ABSTRACT

The present work is the first instance where a novel multiple-particle tracking microrheology technique has been applied to study molecular interactions of clinical significance. Herein we describe the molecular changes due to lubricin-hyaluronate interaction and their effect on mechanical properties of synovial fluid. Along with bulk rheology studies it was found that lubricin, a boundary lubricant, increases the HA network formation conducive to the enhanced molecular layering of HA under stress which results in increased shear thinning. This interaction may also allow HA molecules to bind to the cartilage surface, providing boundary lubrication, by virtue of its interaction with lubricin.

INTRODUCTION

Synovial fluid is a semi-dilute solution of hyaluronate (HA) with additional constituents that play a wide variety of biological roles, including the regulation of the molecular structure of the fluid and lubrication of cartilaginous joints. HA, a polyelectrolyte molecule with rotational bonds and a molecular weight of approximately $1 \times 10^6$ daltons, is the principal constituent of synovial fluid giving rise to its viscoelastic characteristics and also serves as a boundary lubricant when attached to a surface. This attachment is not easily attained [1]. The rheology of hyaluronate depends on aggregates and proteins present in the fluid [2-4]. Hyaluronate and lubricin, a glycoprotein expressed by synovial fibroblasts [5], synergistically reduce friction under high loads [6, 7].

The synovial fluid of patients with camptodactyly-arthropathy-coxa vara-pericarditis (CACP) syndrome lacks lubricin and thus fails to provide lubrication [8]. CACP synovial fluid provides an opportunity to identify the rheological contributions of lubricin, as well as the tribological contributions of lubricin-hyaluronate interaction, to diarthrodial lubrication. Previous studies of the lubricin-hyaluronate interaction relied on bulk rheology methods, which induced a deformation that disrupted the molecular network of the fluid. These showed a decrease in hyaluronate viscosity in the presence of lubricin.

METHODS

**Multiple-Particle Tracking Microrheology** Particles (200nm mean-diam.) in the sample were tracked separately. The time-dependent ensemble-averaged mean squared displacement (MSD) of all particles was measured and analyzed over a range of frequencies using MATLAB technical computing language software. A Gaussian distribution is fitted to the Airy disks formed by the intensity of the light emitted from the fluorophores in each particle. The center of approximately 100 particles was individually tracked, for 12 seconds at a rate of 16 frames per second, with a subpixel interpolation that measures the time-dependent MSD with approximately 5nm spatial resolution. The structural and mechanical heterogeneity of the network in the dilute solutions were probed by observing the time-dependent distribution of MSD of individual particles at different locations throughout the sample.

**Bulk Rheology Studies** The rheological studies of bovine synovial fluid (BSF), enzyme-digested BSF (ET-BSF) and human synovial fluid from a patient with CACP (CACP-HSF)
were performed in the AR 2000 (TA Instruments, New Castle, DE) rheometer at 21°C. A quantity of 200µL was needed of each fluid to fill the 19µm gap between the stage and cone. Shear flow tests were performed on each fluid sample to measure the shear rate-dependent dynamic viscosity at a shear rate range of $10^1$ to $2 	imes 10^5$ s$^{-1}$ and 5% strain. Unsteady or oscillatory shear flow measurements were obtained at an angular frequency range of $10^0$ to $10^3$ rad/s. The hyaluronate concentration was 3.5mg/ml across all samples.

**RESULTS**

The time-dependent ensemble-average MSD of probes embedded in polymeric viscoelastic fluids adopts a power law behavior, $\langle \Delta r^2(\tau) \rangle \sim \tau^\alpha$, where at low time lags (<300ms) BSF shows a subdiffusive behavior ($\alpha < 1$) due to particle entrapment in the HA network. At higher time lags BSF shows a mostly diffusive behavior, which is evident by a slope that approaches unity ($\alpha \approx 1$). Enzyme-treated BSF and CACP-HSF exhibited a diffusive behavior at both low and high time lags for the same bead-size (200nm). This behavior resembles that of 4:1 glycerol-DIW solution, a Newtonian fluid.

The HA solutions lacking lubricin (both UHA and CACP-HSF) exhibited the viscoelastic moduli of an untangled polyelectrolyte solution. The relaxation time for CACP-HSF and umbilical cord hyaluronate (UHA) was an order of magnitude higher than that of both the BSF and the ET-BSF solutions (Table 1).

**DISCUSSION**

The absence of subdiffusive and elastic behavior of the CACP-HSF, at the physiological shear rates, is evidence for an interaction of lubricin-hyaluronate where lubricin increases the degree of entropy of an otherwise stiffer HA polymer. A decrease in the persistence length due to this interaction would lead to a more flexible hyaluronate molecule that is likely to form entanglements. Subdiffusive and elastic behavior in BSF is evident by the particle entrapment of the 200nm probes. The diffusive behavior and the little change in bulk elasticity after enzymatic treatment of BSF point to an increase in the characteristic mesh size of the molecular network, whereby the probes move freely through the fluid. Although in the absence of lubricin, synovial fluid is unable to store and dissipate the energy of impact during normal walking gait, and therefore lacks this chondroprotective feature distinct from lubrication, the influence of other HA-binding proteins cannot be disregarded. Lubricin increases the HA network formation conducive to the enhanced molecular layering of HA under shear stress which results in increased shear thinning. This interaction may also allow HA molecules to bind to the cartilage surface, providing boundary lubrication, by virtue of its interaction with lubricin.

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