EFFECT OF GLYCOSAMINOGLYCAN LOSS ON THE DEFORMATION AND FRICTION PROPERTIES OF ARTICULAR CARTILAGE

INTRODUCTION
Glycosaminoglycans (GAGs) have been shown to be responsible for the Donnan osmotic swelling pressure or the interstitial fluid pressurization of articular cartilage and hence its biphasic nature, compressive stiffness and load bearing properties. Contradictory results have, however, been presented in the literature about the role of GAGs on cartilage friction properties5–7. The current study explored the role of GAGs on the deformation properties of cartilage and its friction properties under various tribological conditions in a cartilage sliding against cartilage configuration.

MATERIALS AND METHODS
Materials: Cartilage plates (20mm x 15mm) and pins (9mm dia) with the underlying cancellous bone (10mm thickness) were obtained from the patellofemoral grooves of 18 month old bovine knee joints. Samples were stored frozen in PBS and used within a month of acquiring them.

Enzymatic Treatment: The effect of depleting GAGs from the cartilage tissue was studied by treating the samples with chondroitinase ABC (CABC). The enzyme was used at a concentration of 0.1 units/ml with an aseptically prepared buffer solution containing 50mM Tris-HCl, 60mM sodium acetate, 0.02% (w/v) bovine serum albumin, protease inhibitors and antibiotics to inhibit microbial growth during the incubation of cartilage samples at 37°C for 24 hours.

Friction Tests: A reciprocating motion pin-on-plate friction rig was used to perform the study. Tests were divided into three models (n=6 each) based on the sliding conditions – Dynamic 4 (4mm/s sliding velocity; 4mm stroke length), Dynamic2 (2mm/s sliding velocity; 2mm stroke length), and Static model (4mm/s startup velocity) to provide different loading and unloading conditions on articular cartilage.

For each pair of cartilage pins and plates, the baseline friction curve was determined under 25N load with PBS as the lubricant. The samples were then treated with CABC as described above. The treated samples were washed with PBS and the friction test repeated under identical conditions to determine the friction curve after CABC treatment. Controls were subject to the same procedure but treated only with the buffer solution minus enzyme. The ratio of friction coefficients for each pair of cartilage pin and plate before and after enzyme treatment were calculated and compared to similar ratios from their respective controls. Results were analyzed by ANOVA.

Roughness Measurements: 7 cartilage pins and 3 cartilage plates were measured for their surface roughness before and after CABC treatment using a TalySurf stylus profilometer. Each Ra reading was an average of data collected over four 8mm traces drawn on each sample surface.

Indentation Tests: Indentation tests were carried out on cartilage plates (20mm x 15mm; n=5) with a 3mm diameter flat-ended indenter at a load of 1.9N in a PBS bath before and after CABC treatment to determine the loss of any compressive and biphasic properties after the loss of GAGs from the tissue.

GAG Assay and Staining: The amount of GAGs lost due to CABC treatment was evaluated by 1,9-dimethylmethylene blue (DMB) assay for sulfated proteoglycans and Alcian blue staining of cartilage samples (n=3) before and after CABC treatment of cartilage samples.

RESULTS
The DMB assay showed that CABC treatment reduced the % wet weight of GAGs from 2.42±0.23 (mean±SE) in the native tissue to 1.45±0.08 in the enzyme treated tissue and the loss of GAGs was confirmed by the Alcian blue staining of the cartilage sections (fig 1). CABC treatment did not have any significant effect on the surface roughness of cartilage samples. The ten cartilage samples had an average Ra of 1.01±0.147µm (mean±95% CI) compared to the average Ra of 1.05±0.197µm (mean±95% CI) after chondroitinase ABC treatment. CABC treatment did not have any significant effect on the surface roughness of cartilage samples. The ten cartilage samples had an average Ra of 1.00±0.147µm (mean±95% CI) compared to the average Ra of 1.04±0.197µm (mean±95% CI) after chondroitinase ABC treatment. The baseline friction levels were the lowest in the Dynamic 4 model, and highest in the Static model with Dynamic 2 model lying in between. Depleting GAGs from the cartilage tissue by CABC treatment increased the friction levels in Dynamic 4 model by more than 50% at the end of one hour tests (fig 2). No statistically significant changes were observed in Dynamic 2 and Static models. Indentation tests showed that the deformation in GAG depleted samples was almost six times that of native cartilage (fig 3) indicating a substantial loss in compressive stiffness.

DISCUSSION
The surface roughness of cartilage samples did not increase after CABC treatment indicating that the increase in friction levels in Dynamic 4 model could only have been due to the loss of interstitial fluid pressurization and hence a loss in biphasic lubrication, as a result of loss of GAGs from the tissue. Similar increase in friction levels were found in the study by Basalo et al2. In static and Dynamic 2 models, where the contact zone was loaded most of the time and boundary lubrication was active, friction levels were found to be unchanged consistent with the findings of Pickard et al1. In these models, macromolecules in the superficial zone of cartilage unaffected by CABC treatment, and/or large compressive strains induced in the matrix of the CABC treated samples appear to control the friction properties.

The large deformation of the CABC treated samples appears to be due to the loss of the compressive modulus of the samples, which would be mainly dependent on the interstitial water content and the integrity of proteoglycan network in the samples. Normalized deformation curves (not shown) suggested that CABC treated samples deformed at a higher rate than untreated samples indicating an increase in the permeability of the GAG depleted samples.

The study is the first to report the role of GAGs in cartilage friction properties under various tribological conditions in a cartilage-on-cartilage model, and also resolves the contradicting results presented in the literature1,2. The increase in friction levels and the loss of compressive stiffness of the tissue due to a loss in GAGs can compromise the mechanical integrity of the tissue and can lead to further damage similar to that found in clinical OA of articular cartilage.