INTRODUCTION: Atomic force microscopy (AFM) techniques are increasingly used for tribological studies of engineering and biological surfaces at microscale levels [1-3]. To the best of our knowledge, the frictional coefficient of articular cartilage has not been characterized at the microscale level, yet such measurements may yield valuable insight into the frictional response of the tissue and the mechanisms responsible for its remarkable tribological properties. The objective of the present study is to compare micro- and macroscale frictional coefficients of bovine articular cartilage by using a relatively large AFM spherical probe tip and applying previously reported methodologies [4,6].

METHODS: Twelve cylindrical osteochondral plugs were harvested in pairs from adjacent positions in three fresh bovine humeral heads (4-6 months old), and divided into two groups for AFM (Ø 8 mm) and macroscope (Ø 4.78 mm) friction measurements. All samples were refrigerated at 4°C and tested within 3 days, but never frozen. AFM Measurements: Samples in the AFM group were glued on the bony side to 60 mm polystyrene petri dishes using cyanoacrylate glue. Frictional signal (trace and retrace) and height imaging was conducted with samples submerged in PBS on a Bioscope AFM (Digital Instruments). Novascan polystyrene Ø 5 µm spherical AFM probes (Veeco Metrology) with nominal spring constants of k=0.32 N/m were used. The mounted probe was submerged in PBS for at least 20 min prior to imaging to allow for thermal equilibration at room temperature. The sample was moved in the perpendicular direction to the cantilever beam at a velocity of 200 µm/s and slowly stepped (by 0.39 µm) in the parallel direction (1.56 µm/s) to scan a 100 x 100 µm area (128x128 pixels). The friction voltage signal (half of the difference between trace and retrace scans, Fig. 1) was converted to units of force using a conversion factor based on a calibration test on platinum [4], and the applied normal deflection voltage signal was converted to units of force using the AFM deflection sensitivity (53.88 nm/V) and k of the cantilever beam. The measurement of the frictional force was performed at 5 step increments of the normal load at each location and the friction versus normal force response was fitted with a straight line whose slope is the frictional coefficient (μAFM, Fig. 2). The surface roughness (Rq) was measured over the same 100 x 100 µm test area (n=18). Finally, the applied stress and effective modulus at each location were estimated from additional indentation tests at three different locations in the test area, using a Hertz contact model. All these measurements were repeated at three different locations on each sample.

Macrosopic Measurements: Macrosopic frictional measurements were performed on the paired samples versus a custom friction device [5]. Sliding motion (200 µm/s) was provided by a computer controlled translation stage (Model PM500-IL, Newport Corporation, CA). Normal loads (3.6 N, 200 kPa) were prescribed via a voice-coil force actuator (B&H Kimco Magnetics Division, CA) connected in series with an LVDT for displacement measurements (Schaeve Sensors, VA). All loads were measured with a multiaxial load cell mounted on the translation stage (Model 20E12A-M25B, JR3 Inc., CA). Specimens were loaded using a polystyrene plate (same material as AFM probe tip). From the measured time-dependent normal and frictional force, the minimum (μmin) and equilibrium (μeq) frictional coefficient can be calculated (Fig. 3). One-way ANOVA with repeated measures was performed to investigate statistical differences between μmin, μeq, and μAFM (SAS Institute Inc., NC) with an α= 0.05. Posthoc testing of means was performed using Bonferroni adjustments.

RESULTS: Average±standard deviation values of μAFM (0.124±0.048, n=4) and μeq (0.111±0.021, n=6) exhibited no statistical differences (p=0.48). However, μmin (0.005±0.001, n=6) was significantly smaller than μeq (p=0.0001) and μAFM (p=0.0007). The surface roughness Rq was 452±203 nm (n=18). There was no significant correlation between Rq and μAFM, μeq, or μmin (R²=0.17, 0.04, and 0.14, respectively). The AFM applied stress ranged from 8.3±2.2 kPa to 15.0±2.9 kPa and the load-dependent effective modulus ranged from 49.7±17.0 kPa to 63.1±18.5 kPa (n=3x3x6=54).

DISCUSSION: Standard AFM techniques for measuring friction at the microscale were successful with articular cartilage (Figs. 1&2). Though different stress levels were applied at the microscale (~15 kPa) and macroscale (200 kPa), μAFM was comparable to μeq. These results suggest that the AFM frictional coefficient is a measure of the macroscope equilibrium and not the minimum frictional coefficient. It is known that the macroscope frictional coefficient increases with decreasing cartilage interstitial fluid load support as shown in our recent study [5]. This time-dependent response is governed by the gel time constant for interstitial fluid flow, which is ~1000s at the macroscope (Fig. 3). At the microscale however, fluid pressurization drops rapidly after load application because the small size of the AFM probe (2.5 µm radius) yields a very short gel time constant (estimated at ~10 ms), which is significantly smaller than the friction testing duration with AFM. Hence AFM results yield the frictional coefficient in the absence of interstitial fluid pressurization and can be used to assess the boundary lubrication properties at the surface of the cartilage matrix. In this study no significant relationship between surface roughness and frictional coefficient was observed, consistent with findings in other materials [6,7]. The compressive modulus of cartilage estimated from AFM (~56 kPa) is also consistent with alternative measurement methods yielding a modulus on the order of 100 kPa in the most superficial zone of bovine articular cartilage [8]. In future studies AFM probes may be coated with various boundary lubricants to assess their effectiveness on cartilage surfaces.

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