INTRODUCTION:
Loss of glycosaminoglycans (GAGs) is indicated in the etiology of osteoarthritis and other joint diseases. In such cases, supplementing these joints with chondroitin sulphate (CS), may provide biomechanical and tribological benefits. This in vitro study investigated the effect of CS treatment on the friction properties of native and GAG deficient articular cartilage under boundary and biphasic lubrication conditions. The transport of CS into the cartilage tissue was studied using biochemical assays and fluorescence microscopy.

MATERIALS & METHODS:
Cartilage plates (ca. 20mm x 15mm x 10mm) and pins (9mm Φ) with the underlying cancellous bone (10mm thickness) were collected from the patello-femoral grooves of 18 month old bovine knee joints. A reciprocating motion pin-on-plate machine was used for friction tests at a load of 25 N (0.4 MPa nominal contact stress). Two friction models were used (n=6 each) – Dynamic (4mm/s sliding velocity; 4mm stroke length) that measured the coefficient of friction (COF) under biphasic conditions, and Static model which recorded the startup COF after a constant period of loading, under boundary lubrication conditions. Effect of CS treatment on native cartilage: For each cartilage pin and plate couple, the COF was determined under three consecutive tests – (1) baseline COF in PBS lubricant (2) COF in CS lubricant and (3) the same cartilage samples were treated for 24 hours with CS solution, and COF determined again in PBS lubricant. Chondroitin sulfate (CS A: CS B: CS C – 1: 0.1:1) was used at 50 mg/ml in PBS. COF was determined under both Static and Dynamic models. Effect of CS treatment on GAG deficient articular cartilage: Initially, for each cartilage pin and plate couple, the baseline COF was determined in PBS (Dynamic model). The cartilage samples were then treated with 0.1 U/ml chondroitinase ABC (CaseABC) enzyme for 24 hours at 37°C to deplete GAGs in the tissue [1], and the COF measured in PBS. The samples were then treated for 24 hours with CS solution (50mg/ml) at 4°C and the friction test repeated in PBS lubricant to measure the COF. Dimethylmethylene blue (DMB) assay: DMB Assay was used to estimate the amount of sulphated sugars in native cartilage, CaseABC treated cartilage and samples subsequently treated with CS 50 mg/ml. Fluorescence Microscopy: The transport of CS into the cartilage tissue was visualized using fluorescein conjugated chondroitin sulphate (CSF). Thin transverse (~50 μm) sections of native cartilage and GAG deficient cartilage treated with 50 mg/ml CS (CS:CSF – 9:1) for 24 hours, were viewed under a Zeiss upright LSM510 microscope (Argon 488 nm laser, Standard FITC filter).

RESULTS:
DMB assay results showed that native cartilage had a GAG content (% wet weight of tissue; mean (n=3);±SE) of 1.37±0.21, while CaseABC treatment reduced it to 0.82±0.39, and subsequent treatment with CS 50mg/ml solution raised it to 3.76±0.43. Fluorescence microscopy with fluorescein conjugated CS confirmed the transport of CS into both native (Figure 1a) and GAG deficient cartilage tissue (Figure 1b).

DISCUSSION:
In the Static friction model, due to the loss of fluid load support due to the continuous translation of contact area. Under such conditions, CS reduced the startup COF levels and proved to be an efficient boundary lubricant for native cartilage, as shown in an earlier study [2].

Dynamic friction model ensures biphasic lubrication regime and very high fluid load support due to the continuos translation of contact area. Under such conditions, CS in the lubricant or treating the cartilage samples for 24 hours with CS provided no additional reduction in the COF of native cartilage. While fluorescence microscopy showed that supplemental CS penetrated deeper into GAG deficient cartilage when compared to native cartilage, it still failed to lower the increased COF of GAG deficient cartilage to that of native cartilage. These findings suggest that the supplemental CS that diffused into the samples may have failed to conjugate with the extracellular matrix of cartilage leading to its exudation under load and having no influence on the fluid load support of cartilage. Future studies will investigate methods of integrating supplemental CS to the ECM of cartilage.

REFERENCES: