RHEOLOGICAL STUDY OF VISCOSUPPLEMENTS AND SYNOVIAL FLUID IN PATIENTS WITH OSTEOARTHRITIS

Petcharatana Bhuanantanondh[1, 2], Dana Grecov [1], Ezra Kwok [2]

[1] *Department of Mechanical Engineering*

[2] *Biomedical Engineering Program*
University of British Columbia

ABSTRACT

A detailed rheological characterization of synovial fluid from 22 patients undergoing total knee arthroplasty and three commercially available viscosupplements was performed. The results showed that synovial fluid in patients with osteoarthritis (OA) exhibited a non-Newtonian shear thinning behavior and viscoelastic properties. Aspirates of the knees from the same individual show very different viscosity and viscoelasticity. Moreover, rheopectic behavior was observed in OA synovial fluid at 37 ºC. All three viscosupplements exhibited a non-Newtonian shear thinning behavior. The viscosupplement with cross-linked hyaluronic acid has higher viscosity than that with the non-cross-linked ones. Moreover, high molecular weight viscosupplements have greater viscoelasticity than low molecular weight viscosupplements.

INTRODUCTION

Osteoarthritis (OA), the most common form of arthritis, is a degenerative joint disease that is characterized by the breakdown of articular cartilage [1] causing pain, swelling and limited mobility in the joint. In healthy individuals, articular cartilage and a thin film of synovial fluid are closely linked in providing protective barrier between the ends of the bones and lubricating the joint. In osteoarthritic joint, damage to articular cartilage may result in changes in rheological properties of the synovial fluid. Synovial fluid becomes less viscous and therefore less effective in joint lubrication [2, 3] and eventually will have an effect on the performance of the joint. A thorough elucidation of the rheological properties of the synovial fluid is necessary in order to better understand its role in joint lubrication.

The goals for treatment of OA are to minimize pain and maintain joint mobility [4]. One of the non-operative treatments for OA is intra-articular injections of hyaluronic acid known as viscosupplementation. There are different formulations of viscosupplements that are commercially available. Viscosupplements can be derived either from animal or from biological fermentation of streptococcal origin [5]. There is also the family of cross-linked hyaluronic acid derivatives name hylans. Hylans are polymers of hyaluronan that have been cross-linked through their hydroxyl group [6]. Therefore, further studies are warranted to examine rheological properties of different viscosupplements.

The objectives of this study were to determine rheological behavior of synovial fluid in patients with OA and to examine whether there are any differences between different formulations of viscosupplements in rheological behavior and their effects related to joint lubrication.

MATERIALS AND METHODS

Synovial fluid samples were obtained from 22 patients during knee arthroplasty for OA. Patients ranged from 44 to 85 years old, with a mean age 64 years. Synovial fluid samples came from the orthopedic reconstructive service at Vancouver Coastal Health Region (University of British Columbia Hospital or Vancouver General Hospital) in accordance with a protocol approved by the University of British Columbia Clinical Research Ethics Board and Vancouver Coastal Health Research Institute. Informed consent was obtained from each patient prior to the surgery. Synovial fluid was aspirated from each patient's knee joint into a test tube by an experienced surgeon under sterile condition at the time of the surgery.

In addition, rheological properties for 3 different commercially available viscosupplements were examined. The viscosupplements are (1) a non-cross-linked high molecular weight hyaluronic acid (VS1), (2) a non-cross-linked low molecular weight hyaluronic acid (VS2), and (3) a cross-linked hyaluronic acid (VS3).

Rheological properties of each synovial fluid sample and each viscosupplement were determined using a Bohlin Gemini HR^{nano} rheometer and a

Kinexus rheometer (Malvern Instruments Ltd., Worcestershire, UK), respectively, at 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 ranged from 0.01 to 1000 s^{-1} were applied to each sample. In addition, a constant shear rate at 0.05 s^{-1} was applied to the samples in order to examine the changes in shear stress over time. In the small amplitude oscillatory shear test, preliminary strain sweep test 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 5% strain, over a frequency range of 0.1-10 Hz.

RESULTS AND DISCUSSION

Rheological Behavior of Human Synovial Fluid

Synovial fluid in OA exhibited a non-Newtonian shear thinning behavior (i.e. viscosity decreases with increasing shear rate) and viscoelastic properties. In two of the patients, synovial fluid samples were obtained from both knees during bilateral total knee arthroplasty. For these two patients, the rheological properties of synovial fluid from left and right knees were compared. The results showed that the viscometric and viscoelastic parameters of the two knees were very different (Figure 1 & 2).

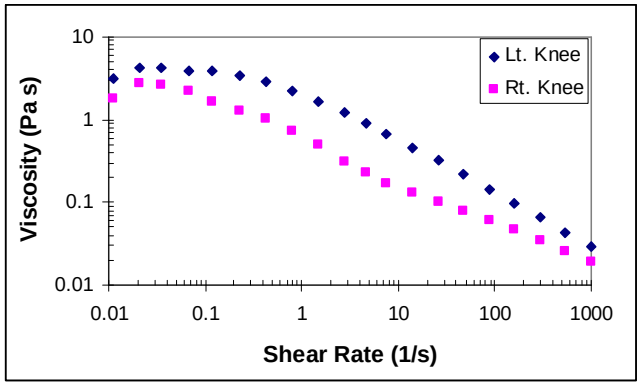

Figure 1: Viscosity as a function of shear rate: Viscometric parameter of left knee vs. right knee.

In a study by Mazzucco et al. [7], the flow parameters of synovial fluid were evaluated at 25 ºC. They reported that viscosity of synovial fluid between the left and right knees differ substantially but also their viscoelastic properties of synovial fluid are different. This implies that OA is more than a systemic disorder. Alterations within the joint determine the properties of the synovial fluid to a larger extent. Although only two cases were reported to have significance difference between the knees of the same patient, it is intuitive that many factors contribute to the variability of the rheological properties of synovial fluid within a single patient, including left- or right-sided dominance, joint geometry, and trauma history.

Moreover, rheopectic behavior (i.e. shear stress increases over time at a constant shear rate) was observed in OA synovial fluid at the physiological temperature of 37 ºC (Figure 3).

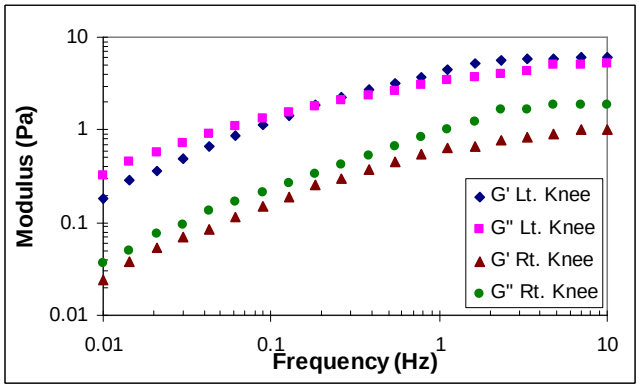

Figure 2: Elastic (G') and viscous (G") moduli as a function of frequency: Viscoelastic parameters of left knee vs. right knee.

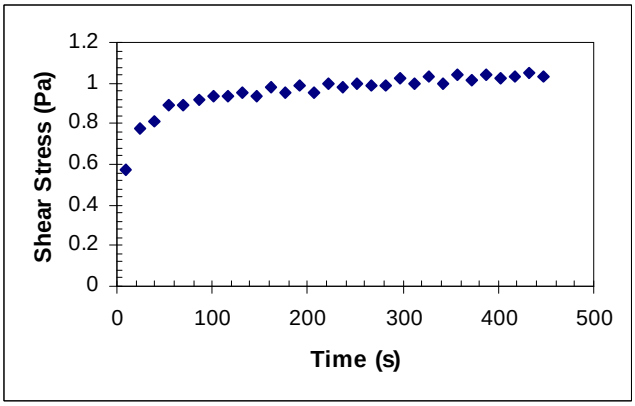

Figure 3: Shear stress as a function of time at shear rate 0.05 s^{-1}

Rheopexy in synovial fluid has previously been observed in several studies [8-11]. O'neill and Stachowiak [11] reported that OA synovial fluid exhibited rheopectic behavior at the temperature of 20 ºC or less. However, in the present study, the rheopectic behavior was observed in OA synovial fluid

samples at 37 ºC. The differences of the findings may be caused by several factors which include the type of instrument, the type of measuring geometry and the constant shear rate that was applied to the sample. It should be noted that in the O'neill and Stachowiak [11] study, the constant shear rate applied to the sample was not reported. Oates et al. [10] asserted that rheopectic behavior is attributed to protein aggregation which appears to play an important role in enhancing the viscoelastic properties of synovial fluid.

Rheological Behavior of Viscosupplements

As stated in the previous section, three commercially available viscosupplements for joint injection were analyzed. The results showed that different formulations of viscosupplements used in this study also exhibited a non-Newtonian shear thinning behavior (Figure 4), similar to human synovial fluid. The one with non-cross-linked low molecular weight hyaluronic acid (VS2) had the lowest viscosity throughout the entire range of shear rates. This finding is consistent with a study by Prieto et al. [12]. At lower shear rates (~ 0.01 – 1 s^{-1}), the one with cross-linked hyaluronic acid (VS3) had higher viscosity than the one with non-cross-linked high molecular weight hyaluronic acid (VS1), but had lesser viscosity at higher shear rates. At shear rate 0.01 s^{-1}, the viscosity of the one with cross-linked hyaluronic acid (VS3) was about two orders of magnitude higher than that of the one with non-cross-linked low molecular weight hyaluronic acid (VS2)

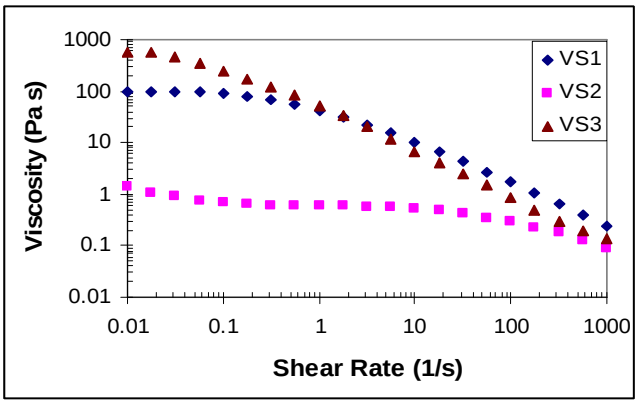

Figure 4: Viscosity as a function of shear rate for three different viscosupplements (VS1: a non-cross-linked high molecular weight hyaluronic acid, VS2: a non-cross-linked low molecular weight hyaluronic acid, VS3: a cross-linked hyaluronic acid).

The results from the present study indicated that viscosity of the viscosupplement highly depends on molecular weight of the hyaluronic acid. In addition, it was observed that, as shear rate increases, the one with cross-linked hyaluronic acid (VS3) showed a sharper decrease in viscosity than that with the non-cross-linked ones. This finding is consistent with the results from previous study which reported that the decrease in apparent viscosity was more pronounced in high molecular weight hyaluronic acid than low molecular weight hyaluronic acid [13].

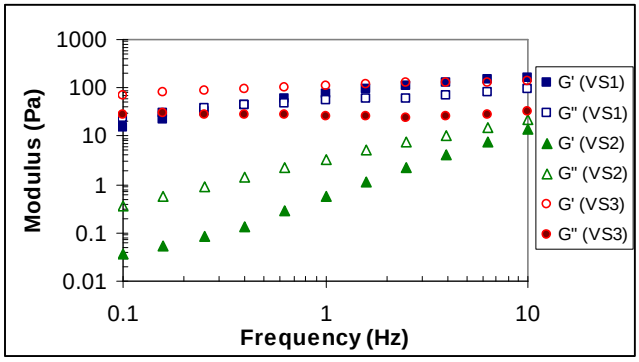

Figure 5: Elastic (G') and viscous (G") moduli for three different viscosupplements (VS1: a non-cross-linked high molecular weight hyaluronic acid, VS2: a non-cross-linked low molecular weight hyaluronic acid, VS3: a cross-linked hyaluronic acid).

Figure 5 shows that each viscosupplement exhibited different viscoelastic behavior. For the one with non-cross-linked high molecular weight hyaluronic acid (VS1), it was found that at low frequencies, viscous modulus (G") was higher than elastic modulus (G'). At higher frequencies, elastic modulus (G') exceeded viscous modulus (G"). The cross over frequency was also observed. The results also showed that the one with non-cross-linked low molecular weight hyaluronic acid (VS2) exhibited a viscous-like behavior; that is viscous modulus (G") remained larger than elastic modulus (G') [14] over the entire range of oscillation frequency. The one with cross-linked hyaluronic acid (VS3), however, exhibited a gel-like behavior; that is elastic modulus (G') remained larger than viscous modulus (G") [14] throughout the range of oscillation frequency. Furthermore, the result showed that the one with non-cross-linked low molecular weight hyaluronic acid (VS2) had the lowest elastic modulus (G') and viscous modulus (G") over the range of frequency.

These findings suggested that the differences in rheological behavior are related to molecular weight of the viscosupplements and its network forming ability. Previous studies reported that at high molecular weight a transient entanglement network is formed, but it was absent for hyaluronic acid at low molecular weight [8, 15]. The network-forming ability of hyaluronic acid in solutions affects the viscoelasticity of the solution [13].

Table 1: Viscoelastic properties of different viscosupplements at 0.5 Hz. and 2.5 Hz.

VS	0.5 Hz.		2.5 Hz.	
	G'	G''	G'	G''
1	51.2	45.9	111.2	61.5
2	0.2	1.8	2.2	7.4
3	91.9	26.1	118.1	22.5

Viscoelastic properties are related to the function of shock absorber during walking and running. Table 1 summarizes the elastic (G') and viscous (G'') moduli of viscosupplements tested at the frequencies of 0.5 and 2.5 Hz. These frequencies, 0.5 and 2.5 Hz., correspond to joint movement during walking and running, respectively [16]. The results showed that the dynamic moduli, G' and G'', at both 0.5 and 2.5 Hz. were smallest in the one with non-cross-linked low molecular weight hyaluronic acid (VS2). It was also observed that the elastic modulus (G') of the one with cross-linked hyaluronic acid (VS3) was largest at both frequencies. On the other hand, the one with non-cross-linked high molecular weight hyaluronic acid (VS1) was found to have the largest viscous modulus (G'').

CONCLUSION

Rheological behavior of synovial fluid varied widely in OA. Synovial fluid in OA exhibits a non-Newtonian shear thinning behavior and viscoelastic properties. Aspirates of the knees from the same individual show very different viscosity and viscoelasticity. It has been reported previously that OA synovial fluid exhibited rheopectic behavior only at the temperature of 20 ºC or less. In the present study, the rheopectic behavior was also observed in OA synovial fluid at the physiological temperature of 37 ºC. The findings from this study will lead to a better understanding of the role of synovial fluid in joint lubrication.

In addition, the results from this study indicate that there are differences in rheological behavior between different formulations of viscosupplements. The viscosupplement with cross-linked hyaluronic acid has higher viscosity than that with the non-cross-linked ones. Moreover, high molecular weight viscosupplements have greater viscoelasticity than low molecular weight viscosupplements. Viscosity and viscoelasticity of each viscosupplement are related to its effect on joint lubrication. Therefore, the findings from this study may help in selecting which viscosupplement will be the most efficient in relation to joint lubrication.

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