LUBRICIN AS AN OCULAR SURFACE-CONTACT LENS BOUNDARY LUBRICANT: DOSE-DEPENDENT & SYNERGISTIC EFFECTS

S. Morrison1, B. Snider1, B. Sullivan2, E. Truit III3, D. Sullivan4, T. Schmidt1
1 University of Calgary, Calgary, Canada; 2 TearLab Corp., San Diego, CA; 3 Singularis, Inc., San Diego, CA; 4 Schepens Eye Research Institute, Boston, MA.

INTRODUCTION
- Lubricin:
  - Mucin-like glycoprotein from proteoglycan 4 (PRG4) gene
  - Secreted by chondrocytes and synoviocytes lining joint surfaces
  - Present in synovial fluid and on the surface of articular cartilage
  - Cartilage boundary lubrication function
  - Decreases friction during surface-to-surface contact
  - Synergism with hyaluronan (HA)
- Ocular surface:
  - Significant shear/friction forces from eyelid blink and contact lens wear
  - Lubricin expression
  - Molecules on ocular cornea and conjunctival epithelial cells
  - Protein in human meibomian gland secretions
  - Lubricin function: reduce friction at cornea-eyelid biointerface
  - Dry eye disease: decreased lubrication leads to pain and discomfort during contact lens wear
  - No effective therapeutic treatment

OBJECTIVE
1) Assess boundary lubricating ability of lubricin at a contact lens-cornea biointerface, 2) Determine if lubricin protein is present at the ocular surface

METHODS

Ocular Surface Materials
- Human corneas: Southern Alberta Lions Eye Bank
  - Fresh, preserved in Optisol-GS @ 4°C, used within 2 weeks
  - Age: 24-90 years old
- Contact lens materials: polydimethylsiloxane (PDMS, from the Dr. Heather Sheardown, McMaster University)

Lubricin Preparation, Purification, & Characterization
- Preparation:
  - Cartilage disks from patellofemoral groove of mature bovine stifle joints
  - Cultured for 15 days in Dulbecco’s modified Eagle’s medium, 0.01% BSA, 25 μg/ml ascorbic acid, 10 ng/ml recombinant TGF-β1
- Purification:
  - Spent medium fractionated by DEAE anion exchange chromatography
  - DEAE-Sepharose column
  - Concentrate and buffer exchange PRG4 rich 0.3-0.6 M NaCl eluate

Characterization:
- 3-8% Tris Acetate SDS-PAGE Western Blot & protein stain
- C terminal anti-peptide antibody
- Gel protein stain
- BCA protein assay

Ocular Surface Boundary Lubrication Test
- Test Samples (Fig. 1A)
  - Cornea with <3 mm sclera
  - Contact lens: R = 3.2 mm, Rr = 1.5 mm, Rv = 2.4 mm
- Test Setup (Fig. 1B)
  - Cornea-contact lens biointerface, BOSE ELF3200 biomechanical tester
  - Rotate (μ radians) / 4 rev at linear sliding velocity: 30, 10, 1, 0.3 mm/s
  - 12 s pre-sliding duration (dwell time) between rotations
  - Measure normal load N, torque τ
  - Normal stress σN = N/Rr / [R(Rv - Rv)]; avg 15.7±1.2 kPa

Data Analysis:
- Friction coefficients μN = (τ/2N) kPa
  - μN = resistance to motion: peak μN
  - μN = resistance to steady state motion: average steady μN
- Mean ± SEM, repeated measures ANOVA

Test Lubricants
- Test 1: Lubricin Dose Response (n=4)
  - Lubricin (PRG4) @ 30,100,300 μg/ml in saline
  - Lubrication test samples (A, B)
- Test 2: Lubricin + HA (n=5)
  - CIBA Vision Aquify ~ 1mg/ml HA (AQ)
  - Lubricin @ 300 μg/ml in saline (PRG4)
  - Lubricin @ 300 μg/ml in Aquify (PRG4+AQ)
- Test 3: Lubricin + HA vs. Systane (n=5)
  - Alcon Systane (Systane)
  - Systane @ pH 8 (Systane 8)
  - Lubricin @ 300 μg/ml in saline (PRG4)
  - Lubricin @ 300 μg/ml in Aquify (PRG4+AQ)

Lubricin Presence At Ocular Surface
- Fresh porcine corneas
- Epithelial scrape, RIPA buffer extraction, and homogenization
- SDS-PAGE Western Blot

RESULTS
- Test 1: Lubricin lowers friction in a dose dependent manner (30-300 μg/ml)
  - Static μN lubricant (p<0.01), kinetic μN lubricant (p<0.01) (Fig. 2A)
  - Lubricin @ 300>100>30 (p<0.05), Saline – Lubricin @ 30 (p=0.99)
  - Kinetic μN lubricant (p<0.01), Vel (p<0.74) (Fig. 2B)
  - Lubricin @ 300>100>30 (p<0.05), Saline – Lubricin @ 30 (p=0.33)

Test Lubricants (Fig. 2)
- Test 1: Lubricin + HA synergy (p<0.005)
  - Static μN lubricant (p<0.01), kinetic μN lubricant (p<0.01) (Fig. 2A)
  - Lubricin@ 300>100>30 (p<0.05), Saline – Lubricin @ 30 (p=0.99)
  - Kinetic μN lubricant (p<0.01), Vel (p<0.74) (Fig. 2B)
  - Lubricin@ 300>100>30 (p<0.05), Saline – Lubricin @ 30 (p=0.33)

Test 2: Lubricin+HA synergism (e.g. Aquify)
  - Lubricin in Aquify (PRG4+AQ)
  - Lubricin+HA in Aquify (PRG4+AQ)

Ocular Surface Boundary Lubrication Test Samples (A, B)
- Lubricin in Aquify (PRG4+AQ)
- Lubricin+HA in Aquify (PRG4+AQ)

DISCUSSION

Conclusions:
- Developed a novel boundary lubrication test: cornea-contact lens biointerface
- Lubricin functions as an effective ocular surface boundary lubricant
- Dose dependent (30-300 μg/ml)
- Synergism with HA superior to commercially available drops
- Potential new & improved dry eye biotherapeutic
- Lubricin protein exists at the ocular surface

Future Studies:
- Elucidation of lubricin+HA synergism
- Evaluation of other contact lens materials & coating +/- HA applications

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