Characterization of Recombinant Human PRG4 as an Ocular Surface Boundary Lubricant

N Masala[1], S Morrison, BSc[1]; M Samsom[1], BSc; H Sheardown[2], PhD; T Schmidt[1], PhD.
[1]*University of Calgary (2500 University Dr. NW, Calgary, Alberta, Canada T2N 1N4)*
[2]*McMaster University (Main Street West, Hamilton, Ontario, Canada L8S 4L8)*

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 dominant[1]. Motivated by the recent discovery that proteoglycan 4 (PRG4, a mucin-like glycoprotein originally discovered in synovial fluid as a boundary lubricant[2]), functions as an ocular surface boundary lubricant[3], advances in recombinant protein expression technology[4], 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±0.06 to 0.40±0.06, mean±SEM), were similar in rh-PRG4 (0.12±0.01 to 0.25±0.03) and nPRG4 (0.19±0.02 to 0.28±0.03) across all velocities. Autoclaved rh-PRG4 had similar values to rh-PRG4 as well (0.19±0.02 to 0.26±0.04, 0.16±0.02 to 0.26±0.02, 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

1. Holly FJ and Holly TF. "Advances in Ocular Tribology" in *Lacrimal Gland, Tear Film, and Dry Eye Syndromes*. Sullivan DA, Ed. New York: Plenum Press, 1994, pp. 275-283.
2. Swann DA, Slayter HS, Silver FH, "The molecular structure of lubricating glycoprotein-I, the boundary lubricant for articular cartilage", *J Biol Chem*, vol. 256, no. 11, 1981. pp. 5921-5925.
3. Morrison S, Sullivan DA, Sullivan BD, Sheardown H, Schmidt TA, "Dose-dependent & synergistic effects of proteoglycan 4 (PRG4) on boundary lubrication at a human cornea – contact lens biointerface", *Eye & Contact Lens* (in revision).
4. Girod P, Nguyen D, Calabrese D, Puttini S, Grandjean M, Martinet D, Regamey A, Saugy D, Beckmann JS, Bucher P, Mermod N, "Genome-wide prediction of matrix attachment regions that increase gene expression in mammalian cells", *Nature Methods*, vol. 4, no. 9, 2007. pp. 747-753.