Characterization of Recombinant Human PRG4 as an Ocular Surface Boundary Lubricant

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Introduction: Dry-eye disease involves tear film instability that can result in surface-to-surface contact between the cornea and eyelid or contact lens, where boundary lubrication can be dominant¹. Motivated by the recent discovery that proteoglycan 4 (PRG4, a mucin-like glycoprotein originally discovered in synovial fluid as a boundary lubricant²), functions as an ocular surface boundary lubricant³, advances in recombinant protein expression technology⁴, and PRG4's potential use as a friction-reducing contact lens coating, the objectives of this study were to: 1) biochemically characterize recombinant human PRG4 (rh-PRG4); and 2) assess the boundary lubricating properties of rh-PRG4, both before and after autoclave sterilization, at a cornea-contact lens material (PDMS) biointerface.

Methods: SDS-PAGE western blot analysis using a variety of anti-PRG4 antibodies and lectins was performed on native PRG4 (nPRG4) and rh-PRG4 samples, both nonreduced and reduced, with and without enzymatic removal of O-linked glycosylations. Human corneas and PDMS were articulated against each other, subject to physiological loads of 8-25 kPa, at effective sliding velocities of 0.3-30 mm/s. Test lubricant sequences were A) saline, rh-PRG4 @300µg/mL, nPRG4 @300µg/mL, and saline; and B) saline, autoclaved rh-PRG4 @300µg/mL, rh-PRG4 @300µg/mL, and saline. Static and kinetic coefficients of friction were calculated.

Results: rh-PRG4 demonstrated similar immunoreactivity to nPRG4, and effectively lowered friction at the cornea-PDMS biointerface. Western blotting indicated immunoreactive rh-PRG4 bands had a similar apparent molecular weight (MW) to nPRG4, and decreased appropriately upon reduction as well as enzymatic removal of glycosylations. Kinetic friction coefficients, which were highest in saline $(0.31\pm0.06 \text{ to } 0.40\pm0.06, \text{ mean}\pm\text{SEM})$, were similar in rh-PRG4 ($0.12\pm0.01 \text{ to } 0.25\pm0.03$) and nPRG4 ($0.19\pm0.02 \text{ to } 0.28\pm0.03$) across all velocities. Autoclaved rh-PRG4 had similar values to rh-PRG4 as well ($0.19\pm0.02 \text{ to } 0.26\pm0.04, 0.16\pm0.02 \text{ to } 0.26\pm0.02$, respectively).

Conclusions: rh-PRG4 demonstrates similar biochemical and ocular surface lubricating properties to nPRG4, and may function as an effective friction-reducing contact lens coating.

References

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