Effect of Knee Osteoarthritis on the Boundary Lubricating Molecules and Function of Human Synovial Fluid

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INTRODUCTION: Osteoarthritis (OA) has been reported to have variable effects on the lubricating molecules and functions of human synovial fluid (hSF)[1-3]. The variability in lubricating function of OA-hSF may be due, in part, to the differing counterface materials and methods of biomechanical testing, resulting in multiple modes of lubrication[1-4]. Recent studies have enabled testing of fluid samples to assess friction-lowering properties in the boundary mode of lubrication[5]. The variability in lubrication function of OA-hSF may also reflect patient-specific differences in the concentration and quality of lubricating molecules, including proteoglycan 4 (PRG4)[6] and hyaluronan (HA)[4]. HA has a broad molecular weight (MW) distribution in hSF[7-9], and has size-dependent rheological properties[9,10]. Thus, the objectives of this study were to determine if the lubricating molecules and functions of hSF were altered in OA knees as compared to normal knees, and if the lubricating functions of such fluids were related to the concentrations of PRG4 and HA of various MW.

METHODS: ***Lubricant Solutions.*** Following IRB-approved human subject protocols, SF was obtained from subjects undergoing total knee arthroplasty for OA (OA-hSF, n=24, age 72±2 yrs, mean±SE), who had granted informed consent. In addition, SF from radiographically normal knees of patients undergoing surgery on other joints (NL-hSF, n=8-10, age 46±4 yrs)[11] were analyzed. 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.

Some samples of OA-hSF were further analyzed to assess the role of the protein (protease-sensitive) and HA (protease-resistant) components of OA-hSF. Portions of some OA-hSF were protease digested by treating with 0.5mg/ml proteinase-K, incubation at 37ºC overnight, and subsequently treating with 5mM AEBSF to inhibit residual enzyme activity (ProK-treated). Other portions of OA-hSF were sham-digested by mixing an equivalent amount of AEBSF-inhibited proteinase-K. Inhibition was verified based on the sensitivity of cartilage to protease-induced GAG depletion.

Biochemical Analysis of Putative Boundary Lubricants. Portions of OA-hSF and NL-hSF samples were assayed for the concentrations of total protein, PRG4-immunoreactive proteins and HA, as well as the MW distribution of HA. Total protein was quantified with the BCA assay. HA was quantified with an ELISA-like assay[12]. The concentrations of HA in MW ranges of 0.03-1, 1-3, and 3-7 MDa were determined by proteinase-K digestion of a portion of sample and assessing the *S. Hyaluronidase*–sensitive portion on electrophoresis in 1% agarose gels[13]. PRG4 was quantified by *S. Hyaluronidase* digestion of a portion of sample and analyzed by Western Blot using an antibody directed against the C-terminal of the human molecule[14] and PRG4 standards purified from conditioned medium of human cartilage explants[15].

Friction Test of Boundary Lubrication. Portions of OA-hSF and NL-hSF, ProK-treated OA-hSF, and sham-digested OA-hSF were analyzed for coefficients of friction as measures of boundary lubrication function in a cartilage-on-cartilage articulation test[6]. 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 2 min pause) and steady-state friction coefficient in the boundary mode at a sliding velocity of 0.3mm/s as calculated from the measured torque and equilibrium axial load.

Statistics. Data are expressed as mean±SEM. Differences between NL-hSF and OA-hSF properties, as well as between ProK-treated and sham-digested OA-hSF, were assessed by t-test.

RESULTS: The volume of recovered SF was markedly elevated for OA-hSF (8.8mL) over that of NL-hSF (1.5mL, p<0.05).

The concentrations of HA differed between OA-hSF and NL-hSF in a manner that varied with MW (**Fig. 1A**). HA concentration in OA-hSF relative to NL-hSF was lower in the 3-7MDa MW range (–64%, p<0.005**, Fig. 1B**), similar in the 1-3MDa MW range (1.1X, p=0.3**, Fig. 1C**), and higher in the 0.03-1MDa MW range (5.8X, p<0.005**, Fig. 1D**). Overall HA concentration was similar (~2.3 mg/mL) for NL-hSF and OA-hSF (p=0.4**, Fig. 1E**).

The concentration of PRG4 was markedly elevated (to 1.8X) in OA-hSF over that of NL-hSF (p<0.005, **Fig. 2A, B**).

The startup friction coefficient was lower for OA-hSF than NL-hSF samples (–33%, p<0.005, **Fig. 2C**). The steady-state friction coefficient was similar for OA-hSF and NL-hSF (p=0.5, **Fig. 2D**). Digestion of the protein component of OA-hSF with ProK resulted in an elevation of steady-state friction coefficient (to 2.0X) over sham-digested controls (p=0.06).

DISCUSSION: The finding that the friction-lowering lubricant functions of OA-hSF are similar to, and in some test situations better than, those of NL-hSF, despite markedly different SF biochemical composition, indicates a remarkable functional biomechanical adaptation of SF in established OA. The impairment of OA-hSF function due to protease digestion is consistent with a contribution to lubricating properties by PRG4 protein. Indeed, if the shift in HA to a lower MW form is deleterious for lubrication function in OA, the increase in PRG4 concentration may fully compensate for the HA deficiency. The OA-associated increase in concentration of PRG4 together with the increased volume of SF suggests exuberant production and retention of PRG4 lubricant. If such a PRG4 response is protective of cartilage in established OA, a useful therapeutic strategy may be to trigger or mimic this response early in the disease process, before significant cartilage damage occurs.

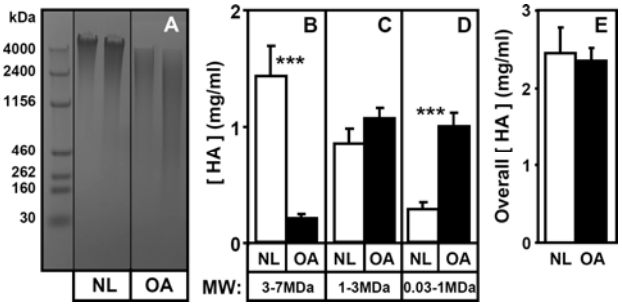

Figure 1. Characteristics of hyaluronan in NL-hSF and OA-hSF. **(A)** Electrophoretic separation of typical samples. Concentration of HA in (**B-D**) 0.3-1, 1-3, and 3-7 MDa ranges and (**E**) overall. ***p<0.005.

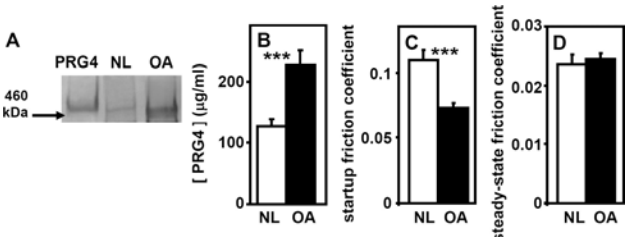

Figure 2. PRG4 content and lubrication properties of NL-hSF and OA-hSF. (**A**) Typical Western Blot of PRG4 standard, NL-hSF, and OA-hSF, (**B**) concentration of PRG4, and (**C**) start-up and (**D**) steady-state friction coefficients.

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