml for 24 h at 4°C under mild agitation (DMB1). Nine previously unused cartilage pins from three different animals (three pins per animal) were used in the assay.

In a second assay (DMB2), three native cartilage pins (all from different animals; previously unused) were treated with CaseABC for 24 h at 37°C followed by CS 50 mg/ml for 24 h at 4°C. GAG content was estimated in these specimens while they were in their native condition, after CaseABC treatment and after CS 50 mg/ml treatment. Prior to both the assays, cartilage tissue was lyophilized and treated with Papain buffer for 48 h at 60°C to hydrolyse the tissue. Previous studies have reported a GAG content between 2% and 5% wet weight for native bovine cartilage[8–10,25]. Data was analyzed using ANOVA between-subject and within-subject designs for DMB1 and DMB2, respectively.

Fluorescence microscopy

The localization of CS in the cartilage tissue was visualized using Fluorescein-amine (MW = 347.32 g/mol) conjugated chondroitin sulphate (CSF) and fluorescence microscopy techniques. The conjugation was achieved by activating random OH groups of the CS with cyanogen bromide to form very reactive cyanate ester intermediates on the surface of CS which were immediately reacted with Fluorescein-amine to form stable isourea bonds between the carbohydrate and the dye[26–29].

Native cartilage pins treated with plain CS (50 mg/ml); native and GAG deficient cartilage treated with 10 mg/ml and 50 mg/ml CSF for 24 h, were sectioned transversely through the depth (~50 μm sections) and viewed under a Zeiss upright LSM510 microscope (Argon 488 nm laser, Standard FITC filter) to record the localization of CS.

Rheology

The shear viscosity of PBS, CS 10 mg/ml, and CS 50 mg/ml was determined using a Bohlin CVO 120 rheometer in a cone on plate configuration (cone angle: 1°, diameter: 60 mm) at 20°C. Approximately 0.8−1 ml of the sample was loaded on the rheometer plate and the cone lowered to a predetermined level of 30 μm above the plate. The shear viscosity experiments were carried out in 10−1000 s^{-1} shear rate range and the viscosity was measured as a function of increasing shear rate. Earlier studies have indicated that the viscosity of a lubricant can influence its ability to lubricate cartilage tissue[22,30,31].