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Effects of Concentration on Synergistic PRG4-Hyaluronan Cartilage Boundary Lubrication

Hunter, Miles

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Miles Hunter[1], Taryn Ludwig[2], Tannin Schmidt[1,2]
[1] Faculty of Kinesiology, [2] Biomedical Engineering Graduate Program,
Human Performance Lab, University of Calgary

INTRODUCTION

- Proteoglycan 4 (PRG4) and hyaluronan (HA) are key constituents of synovial fluid (SF)[1]
  - Present at articular cartilage surfaces
  - Contribute to boundary lubrication (cartilage surfaces are in contact)
  - Act synergistically to reduce friction

↓ HA[2] or PRG4[3] = ↓ Boundary lubrication + ↑ Cartilage wear[4]

- ↑ cartilage wear may degenerate cartilage, leading to osteoarthritis (OA)[5]
- Effects of varying PRG4 and HA concentrations on friction is unknown

OBJECTIVE

Examine how varying concentrations of PRG4 & HA interact to reduce friction

1. Vary [PRG4] while keeping [HA] physiologically normal
2. Vary [HA] while keeping [PRG4] physiologically normal

METHODS

Lubricants

- PRG4 purified from culture medium conditioned by bovine cartilage explants
- 1500 kDa HA obtained from Lifecore Biomedical

Samples

- Osteochondral samples were harvested from the patellofemoral groove of mature bovine knee joints
- Sample pairs consisted of a core and annulus
  - Soaked in the lubricant of interest overnight before testing

Cartilage Lubricating Ability

- Lubricating ability was evaluated in a cartilage-cartilage friction test[6] (Fig. 1)
  - 18% cartilage compression
  - 40 minute stress relaxation period
  - 0.3 mm/s effective sliding velocity
  - Each core/annulus pair was tested in 5 lubricants (Fig. 2, 3)

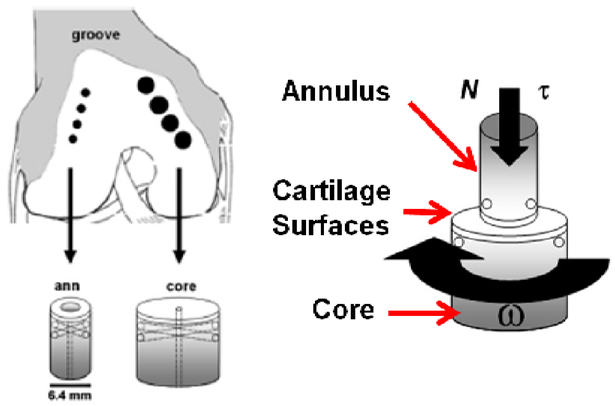

Figure 1. Boundary lubrication test setup

Friction Coefficient (μ)

- Kinetic friction coefficient $\langle\mu_{kinetic,Neq}\rangle$ represents resistance to steady motion[6]
  - Lower $\langle\mu_{kinetic,Neq}\rangle$ values indicate increased lubricating ability

- Two sets of tests performed to characterize PRG4-HA interaction
  Test 1: PRG4 dose response + constant HA = 3.33 mg/mL, n = 6 (Fig. 2)
  Test 2: HA dose response + constant PRG4 = 450 μg/mL, n = 5 (Fig. 3)

PBS → 150 μg/mL PRG4 +HA → 450 μg/mL PRG4 +HA → 1500 μg/mL PRG4 +HA → SF

Figure 2. Test 1: PRG4 dose response

PBS → 0.3 mg/mL HA +PRG4 → 1.0 mg/mL HA +PRG4 → 3.3 mg/mL HA +PRG4 → SF

Figure 3. Test 2: HA dose response

Statistical Analysis

- Data is presented as mean ± SEM
- ANOVA with Tukey post hoc was used to asses the effect of various lubricants

RESULTS

Cartilage Lubricating Ability

Test 1: PRG4 Dose Response

- Boundary lubricating ability was not significantly different between varying PRG4 concentrations combined with normal HA concentration
  - Not significantly different from SF (Fig. 4)

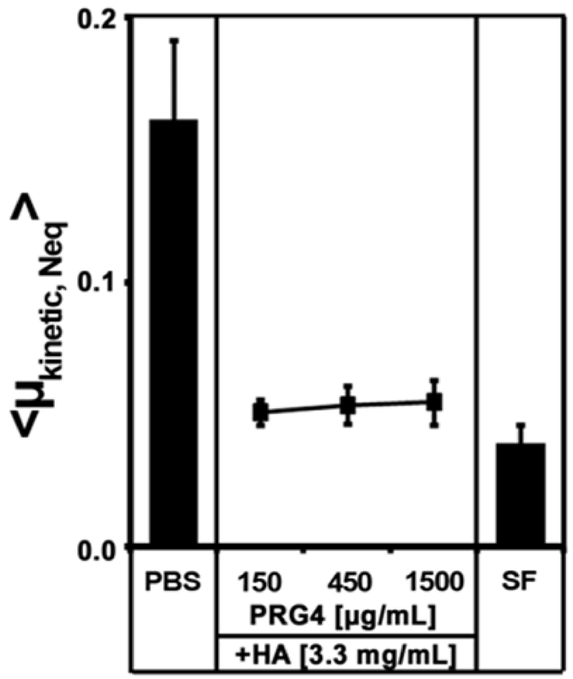

Figure 4. Kinetic, $\langle\mu_{kinetic,Neq}\rangle$ friction coefficients for PRG4 at 150, 450 and 1500 μg/mL + constant HA at 3.3 mg/mL, n = 6

Test 2: HA Dose Response

- Boundary lubricating ability was not significantly different between varying HA concentrations combined with normal PRG4 concentration
  - Not significantly different from SF
  - Apparent trend toward a lower friction coefficient with increasing HA concentrations (Fig. 5)

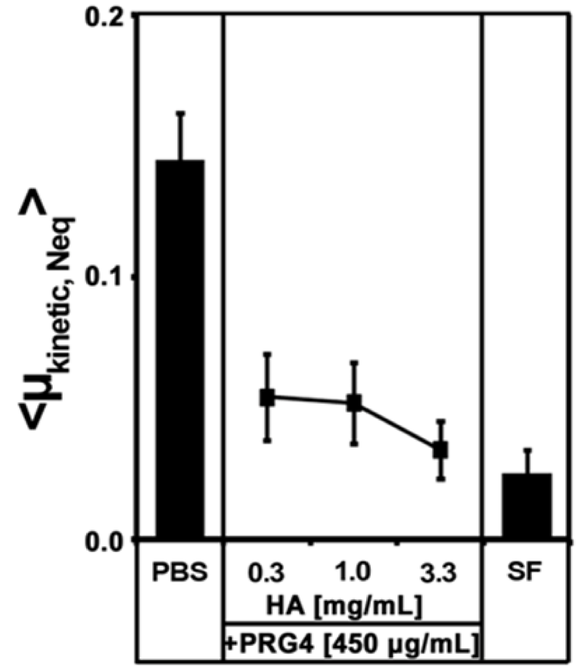

Figure 5. Kinetic, $\langle\mu_{kinetic,Neq}\rangle$ friction coefficients for HA at 0.3, 1.0 and 3.3 mg/mL + constant PRG4 at 450 μg/mL, n = 5

CONCLUSIONS

- PRG4-HA lubrication synergism is maintained, provided that either PRG4 or HA is present at a physiologically normal concentration
  - Provides cartilage boundary lubricating ability approaching that of SF
- Future tests can clarify the apparent trend of increasing [HA] improving lubricating ability in the presence of PRG4
- Clarifying the PRG4-HA synergism could contribute to improved biotherapeutic treatments for OA and other joint lubrication disorders[7-10]
  - Pain alleviation
  - Reduced rate of cartilage degradation

REFERENCES

[1]Schmidt+ *A&R* '07, [2]Antonacci+ *A&R* '12, [3]Ludwig+ *A&R* '12, [4]Jay+ *A&R* '07, [5]Teeple+ *J. Orthop. Res.* '07, [6]Schmidt+ *OAC* '07, [7]Flannery+ *A&R* '09, [8]Jay+ *A&R* '10, [9]Jay+ *A&R* '11, [10]Elsaid+ *OAC* '12

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