

PRG4/LUBRICIN/SZP AND THE SLIPPERY SIDE OF CARTILAGE

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INTRODUCTION: Proteoglycan-4 (PRG4), lubricin, superficial zone protein (SZP), and megakaryocyte stimulating factor (MSF) are synonyms for a proteoglycan which 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 molecule have been studied by several laboratories including those of Swann¹, Jay², Sah³, Bonasser⁴ and my own. Here we report that a custom tribometer using a cartilage-plastic interface is able to detect dramatic changes in the coefficient of friction when microgram quantities of PRG4 are coated on the plastic surface and tested in a PBS solution.

METHODS: PRG4 was purified from human synovial fluid by a combination of ion exchange, gel filtration and affinity chromatography. PRG4 was coated on Falcon tissue culture dishes (35 mm diameter) in 2 ml of 50 mM sodium carbonate buffer pH 9.0 for 18 h at room temperature. Unbound PRG4 was removed by washing in PBS and the plates were tested in PBS. Additional plates were coated with bovine serum albumin (BSA) or hyaluronan. In other experiments the PRG4 was reduced with various concentrations of dithiothreitol and alkylated with an excess of iodoacetamide before coating the plates. The ability of PRG4-coated plates to lower the coefficient of friction was tested in a custom pin-on-disc tribometer using an adult rabbit phalangeal bone with its articular cartilage opposed to the coated plastic surface. All tests were performed in PBS. The normal force was 0.4 MPa and the frictional force measured with a load cell. Tests were performed at several sliding velocities for 5 or 10 minutes each.

RESULTS: Falcon tissue culture dishes were coated with purified PRG4 at different concentrations and the coefficient of friction measured between rabbit articular cartilage and the coated plastic surface in PBS. Figure 1 shows low concentrations of PRG4 in the coating solutions were unable to lubricate the cartilage-plastic interface. However a threshold

coating concentration is reached between 3-4 ug per plate which caused a dramatic reduction in the coefficient of friction. When petri dishes or Primaria plates were coated, more PRG4 bound to the plates and lower coefficients of friction were obtained. This lubrication assay was used to test if reduction and alkylation affected the lubricating activity of PRG4. Titration experiments showed 20 mM dithiothreitol treatment almost completely eliminated the lubricating activity of PRG4 in these assays.

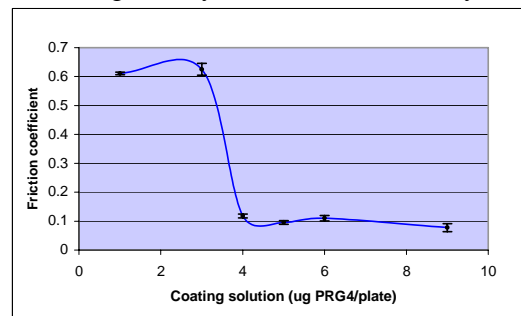


Fig. 1: Effect of [PRG4] coating concentration on friction coefficient between cartilage and plastic.

DISCUSSION & CONCLUSIONS: PRG4 has been shown to reduce the coefficient of friction in cartilage-glass^{1,4}, rubber-glass² and cartilage-cartilage³ test systems. Usually the [PRG4] needed to produce this effect is relatively high (50-450 ug/ml)¹⁻⁴. We found very low concentrations (~5 ug/ml) were necessary to coat plastic surfaces and generate similar effects. When PRG4 was reduced and alkylated it lost its ability to lubricate the cartilage-plastic interface. This lubrication assay should be useful to dissect the molecular features of PRG4 which support its lubricating function.

REFERENCES: ¹D.A. Swann et al (1981) *Arthritis Rheum* 24:22-30. ²G.D. Jay (1992) *Conn Tiss Res* (1992) 28:71-88. ³T.A. Schmidt et al (2006) *Trans Orthop Res Soc* 52:84. ⁴J.P. Gleghorn et al (2006) *Trans Orthop Res Soc* 52:1502.

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