BOVINE ARTICULAR CARTILAGE SURFACE TOPOGRAPHY AND ROUGHNESS IN FRESH VERSUS FROZEN TISSUE SAMPLES USING ATOMIC FORCE MICROSCOPY

Bryte J. Moa-Anderson, Kevin D. Costa, Clark T. Hung, and Gerard A. Ateshian

Department of Biomedical Engineering
Columbia University
New York, NY

INTRODUCTION

The surface roughness and physical characteristics of the superficial layer of cartilage play important roles in understanding the frictional properties and load bearing mechanisms in articulating joints. Several fluid film and boundary lubrication models for the frictional response of articular cartilage have been proposed. Fluid film lubrication, which includes hydrodynamic, elastohydrodynamic, and micro-elastohydrodynamic modes, requires a minimum fluid film thickness of three times the surface roughness of cartilage to remain viable [1,2]. Other lubrication models, such as weeping and boosted lubrication [3,4], are premised on the existence of peaks and valleys on the articular surface where synovial or cartilage interstitial fluid is trapped. Under loading conditions detrimental to fluid film lubrication, some form of boundary lubrication is also believed to exist [5]. Boundary lubrication theories assume that the boundary lubricant is contained in synovial fluid and is adsorbed onto the cartilage surface, or is synthesized by chondrocytes in the superficial zone [6-8]. The superficial layer of cartilage has been physically described as a highly viscous, electron dense, non-fibrous, superficial layer between 0.3 and 1μm thick [9]. In order to evaluate the plausibility of these lubrication theories, the characteristics of uppermost layer(s) of cartilage must be further investigated. Furthermore, the effects of sample preparation and testing methods must be carefully considered. In this study, we report measurements of articular surface roughness using atomic force microscopy (AFM), and investigate the effect of freezing on these surface characteristics.

MATERIALS AND METHODS

Test Materials

2-4 month old bovine humeral head cartilage explants were harvested from fresh shoulder joints. The intact joints were refrigerated, but never frozen, for a maximum of two days. Two samples from each of six joints were cored from adjacent positions on the articular surface of the humeral head, immediately upon resection of the joint capsule. The bony substrate was trimmed from the samples such that 2-3mm remained under the articular layer. The samples were then glued on the bony side to 60 mm polystyrene petri dishes using a cyano-acrylate glue. During specimen harvesting, samples were irrigated with phosphate buffered saline (PBS), and were subsequently immersed in PBS solution within 15 seconds of gluing. One of the samples from each pair was frozen at -28°C for 24 hours before being imaged, and the other was imaged within 3 hours of harvesting.

AFM Imaging: Imaging was conducted with samples submerged in PBS on a Bioscope AFM (Digital Instruments). Unsharpened Microlever AFM probes (Veeco Metrology) with nominal spring constants of 0.01 - 0.02 N/m were used. The mounted probes were submerged in PBS for at least 20 min prior to imaging to allow for thermal equilibration at room temperature. The scan rate was 1 line/sec for all samples with a scan size of 100×100×12 μm and an image size of 512×512 pixels. The contact force was sufficiently small to avoid detectable damage to the samples, as verified by repeated scanning. Height images were recorded in contact mode at 3 to 5 locations on each sample, and rendered in grayscale with higher features displayed brighter.

Analysis: Average surface roughness, $R_a = \frac{1}{N}\sum_{j=1}^{N}\left|z_j\right|$, where z_j is the height deviation from the mean plane and N is the number of pixels in the region of interest [10], was measured for all height images using Digital Instruments Nanoscope III software. No pre-processing of the original image was performed. R_a was determined for the entire 100×100μm image ($R_{a\text{-}100}$), and for 10×10μm sub-regions sampled at 9 locations across the image and averaged ($R_{a\text{-}10}$). Each image was also characterized according to its surface structure as described below. Surface roughness measurements were compared using ANOVA to examine the effects of storage (fresh n=22, frozen n=25) and surface structure (amorphous n=14, fibrillar n=12, mixed n=21). Post hoc analysis was based on Sheffe's S test, and statistical significance was accepted for $p<0.05$. Results are presented as mean±SD.

RESULTS

The full-image roughness, $R_{a\text{-}100}$, was 450±237 nm for fresh samples and 495±189 nm for frozen samples. Local roughness, $R_{a\text{-}10}$, was

72±23 nm and 65±24 nm for fresh and frozen samples, respectively. No significant difference in either surface roughness was found between fresh and frozen samples (p>0.3).

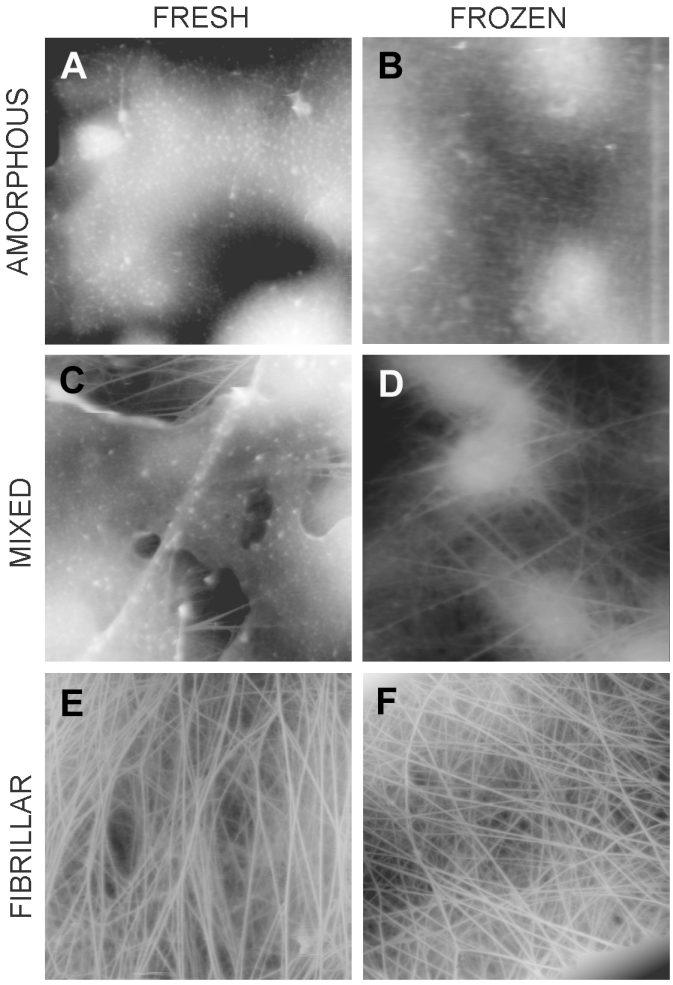

Figure 1: AFM images of articular cartilage surface. (100x100µm field of view, gray scale = 8 µm)

The fine surface structure varied among samples and was broadly classified into one of three categories. An amorphous superficial layer was observed in some of the samples (Fig. 1a-b), which obscured the underlying collagenous tissue. In other samples, the surface structure was highly fibrillar, with no evidence of the amorphous layer (Fig. 1e-f). And in some cases the structure was a mix of fibrillar and amorphous features (Fig. 1c-d) [9]. Local roughness, $R_{a\text{-}10}$, was significantly lower for the amorphous (51±15 nm) and mixed (65±20 nm) samples than for the fibrillar samples (94±13 nm, $p<0.0005$), though the difference between amorphous and mixed samples was not significant at the accepted level (p=0.062). The gross surface structure also varied among samples. For most images (34 of 47) multiple surface protrusions varying from 2 to 6 µm in height were observed in the 100×100µm region (Fig. 2). These features dominated $R_{a\text{-}100}$, which was significantly greater with protrusions than without (552±187nm vs 271±120nm, p=0.0001). It was also noted that of the 34 images with protrusions, 32 images were classified as either amorphous or mixed (94%), and only 2 were classified as fibrillar (6%). In contrast, of the 13 images without protrusions, only 3 were classified as amorphous or mixed (23%) and 10 were classified as fibrillar (77%). Consequently, local $R_{a\text{-}10}$ was significantly lower with protrusions than without (62±19nm vs 87±26nm, p=0.001).

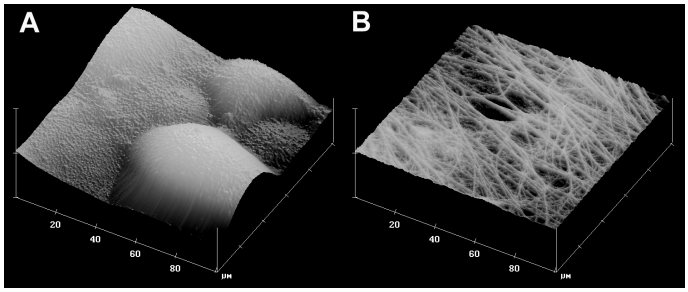

Figure 2: 3-D rendering of surface regions with (A) and without (B) large surface protrusions. Prominent feature in (A), presumably a chondrocyte, is 5µm high and 60µm long. (vertical scale=8µm)

DISCUSSION

Analysis showed a hierarchy of surface roughness which must be differentiated based on scale. The local roughness in a 10µm region is dominated by the surface structure, and ranged from approximately 50 to 100 nm. However, the roughness of a 100µm region was typically ~500nm, comparable to measurements on newborn calf cartilage using traditional testing methods [11]. This measurement is dominated by features that are 100 times the scale of collagen fibrils. These larger protrusions on the cartilage surface appear to signify the presence of chondrocytes residing below the articular surface (Fig. 2). From images such as Fig. 1c, the superficial layer was found to be approximately 300-800 nm thick, consistent with previous studies [9]. This layer was not disrupted by multiple AFM scans of varying contact force. However, wiping the sample with a latex-gloved finger was sufficient to reveal the underlying collagen fibril network during the subsequent scan, indicating the delicate nature of this superficial layer. The presence of the amorphous superficial layer seems strongly correlated with the observation of the surmised chondrocyte structures. In conclusion, freezing of articular cartilage was shown to have no significant effect on surface roughness or superficial structure. However, contact with the cartilage surface could significantly disturb the superficial layer. While this may not affect large-scale surface roughness measurements, it may be critical to investigations of micro-scale roughness and frictional properties.

Acknowledgements: National Institutes of Health AR43628.

REFERENCES

1. Dowson, D., and Jin, Z-M. (1986) J. Eng. Med., 15:63-65.
2. Hamrock B.J. (1994) Fundamentals of Fluid Film Lubrication. McGraw-Hill, New York.
3. McCutchen, C.W. (1962) Wear, 5:1-17.
4. Walker, P.S., Dowson, D., Longfield, M.D., and Wright, V. (1968) Ann. Rheum. Dis., 27:512-520.
5. Charnley, J. (1959): The lubrication of animal joints. In: Symposium on Biomechanics. pp.12-22. IMechE, London.
6. Radin, E.L., Swann, D.A., and Weisser, P.A. (1970) Nature (London), 228:377-378.
7. Schumacher, B.L., Hughes, C.E., Kuettner, K.E., Caterson, B., and Aydelotte, M.B. (1999) J. Orthop. Res., 17:110-120.
8. Hills, B.A. (2000) Proc. Instn. Mech. Engrs. Part H, 214:83-94.
9. Jurvelin, J.S., Muller, D.J., Wong, M., Studer, D., Engel, A., and Hunziker, E. (1996) J. Struct. Biol., 117:45-54.
10. Surface texture, surface roughness waviness and lay, ASME B46.1, 1995
11. Sayles, R.S., Thomas, T.R., Anderson, J., Haslock, I. and Unsworth, A. (1979) J. Biomech., 12:257-267.