Internal Derangement of the Knee is Associated with Impaired Synovial Fluid Lubricant Function and Composition

INTRODUCTION: Injuries to the knee are a major risk factor for the initiation and progression of osteoarthritis (OA)1. The pathogenesis of knee OA in such individuals may involve a variety of factors including acute injury to cartilage, subchondral bone changes, joint instability2 as well as impaired lubrication quality of synovial fluid (SF). SF lubricant function is impaired in humans and animal models following acute3 or traumatic4 injury, possibly in association with decreased SF concentration of proteoglycan 4 (PRG4). PRG4 as well as hyaluronan (HA) provide boundary lubrication function5, and HA is present in SF over a broad size distribution6. In the more general setting of internal derangement of the knee, treated and evaluated by arthroscopic surgery, the possible alteration of SF lubricant function and components is unknown. The objective of this study was to investigate the lubricating ability of SF from human subjects undergoing arthroscopic knee surgery for internal derangement, and the associated lubricant composition in these SF samples.

METHODS: Patient Samples. Patients that were undergoing orthopaedic surgery under an IRB-approved procedure gave informed consent and were considered candidates for this study. Experimental Group: These patients were being treated surgically for the knee (n=24, age 44±2 yrs, mean±SE), and SF was collected prior to arthroscopic knee surgery (INJ-hSF). The fast was normal in all cases. The diagnosis included (in order of frequency): Meniscal tear, cartilage lesion, ACL injury, Synovitis, and Plica syndrome. Control Group: Patients were undergoing orthopaedic surgery of a knee or ankle joint under general anesthesia, and SF was collected from an unaffected (radiographically normal) knee of each of these patients (NL-hSF, n=10, age 46±3 yrs) as obtained previously7. The volume of recovered SF was noted. SF samples were clarified of cells and debris by centrifugation (3,000g, 30min, 4ºC), and the resultant samples were stored at ~70°C before subsequent analysis.

Biochemical Analysis of Putative Boundary Lubricants. Portions of INJ-hSF and NL-hSF samples were assayed for the concentrations of HA, PRG4, and PRG4-immune-reactive proteins. HA was quantified using an ELISA-like assay7. The concentration of HA in MW ranges of 0.03-1, 1-3, and 3-7 MDa was determined by proteinase-K digestion of a portion of sample and then Western Blot (WB) using an antibody recognizing the C-terminal of the human molecule8 and PRG4 standards purified from conditioned medium of human cartilage explants9.

Friction Test of Boundary Lubrication. Portions of INJ-hSF and NL-hSF were analyzed for coefficients of friction as measures of boundary lubrication function in a cartilage-on-cartilage articulation test10. Lubricant solutions, as well as PBS controls, were tested by addition of protease inhibitors, incubation with normal cartilage substrates, and assessment of startup (after a 2min pause) and steady-state friction coefficient in the boundary mode at an effective sliding velocity of 0.3 mm/s as calculated from the measured torque and equilibrium axial load.

Statistics. Data are expressed as mean±SEM. Differences between NL-hSF and INJ-hSF properties were assessed by t-test. The relationship between friction coefficient and biochemical constituents were analyzed by principal components analysis (PCA) as well as multivariate and univariate linear regression.

RESULTS: Patients of the experimental group had a broad range of knee injury. Meniscus was damaged in isolation in 14 (58%) and with ACL rupture in 4 (17%), and normal in the remaining 6 (25%). ACL rupture without meniscus damage was present in 2 (8%) and intact in 18 (75%). Synovitis was pathologic with plica syndrome alone in 6 (25%), together with synovitis in 1 (4%), and with synovitis alone in 3 (13%) The volume of SF recovered was significantly higher for INJ-hSF (5.0mL) than NL-hSF (1.5mL, p<0.01).

The concentrations of HA differed between INJ-hSF and NL-hSF in a manner dependent on MW range (Fig. 1A). HA concentration in INJ-hSF relative to NL-hSF was lower in the 3.7MDa MW range (0.40X, p<0.05, Fig. 1B), similar in the 1-3MDa MW range (1.3X, p=0.12, Fig. 1C), and higher in the 0.03-1MDa MW range (2.8X, p<0.001, Fig. 1D).

Overall HA concentration was similar, ~2.4mg/mL, for NL-hSF and INJ-hSF (p=0.95, Fig. 1E).

The concentration of PRG4 was also similar, ~0.14mg/mL, for INJ-hSF and NL-hSF (p=0.82, Fig. 2).

The steady-state friction coefficient of INJ-hSF was elevated by 21% above that of NL-hSF samples (p<0.05, Fig. 2C).

PCA identified positive associations between HA concentration in the 0.03-1 MW range (+0.91) and steady state friction coefficient (+0.81). Regression analysis confirmed that steady-state friction coefficient increased with increased low MW HA (slope=0.014mg/mL)2, p<0.005, R²=0.32).

DISCUSSION: The finding that patients treated by arthroscopy for internal derangement of the knee have SF that is impaired in terms of lubricant function suggests that abnormal joint mechanics associated with deficient SF may be a contributing factor to accelerated cartilage deterioration in this broad patient population. Whether friction-lowering and wear-protective boundary functions are related in these SF samples, as they appear to be in others2, remains to be established. In addition, the molecular basis of impaired SF lubrication function remains to be fully elucidated but may involve a shift in HA to low MW forms but not diminished PRG4, based on the accompanying biochemical characteristics of the SF samples and correlative analysis.


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