Tissue

• Ocular Tissues
  • assess the boundary lubricating ability of lubricin at a cornea-eyelid biointerface
  • dry eye disease
  • decreased lubrication => pain and discomfort
  • no effective therapeutic treatment

OBJECTIVE

METHODS

Ocular Tissues

• Human cornea: Southern Alberta Lions Eye Bank
  • fresh, obtained within 8 hr after death
  • preserved in Optisol-GS @ 4°C, used within 2 weeks
  • age: 24-83 years old
• Human eyelids: U Calgary Body Donation Program
  • fresh, obtained within 1-2 days after death
  • used immediately, or stored in saline @ -20°C and used within 1 week

Lubricin Preparation, Purification, & Characterization (Fig. 1)

• Preparation:
  • cartilage disks from patellofemoral groove of mature bovine stifle joints
  • 6 mm diameter, ~0.3 mm thick including the articular surface
  • 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
  • medium changed every 3 days
  • collected and stored at -20°C
• Purification:
  • spend medium fractionated by DEAE anion exchange chromatography
    • DEAE-Sepharose column
    • PRG4 rich 0.3-0.6 M NaCl eluate
    • concentrated with a 30 kDa MW cutoff filter
    • buffer exchanged into water
• Characterization:
  • SDS-PAGE Western Blot & protein stain
  • 3-8% Tris Acetate gels
  • C terminal anti-peptide antibody5: LPN
  • gel protein stain
  • BCA protein assay

RESULTS

Ocular Surface Boundary Lubrication Test

• consistent test setup achievable ➔ accurate τ & N data collected (Fig. 4)
• normal stress σ = N/(n (R_e^2 - R_i^2)) range 13.1 - 23.4 kPa (avg 19.0±1.2 kPa)

Test Samples (Fig. 2A)

• cornea with ~3mm sclera
  • eyelid: R_e = 3.2mm, R_i = 1.5mm ➔ R = 2.4mm

Test Setup (Fig. 2B)

• cornea-eyelid biointerface, BOSE ELF3200 biomechanical tester
  • rotate (ω 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

Data Analysis

• friction coefficients μ = (τ/ NR)
• static μ - resistance to onset of motion: peak τ
• kinetic μ - resistance to steady state motion: average steady τ
• mean ± sem
• repeated measures ANOVA, posthoc with Sidak

Figure 2. Ocular surface boundary lubrication test samples (A) and setup (B).

Test Lubricants (Fig. 5)

• lubricant bath
• sequential testing with Saline rinses in between

Test 1 (n=6)

• Saline (Sterile Plus Saline, Bausch & Lomb)
• Aquify (CIBA Vision)
• Lubricin @ 300 μg/ml in Saline (Fig. 3)

Test 2 (n=3)

• Saline
• Bovine Serum Albumin (BSA) @ 300 μg/ml in Saline

Figure 3. Lubricin test lubricant.

DISCUSSION

Conclusions

• novel ocular surface boundary lubrication test
• lubricin functions as an effective ocular surface boundary lubricant
  • specific effect at a low concentration (300 μg/ml)
  • possibly better than commercially available eye drops
  • potential new & improved dry eye biotherapeutic

Future Studies

• dose-dependent ability of lubricin6
• synergistic friction lowering effect with hyaluronan6 (e.g. Aquify)

REFERENCES


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Figure 4. Ocular surface boundary lubrication test representative data.

Figure 5. Effect of lubricant and sliding velocity on static (A, C) and kinetic (B, D) friction at a human cornea-eyelid biointerface. Test 1: Saline, Aquify, PRG4 @ 300 μg/ml in A, B, Test 2: Saline, BSA @ 300 μg/ml in C, D.