RHEOLOGICAL AND STRUCTURAL CHARACTERIZATION OF SYNOVIAL FLUID FROM PATIENTS WITH OSTEOARTHRITIS

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INTRODUCTION

Osteoarthritis (OA) is one of the most disabling degenerative diseases and is characterized by the breakdown of articular cartilage resulting in joint pain and stiffness [1]. In healthy individuals, articular cartilage and a thin film of synovial fluid (SF) are closely linked in providing a protective barrier between the ends of the bones and lubricating the joint. SF has a major role in joint lubrication, shock absorption and load bearing. Healthy SF is a viscoelastic fluid, highly viscous at low strain rate and highly elastic at high strain rate [2]. SF is a plasma dialysate consisting mainly of hyaluronic acid (HA), proteins, water and lubricin. In an osteoarthritic joint, damage to articular cartilage causes modifications in rheological properties of the SF and its chemical composition. As a result of these chemical composition changes, SF losses its viscoelasticity properties and becomes a less effective lubricant [3, 4].

Synovial fluid rheological behavior is mostly determined by its HA content [5]. As a result of HA degradation, the SF becomes less viscous [6] and, therefore, less effective in lubricating the joint [2, 7]. In addition, the reduction in molecular weight and concentration of HA reduces the SF viscoelasticity and sequentially its ability to protect the joint [8]. The protein content may also affect the rheological properties of synovial fluid [4, 9]. A recent study suggested that protein may interact with HA [10]. However, the interaction mechanism, and the protein role in synovial fluid lubrication are not totally elucidated. Lubricin may also have a role in synovial fluid lubrication ability, especially in boundary lubrication [11].

A thorough elucidation of the rheological properties of SF is necessary in order to better understand its role in joint lubrication. The objective of this study is to provide a comprehensive shear and extensional rheological characterization of OA SF, and correlate the rheological properties with synovial fluid chemical composition leading to a better understanding of its lubrication properties.

MATERIALS AND METHODS

SF samples have been collected from osteoarthritis patients who are undergoing total knee replacement surgery. SF samples are obtained from the orthopedic reconstructive service at Vancouver Coastal Health Region in accordance with a protocol approved by the University of British Columbia Clinical Research Ethics Board and the Vancouver Coastal Health Research Institute. Informed consent is obtained from each patient prior to surgery. Synovial fluid is aspirated from each patient's knee joint into a test tube by an experienced surgeon under sterile conditions.

Shear rheological properties of each synovial fluid sample are determined using a Kinexus Ultra rheometer (Malvern Instruments Ltd., Worcestershire, UK) at 25 ºC and/or 37 ºC using a stainless steel cone and plate geometry (40 mm diameter cone with a 1º cone angle). Each rheometer was first calibrated with Cannon Certified Viscosity Standard oil. The shear rates ranging from 10^{-2} to 10^{3} s^{-1} were applied to each sample. It is worth noting that the shear rate in human joints can reach more than 10^{5} s^{-1} [12]; however, measurements over 1000 s^{-1} are not possible due to an inherent limitation of the rheometer. In the small amplitude oscillatory shear test, preliminary strain sweep tests were performed on the samples in order to identify the linear viscoelastic response range of the samples. Then, frequency sweep measurements were conducted in the linear region, at 1-5% strain, over a frequency range of 10^{-2}-10 Hz.

Extensional rheological properties of each synovial fluid sample are determined using a CaBER 1 extensional rheometer (Thermo Electron, Karlsruhe, Germany) with 6 mm circular endplates. A small volume of the sample is applied between two plates forming a cylindrical liquid bridge with an initial distance of 3 mm. Then, the upper plate is pulled to a predefined final axial height of 10 mm. CaBER measures the extensional relaxation time and the steady terminal extensional viscosity.

The protein concentrations of synovial fluid are determined using a bicinchoninic acid (BCA) protein assay kit (Thermo Scientific, Rockford, IL). Each SF sample is diluted using Phosphate buffered saline (PBS) to within the BCA working range of 20 to 2000 µg/mL.

RESULTS AND DISCUSSION

Shear Rheology of Synovial Fluid

Osteoarthritis synovial fluid exhibits non-Newtonian shear thinning behavior (i.e. the viscosity decreases with increasing shear rate) as shown in Figure 1. These results are in agreement with previous studies [2, 3, 13-17]. Moreover, these results show that OA SF is less viscous than healthy SF [2]. Therefore, OA SF is a less effective lubricant for articulating joints. Rheological testing is done at 25 ºC and 37 ºC to witness the temperature effects on SF rheological properties. Previous rheological studies were conducted at either 25 ºC or 37 ºC. SF temperature inside the joint varies depending on the surrounding environment and activity [18]. Therefore, neither 25 ºC nor 37 ºC would reflect the SF physiological temperature; however, as human body temperature is 37 ºC, it may be a more relevant reference temperature. Figure 1 shows that as temperature increases, SF viscosity decreases. That suggests the SF becomes a less effective lubricant in high temperature environments or with excessive physical activity.

OA SF viscoelastic properties are investigated by oscillatory flow. Figure 2 shows the elastic and viscous moduli at 25 ºC and 37 ºC, as well as the crossover frequency. As with previous steady shear rheological results, OA SF viscoelastic properties decreases as temperature rises. OA SF viscoelasticity varies from sample to sample as shown in Figure 3. Elastic and viscous moduli are significantly lower than those reported for healthy SF [2, 3, 13]. An increase in crossover frequency in OA samples is also observed, and some samples do not show a crossover in the investigated frequency range. Crossover frequency relates to the disentanglement rate of the HA chain, and depends on the molecular weight and concentration of HA as it reflects the mobility of the HA chain [19]. The reduction in the elastic properties and the increase of crossover frequency can indicate a decrease in the molecular weight and the concentration of HA [19].

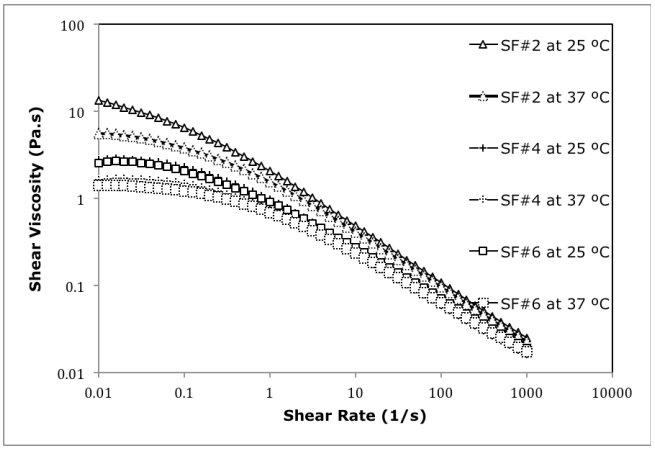

Figure 1: Viscosity as function of shear rate for three SF samples at 25 ºC and 37 ºC.

Healthy SF has a high viscous modulus at low frequencies and a high elastic modulus at high frequencies which makes SF a suitable lubricant at low frequency activity like walking, and a good shock absorber at high frequency activity like running. However, OA SF tends to lose its elastic properties as shown in Figures 2 and 3. These results suggest that OA patients suffer more when they preform activity at high frequency because their SF losses its shock absorption properties.

Extensional Rheology of Synovial Fluid

Steady shear and oscillatory flow can not fully describe the SF rheological behavior in joint movement [20]. Extensional flow can significantly stretch HA molecules, which result in a significant increase in elastic forces and extensional viscosity [21]. The extensional

viscosity is potentially dominant at high shear rate (500 s^{-1}) [20]. Thus, measurement of extensional rheology of SF is important.

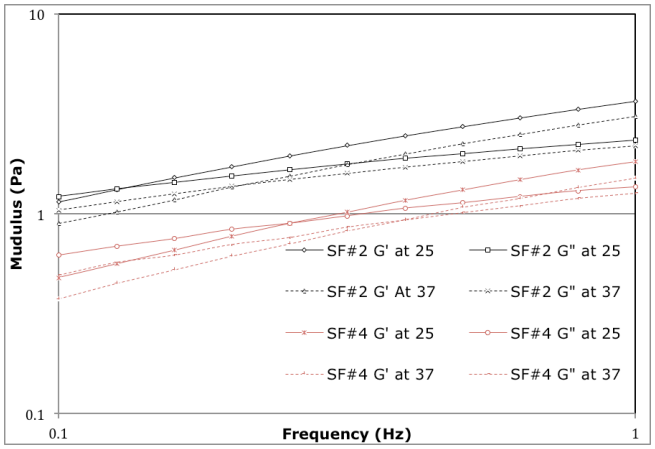

Figure 2: Elastic (G') and viscous (G") moduli as function of frequency for two SF samples at 25 ºC and 37 ºC.

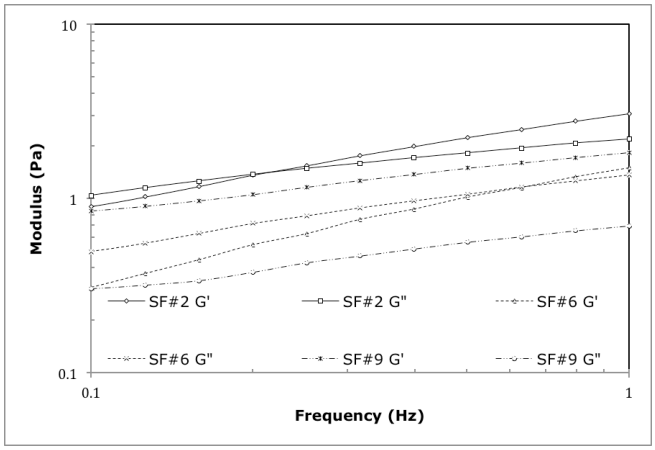

Figure 3: Elastic (G') and viscous (G") moduli as function of frequency for three SF samples.

The steady state extensional viscosity and the relaxation time of osteoarthritis synovial fluids are measured by CaBER. Table 1 shows the results of five OA SF samples. The extensional viscosity is significantly higher than the shear viscosity. The extensional relaxation time is also significantly lower than shear relaxation time. This suggests that HA macromolecules are stretched faster in extensional flow. Bingöl et al. [22] studied the extensional rheology of human SF taken post mortem from knee joints. Their results showed higher rheological properties than the current study. These results suggest that a more degenerate OA disorder leads to less effective lubrication of the joint.

Table 1: Terminal extensional viscosity and relaxation time for 5 OA SF.

Sample No.	Terminal Extensional Viscosity (Pa.s)	Relaxation Time (s)
1	55	0.206
2	27	0.105
3	28	0.125
4	12	0.06
5	30	0.114

Protein Concentrations of Synovial Fluid

Protein concentration tests are carried out on SF samples to find if SF protein concentration correlates with SF rheological properties. Our preliminary results are shown in Table 2. The protein concentration for healthy SF falls between 16 and 22 mg/mL [13]. Most of the samples shown in Table 2 have higher protein concentrations than those found in healthy SF. This indicates that OA causes an increase in the SF protein concentration, and these results agree with previous studies [3].

Table 2: Total protein concentration and shear rheological properties for OA SF samples.

Sample No.	Protein Concentration (mg/mL)	Zero Shear Viscosity at 37 ^{0}C (Pa.s)	Cross-over Frequency (Hz)
1	12.08	2	0.65
2	22.77	5.6	0.2
3	32.8	0.1	No cross-over
4	26.07	1.7	0.4
5	29.4	0.3	No cross-over
6	30.74	1.4	0.7
7	21	0.17	No cross-over
8	23.87	0.3	No cross-over

The effect of protein concentration on SF rheology is still not completely understood. Balazs did not find evidence indicating that protein concentration considerably alters the sodium salt of HA viscoelasticity [13]. However, a recent study suggests that protein may play a role in the SF rheopexy effect [9].

CONCLUSION

Rheological behavior of OA SF differed from healthy SF. OA SF is less viscous than healthy SF, and suffers a reduction of viscoelastic properties. As a result, it becomes a less efficient lubricant. SF rheological behavior is affected by the environment and physical activity. At high temperature or excessive physical activity, SF becomes less viscous, and its viscoelastic properties decrease as well. In addition to shear rheology, extensional rheological behavior of OA SF have been studied. Extensional rheological properties are significantly higher than shear rheological properties.

The results presented in this paper are preliminary results. Therefore, it is still too early to conclude any information about protein effects on SF rheology. Many tests are still underway. Our aim is to measure protein concentration of SF samples and correlate it with shear and extensional rheology. The results from this study will lead to a better understanding of the role of synovial fluid in joint lubrication.

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