Rheological Study of Viscosupplements and Synovial Fluid in Patients with Osteoarthritis

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Abstract

A detailed rheological characterization of synovial fluid from 22 patients undergoing total knee arthroplasty and three commercially available viscosupplements is performed. The results show that synovial fluid in osteoarthritis (OA) patients exhibits non-Newtonian shear thinning behavior and viscoelastic properties. Synovial fluid of the knees aspirated from one individual show very different viscosities and viscoelasticities. Moreover, rheopexic behavior is observed in OA synovial fluid at 37°C. All three viscosupplements exhibit non-Newtonian shear thinning behavior. The viscosupplement with cross-linked hyaluronic acid has a higher viscosity than that of with non-cross-linked hyaluronic acid. Moreover, high-molecular-weight viscosupplements have greater viscoelasticity than that of low-molecular-weight viscosupplements.

Keywords: Rheology, Viscosupplements, Osteoarthritis, Synovial fluid

1. Introduction

Osteoarthritis (OA), the most common form of arthritis, is a degenerative joint disease that is characterized by the breakdown of articular cartilage, causing pain, swelling, and limited mobility in the joint [1]. In healthy individuals, articular cartilage and a thin film of synovial fluid provide a protective barrier between the ends of the bones and lubricate the joint. In osteoarthritic joints, damage to articular cartilage may result in changes in the rheological properties of the synovial fluid. Synovial fluid becomes less viscous and therefore less effective in joint lubrication [2,3], which eventually affects 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 of OA treatment are to minimize pain and maintain joint mobility. One of the non-operative treatments for OA is the intra-articular injection of hyaluronic acid, known as viscosupplementation [4]. Commercially available viscosupplements have different formulations. Viscosupplements can be derived from either animals or the biological fermentation of streptococcal bacteria [5]. A family of cross-linked hyaluronic acid derivatives called hylans are polymers of hyaluronan that have been cross-linked through their hydroxyl group [6].

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

2. Materials and methods

2.1 Rheological measurements

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 of 64 years. Patients who participated in this study were recruited from the patient lists of two surgeons at the orthopedic reconstructive service at Vancouver Coastal Health Region (University of British Columbia Hospital and Vancouver General Hospital). Patients between the ages of 30 and 85 who were diagnosed with knee OA and required knee replacement were included in the study. Patients diagnosed with other arthritic conditions (e.g., inflammatory arthritis) and those who had surgery on their knees within 10 years prior to enrollment in the study were excluded. Diagnosis of the severity of OA was performed by the surgeons according to the Kellgren-Lawrence radiographic grading system [7]. Of the 22 patients, 20 were diagnosed with grade 4 OA, 1 with grade 3 OA, and 1 with grade 2 OA. In two of the patients, synovial fluid samples were obtained from both knees during bilateral total knee arthroplasty. In the bilateral cases, patients were diagnosed with grade 4 OA.
Synovial fluid samples came 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 Vancouver Coastal Health Research Institute. Informed consent was obtained from each patient prior to surgery. Synovial fluid was aspirated from each patient’s knee joint into a test tube by an experienced surgeon under sterile conditions at the time of surgery. The rheological behavior of most synovial fluid sample was evaluated within 2 hours after aspiration. For cases where the measurement could not be performed within 2 hours, aspired synovial fluid was kept in a refrigerator at approximately 4°C [8] for testing within 2 days after aspiration.

In addition, the rheological properties of 3 commercially available visco-supplements were examined. The visco-supplements were 2 brands of non-cross-linked hyaluronic acid (Orthovisc, Anika Therapeutics, Woburn, MA; Suplasyln, Bioniche Teo, Galway, Ireland) and a cross-linked hyaluronic acid (Synvisc, Genzyme Corp, Ridgefield, NJ).

The rheological properties of each synovial fluid sample and each viscosupplement were determined using a Bohlin Gemini HR<sup>nano</sup> rheometer and a Kinexus rheometer (Malvern Instruments Ltd., Worcestershire, UK), respectively, at 37°C using a stainless steel cone and plate geometry (40-mm-diameter cone with a 1° cone angle). Each rheometer was first calibrated with Cannon Certified Viscosity Standard oil. In the steady shear test, which measures viscosity as a function of shear rate, shear rates of 0.01 to 1000 s<sup>-1</sup> were applied to each sample. A constant shear rate of 0.05 s<sup>-1</sup> was also applied to the samples in order to examine the changes in shear stress over time.

The viscoelastic properties of the samples were determined by performing the small-amplitude oscillatory shear (SAOS) test. The SAOS test measures both components of stress, elastic and viscous, when the material is subjected to sinusoidal stress or strain. The elastic modulus ($G'$) represents the energy stored in the elastic structure of the material. The viscous modulus ($G''$) represents the amount of energy dissipated in the material [9]. For the SAOS 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 5% strain, over a frequency range of 0.1-10 Hz.

2.2 Model fitting

For a Newtonian fluid, the constitutive equation can be expressed as follows [9]:

$$
\begin{bmatrix}
\tau_{xx} \\
\tau_{xy} \\
\tau_{yx} \\
\tau_{yy} \\
\tau_{xz} \\
\tau_{zy} \\
\tau_{yx} \\
\end{bmatrix}
= \mu \begin{bmatrix}
\frac{\partial v_x}{\partial x} + \frac{\partial v_y}{\partial y} \\
\frac{\partial v_x}{\partial y} + \frac{\partial v_y}{\partial x} \\
\frac{\partial v_x}{\partial z} + \frac{\partial v_y}{\partial z} \\
\frac{\partial v_y}{\partial x} + \frac{\partial v_z}{\partial y} \\
\frac{\partial v_x}{\partial z} + \frac{\partial v_z}{\partial x} \\
\frac{\partial v_y}{\partial z} + \frac{\partial v_z}{\partial y} \\
\end{bmatrix}
$$

where $\tau$ represents the components of the stress tensor, ($v_x, v_y, v_z$) are the components of velocity, and $\mu$ is the viscosity.

For a Newtonian fluid, the viscosity is constant in steady shear. Thus, the generalized Newtonian constitutive model was developed for materials for which viscosity is not constant [9].

For generalized Newtonian constitutive models, the relationship between the stress tensor and the rate of deformation tensor is:

$$
\begin{bmatrix}
\dot{\tau}_{xx} \\
\dot{\tau}_{xy} \\
\dot{\tau}_{yx} \\
\dot{\tau}_{yy} \\
\dot{\tau}_{xz} \\
\dot{\tau}_{zy} \\
\dot{\tau}_{yx} \\
\end{bmatrix}
= \mu(\dot{\gamma}) \cdot \begin{bmatrix}
\dot{\gamma}_{xx} \\
\dot{\gamma}_{xy} \\
\dot{\gamma}_{yx} \\
\dot{\gamma}_{yy} \\
\dot{\gamma}_{xz} \\
\dot{\gamma}_{zy} \\
\dot{\gamma}_{yx} \\
\end{bmatrix}
$$

where $\dot{\gamma}$ is the shear rate, $\dot{\gamma}_0$ represents the components of the rate of deformation tensor, and $\mu(\dot{\gamma})$ is the viscosity function of the local shear rate.

The rate of deformation tensor can be calculated using:

$$
\begin{bmatrix}
\dot{\gamma}_{xx} \\
\dot{\gamma}_{xy} \\
\dot{\gamma}_{yx} \\
\dot{\gamma}_{yy} \\
\dot{\gamma}_{xz} \\
\dot{\gamma}_{zy} \\
\dot{\gamma}_{yx} \\
\end{bmatrix}
= \begin{bmatrix}
2 \frac{\partial v_x}{\partial x} + \frac{\partial v_y}{\partial y} + \frac{\partial v_z}{\partial z} \\
\frac{\partial v_x}{\partial y} + \frac{\partial v_y}{\partial x} + 2 \frac{\partial v_z}{\partial z} \\
\frac{\partial v_x}{\partial z} + \frac{\partial v_y}{\partial z} + \frac{\partial v_z}{\partial x} \\
\frac{\partial v_y}{\partial x} + \frac{\partial v_y}{\partial y} + \frac{\partial v_z}{\partial z} \\
\frac{\partial v_x}{\partial z} + \frac{\partial v_z}{\partial y} + \frac{\partial v_z}{\partial x} \\
\frac{\partial v_y}{\partial z} + \frac{\partial v_z}{\partial z} + \frac{\partial v_z}{\partial x} \\
\end{bmatrix}
$$

The viscosity of the generalized Newtonian model is a function of the shear rate. The generalized Newtonian constitutive equation can capture the non-Newtonian behavior with sufficient accuracy for inelastic fluids. The viscosity as a function of the local shear rate can be determined by fitting with the viscosity experimental data. Several models can be used to fit the material’s characteristics. In this study, two models were used for fitting the data, namely the Cross model [10] and the Carreau-Yasuda model [9].

Cross model:

$$
\frac{\eta(\dot{\gamma}) - \eta_\infty}{\eta_0 - \eta_\infty} = \frac{1}{1 + (\dot{\gamma} \lambda)^n}
$$

where $\eta$ is the viscosity, $\eta_0$ is the zero-shear viscosity, $\eta_\infty$ is the infinite-shear viscosity, $\lambda$ is a time constant, and $n$ is a rate constant that describes the slope.

Carreau-Yasuda model:

$$
\frac{\eta(\dot{\gamma}) - \eta_\infty}{\eta_0 - \eta_\infty} = [1 + (\dot{\gamma} \lambda)^n]^{\frac{a-1}{\alpha}}
$$

where $\eta$ is the viscosity, $\eta_0$ is the zero-shear viscosity, $\eta_\infty$ is the infinite-shear viscosity, $\lambda$ is a time constant, $n$ is a rate constant that describes the slope, and $\alpha$ is a constant.

3. Results and discussion

3.1 Rheological behavior of human synovial fluid

Synovial fluid in OA joints exhibited non-Newtonian shear thinning behavior (i.e., viscosity decreases with increasing shear rate) and viscoelastic properties (Figs 1 and 2). The zero-shear viscosity ($\eta_0$) varied from 0.28 to 10.59 Pa·s (mean ± SD = 3.40 ± 2.90). At a frequency of 0.5 Hz, the elastic modulus ($G'$) of the synovial fluid samples ranged from 0.26 to 5.79 (mean ± SD = 2.14 ± 1.7) and the viscous modulus ($G''$) ranged from 0.34 to 3.84 (mean ± SD = 1.63 ± 1). At a
frequency of 2.5 Hz, the elastic modulus (\(G'\)) of the synovial fluid samples ranged from 0.36 to 9.63 (mean ± SD = 3.55 ± 2.72) and the viscous modulus (\(G''\)) ranged from 0.65 to 5.09 (mean ± SD = 2.51 ± 1.25). The frequencies 0.5 and 2.5 Hz correspond to joint movement during walking and running, respectively [11].

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 the left and right knees were compared. The results show that the viscometric and viscoelastic parameters of the two knees were very different (Figs 3 and 4), that not only does the viscosity of the synovial fluid between the left and right knees differ substantially, but also that the viscoelastic properties of the synovial fluid are different. This may suggest that OA is more than a systemic disorder. Alterations within the joint determine the properties of the synovial fluid to a large extent. Many factors may 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°C (Fig. 5). Rheopexy in synovial fluid has previously been observed in several studies [13-16]. O’Neill and Stachowiak [16] reported that OA synovial fluid exhibits rheopexic behavior at temperatures of 20°C or below. However, in the present study, rheopexic behavior was observed in OA synovial fluid samples at 37°C. This discrepancy may be caused by several factors, including the types of instrument used and the value of the constant shear rate applied to the sample. It should be noted that in the study by O’Neill and Stachowiak [16], the constant shear rate applied to the sample was not reported. Oates et al. [15] asserted that rheopexic behavior is attributed to protein aggregation, which appears to play an important role in enhancing the viscoelastic properties of synovial fluid.

In a study by Mazzucco et al. [12], the flow parameters of synovial fluid were evaluated at 25°C. They reported that the viscosity of synovial fluid between the left and right knees for a single patient differed substantially. In the present study, the rheological properties were examined at 37°C. The results show...
3.2 Rheological behavior of visco-supplements

Three commercially available viscosupplements for joint injection were analyzed. The results show that the viscosupplements also exhibit non-Newtonian shear thinning behavior (Fig. 7), similar to that of human synovial fluid. The non-cross-linked hyaluronic acid with the lower molecular weight had the lowest viscosity throughout the entire range of shear rates, which is consistent with a study by Prieto et al. [17]. At lower shear rates (0.01-1 s\(^{-1}\)), the cross-linked hyaluronic acid had higher viscosity than that of the non-cross-linked hyaluronic acid with the higher molecular weight, but at higher shear rates, it had a lower viscosity. At a shear rate of 0.01 s\(^{-1}\), the viscosity of the cross-linked hyaluronic acid was about two orders of magnitude higher than that of the non-cross-linked hyaluronic acid with the lower molecular weight.

![Figure 6. Model fitting for synovial fluid.](image)

The results from the present study indicate that the viscosity of viscosupplements highly depends on the molecular weight of the hyaluronic acid. In addition, it was observed that as the shear rate increases, the viscosupplement with cross-linked hyaluronic acid showed a sharper decrease in viscosity than that of those with non-cross-linked hyaluronic acid. This finding is consistent with the results from a previous study which reported that the decrease in apparent viscosity was more pronounced in high-molecular-weight hyaluronic acid than that in low-molecular-weight hyaluronic acid [18].

![Figure 7. Viscosity as a function of shear rate for three viscosupplements (VS1: a non-cross-linked hyaluronic acid with the higher molecular weight, VS2: a non-cross-linked hyaluronic acid with the lower molecular weight, VS3: a cross-linked hyaluronic acid).](image)

Figure 8 shows that the viscosupplements exhibited different viscoelastic behaviors. For the non-cross-linked hyaluronic acid with the higher molecular weight, it was found that at low frequencies, the viscous modulus (\(G''\)) was higher than the elastic modulus (\(G'\)). At higher frequencies, the elastic modulus (\(G'\)) exceeded the viscous modulus (\(G''\)). A cross-over frequency was observed to be 0.39 Hz. The results also show that the non-cross-linked hyaluronic acid with the lower molecular weight exhibited viscous-like behavior; its viscous modulus (\(G''\)) remained larger than the elastic modulus (\(G'\)) [9] over the entire range of oscillation frequencies. In contrast, the viscosupplement with cross-linked hyaluronic acid exhibited gel-like behavior; its elastic modulus (\(G'\)) remained larger than the viscous modulus (\(G''\)) [9] throughout the range of oscillation frequencies. Furthermore, the results show that the non-cross-linked hyaluronic acid with the lower molecular weight had the lowest elastic modulus (\(G'\)) and viscous modulus (\(G''\)) over the range of frequencies.

![Figure 8. Elastic (\(G'\)) and viscous (\(G''\)) moduli for three viscosupplements (VS1: a non-cross-linked hyaluronic acid with the higher molecular weight, VS2: a non-cross-linked hyaluronic acid with the lower molecular weight, VS3: a cross-linked hyaluronic acid).](image)

These findings suggest that the differences in rheological behavior are related to the molecular weight of the viscosupplements and their network forming ability. Previous studies reported that at high molecular weight, a transient entanglement network forms [13,19]. The network forming ability of hyaluronic acid in solutions affects the viscoelasticity of the solution [18].

Viscoelastic properties are related to the function of shock absorption during walking and running. Table 1 summarizes the elastic (\(G'\)) and viscous (\(G''\)) moduli of viscosupplements tested at frequencies of 0.5 and 2.5 Hz, which correspond to joint movement during walking and running, respectively [11]. The results show that the dynamic moduli, \(G'\) and \(G''\), at both 0.5 and 2.5 Hz were smallest for the non-cross-linked hyaluronic acid with the lower molecular weight. It was also observed that the elastic modulus (\(G'\)) of the viscosupplement with cross-linked hyaluronic acid was largest at both frequencies. The non-cross-linked hyaluronic acid with the higher molecular weight was found to have the largest viscous modulus (\(G''\)).
Table I. Viscoelastic properties of three viscosupplements at 0.5 and 2.5 z.

<table>
<thead>
<tr>
<th>Viscosupplement</th>
<th>0.5 Hz G'</th>
<th>2.5 Hz G'</th>
<th>0.5 Hz G″</th>
<th>2.5 Hz G″</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS1</td>
<td>51.2</td>
<td>111.2</td>
<td>61.5</td>
<td></td>
</tr>
<tr>
<td>VS2</td>
<td>0.2</td>
<td>2.2</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>VS3</td>
<td>91.9</td>
<td>261.1</td>
<td>118.1</td>
<td>22.5</td>
</tr>
</tbody>
</table>

VS1: a non-cross-linked hyaluronic acid with the higher molecular weight, VS2: a cross-linked hyaluronic acid with the lower molecular weight, VS3: a cross-linked hyaluronic acid.

4. Conclusion

The rheological behavior of synovial fluid in OA joints varies widely. Synovial fluid in OA exhibits non-Newtonian shear thinning behavior and viscoelastic properties. Synovial fluid of the knees aspirated from an individual show very different viscosities and viscoelasticities. It has been reported previously that OA synovial fluid exhibits rheopetic behavior only at temperatures of 20°C or below. In the present study, the rheopetic behavior was observed in OA synovial fluid at a physiological temperature of 37°C. The findings from this study will lead to a better understanding of the role of synovial fluid in joint lubrication.

The results 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 of those with non-cross-linked hyaluronic acid. Moreover, high-molecular-weight viscosupplements have greater visco-elasticity than that of low-molecular-weight viscosupplements. The viscosity and viscoelasticity of viscosupplements affect their joint lubrication function. Therefore, the findings from this study may help the selection of the most suitable viscosupplement.

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