Both lubricin, expressed by synovial fibroblasts [1], and superficial zone protein (SZP), expressed by surface chondrocytes [2], serve to lubricate the pressurized cartilaginous joint articular surfaces in the boundary mode. Boundary lubrication is the regime of lubrication where the film of lubricant does not exceed the height of the surface asperities. These films are often characterized by molecular monolayers that may form as a result of a chemical reaction between the surface and lubricant. It is this interaction rather than the bulk properties of the lubricant that limit friction between contact surfaces [3]. Boundary lubricants contain a domain that reacts with and binds to the surface, and a domain that generates a repulsive force that will lower the friction coefficient between the flattened asperities. Lubricin is an amphipathic molecule that has been shown to bind to hydrophobic surfaces and lower the friction coefficient when apposed by a hydrophilic surface [4]. Its ability to lubricate is diminished by the removal of saccharides in the molecule, pointing to hydration forces as the mechanism for repulsion between lubricin-coated surfaces.

In order to test the surface modifying ability of lubricin we have employed the atomic force microscope (AFM) in dry conditions. The surface adhesive forces were measured as the piezoelectric scanner, on which the hydrophobic substrate rested, extends to make contact with the tip of a cantilever beam. After the scanner retracts the adhesive forces are measured as cantilever deflection, as it comes out of contact with the substrate, this is referred to as the “snap-out,” (SO) see figure 1a.

Methods

Atomic Force Microscopy

Lubricin, purified from human synovial fluid, at concentrations of 10, 100, 200, 250 and 300μg/ml in physiological saline, was studied in the atomic force microscope in the contact mode. Solutions were deposited on highly ordered pyrolytic graphite (HOPG) to allow for equilibrium in the solution as the lubricin molecules settle on the hydrophobic surface of the HOPG. After 10 minutes the excess solution was removed with a pipette and the remaining fluid was extracted through capillary action without disturbing the surface. Silicon-tipped cantilevers with spring constants of 0.26 and 0.4N/m were used to generate a 30μm x 30μm topography image of the surface and subsequently to generate 16-force versus distance (FvD) curves across the sample. The FvD and adhesive force calculations were performed using MATLAB technical computing language software.

Results

FvD curves were generated for the clean HOPG substrate and subsequently for each solution of lubricin. During the extension of the scanner, eventual contact was attained. After contact of the cantilever tip and the substrate, additional extension provided a load of up to 300nN (Figure 1). During retraction it was evident that both the clean HOPG and the 200μg/ml solution of lubricin showed a high magnitude negative force, shown by the SO in the retraction curve (red line) at the end of the contact. This was also the case for the 10 and 100μg/ml solutions (not shown). In the substrate coated with the 250 and 300μg/ml solutions of lubricin, the magnitude of the negative force is significantly less than that for lower concentrations. Differences in the contact extension distance are due to the use of two cantilevers with different spring constants.

The adhesive force between cantilever and substrate varied as a sigmoid function against lubricin concentration. The HOPG substrate coated with 10 and 100μg/ml displayed the same adhesive force as that of clean HOPG (Figure 2). As the concentration of lubricin increases, the force of adhesion decreases, reaching a plateau at a concentration of 250μg/ml.

Discussion

These results are in agreement with previous experiments in which a critical concentration of lubricin was necessary to enable lubrication of a hydrophobic-hydrophilic bearing, this was also the concentration required to reduce the interfacial tension of an aliphatic-aqueous interface [4]. At concentrations in excess of 200μg/ml, lubricin is able to aggregate on a hydrophobic surface in such a way as to reduce the adhesive force between contact surfaces. These data also support emerging scale bridging concepts of surface phase fields, which provide a nanotribological understanding of boundary lubrication. HOPG proved to be a suitable substrate in which to study lubricin by AFM.

References