

Lubricin

Lubricin is a mucinous glycoprotein that provides lubricating properties within synovial joints. It is synthesized by cells lining synovial joints, including synovial cells, superficial zone chondrocytes, and tendon cells. The lubricating properties of the molecules have been studied in several laboratories and have been shown to reduce the coefficient of friction in cartilage-on-glass, rubber-on-glass, and cartilage-on-cartilage test systems.

A PRG4 null mouse colony exists at Rhode Island Hospital which has provided important insights into the role of lubrication in chondroprotection. We recently established that the absence of this protein leads to premature and significant joint wear (Jay, et al., 2007). This is a very important finding as lubrication does not always prevent wear. For example, a graphite pencil provides lubrication but does not prevent wear, enabling a pencil to leave a residue (writing). Lubricin lubricates via the boundary mode which is a regime in the leftward area of the Striebek curve (Márton & Lantos, 2006). This means that boundary lubrication functions independently of synovial fluid viscosity. These are seminal observations first made by Charles McCutchen a number of years ago who digested synovial fluid with hyaluronadase which eliminated viscosity. This modified synovial fluid continued to lubricate. By contrast, digestion of synovial fluid with trypsin results in a synovial fluid which remains viscous but fails to lubricate.

The important role that this mucinous glycoprotein plays is now being studied in a new animal model. Rat joints traumatized in vivo via a surgically induced ACL injury have been studied using whole joint friction ex vivo (Crisco, et al., 2007) and immuno-histochemistry of articular cartilage. The same techniques used to study lubricin null murine joints. The ACL injury results in a permanent decrease in lubricating ability and early damage to articular cartilage. This is in contrast to a transient synovitis induced by injected adjuvant (Elsaid, et al. 2007). The translational relevance of these findings to acute orthopedic problems have been confirmed in humans. Lubricin levels are indeed low in synovial fluid aspirated from patients who are within a 60 day peri-injury period and remain low up to a year post injury (ORS abstract from 2008). This work is ongoing to identify ways to supplement lubricin levels in traumatized joints.

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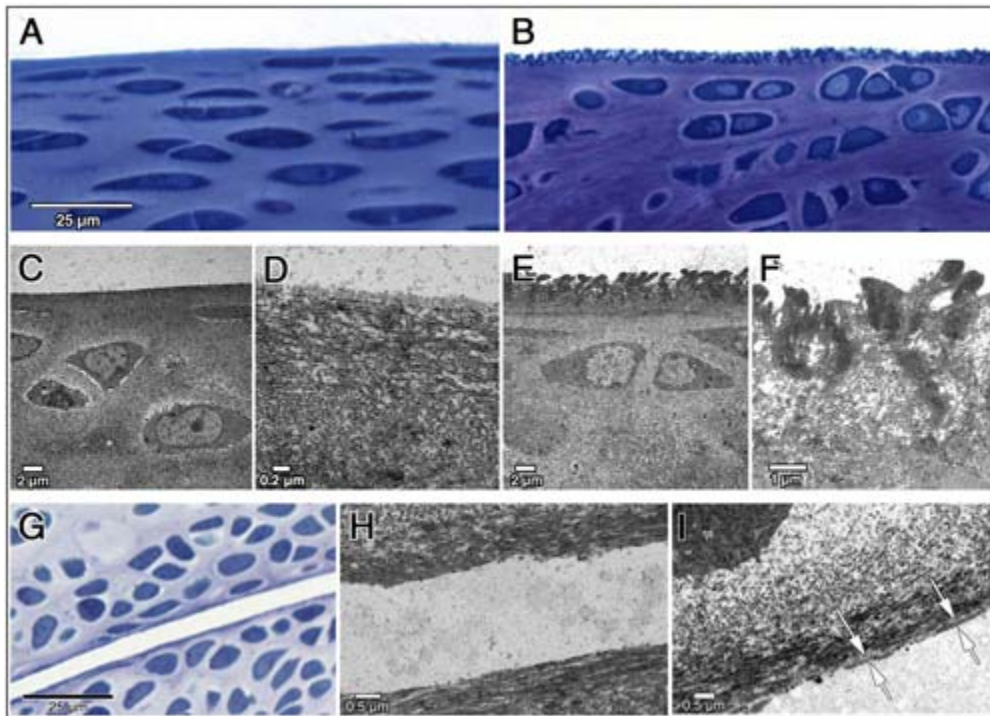


Figure 1. Microscopy of heterozygote (Prg4^{+/-}) and lubricin null (Prg4^{-/-}) cartilage. (A & B) Representative photomicrographs of Toluidine blue stained knee joint cartilage sections from 2-week-old Prg4^{+/-} (A) and Prg4^{-/-} (B) mice, which demonstrate differences in surface smoothness. (C & D) Representative electron micrographs of the cartilage surface in 2-week-old Prg4^{+/-} mice, revealing a smooth, uniform surface and a clearly visualized lamina splendens. Note the parallel orientation of collagen fibrils adjacent to the cartilage surface. (E & F) Representative electron micrographs of the cartilage surface in 2-week-old Prg4^{-/-} mice, revealing roughening of the surface, lack of a recognizable lamina splendens, and disruption of the parallel orientation of the collagen fibrils. (G & H) Toluidine blue stained section and electron micrograph of the cartilage surface in a newborn Prg4^{-/-} mouse. (I) electron micrograph of the cartilage surface in a newborn Prg4^{+/-} mouse. Note that there is no difference in collagen fibril orientation between newborn Prg4^{+/-} and Prg4^{-/-} mice, but only the Prg4^{+/-} mouse has a detectable lamina splendens (arrows). (Arthritis Rheum 56(11):3662-9, 2007.)

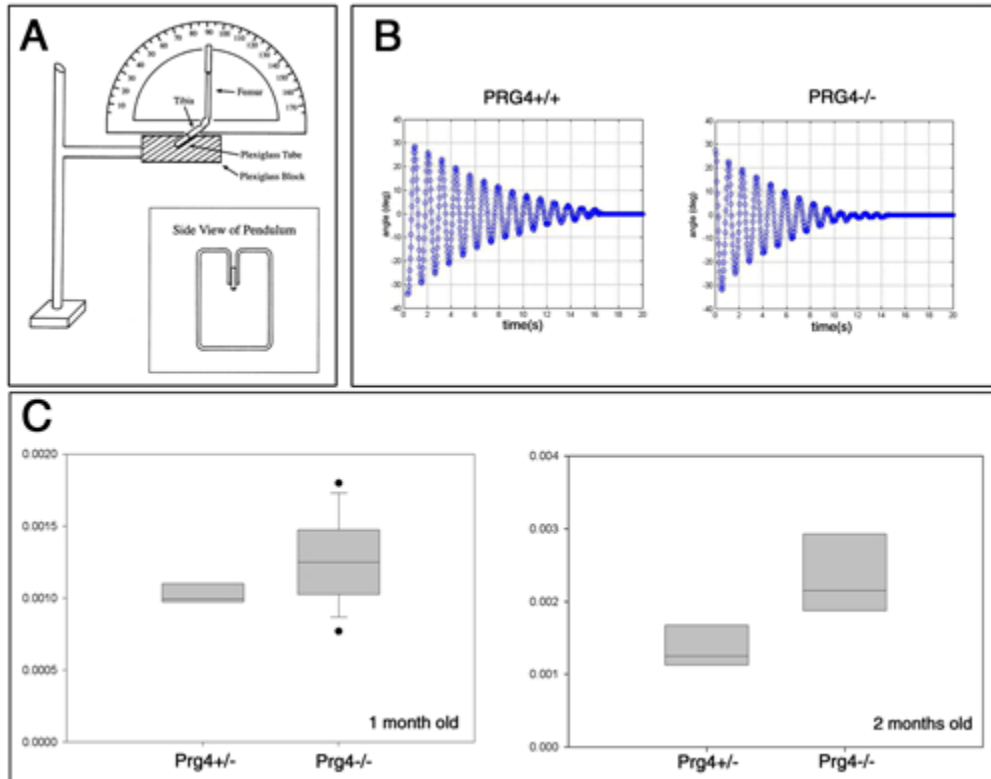


Figure 2. Schema of the pendulum apparatus used to measure whole joint coefficient of friction and representative data. (A) Whole joint measurements use mouse knee joints, in which muscles are removed and the joint capsule is left intact. The tibia is fixed at a 45° angle so that the knee joint angle is 120° when the femur is held perpendicular. The joint is loaded with 20 gm weight, simulating the weight of an adult mouse standing on one limb, with the fulcrum of the pendulum located at the knee joint. The femur is manually deflected from the perpendicular by 30° and allowed to oscillate freely at the knee joint until joint motion stops. (B) Representative raw data using a modified Moiré encoder technique, measuring the oscillation of Prg4+/+ and Prg4 -/- knee joints. Deceleration of the pendulum is calculated from the pendulum decay and divided by G, the earth's gravitational constant, to determine the coefficient of friction (m). Note that the Prg4+/+ knee oscillates longer and for more cycles than the Prg4-/- knee. (C) Box plots of values of m from 1-month-old and 2-month-old Prg4-/- and Prg4+/+ mice measured with the pendulum apparatus. The boxes in these plots have lines at the lower quartile, median, and upper quartile values. The whiskers are lines extending from each end of the box to show the extent of the data. Outliers are data with values beyond the ends of the whiskers. (Arthritis Rheum 56(11):3662-9, 2007.)

Figure 3. Atomic force microscopy of lubricin coated onto HOPG (A to F) Topographic images (A, C, E) and adhesion images (B, D, F) of lubricin networks formed from 10, 50, or 300 mg/ml lubricin concentrations. In the topographic images (A, C, E) the networks of lubricin molecules are pseudocolored to appear bright. In the adhesion images (B, D, F) regions of low adhesion are pseudocolored to appear dark. Note that at all concentrations the lubricin network in the topographic images (bright areas in A, C, and E) corresponds to reduced adhesion (dark areas in B, D, and F). (A & B) At 10 mg/ml, lubricin forms a loose, mesh-like, network structure. At higher concentrations of lubricin, 50 mg/ml (C & D) and 300 mg/ml (E & F) the network remains mesh-like, although the mesh-size decreases as the concentration increases. Arrows indicate an example of openings in the mesh-like network at each concentration. Boxed areas indicate the identical regions in a topographic and adhesive image, demonstrating the relationship between the presence of lubricin on the HOPG surface and the decrease in adhesion between the surface and the AFM probe. **(G)** AFM force vs. distance curves for the clean HOPG substrate and for substrates coated with different concentrations of lubricin. Note the sigmoidal shape of the curve, which is compatible with lubricin undergoing a phase change when the concentration is ~ 250 mg/ml. Error bars correspond to mean±SD. (Arthritis Rheum 56(11):3662-9, 2007.)

