OARSI 2015
Seattle, WA

Lubricin

and its Potential as an OA Therapy

Disclosure:
Carl R. Flannery, Ph.D.
is a Sanofi employee & holds stock/options in
Sanofi and Pfizer

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New Therapeutics for Osteoarthritis May Be in Sight

by Antonios Aliprantis, MD, PhD

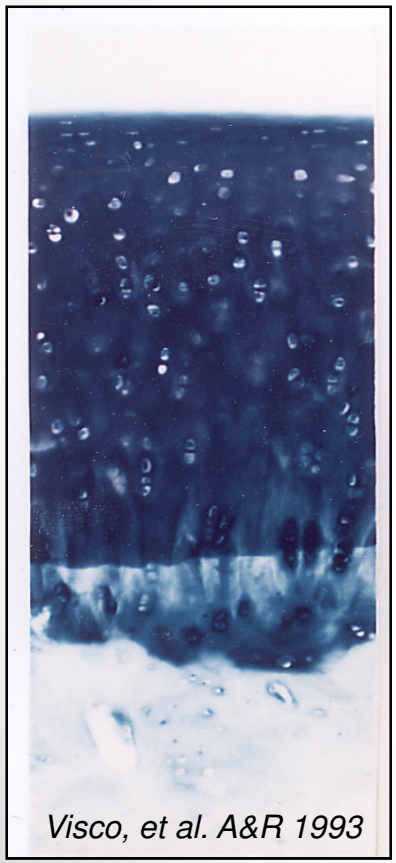
Biolubricants

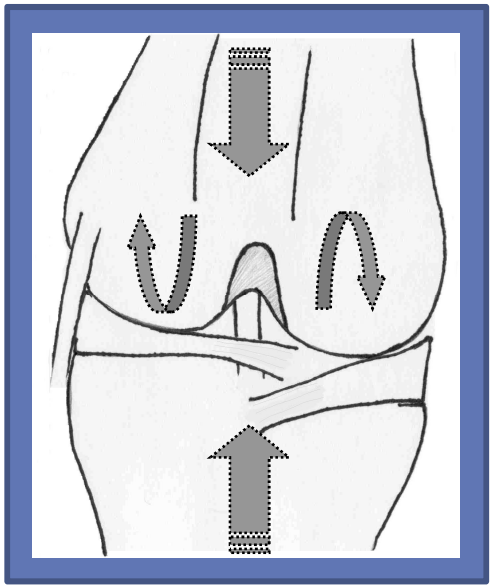
The high viscosity of synovial fluid and boundary lubricants on the articular surface maintain healthy cartilage as a near frictionless biomaterial.[17] One of the most important boundary lubricants is a protein by the name of lubricin, encoded by the Proteoglycan 4 (PRG4) gene.[7]

Cartilage is subjected to high load & shear forces

Highly efficient lubrication is imperative for healthy joint function

Normal



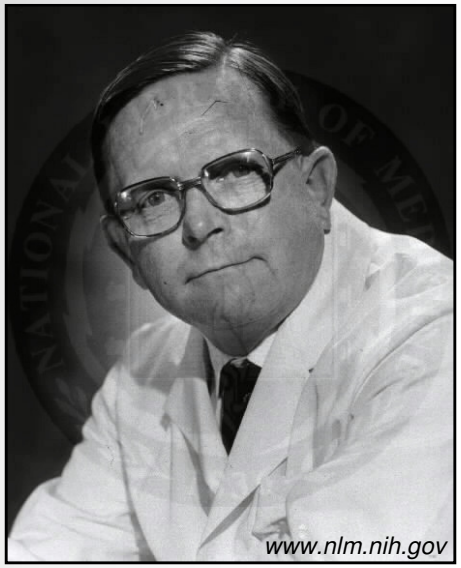


Injury/disease progression

Compromised Lubrication

OA

Cartilage lubrication: historical perspective



Prof. Sir John Charnley (1911-1982)
FRS, FRCS; 1974 Lasker Award Winner

"… Nature has conveniently covered the rubbing surfaces of the bones with a highly polished layer of cartilage… and has provided a viscous liquid (synovial fluid) to moisten these surfaces."

"It might seem unduly contentious… to suggest that the synovial fluid of a joint is not a lubricant; this is, however, the object of this paper."

from: Charnley J. 1959. The lubrication of animal joints. In: Symposium on Biomechanics. Institution of Mechanical Engineers, London, pp. 12-22.

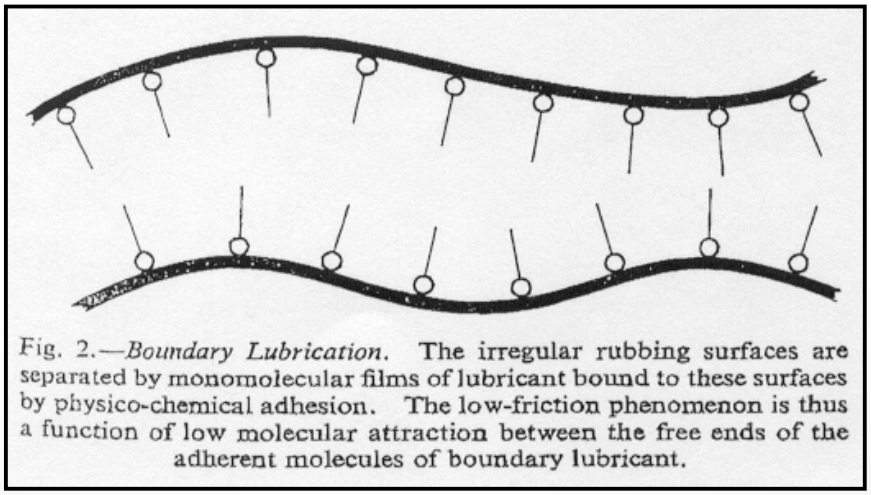
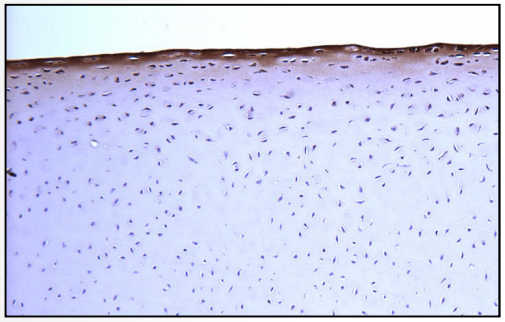


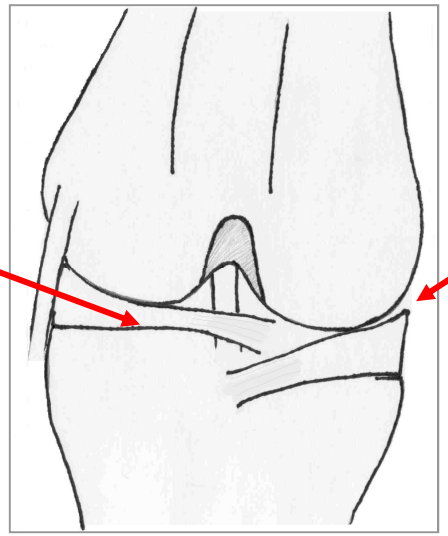
Fig. 2.—*Boundary Lubrication.* The irregular rubbing surfaces are separated by monomolecular films of lubricant bound to these surfaces by physico-chemical adhesion. The low-friction phenomenon is thus a function of low molecular attraction between the free ends of the adherent molecules of boundary lubricant.

Lubricin is a synovial glycoprotein which localizes to and protects cartilage surfaces

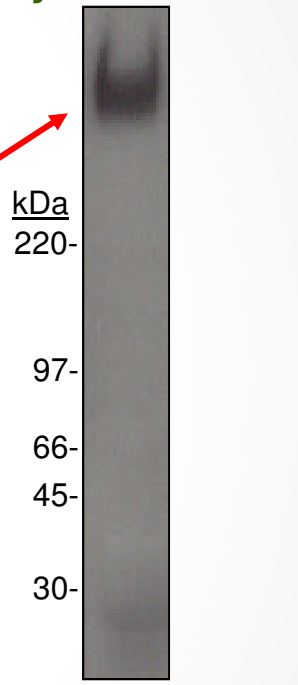
Lubricin at cartilage surfaces



Immunohistochemistry



Lubricin in synovial fluid

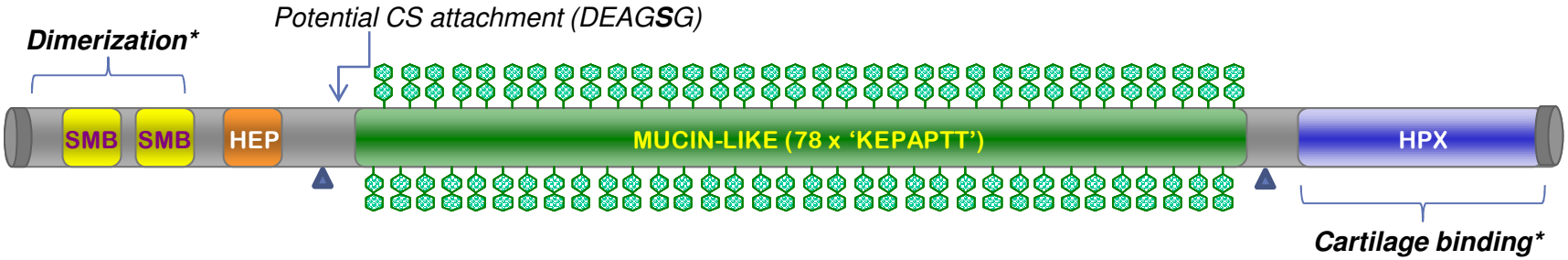


Western Blot (reduced SDS-PAGE)

Lubricin functions as a boundary lubricant and anti-adhesive at joint tissue surfaces, to help prevent wear and degeneration

Lubricin

- **1970-80s: Isolated from synovial fluid and characterized as a cartilage lubricating glycoprotein (Swann & Radin, et al.).**
- **Early 1990s: Further characterized by Jay, et al. Gene cloned at Genetics Institute; described as 'Megakaryocyte Stimulating Factor (MSF)' precursor.**
- **Late 1990s: Identity of MSF precursor with lubricin/superficial zone protein (SZP) established. Gene name assigned: Proteoglycan 4 (PRG4).**



SMB = somatomedin-B (vitronectin)-like
HEP = heparin-binding
HPX = hemopexin-like

1,404 amino acids
Mw: ~148 kDa (protein core)
Mw: >300 kDa (observed)

▲ = N-linked oligo attachment site

= Lubricating O-linked oligosaccharide: β-(1-3)-Gal-GalNAc (+/- sialic acid)

**Jones, et al. JOR 2007*

Altered lubricin metabolism and relation to joint pathology

Human lubricin gene mutations are associated with absence of lubricin synthesis (CACP syndrome). Patients exhibit noninflammatory synovial hyperplasia/hypercellularity and subintimal capsular fibrosis, and early onset cartilage degeneration.
(Marcelino, et al. Nature Genet 1999)

Lubricin null mice recapitulate human CACP syndrome phenotype, and exhibit tendon abnormalities as well as elevated joint friction levels.
(Rhee, et al. JCI 2005; Jay, et al. A&R 2007; Coles, et al. A&R 2010)

Perturbations in lubricin expression/function observed in models of joint injury/OA, and in patients with joint injury/OA.
(Elsaid, et al. A&R 2005, 2007, 2008, 2009; Young, et al. ART 2006; Teeple, et al. JOR 2007; Neu, et al. A&R 2010; Wong, et al. OAC 2010; Antonacci, et al. A&R 2012)

Implications: lubricin supplementation could be beneficial in treating joint disease

Efficacy of intraarticular (IA) lubricin supplementation in preclinical joint disease/OA models

OA Model	Lubricin/dose	Dosing protocol	Results	References
Rat Meniscal Tear (MT)	rhLubricin (LUB:1); 20µg/joint	1X/wk or 3X/wk for 4 wks starting 1 wk post-Sx	Significantly reduced joint histopathology scores	Flannery, et al. A&R 2009
Rat ACLT	Human synoviocyte or SF lubricin; rhLubricin; 10µg/joint	2X/wk starting 1 wk post-Sx; 32 day study duration	Significantly reduced joint histopathology scores	Jay, et al. A&R 2010
Rat ACLT	Human SF lubricin; 8µg/joint (+/- HA)	2X/wk for 4 wks starting 1 wk post-Sx	Significantly reduced histopathology scores	Teeple, et al. Am J Sports Med 2011
Rat ACLT	Human synoviocyte lubricin; 80µg/joint	Single dose 1 wk post-Sx; 70 day study duration	Significantly reduced uCTX-II levels; gait normalization	Jay, et al. A&R 2012
Rat ACLT + forced exercise	Human SF lubricin; 40µg/joint	Single dose 1 wk post-Sx; 5 wk study duration	Significantly reduced uCTX-II levels; inhibition of chondrocyte apoptosis	Elsaid, et al. OAC 2012
Rat ACLT	rhLubricin; 8µg/joint (+/- IL1ra)	Single dose 1 wk post-Sx; 5 wk study duration	Significant inhibition of chondrocyte apoptosis	Elsaid, et al. OAC 2015
Rat OVX	rhLubricin; 10µg/joint	2X/week for 4 wks starting day of Sx or 2 wks post-Sx	Significantly reduced joint histopathology and uCTXII levels, sig. inhibition of MMP13 & COLX levels & vascularization in cartilage and TRAP & OSX levels in bone, and normalized bone CT parameters	Cui, et al. Bone 2015

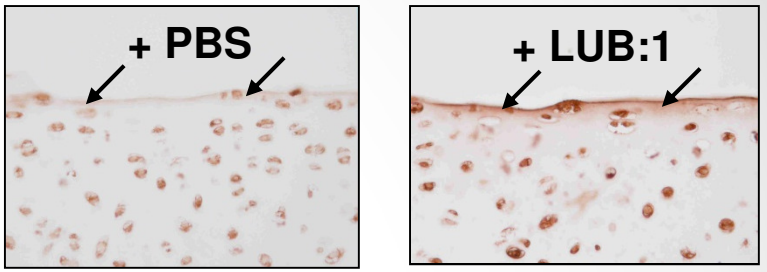
Recent study also demonstrates chondroprotective effects of helper-dependent adenoviral vector (HDV)-mediated lubricin expression after IA injection in a mouse CLT OA model. Therapeutic model = HDV delivered 2 wks post-Sx, significantly reduced histopathology scores + cartilage volume & surface area at 6 wks post-treatment (Ruan, et al. Sci Transl Med 2013).

Assessing LUB:1 functionality ex vivo

LUB:1 Binds to Cartilage Surfaces

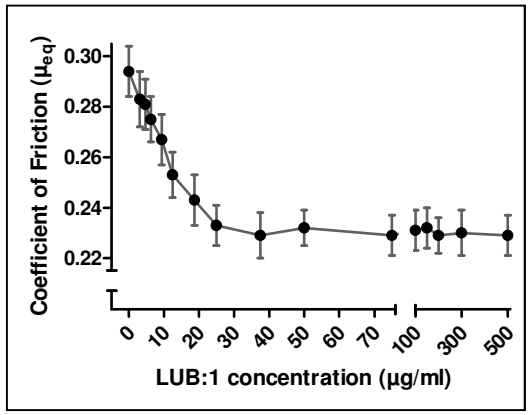
Immunostaining of LUB:1

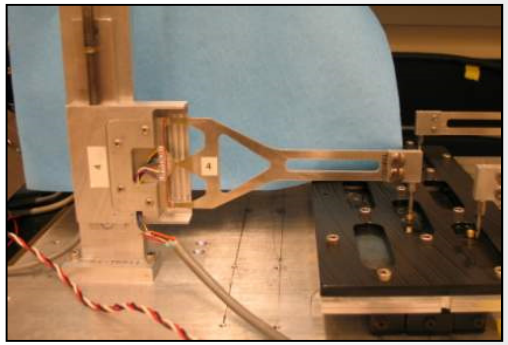
Pre-extracted cartilage



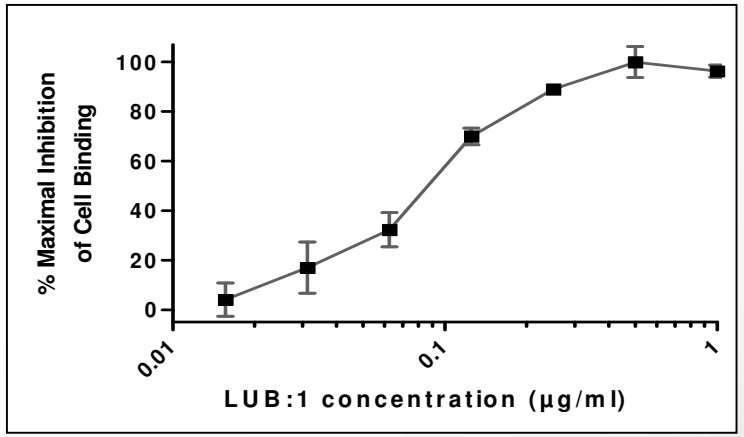
LUB:1 Lubricates Cartilage

Custom cartilage friction testing apparatus
Bonassar Lab, Cornell University





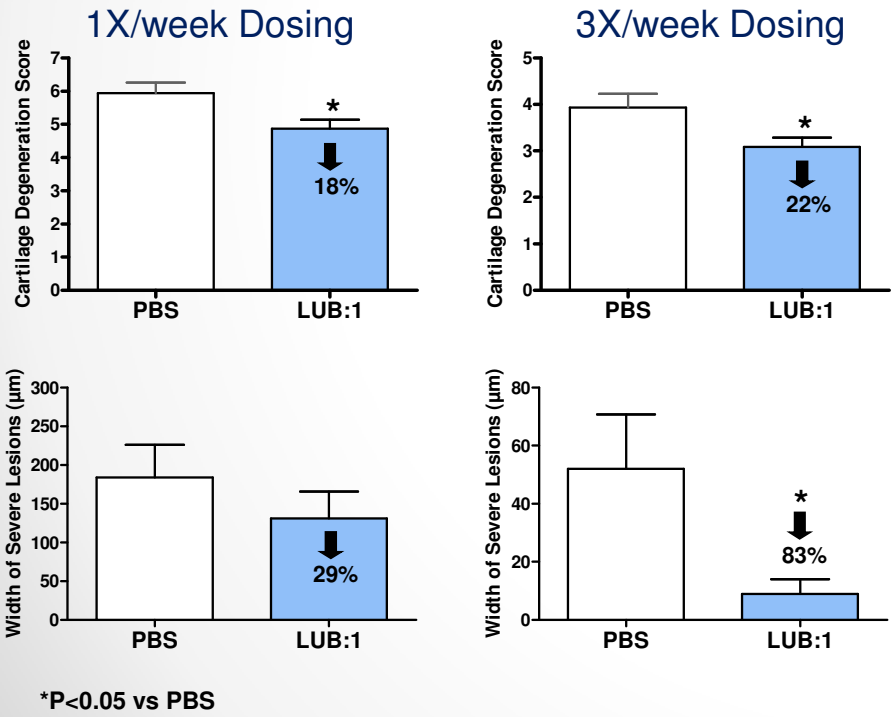
LUB:1 is Anti-Adhesive for Synoviocytes



Therapeutic efficacy of LUB:1 in vivo

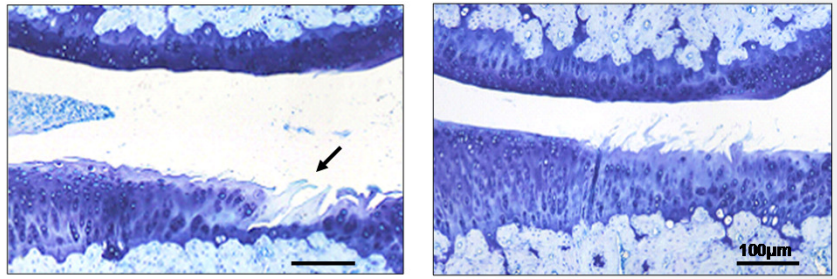
Our first proof-of-principle study demonstrated that IA treatment with LUB:1 is chondroprotective in rat meniscal tear OA model

Dosing with 20µg/joint of LUB:1 for 4 weeks starting 1 week post-surgery



*P<0.05 vs PBS

Histology: 3X/week Treatment



PBS-treated

Cart. Degen. Score = 4.00

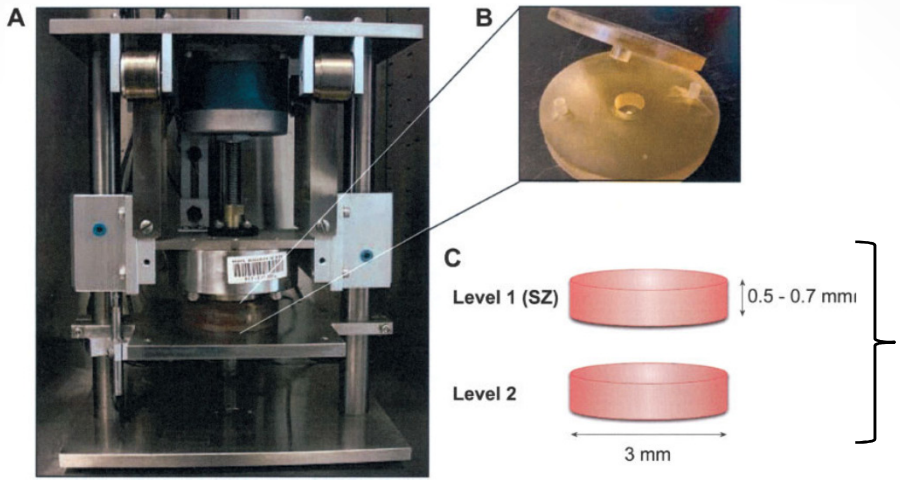
(Group mean = 3.93 ± 0.29)

LUB:1-treated

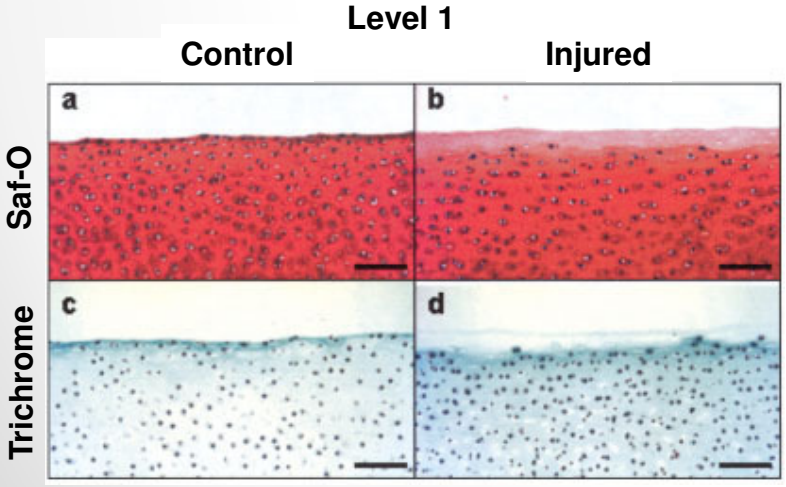
Cart. Degen. Score = 3.00

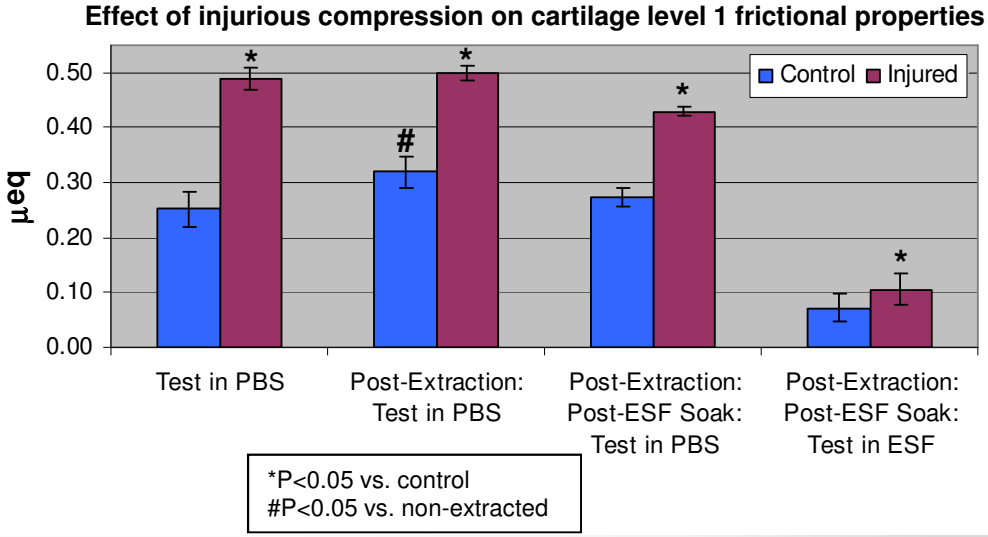
(Group mean = 3.08 ± 0.20)

Focus on cartilage surface – ex vivo injury model



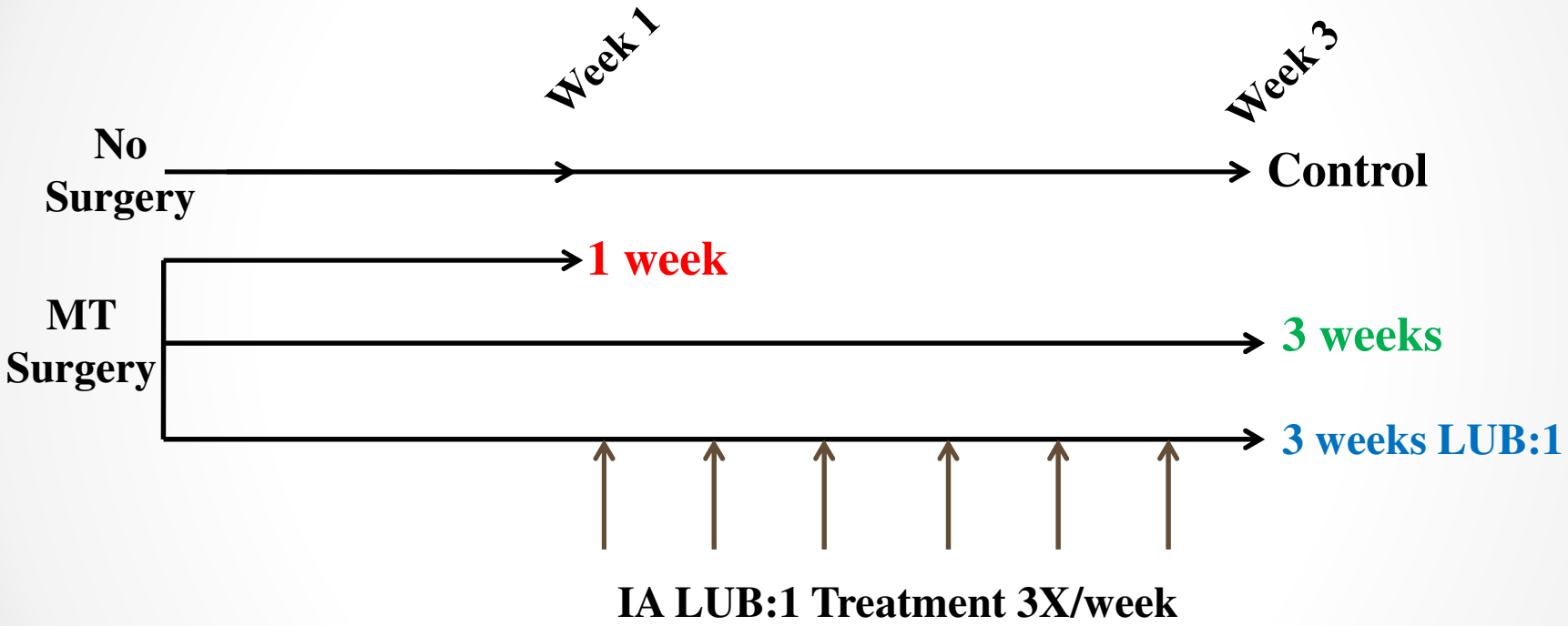
50% compression; 100%/sec → 2d culture post-injury





Jones, et al. A&R 2009 (Collaboration with Grodzinsky & Bonassar Labs)

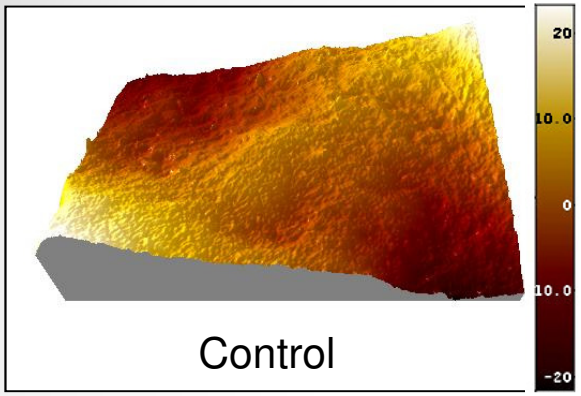
Assessing cartilage surface damage in rat meniscal tear (MT) model and effect of LUB:1 treatment



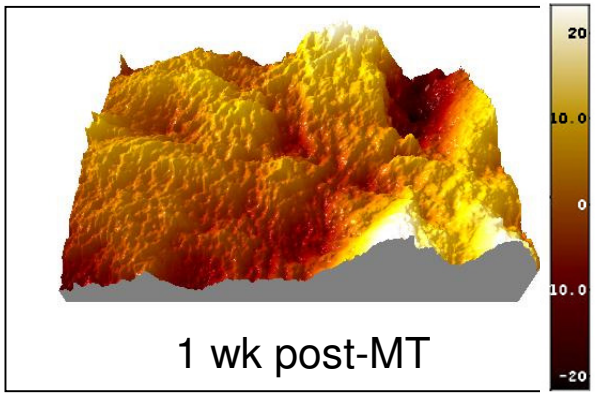
3mm osteochondral cores harvested from Medial Tibial Plateau for testing

Collaboration with Bonassar Lab
(Galley, et al. ORS 2011)

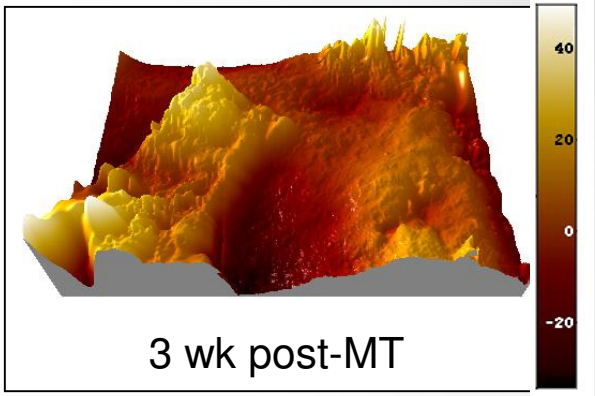
Cartilage surface damage in rat MT model (profilometry)



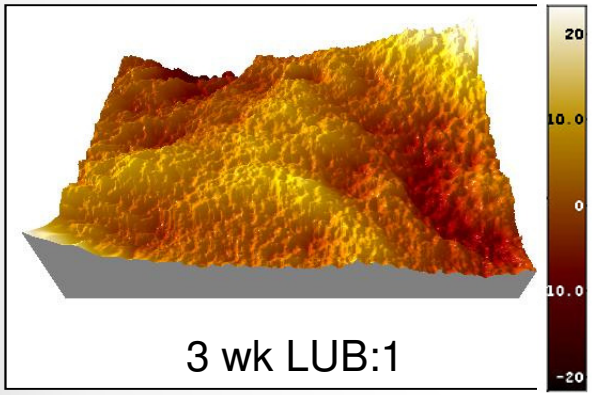
Control



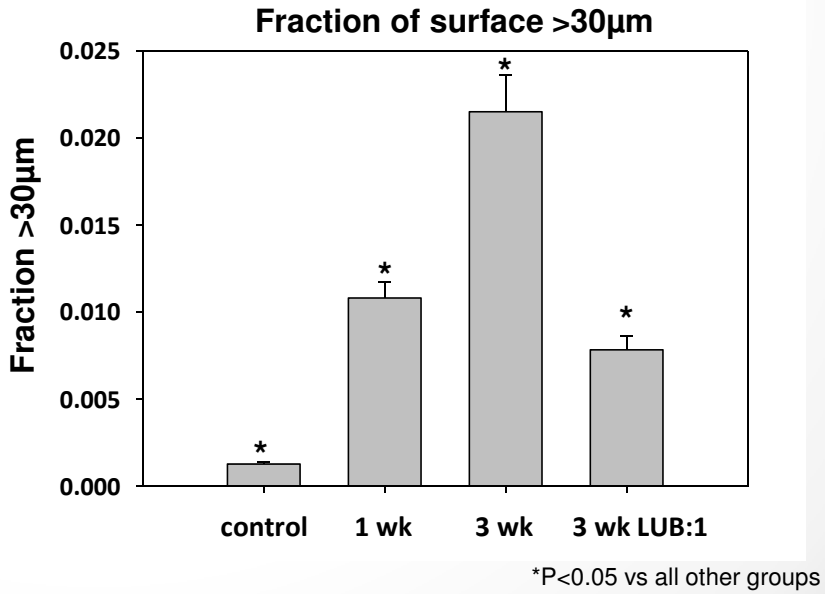
1 wk post-MT



3 wk post-MT



3 wk LUB:1

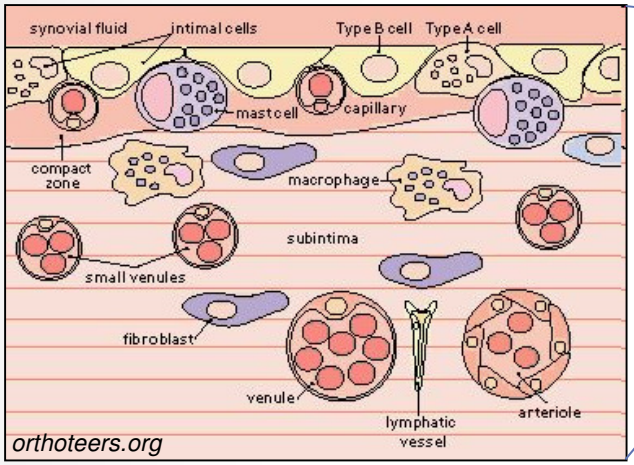


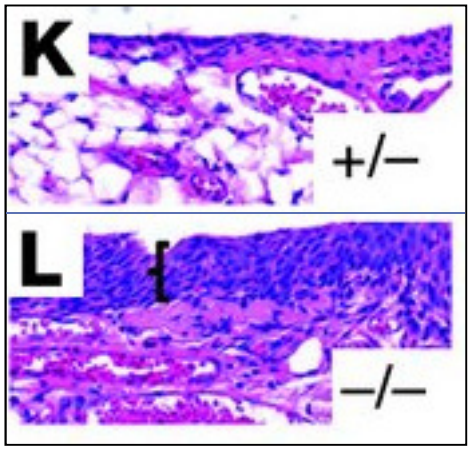
*P<0.05 vs all other groups

Collaboration with Bonassar Lab
(Galley, et al. ORS 2011)

Mechanism(s) of action: beyond lubrication (?)

A prominent feature of lubricin deficiency is synovial hyperplasia/hypercellularity



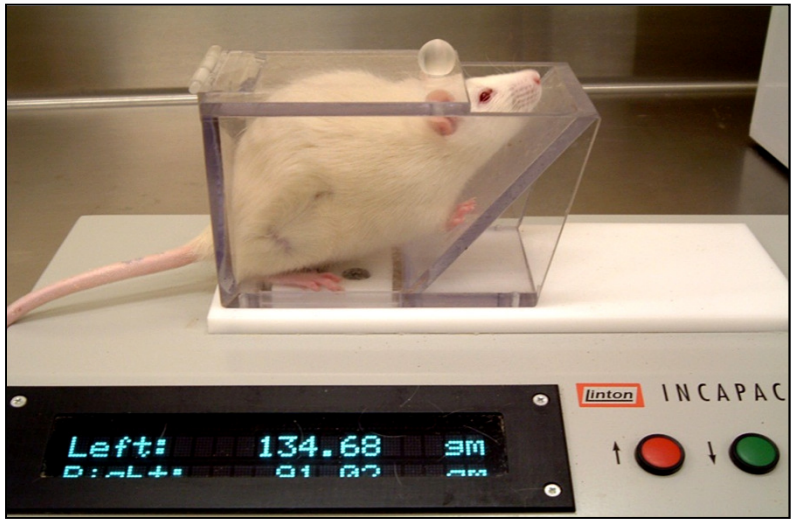


Synovium of 6 month old lubricin +/- and -/- mice (Rhee, et al. JCI 2005)

Synovium from CACP syndrome patients contains a dominant number of infiltrating CD68+ macrophages/giant cells *(Shayan, et al. Pediatr Dev Pathol 2005).*

- Could lubricin inhibit macrophage infiltration, and thereby influence levels and activities of proinflammatory/pain modulators in the joint?

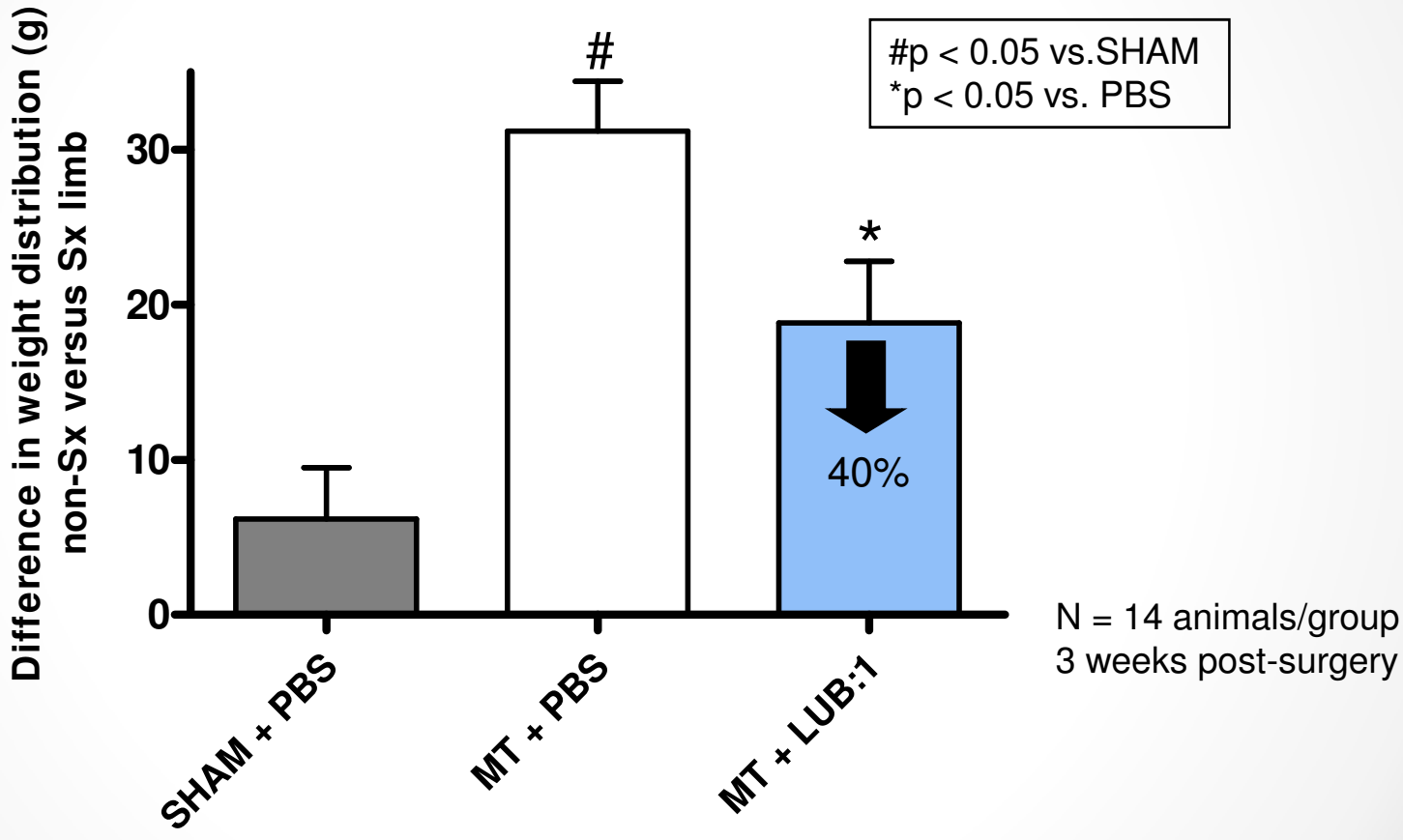
Assessment of LUB:1 effect on pain (weight-bearing) in rat MT model



- Perform unilateral hind-limb surgery (sham or meniscal tear).
- IA treatment with PBS (vehicle) or LUB:1.
- Measure hind limb weight-bearing distribution.

Collaborative studies with:
Lilly Mark, Garth Whiteside, Wyeth Neuroscience

LUB:1 treatment significantly improves weight bearing in the rat MT model



Collaborative studies with:
Lilly Mark, Garth Whiteside, Wyeth Neuroscience

Further considerations for the development of lubricin treatment as an OA therapy

- **Clinical trials for testing disease-modifying OA drugs (DMOADs) are time consuming → expensive. And to date, unsuccessful…**
- **Clinical trials for symptom-modifying drugs offer good potential. Relatively rapid; validated and accepted endpoints for pain/function (i.e. WOMAC OA index).**
- **Local IA dosing advantages include limited systemic exposure and patient compliance. However, turnover/clearance rate must be considered.**
- **Frequency of administration is a critical factor – regulatory agencies, health authorities/payers advocate minimum 3 mo duration of action.**
  - PK studies conducted in rats using [^{125}Iodine]-LUB:1 demonstrate tri-phasic disposition profile after single (20μg) IA dose: $T_{1/2}$ = 4.5h, 1.5d and 2.1 wks *(Vugmeyster, et al. AAPS J 2012).*
    - 6% (1.2μg) of LUB:1 remaining at 48h.
    - At 28d, ~0.05% (0.01μg) of LUB:1 remaining, with localization to joint tissues/cartilage surface.
    - M*eff* prediction of 5.6μg/knee suggests need to explore extended release formulations.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Acknowledgments

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Cornell University
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Bruce Caterson, Clare Hughes & Team

MIT
Al Grodzinsky & Team

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and new (ad)ventures at Genzyme/Sanofi