Osteoarthritis and Cartilage

Oral Presentations

Oral Abstracts

1

LUBRICIN AND HOW ITS CARBOHYDRATE-PROTEIN AND PROTEIN-PROTEIN INTERACTIONS PROVIDE ITS FUNCTIONALITY


Purpose: Biolubrication is key for sustaining the mobility of joints. The consequence of faulty biolubrication is pronounced in pathological conditions such as osteoarthritis (OA) and rheumatoid arthritis (RA), where degradation of the joint is exacerbated by defect of the lubricating superficial layer on the cartilage. For OA and RA, molecular investigation of this layer and its molecules in healthy and disease conditions is crucial.

Methods: We have characterised lubricin in the synovial fluid using proteomic and glycomic techniques. Lubricin was found to be associated with extracellular matrix (ECM) proteins of joint tissue and this association was verified in vitro using recombinant protein constructs of lubricin and its identified binding partners. Immunohistological staining was also used to identify the specific staining of lubricin to cartilage (protein interaction) and to synovial neutrophils (carbohydrate interaction).

Results: Our data suggest that part of lubricin becomes linked to the Cartilage Oligomeric Matrix Protein (COMP) via covalent and non-covalent interaction. This association to a cartilage located protein explains how lubricin can provide efficient boundary lubrication even under high stress conditions in a healthy joint by its specific interaction to cartilage. This organization allows the glycosylated mucin domain of lubricin to generate a friction free joint surface. Analysis of oligosaccharides from OA patients suggests that there are pathological changes that could influence the lubrication property. Also, identification of complex oligosaccharides present in healthy and diseased state indicates that its glycosylation may have additional function as an immune regulator.

Conclusions: The data suggest that the mechanisms for localization of the surface active lubricin to synovial surfaces provide insight into transformation from a healthy state to pathological state, including also changes of the glycosylation that would directly link to the pathology found in OA and RA.

2

LUBRICATION OF CARTILAGE — MENISCUS BIOINTERFACE BY PROTEOGLYCAN 4 AND HYALURONAN; EFFECT OF SLIDING VELOCITY

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Purpose: The meniscus is a fibrocartilaginous pad in the knee joint that has load bearing and lubricating properties. The menisci provide these properties during joint loading through articulating against the cartilage of the femur superiorly and the tibia inferiorly. Removal or damage of the meniscus can lead to changes in joint loading and sub-chondral bone structure, as well as higher prevalence of chronic bone degradation, or osteoarthritis (OA). Meniscal lesions are prevalent in the middle-aged and elderly (ranging 1% of women aged 50-59 to 56% of men aged 70-90); similarly 50% of people aged over 65 suffer from OA. The meniscus forms a biointerface with articular cartilage within the synovial joint. Friction between the apposing surfaces is modulated through several distinct modes of lubrication. Boundary mode lubrication occurs during surface-to-surface contact where surface bound molecules effect lubrication, generally in high load and low speed conditions.

Proteoglycan 4 (PRG4) and hyaluronan (HA) are lubricating molecules present within synovial fluid (SF). PRG4, or lubricin, is a lubricating glycoprotein that contributes to, and is necessary for, proper joint function. PRG4 is synthesized and secreted by cells near the surface of cartilage, as well as the meniscus, and is present at the articulating surfaces of these tissues. Both PRG4 and HA, a repeating disaccharide, have been shown to be effective, dose-dependent boundary lubricants at an articular cartilage-cartilage biointerface in vitro. HA and PRG4 also interact synergistically to lower friction to levels similar to that of whole SF. However, the lubricating properties of these two lubricants at an articular cartilage-meniscus biointerface are currently unknown. Therefore, the objective of this study was to characterize the boundary lubricating properties of PRG4 and HA, both alone and in combination, using an in vitro cartilage-meniscus boundary lubrication friction test.

Methods: A custom cartilage-on-cartilage friction test setup was modified to articulate the surface of meniscus tissue against that of articular cartilage. Tissue samples were harvested from fresh mature bovine stif joints. Osteochondral annuli were harvested from the patellofemoral groove. Meniscal tissues, with the articular surface in tact, were fashioned into ~12mm diameter disks from the inferior surface. The effect of sliding velocity on the friction coefficients at this articular cartilage-meniscus biointerface was evaluated to identify conditions where a boundary mode of lubrication is operative. PRG4 at 0.450 mg/ml, 1500 kDa HA at 3.33 mg/ml, both alone and in combination, were the lubricants of interest. Phosphate-buffered saline (PBS) and SF served as negative and positive controls, respectively. Static friction, μ static, Neq, and kinetic friction, μ kinetic, Neq, coefficients were calculated. Data is presented as mean±sem, N=5–17.

Results: PRG4 and HA demonstrated lubricating function, both alone and in combination. PBS had highest values of μ kinetic, Neq, at all sliding velocities, while SF had the lowest. PRG4 lowered friction at low sliding velocities (0.01–1 mm/s), while both HA and PRG4+HA lowered friction approaching the level of SF (Fig. 1). At a sliding velocity of 0.03 mm/s, PBS had a μ kinetic, Neq, of 0.145±0.015, which decreased to 0.105±0.018 in PRG4 and 0.083±0.009 in HA. PRG4+HA was 0.070±0.010, approaching 0.046±0.003 of SF. Similar trends were observed for μ static, Neq.
Conclusions: This study employed a novel in vitro articular cartilage-meniscus friction test and demonstrated that PRG4 and HA function as effective boundary lubricants at lower sliding velocities. At higher velocities, PRG4 did not reduce friction compared to PBS, consistent with PRG4 being a boundary lubricant. When combined, PRG4 acted synergistically with HA to reduce friction and function as a boundary lubricant similar to that of whole SF. Collectively these results demonstrate PRG4 and HA’s boundary lubricating properties at a cartilage-meniscal biointerface. Given the increased risk for post traumatic osteoarthritis development following a meniscal tear or injury, these results provide the framework for future evaluation of both biomaterial development or biotherapeutic treatment of osteoarthritis.

3 SUB-CRITICAL IMPACT INHIBITS CARTILAGE LUBRICATION MECHANISMS

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Purpose: This study evaluated the effects of sub-critical impact (i.e., an impact that produced surface fissuring but not full-thickness defects) on the morphology and lubrication behavior of cartilage explants to understand how trauma can initiate mechanical mechanisms that lead to further degeneration.

Methods: Cartilage explants taken from the patellofemoral groove of neonatal bovids were impacted using a custom, spring-loaded impactor. The sub-critical damage model was validated by analyzing surface damage as a function of peak stress and stress rate. After determination of the stresses and stress rates that produce surface fissuring but not full-thickness defects in the explants, cartilage plugs were impacted at 17 MPa peak stress and 30 GPa’s peak stress rate. These impacted samples were then tested in a custom friction testing device and compared to healthy tissue. To determine lubrication mechanisms, samples were tested at a range of sliding speeds and in lubricants over a large range of viscosity (PBS 1 mPas, HA 150 mPas, HYADD4 70000 mPas) to reveal lubrication mode transitions. Damage after impact and impact + sliding were analyzed histologically (Safranin O and Picrosirius Red), topographically (white light interferometry) and biochemically (DMMB assay).

Results: Stress and stress rate provided significant correlations with degree of surface damage (Fig 1 A-D), quantified as the percent of surface staining positive for India ink (Fig 1 E) while strain, strain rate, and strain energy density did not (data not shown). The applied impacts produced surface fissures that were on the order of 50 μm wide and 25 μm deep and increased the surface roughness (S_d) of the cartilage by over 50% (Fig 1 FG). After sub-critical impact, the lubrication mechanisms of cartilage were altered. Friction was presented as a function of the Sommerfeld number which combines sliding speed, lubricant viscosity, contact width, and normal load to reveal transitions in lubrication modes. The friction curves (Fig 2 AB) revealed an increase in boundary mode friction (μ_b; the left plateau of the friction curve) (Fig 2 C). More clearly, the transition away from boundary mode friction was inhibited as revealed by increases in the transition number (S_t; the midpoint between boundary and minimum friction) and the minimum friction coefficient (μ_min; the right plateau of the friction curve) (Fig 2 DE). These alterations indicate that impacted cartilage will operate more in the damaging boundary mode of lubrication where friction and wear are high. The impacts alone did not alter proteoglycan content or the collagen structure, but surface fibrillation was evident (Fig 2 F-K). After impact and subsequent sliding, proteoglycan loss and early signs of delamination were evident in the histology, and the proteoglycan content of the impacted and slid plugs was decreased more compared to healthy samples that were slid for an equal amount of cycles (Fig 2 L).

Conclusions: This study revealed that sub-critically impacted cartilage is predisposed to damage propagation due to alterations in lubrication. Specifically, we found that although the boundary mode friction coefficient was elevated after impact, the transition away from boundary mode was more severely altered. This phenomenon is likely due to the alterations in surface topography such as the formation of the fissures that serve as channels through which fluid may flow. By allowing fluid to flow away from areas of high contact stress, the surfaces are less effective at separating and reducing friction. This alteration in surface roughness predisposed cartilage to operating more in boundary mode lubrication, where cartilage is more likely to experience proteoglycan loss and delamination.