RHEOLOGY OF SYNOVIAL FLUID WITH AND WITHOUT VISCOSUPPLEMENTS IN PATIENTS WITH OSTEOARTHRITIS: A PILOT STUDY

by

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ABSTRACT

Osteoarthritis (OA) is a degenerative joint disease that is characterized by the breakdown of articular cartilage. Over 80% of people over the age of 60 show radiographic evidence of OA. Rheology of synovial fluid is of interest because of its significance in the joint lubrication. However, there are still many questions related to synovial fluid rheology and its relation with OA.

Although viscosupplementation has been used as a treatment for OA for many years, its clinical effect remains controversial. Therefore, the purposes of this pilot study were to determine rheological behavior of synovial fluid in patients with OA to better understand its role in joint lubrication, and to determine in vitro the effect of different viscosupplements on the rheological properties of synovial fluid.

A detailed rheological characterization of synovial fluid from 22 patients undergoing total knee arthroplasty was performed. The results showed that synovial fluid in OA exhibited a non-Newtonian shear thinning behavior and viscoelastic properties. Within an individual, rheological properties of synovial fluid from the left knee differed substantially from the right knee. Moreover, rheopectic behavior (i.e. shear stress increases over time at a constant shear rate) was observed in OA synovial fluid.

All three viscosupplements considered in this study (i.e. Orthovisc®, Suplasyn®, and Synvisc®) exhibited a non-Newtonian shear thinning behavior. Within the range of frequency from 0.1 to 10 Hz., Orthovisc® exhibited a linear viscoelastic behavior, whereas Synvisc® and Suplasyn® exhibited a gel-like behavior and a viscous-like behavior, respectively. By adding viscosupplements to OA synovial fluid, the results showed cross-linked high molecular weight viscosupplement is more efficient than the non cross-linked

ones in improving the overall rheological properties of synovial fluid. Furthermore, rheological properties of synovial fluid mixed with viscosupplements in vitro were nearly unchanged over 2 weeks.

In conclusions, synovial fluid in OA exhibited a non-Newtonian shear thinning behavior, viscoelastic properties, and rheopectic behavior. Cross-linked viscosupplement is more efficient than the non cross-linked ones in improving the overall rheological properties of synovial fluid. The rheology of synovial fluid mixed with viscosupplements was nearly unchanged over 2 weeks.

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CHAPTER 1

INTRODUCTION

1.1 Overview

Osteoarthritis (OA) is the most common joint disorder associated with aging, affecting the quality of life of people worldwide. OA is a degenerative joint disease that is characterized by the breakdown of articular cartilage [60] resulting in joint pain and stiffness. It is also known as a group of overlapping distinct diseases that affect the entire joint, not only the articular cartilage [21]. OA can affect any synovial joints, but the knee is the most commonly affected weight-bearing joint [74]. It is reported that over 80% of individuals over the age of 60 show radiographic evidence of OA. The prevalence of OA increases with advancing age and does so exponentially after the age of 50 [67]. As the population ages, the incidence and prevalence of OA will continue to rise unless measures are taken to improve disease prevention [125].

Articular cartilage and synovial fluid are closely linked in providing joint lubrication. Damage to the articular cartilage may result in deficient rheological properties of the synovial fluid and eventually will have an effect on the performance of the joint. Rheological characteristics of synovial fluid are of interest because of their significance in the joint lubrication. In a healthy joint, synovial fluid is highly viscous at low shear rates and highly elastic at high shear rates, allowing it to do an excellent job of protecting the articular cartilage from wear [3]. However, in a diseased joint, the composition of the synovial fluid is changed resulting in deterioration of rheological properties. Synovial fluid becomes less viscous and therefore less effective in lubrication [97, 112]. In OA, this

reduction in viscosity results from a decline in both the molecular weight and concentration of hyaluronic acid [81]. In addition, it has been suggested that the loss of viscoelasticity of synovial fluid is directly related to the severity of OA [3]. In order to better understand its role in joint lubrication, a thorough elucidation of the rheological properties of the synovial fluid is necessary. Also, it may become useful as a diagnostic aid for OA.

The goals for treatment of OA are to minimize pain and maintain joint mobility [38]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat OA, but they are associated with significant gastrointestinal side effects [49]. Another non-operative treatment for OA is intraarticular injections of hyaluronic acid known as viscosupplementation. The concept of viscosupplementation for OA was first proposed by Balazs et al. [16]. Viscosupplement is used for reducing pain and improving joint mobility in patients with OA [13] by restoring the physiological homeostasis of the OA joint.

There are different formulations of viscosupplement that are commercially available. Viscosupplement can be derived either from animal or from biological fermentation of streptococcal origin [85]. 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 [12]. Although viscosupplementation has been used as a treatment for OA for many years, its clinical effect remains controversial. Some studies reported the efficacy of intraarticular hyaluronic acid use in selected patients with OA [96, 121]. However, other studies suggest that intraarticular hyaluronic acid has not proven to be clinically effective [5]. Therefore, further studies are warranted to determine possible differences between different commercial viscosupplements and their effects on rheological behavior of synovial fluid.

1.2 Study Purpose

The purposes of this pilot study were to perform a rheological characterization of synovial fluid in patients with OA to better understand its role in joint lubrication, and to determine in vitro the effect of different viscosupplements on the rheological properties of synovial fluid.

1.3 Research Objectives

1. To perform rheological characterization of synovial fluid in patients with severe OA.
2. To determine whether there are any differences in rheological behavior between different viscosupplements.
3. To determine in vitro the rheological changes of synovial fluid attributable to the addition of different formulation of viscosupplements.
4. To determine in vitro the stability of rheological behavior of synovial fluid mixed with cross-linked viscosupplement over time.

1.4 Thesis Organization

The thesis is organized as follows:

In chapter 2, the background information about synovial joint and OA are provided. Principles of tribology and rheology are introduced. A review of the literature regarding rheology of hyaluronic acid and rheology of synovial fluid are also presented. In chapter 3, the demographic of the subjects as well as inclusion and exclusion criteria are presented. Moreover, the details regarding apparatus and viscosupplements used in the study, and experimental procedures are provided. In chapter 4, results from rheological

characterizations of synovial fluid, viscosupplements, as well as synovial fluid mixed with viscosupplements are included. In addition, rheological characterizations of synovial fluid, viscosupplements, as well as synovial fluid mixed with viscosupplements are discussed. Finally, chapter 5 presents the conclusions and recommendations for future work.

CHAPTER 2

LITERATURE BACKGROUND

In this chapter, the background related to synovial joint and its components are provided. Then, the pathology, classification, and treatment of osteoarthritis are described. In addition, the terms "tribology", "biotribology" as well as the principles of rheology are introduced. Finally, literature review on rheological properties of hyaluronic acid, synovial fluid and pathological synovial fluid are presented.

2.1 Synovial Joint

A synovial or diarthrodial joint is a freely movable joint which is characterized by a synovial cavity filled with the lubricating substance known as synovial fluid. The articular surface of a synovial joint is covered completely by a layer of articular cartilage. A synovial joint is a sophisticated system that includes articular cartilage, synovium, and synovial fluid as key components within synovial fluid compartment.

2.1.1 Articular Cartilage

Articular cartilage, the connective tissue that covers on the ends of the bones, serves as both a shock absorber and a smooth load bearing surface to accommodate movement with minimal friction [33]. It is an avascular, aneural, and alymphatic tissue [26]. Articular cartilage can withstand enormous loads with little deformation and is highly wear-resistant [72]. The total cartilage surface area in the human knee joint is from 102 to 163 cm^2, while the thickness of cartilage is from 2-5 mm [41].

The extracellular matrix of articular cartilage is a biphasic material consisting of a solid porous matrix (chondrocytes, collagen fibres, and proteoglycans) and an interstitial fluid phase (water and electrolytes). The cells, known as chondrocytes, are responsible for producing and maintaining the extracellular matrix composing cartilage. These cells are the only cell type in articular cartilage and may occupy as little as less than 5% of the total volume [83].

In the extracellular matrix of articular cartilage, the most abundant constituent is water. Collagen is the second largest component. Although there are several forms of collagen, the most prevalent type in articular cartilage is Type II [26]. Type II collagen contributes to the structural framework of articular cartilage and therefore provides mechanical stability [25]. Another main component of the extracellular matrix is proteoglycan. The majority of the proteoglycans in articular cartilage are found in the aggregated form called 'Aggrecan' [26]. The primary function of proteoglycans is to supply compressive stiffness [109]. Proteoglycans attract water molecules into the matrix and therefore produce a large osmotic pressure giving cartilage its swelling and turgidity.

Articular cartilage is typically divided into three zones: superficial, middle and deep [66]. The superficial zone is approximately 10% of the cartilage thickness. In this zone, the collagen fibers are tightly packed together and arranged parallel to the surface. The concentration of water is high, but proteoglycan concentration is relatively low. The smooth superficial zone of the cartilage helps to reduce friction between the articular surfaces and to distribute forces. In the middle zone, the collagen fibers are randomly oriented. The concentration of water is lower, but higher for proteoglycans as compared to the superficial region. In the deep zone, the fibers form radially oriented bundles and

extend across the tidemark, which is the interface between uncalcified and calcified cartilage, to anchor to the subchondral bone. Water content is the lowest in this zone, but the proteoglycans concentration is quite high.

2.1.2 Synovium

The synovium is the thin, flexible lining of the joint composed of synoviocytes. Most of the cells are macrophages or specialized fibroblasts [8]. The synovium has an extensive extracellular matrix, with a net of capillaries present beneath the cell layer. It is about 50-60 μm thick in the normal human knee [117]. Subsynovium extracellular matrix is mainly composed of Type III collagen, while type I collagen is less prominent. Type V collagen is also present [6, 107]. In addition to collagens, subsynovium extracellular matrix is composed of hyaluronan [126], chondroitin sulphate [127], fibronectin [98], and proteoglycans [29].

A key function of the synovium is to serve as a semi-permeable membrane for exchange of solutes [69]. An ultrafiltrate of plasma and hyaluronan synthesized by type B cells of the synovium [56] pass across its capillary walls, through the extracellular matrix, into the joint cavity. The ultrafiltrate of plasma and hyaluronan form synovial fluid which is contained within the joint cavity. As well as allowing for solute transfer, the synovium must also offer sufficient hydraulic resistance to retain the lubricant macromolecules in synovial fluid within the joint cavity [29]. Since articular cartilage has no blood supply, the joint relies on the synovium to import nutrients and remove waste products from the joint.

2.1.3 Synovial Fluid

Synovial fluid is a dialysate of blood plasma secreted by cells lining the synovium [78] and forms an interface with both the synovium and cartilage. The volume of synovial fluid in normal human knee joint is ~1-2 ml [108]. It plays a crucial role in joint lubrication and bearing functions. It has been shown that synovial fluid has a low coefficient of friction (μ). The coefficient of friction of bovine synovial fluid is reported to be range from 0.002 to 0.01 [71, 73]. Synovial fluid also acts as an important carrier in supplying nutrient to the cartilage and removing catabolic products since articular cartilage is avascular [17].

The concentration of protein in normal synovial fluid was previously studied. Albumin and globulins are the main plasma proteins in normal synovial fluid [68]. The protein content of normal synovial fluid is much lower than that of plasma [108]. In inflammatory and degenerative joint diseases, however, the protein content in synovial fluid increases [12]. Bole [19] reported that, in normal synovial fluid, phospholipids and cholesterol were found in very small amount and the lipid concentration is substantially lower than that of plasma. However, rheumatoid arthritis synovial fluid contained significant amount of phospholipids, cholesterol, and triglycerides as high as 40% to 60% of the lipid concentration in rheumatoid arthritis serum. Cellular components including leukocytes and polymorphonuclear cells are found in small amounts in normal synovial fluid, but increase in joint diseases [78].

Lubricating molecules in synovial fluid include hyaluronic acid and proteoglycan 4. Proteoglycan 4 is also referred to as lubricin. Hyaluronic acid is present in synovial fluid at a concentration of ~2-3 mg/ml [12], while lubricin is present at 0.0291 mg/ml [118]. In

normal joints, the half-life of hyaluronic acid has been reported to be on the order of ~ 24 hours [30]. In diseased joints, both the concentration and molecular weight of hyaluronic acid are lower than that in normal synovial fluid [77]. The concentration of hyaluronic acid in OA and rheumatoid arthritis are 0.7-1.1 mg/mL and 0.8-1.5 mg/mL, respectively. The molecular weight of hyaluronic acid in OA and rheumatoid arthritis are 0.3 MDa and 0.6 MDa, respectively. The pH values of normal synovial fluid are between 7.3 and 7.43 [35], while the pH values of synovial fluid with OA and rheumatoid arthritis ranges from 7.4 to 8.1 and from 6.6 to 7.6, respectively [61].

2.2 Osteoarthritis

The term "arthritis" is used to describe more than 100 different types of inflammatory or degenerative diseases that affect joints and connective tissue. The symptoms are characterized by pain, swelling and stiffness in and around one or more joints and can develop gradually or suddenly. The most common form of arthritis is osteoarthritis.

2.2.1 Pathology of Osteoarthritis

Osteoarthritis (OA), also known as degenerative joint and "wear and tear" disease, is characterized by degeneration of articular cartilage [20]. As the disease progresses, osteophytes and small bony outgrowths form around the margins of the joint. The breakdown of articular cartilage often results in joint pain and loss of mobility, which may lead to long-term disability. The etiology of OA remains elusive. Multiple risk factors including age, gender, nutritional deficiency, estrogen status, bone density and genetic have been found to contribute to this disorder. Local biomechanical factors such as obesity,

joint deformity, meniscus pathology, ligament injury, muscular weakness, overloading by risk sports, and occupations are also associated with degenerative changes [43, 115].

In OA, there is an alteration in the cartilage matrix that permits the cartilage to be more infused with water resulting in cartilage softening. In addition, there are changes in other structures of the joint such as the capsule, inflammatory changes in synovial lining, and arthritic cysts [101]. In advanced stages of OA, the fluid diffusion, which is a major source of nutrition of the cartilage, is reduced and chronic synovial effusion expands the joint space. With further erosion of the articular cartilage, fragments of cartilage matrix and necrotic bone become incorporated into the synovial membrane [21]. Articular cartilage and synovial fluid are closely linked in providing lubrication, cushion, and protective barrier between the ends of the bones [128]. Therefore, damage to articular cartilage may result in deficient rheological properties of the synovial fluid. Changes in either will have an effect on the performance of the joint.

2.2.2 Classification of Osteoarthritis

The clinical diagnosis of OA is based on clinical and radiographs. Radiographs are widely used for classifying the severity of OA. In 1957, Kellgren and Lawrence [59] developed a classification for OA based on radiographic findings. The Kellgren and Lawrence (K/L) grading system is the most widely used system to grade radiographic severity. The K/L system assigns a rating from 0-4 in order of severity (Table 2.1). However, it should be noted that the criteria for grading OA are related to the sequential presence of osteophytes, narrowing of the joint space and subchondral sclerosis.

Therefore, it is unclear how to classify the severity of OA based on the K/L grading system in individuals with decreased joint space but no presence of osteophytes.

Table 2.1: Kellgren-Lawrence grading of severity of knee osteoarthritis

Grading	Kellgren & Lawrence Definition
Grade 0 'Normal'	Definite absence of OA
Grade 1 'Doubtful'	Possible osteophyte lipping, doubtful narrowing of joint space
Grade 2 'Minimal'	Definite osteophytes, possible narrowing of joint space.
Grade 3 'Moderate'	Moderate multiple osteophytes, and definite narrowing of joint space and some sclerosis and possible deformity of bone ends.
Grade 4 'Severe'	Large osteophytes marked narrowing of joint space, and severe sclerosis of subchondral bone.

2.2.3 Treatments of Osteoarthritis

The goals of current treatments for OA are to control pain and maintain articular function rather than alter the natural course of the disease [38]. An initial non-operative treatment of OA consists of patient education, weight loss, physical therapy, occupation therapy, assistive devices followed by pharmacologic intervention [57]. Pharmacologic intervention may include topical and oral analgesics, non-steroidal anti-inflammatory drugs (NSAIDs) and intra-articular corticosteroid injections. While NSAIDs are commonly used for OA treatment, they produce significant gastrointestinal side effects and do not seem to alter the disease process [114]. These gastric side effects have led to a recent development in NSAID therapy known as cyclooxygenase-2 (cox-2) inhibitors. Study reported equal efficacy but significantly decreased gastrointestinal adverse events when compare to most NSAIDs [37].

An alternative treatment for OA is the use of glucosamine sulfate. Glucosamine is a constituent of glycosaminoglycans (GAGs) and proteoglycans that are naturally found in healthy cartilage and synovial fluid. It also helps in synthesizing mucin or mucous secretions which act as lubricant in synovial joints [55]. Results from clinical trials suggested that glucosamine sulfate may potentially delay joint structure changes in OA [94, 103]. Meta-analysis studies also showed that individual with OA of the knee or spine have significantly less symptoms while taking glucosamine than those taking placebo [28, 106]. Natural joint space narrowing in knee OA is slow (< 0.1 mm/year), but can be prevented by glucosamine sulfate [28]. However, there is still uncertainty about its effectiveness.

Another non-operative treatment for OA is intraarticular injections of hyaluronic acid known as viscosupplementation. The concept of viscosupplementation for OA was first proposed by Balazs et al. [16]. There are various commercial products of viscosupplements. Viscosupplements can be extracted either from rooster combs (e.g. Orthovisc®) or from biological fermentation of streptococcal origin (e.g. Suplasyn®) [85]. There is also the family of cross-linked hyaluronan derivatives name hylans (e,g. Synvisc®). Hylans are polymers of hyaluronan that have been cross-linked through their hydroxyl group [12]. Synvisc® (hylan G-F 20) is a mixture of two hylan polymers derived from rooster comb HA, 80% by volume hylan A fluid and 20% by volume hylan B gel [1].

The goal of viscosupplementation is to restore the physiological homeostasis of the OA joint. The hyaluronic acid used in viscosupplement has been shown to stimulate the production of hyaluronic acid in vitro. It restores the homeostasis in the joint [14]. Moreover, the addition of hyaluronic acid may help maintain joint environment by stabilizing and protecting the collagen fibrous network, the cells and pain receptors [15].

The half-life of hyaluronic acid injections in the joint varies from 24 hours to 2 weeks and the longest residence time ranging from 5 to 30 days [85]. Beyond the residence time, there is no longer a significant amount of synthetic fluid in the joint. However, clinical studies have shown that the benefits of viscosupplement can last from 6 months up to a year after the last injection [124]. It is assumed to result from the transient nature of the hyaluronic acid within the joint [22]. Bagga et al. [10] reported 13% increase in synovial fluid hyaluronic acid concentration and increase in complex shear modulus at 3 months after hyaluronic acid injection. This suggests that viscosupplement could promote endogeneous hyaluronic acid production. In addition, the interactions on the molecular level between hyaluronic acid and pain receptors in the joint contribute to analgesic effect [53].

The majority of studies reported the efficacy of hyaluronic acid use in selected patients with OA [96, 121]. A clinical benefit was usually measured by a decrease in pain or improved function. However, some studies reported that intraarticular hyaluronic acid has not proven to be clinically effective [5]. There has been a perception that higher molecular weight hyaluronic acids are more effective in OA treatment [9]. The more recent study, however, suggested that low or high molecular weight hyaluronic acids preparation have similar efficacy [7].

For patients with OA whose noninvasive or nonoperative treatments are ineffective, surgical treatment is the option. Surgical treatment may be helpful in either correcting structural abnormalities or preventing the progression of the disease. Current surgical treatment modalities include osteotomy, debridement, arthrodesis, and arthroplasty. There

are inherent risks associated with surgery. Thus, the risks and benefits must be weighed before considering surgical treatment [51].

2.3 Tribology

The synovial joint is a perfect tribological system with low friction and high wear resistance. The term *tribology* is derived from the Greek word 'tribos', which means 'to rub'. In general, tribology can be defined as the study and application of the principles of friction, wear and lubrication of interacting surfaces in relative motion [49]. Therefore, *biotribology* can be defined as the study of friction, wear, and lubrication in biological systems, e.g. synovial joints. Section 2.3.1 - 2.3.3 are basic principles needed to understand any study of tribology and biotribology.

2.3.1 Friction

Friction can be defined as the resistance to motion which exists when one solid body slides over another [4]. The friction force is a tangential force which acts in a direction directly opposite to the direction of motion. Friction is not a property of material, but, it is often dependent on the surfaces that are in contact. The coefficient of friction, μ, is defined as the ratio of the friction force, F_r, due to the normal load, W. In mathematical terms, it can be expressed as

$$\mu = \frac{F_r}{W} \tag{2.1}$$

2.3.2 Wear

Wear is defined as 'the progressive loss of substance from the operating surface of a body occurring as a result of relative motion at the surface' [89]. There are different types of wear; such as adhesive wear, abrasive wear, fatigue wear and corrosive wear [47]. Adhesive wear occurs when the contacting asperities of two sliding bodies undergo shearing. The adhesion between the two surfaces in contact causes the detachment of fragments from one surface to form wear debris to the other surface. In abrasive wear, the harder material fractures, or plastically deforms the softer material. Fatigue wear occurs when two surfaces meet in a cyclic manner. The cyclic loading may cause surface cracks which in time can break off the surface. Corrosive wear occurs as a result of reaction products, such as oxides, being formed on one or both surfaces. Fretting wear is also considered a wear mechanism. Fretting wear is defined as a combined mechanical and chemical wear which can occur where low amplitude oscillatory motion takes place between two surfaces [122].

2.3.3 Lubrication

Lubrication is a process of reducing friction and/or wear between relatively moving surfaces by the application of a lubricant. Different types of lubrication are discussed in the following sections.

In hydrodynamic lubrication or fluid film lubrication, a viscous fluid film is compressed between two surfaces and a sufficient hydrodynamic pressure is generated to support the load and keeps the sliding surfaces completely separated. The thickness of this fluid film depends upon the bulk physical properties of the lubricants. For rotating disks with parallel axes, the 'simple' Reynolds equation yields

$$\frac{h_0}{R} = 4.9\left(\frac{\eta U}{W}\right) \tag{2.2}$$

where h_0 is the minimum lubricant film thickness, η is the absolute viscosity, U is the average velocity $(U_1 + U_2)/2$, U_1 is the velocity at surface 1, U_2 is the velocity at surface 2, W is the applied normal load per unit width of disk, and R is the reduced radius of curvature $(1/R = 1/R_1 + 1/R_2)$. R_1 is the radius of curvature at surface 1, and R_2 is the radius of curvature at surface 2. The dimensionless term $(\eta U/W)$ is sometimes referred to as the hydrodynamic factor [27, 48].

In elastohydrodynamic lubrication, the surfaces deform elastically. More elastic boundaries allow a greater volume of fluid to be drawn into the converging gap resulting in thicker fluid films. The comparable Dowson-Higginson expression for minimum film thickness is

$$\frac{h_0}{R} = 2.6\left(\frac{\eta U}{W}\right)^{0.7}\left(\frac{\alpha W}{R}\right)^{0.54}\left(\frac{W}{RE'}\right)^{0.03} \tag{2.3}$$

The term E' represents the reduced modulus of elasticity:

$$\frac{1}{E'} = \frac{\left(1-\nu_1^2\right)}{E_1} + \frac{\left(1-\nu_2^2\right)}{E_2} \tag{2.4}$$

where α is the pressure-viscosity coefficient, E is the modulus, ν is Poisson's ratio, and the subscripts 1 and 2 refer to the two solids in contact. All the other terms are the same as previously stated [40, 48].

In addition, squeeze film lubrication can occur when surfaces approach one another. The approaching surfaces tend to squeeze out the intervening fluid, but this action is strongly resisted by viscous forces. In boundary lubrication, the solid surfaces are so close

to each other. The lubricating fluid forms protective layers on the surfaces. Thus, the intimate contact between the rubbing surfaces can be protected and it can minimize damage on contact [119]. In the mixed lubrication, a combination of boundary, hydrodynamic and elastohydrodynamics lubrication is observed.

2.3.4 Biotribology

The goal of many studies in biotribology has been to describe joint operation from the tribological point of view. Synovial joint is the most sophisticated and complex tribological system which involves contacting surfaces (i.e. articular cartilage) as well as the surrounding medium (i.e. synovial fluid). However, until now the mechanisms of the remarkable joint performance are still not fully understood. Degradation of either part of the articular cartilage-synovial fluid system leads to increased friction, wear, and reduction of mobility. A joint disease that is characterized by changes in articular cartilage and bone which results in deformation, increase in friction and, finally, wear of cartilage is called osteoarthritis. Thus, OA may be considered as a tribological problem. Furey [48] suggested possible connections between tribology/normal synovial joint lubrication and degenerative joint disease. A better understanding of the mechanisms of normal joint performance from a tribological point of view could lead to advancement in the prevention and treatment of OA.

2.4 Rheology

Rheology is the study of the deformation and flow of materials under various kinds of stress and strain. Stress is the amount of force exerted per unit area, while shear stress is defined as a stress which is applied parallel to a face of a material. Strain is a measure of

the change of the shape or deformation of a material. Thus, shear strain can be defined as a measure of deformation in shear.

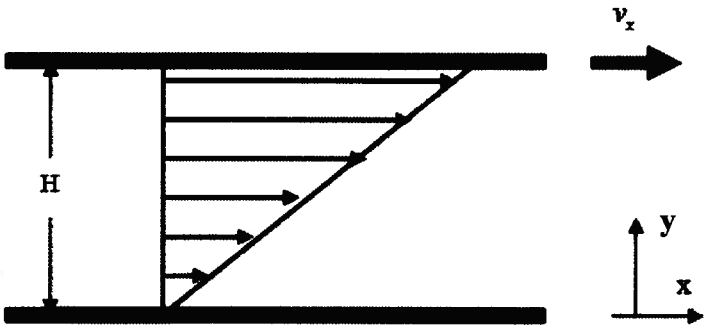

Figure 2.1: Velocity profile for a fluid flowing between two plates

The viscosity is defined as the resistance of a fluid to flow or deform [123]. Using the coordinates shown in Figure 2.1, Newton's law of viscosity is shown in equation

$$\tau_{yx} = -\mu \frac{dv_x}{dy} \tag{2.5}$$

where τ_{yx} is the shear stress, μ is the viscosity, v_x is the velocity component along x axis. Newtonian fluids would behave according to Newton's law where the shear stress exerted on the fluid would be linearly proportional to the shear rate.

In a non-Newtonian fluid, the relation between the shear stress and the strain rate is nonlinear. Non-Newtonian fluids exhibit a variety of different behaviors. Shear thinning is a type of non-Newtonian fluid behavior where viscosity decreases with increasing rate of shear stress. The opposite behavior, where the viscosity increases with increasing rate of shear, is called shear thickening.

Some non-Newtonian fluids show a time-dependent change in behavior. Thixotropy is a time-dependent change in viscosity; that is viscosity decreases over time at

a constant shear rate. On the contrary, a time-dependent change in behavior where the viscosity increases over time at a constant shear rate is called rheopexy.

Fluids also exhibit viscoelastic behavior. Viscoelasticity is the property of materials that exhibit both viscous and elastic behaviors. An inelastic fluid deforms under the action of force. It dissipates all the energy as heat and retains the deformation even when the force is removed. On the other hand, an elastic body deforms under the action of force but it stores all the energy; thus, when the force is removed it regains its original configuration. For a viscoelastic fluid, it deforms under the action of force and retains partially its deformation even when the force is removed. When undergoing deformation, viscoelastic materials store some of the strain energy in the material as potential energy and dissipate some of this energy as heat.

In the study of rheology, rheometry is used to experimentally determine rheological properties of materials. A rheometer is an instrument, which can impose a strain and measures the resulting torque or it can exert a torque on a material and measures its response with time. A rheometer can be of the controlled stress type or the controlled rate type.

To characterize the rheological behavior of the material, different flow test techniques such as steady shear or oscillatory shear could be used. The measuring systems used on the rheometer can be selected from the following geometries based on the material properties:

1. Cone and plate
2. Parallel plate
3. Concentric cylinder

4. Double gap concentric cylinder

2.4.1 Steady Shear Test (Viscometry)

Steady shear test measures the viscosity as a function of shear rate. Steady shear flow can be produced by confining a material between two plates and move one plate at a constant velocity.

A cone and plate geometry (Figure 2.2) is widely used in rheometers. In the cone and plate, the cone rotates at a constant angular velocity with a fixed plate while measuring the torque generated by the tested material. The viscosity at various shear rates can be calculated through the relation formula between torque and viscosity.

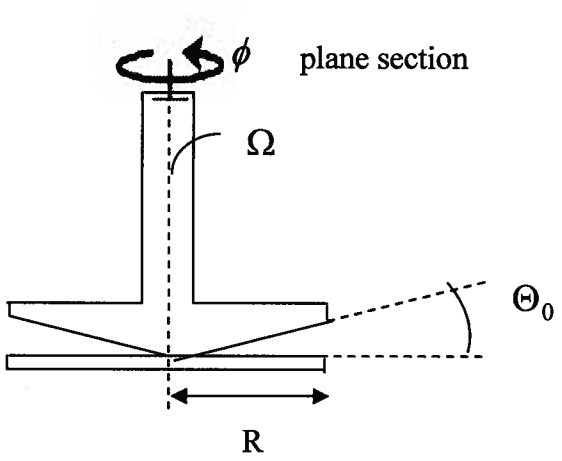

Figure 2.2: Cone and plate geometry

$$T = \tau 2\pi \int_0^R r^2 dr = 2\pi \frac{R^3}{3} \tag{2.6}$$

$$\tau = \frac{3T}{2\pi R^3} \tag{2.7}$$

$$\dot{\gamma} = \frac{\Omega r}{\Theta_0 r} = \frac{\Omega}{\Theta_0} \tag{2.8}$$

$$\mu = \frac{3T}{2\pi R^3} \cdot \frac{\Theta_0}{\Omega} = \frac{T}{\Omega}\left(\frac{3\Theta_0}{2\pi R^3}\right) = \frac{3T\Theta_0}{2\pi R^3 \Omega} \tag{2.9}$$

where Θ_0 is the cone angle, T is the torque on the cone, R is the radius of plate, Ω is the angular velocity of the cone, τ is the shear stress, $\dot{\gamma}$ is the shear rate, and μ is the viscosity.

2.4.2 Small Amplitude Oscillatory Shear Test (SAOS)

Viscoelastic properties of the material can be determined using oscillatory shear test. The velocity field of SAOS flow can be defined as below

$$\begin{cases} v_x = \dot{\gamma}(t)x = \dot{\gamma}_0 \cos \omega t x \\ v_y = 0 \\ v_z = 0 \end{cases} \tag{2.10}$$

where v_x, v_y, v_z are the velocity components along the axis of a Cartesian system of reference (x, y, z), $\dot{\gamma}(t)$ is the oscillatory shear rate, $\dot{\gamma}_0$ is the amplitude of the shear rate oscillation, and ω is the frequency of the shear rate oscillation.

For small strains,

$$\gamma(0,t) = \int_0^t \dot{\gamma}(t')dt' = \int_0^t \dot{\gamma}_0 \cos \omega t' dt' = \frac{\dot{\gamma}}{w} \sin \omega t = \gamma_0 \sin \omega t \tag{2.11}$$

where γ_0 is the strain amplitude.

Generally materials are tested in the 'linear viscoelastic region'. When a material is strained at low strain amplitudes in a sinusoidal way, the material's response to sinusoidal input is sinusoidal with the same frequency. However, the shear stress is usually not in phase with the input strain. There is a phase shift 'δ' between the strain wave and stress response.

The stress response would be,

$$\tau(t) = \tau_0 \sin(\omega t + \delta) \tag{2.12}$$

$$= \tau_0 (\sin \omega t \cos \delta + \sin \delta \cos \omega t) \tag{2.13}$$

$$= (\tau_0 \cos \delta) \sin \omega t + (\tau_0 \sin \delta) \cos \omega t \tag{2.14}$$

where τ_0 is the amplitude of the oscillatory shear stress.

Shear stress has two components. One component is in phase with the imposed strain, and the other component is in phase with the imposed strain rate. SAOS can measures both components of stress, elastic and viscous, when the material is subjected to sinusoidal stress or strain. Storage or elastic modulus (G') represents energy stored in elastic structure of the material. If it is higher than the loss modulus, the material is more solid like. Loss or viscous modulus (G'') represents amount of energy dissipate in the material. If it is greater than the storage modulus, the material is more viscous like.

Storage modulus (elastic modulus):

$$G' = \frac{\tau_0}{\gamma_0} \cos \delta \tag{2.15}$$

Loss modulus (viscous modulus):

$$G'' = \frac{\tau_0}{\gamma_0} \sin \delta \tag{2.16}$$

For an elastic material, δ is zero, and the shear stress is proportional to the imposed strain following Hooke's law. For a viscous material, δ is 90°, and the shear stress response is proportional to the shear rate. For a viscoelastic material, δ is between 0° and 90° and both storage modulus and loss modulus are non zero. SAOS measures elastic modulus and loss modulus as a function of frequency.

In SAOS test with a cone and plate geometry, the elastic modulus and viscous modulus are calculated as follows [82]:

Storage modulus:

$$G' = \frac{3\Theta_0 T_0 \cos\delta}{2\pi R^3 \phi_0} \tag{2.17}$$

Loss modulus:

$$G'' = \frac{3\Theta_0 T_0 \sin\delta}{2\pi R^3 \phi_0} \tag{2.18}$$

where Θ_0 is the cone angle, T_0 is the amplitude of the torque on the cone, δ is the phase difference between torque and torsional angle, and ϕ_0 is the amplitude of torsional angle through which the cone oscillates.

In SAOS test, for a fluid that exhibits viscoelastic behavior, at low frequency the loss modulus G'' is higher than the storage modulus G'. As frequency increases, both moduli increase. At higher frequencies, the storage modulus G' exceeds the loss modulus G''. The frequency at which loss modulus G'' and storage modulus G' are equal is called cross over frequency. The inverse of the cross over frequency is identified with the relaxation time [110]. Relaxation time is the time that characterizes a material's stress relaxation after deformation. Viscoelastic fluids deform when subjected to stress, but when

the stress is removed the internal molecular configuration of the fluid can sustain stress for some time before it relaxes [82].

In SAOS test, a frequency-dependent viscosity function is also determined. The frequency-dependent viscosity is called complex viscosity (η^*) which can be defined as follows [82]:

$$\eta^*(\omega) = \eta' - i\eta'' \tag{2.19}$$

where $\eta' = G''/\omega$ is the dynamic viscosity and $\eta'' = G'/\omega$ is the out of phase component of η^*.

2.4.3 Rheological Models

Constitutive modeling represents the appropriate tensorial expressions for the stress in a flow as a function of deformation matching observed material behavior.

For a Newtonian fluid, the constitutive equation can be expressed as follows [82]

$$\begin{bmatrix} \tau_{xx} & \tau_{xy} & \tau_{xz} \\ \tau_{yx} & \tau_{yy} & \tau_{yz} \\ \tau_{zx} & \tau_{zy} & \tau_{zz} \end{bmatrix} = \mu \cdot \begin{bmatrix} 2\frac{\partial v_x}{\partial x} & \frac{\partial v_y}{\partial x} + \frac{\partial v_x}{\partial y} & \frac{\partial v_z}{\partial x} + \frac{\partial v_x}{\partial z} \\ \frac{\partial v_x}{\partial y} + \frac{\partial v_y}{\partial x} & 2\frac{\partial v_y}{\partial y} & \frac{\partial v_z}{\partial y} + \frac{\partial v_y}{\partial z} \\ \frac{\partial v_x}{\partial z} + \frac{\partial v_z}{\partial x} & \frac{\partial v_y}{\partial z} + \frac{\partial v_z}{\partial y} & 2\frac{\partial v_z}{\partial z} \end{bmatrix} \tag{2.20}$$

where τ_{ij} are the components of stress tensor, v_x, v_y, v_z are the components of velocity. μ is viscosity.

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

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

$$\begin{bmatrix} \tau_{xx} & \tau_{xy} & \tau_{xz} \\ \tau_{yx} & \tau_{yy} & \tau_{yz} \\ \tau_{zx} & \tau_{zy} & \tau_{zz} \end{bmatrix} = \mu(\dot{\gamma}) \cdot \begin{bmatrix} \dot{\gamma}_{xx} & \dot{\gamma}_{xy} & \dot{\gamma}_{xz} \\ \dot{\gamma}_{yx} & \dot{\gamma}_{yy} & \dot{\gamma}_{yz} \\ \dot{\gamma}_{zx} & \dot{\gamma}_{zz} & \dot{\gamma}_{zz} \end{bmatrix} \tag{2.21}$$

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

In equation 2.21, the rate of deformation tensor can be calculated by equation 2.22

$$\begin{bmatrix} \dot{\gamma}_{xx} & \dot{\gamma}_{xy} & \dot{\gamma}_{xz} \\ \dot{\gamma}_{yx} & \dot{\gamma}_{yy} & \dot{\gamma}_{yz} \\ \dot{\gamma}_{zx} & \dot{\gamma}_{zz} & \dot{\gamma}_{zz} \end{bmatrix} = \begin{bmatrix} 2\frac{\partial v_x}{\partial x} & \frac{\partial v_y}{\partial x} + \frac{\partial v_x}{\partial y} & \frac{\partial v_z}{\partial x} + \frac{\partial v_x}{\partial z} \\ \frac{\partial v_x}{\partial y} + \frac{\partial v_y}{\partial x} & 2\frac{\partial v_y}{\partial y} & \frac{\partial v_z}{\partial y} + \frac{\partial v_y}{\partial z} \\ \frac{\partial v_x}{\partial z} + \frac{\partial v_z}{\partial x} & \frac{\partial v_y}{\partial z} + \frac{\partial v_z}{\partial y} & 2\frac{\partial v_z}{\partial z} \end{bmatrix} \tag{2.22}$$

The viscosity of generalized Newtonian is a function of 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 local shear rate can be identified by fitting with the viscosity experimental data. There are several models that can be used to better fit the material's characteristic. Two models are introduced here: Cross model [34] and Carreau-Yasuda model [82].

Cross Model:

$$\frac{\eta(\dot{\gamma}) - \eta_\infty}{\eta_0 - \eta_\infty} = \frac{1}{1 + (\lambda\dot{\gamma})^n} \tag{2.23}$$

where η is the viscosity, η_0 is the zero shear viscosity, η_∞ is the infinite shear viscosity, λ is a time constant, n is a rate constant describes the slope.

Carreau-Yasuda Model:

$$\frac{\eta(\dot{\gamma})-\eta_\infty}{\eta_0-\eta_\infty}=\left[1+(\dot{\gamma}\lambda)^a\right]^{\frac{n-1}{a}} \tag{2.24}$$

where η is the viscosity, η_0 is the zero shear viscosity, η_∞ is the infinite shear viscosity, λ is a time constant, n is a rate constant describes the slope, a is a constant.

2.5 Rheology of Hyaluronic Acid

Hyaluronic acid (HA) is also called hyaluronan and was discovered by Meyer and Palmer in 1934 [79] in the vitreous humor of cattle eyes. HA is present not only in the vitreous humor but also in the extracellular matrix and synovial fluid. It is a linear glycosaminoglycan (GAG) which consists of alternating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine linked by β (1-3) and β (1-4) glycosidic linkages [64].

In normal synovial fluid, HA is a glycosaminoglycan polymer with an average molecular weight of 10^6 - 10^7 Da [95] and a concentration of ~ 3 mg/ml [58]. Both high molecular weight of HA and high concentration of HA is necessary for normal joint function [111]. In solutions at low concentrations (< 1 mg/ml), HA exists as an extended random coil [111] while at higher concentrations (> 1 mg/ml) a transient entanglement network is formed [112]. On the other hand, a study by Scott et al. [113] has shown that HA chains form an irregular honeycomb-like structure of enormous dimensions even at the relatively low concentration of 1 μg / ml. The network-forming ability of HA in solutions

gives rise to the non-Newtonian behavior of HA solutions [64] and affects the viscoelasticity of the solution [80].

Rheological properties of HA have been studied widely. Miyazaki and colleagues [80] observed that measured viscosity values of HA in NaCl solution decreased gradually. The decrease in apparent viscosity was more pronounced in high molecular weight HA than in low molecular weight HA. Ambrosio et al. [2] investigated the effect of molecular weight of HA. The results showed that low molecular weight HA exhibited Newtonian characteristics throughout the shear rate analyzed (10^0-10^3 s^{-1}). For high molecular weight HA, shear thinning behavior was observed at shear rates greater than 1 s^{-1}.

Gibbs et al. [50] measured the dynamic viscoelastic properties of HA solutions. As the oscillation frequency increased, a sharp transition from viscous to elastic behavior occurred. The same relaxation mechanism of HA solutions was observed from time-temperature, time-concentration and time-ionic strength superpositions. The relaxation mechanism involves a breakdown of highly elastic hydrogen-bonded network, followed by viscous flow.

Kobayashi and colleagues [62] investigated the effect of molecular weight of HA on the storage (G') and loss (G'') moduli. They reported that at high molecular weight a transient entanglement network is formed, but it was absent for HA at low molecular weight. For the higher molecular weight HA solutions, a distinct transition from viscous to elastic behavior occurred as the oscillation frequency increased. Another study by Ambrosio et al. [2] also observed that HA solutions of higher molecular weight (1.2 MDa) showed entanglement, whereas lower molecular weight (150 KDa) did not. Also, the low molecular weight HA showed viscous behavior; whereas for the high molecular weight HA

the dynamic moduli G' and G'' exhibited cross-over (i.e. it showed viscous behavior at low frequency and elastic behavior at high frequency).

Another study by Falcone et al.[42] conducted a study on the cohesive and rheological properties of HA at different molecular weights (0.35×10^6 to 1.8×10^6 Da). It was found that the cohesive nature of HA was highly dependent on molecular weight and solution concentration. The study also showed that viscosity strongly depends on concentration and molecular weight of the polymer.

The effect of addition of hyaluronate segments on the viscosity of HA solutions was investigated by Fujii et al. [46]. They reported that longer NaHA was found to increase storage G' and loss G'' moduli whereas shorter NaHA decreased both moduli. Also, addition of sodium glucuronate was found to decrease both the moduli whereas addition of N-acetyl glucosamine was found to increase both the moduli.

The effect of concentration on elasticity of hyaluronan-aggrecan solutions was studied by Nishimura et al. [86]. They determined storage G' and loss G'' moduli using a controlled stress rheometer. Elasticity at different concentrations was studied. Aggrecan solution alone showed little elasticity. Addition of hyaluronan (0.001 to 0.1 mg/ml) markedly increased elasticity, but not viscosity. Elasticity of the hyaluronan-aggregan solution reached a plateau at 500:1 (w/w) ratio.

2.6 Rheology of Synovial Fluid

2.6.1 Viscosity

The rheological propertites of synovial fluid are of interest due to their significant in joint lubrication. Normal synovial fluid is non-Newtonian, in that its viscosity depends

on shear rate and it demonstrates a shear thinning effect [93]. The rheological properties for synovial fluid from normal, osteoarthritic and rheumatoid arthritic joints were investigated in several studies. Normal synovial fluids were found to have the highest viscosity followed by degenerative synovial fluids and inflammatory synovial fluids, respectively [32, 110-112] (Table 2.2). Previous study by Conrad et al. [31] reported that synovial fluid viscosity found to be a good marker for OA severity.

Table 2.2: Viscometric property for normal and pathological synovial fluids.

Reference	η_0 (Pa s)		
	Normal/Postmortem	Osteoarthritis	Inflammatory
Cooke et al.* [32]	> 20 (Normal)	0.1-1	0.1
Schurz & Ribitsch [112]	1-40 (Post Mortem)	0.1-1	0.004-0.07
Schurz [111]	6-12 (Post Mortem)	0.1-1	0.005-0.05
Safari et al. [110]	10-34 (Normal)	NR	0.01-0.1

* The values have been estimated from published charts.
NR = not reported.

A study by Bloch and Distenfass [18] found that synovial fluid from rheumatoid arthritis showed Newtonian characteristics in flow; the viscosity was of the order of 10 centipoises. Synovial fluid from traumatic arthritis, however, showed thixothropic properties; that is, the viscosity decreases with increasing shear rate. Non-Newtonian and shear thinning behavior is characteristic of normal and traumatic arthritis synovial fluid, whereas Newtonian behavior is characteristic of rheumatoid arthritis synovial fluid [39]. A study by Reimann [104] also showed that there are differences in the viscosity of synovial fluid in patients with rheumatoid arthritis, OA (mostly damaged cartilage) and torn meniscus (mostly normal cartilage).

Rainer and Ribitsch [102] examined the viscosity of normal synovial fluid as a function of shear rate. They reported that zero shear rate viscosity, η_0, ranged from 6-175 Pa.s, and the ratio $\eta_0/\eta_{\dot{\gamma}=300}$ ranged from 70-250. Another study by Schurz and Ribitsch [112] reported that η_0 and $\eta_0/\eta_{\dot{\gamma}=300}$ were found to be higher for normal (η_0 = 1-40; $\eta_0/\eta_{\dot{\gamma}=300}$ =100) as opposed to degenerative synovial fluids (η_0 = 0.1-1; $\eta_0/\eta_{\dot{\gamma}=300}$ = 5-40) which were in turn higher than inflammatory synovial fluids (η_0 = 0.004-0.007; $\eta_0/\eta_{\dot{\gamma}=300}$ = 1-4). Schurz and Ribitisch [112] suggested that synovial fluid viscosity can be used to differentially diagnose degenerative and inflammatory joint disease.

The effect of HA concentration on the flow properties of synovial fluid has been investigated in numerous studies. It was found that HA/protein concentration correlated with the apparent viscosity of synovial fluid at a shear rate of 1 s^{-1} [44]. A study by Levine and Kling [70] indicated that the decrease in the intrinsic viscosity matched the decrease in HA concentration in rheumatoid arthritis fluid compared to OA synovial fluid. On the other hand, Stafford and colleagues [116] reported that although the HA concentration in rheumatoid arthritis fluid was less than that of OA fluid; there were no significant difference in the intrinsic viscosity of the two fluids. A more recent study by Praest et al. [99] found that the viscosity of synovial fluid from patients with inflammatory and non-inflammatory joint diseases correlated well with the HA concentration. Furthermore, the viscosity of synovial fluid of various joint diseases showed a better correlation with HA concentration than with average molecular weight.

In addition, a study by O'Neill and Stachowiak [92] showed that synovial fluid exhibits rheopectic behavior; that is stress increases as a function of time during shear at a

constant rate. In their study, a constant shear rate was applied to the sample, which is the synovial fluid from a patient with OA, over a period of time. The results showed that, at temperatures of 20 °C or less, the synovial fluid's viscosity increases with time at a constant shear rate. Another study by Oates et al. [87] also observed rheopectic behavior in a synovial fluid model when applied a constant shear rate of 0.05 s^{-1}. Similar rheopexy also observed in bovine synovial fluid and albumin solutions of similar concentration [88].

2.6.2 Viscoelasticity

The viscoelastic properties of synovial fluid, which are well suited to the joint, were first investigated by Ogston et al. [91]. Myers et al. [84] conducted a study to examine the viscoelastic properties of normal synovial fluid from human knee joints at different frequencies that correspond to different joint speeds. At low frequencies of oscillation, that is characteristic of slow joint motion, synovial fluid acts as a viscous fluid. At high frequencies of oscillation, that is characteristic of rapid joint motion, synovial fluid exhibits elastic-like behavior.

For the "near normal" synovial fluid, viscous modulus G'' is greater than elastic modulus G' at very low frequencies. At higher frequency, synovial fluid has an elastic-like response. For the "near normal" fluid, elastic modulus G' is greater than viscous modulus G'' over most of the frequency range, and cross over frequency can be observed at a fairly low frequency (~0.02 Hz) [110]. However, for pathological synovial fluids, their elastic modulus G' at frequencies above 0.1 Hz is lower than that of normal synovial fluids [105].

In addition, for "near normal" synovial fluid and synovial fluid from patients with ligament defects, the mean values of η'' were higher than the mean values of η'. On the

contrary, for synovial fluids from patients with meniscus lesions, rheumatoid arthritis (RA) seronegative and RA seropositive, the mean values of η' were higher than the mean values of η'' [110]. Similar findings were also found by Anadere et al. [3] for the RA seronegative and RA seropositive fluid groups indicating decreased of elasticity in RA fluids. Furthermore, Anadere et al. [3] also found that both viscous and elastic components were decreased in the diseased fluids. The most pronounced changes were observed in fluids from patients with RA and traumatic diseases, while moderated changes were observed in OA fluids.

Relaxation times for normal synovial fluid were longer compared to pathological synovial fluids [102, 105, 112]. The long relaxation times may impart normal synovial fluid with load bearing capacity [102]. From a study by Schurz and Ribitisch [112], the relaxation time ranges for normal, degenerative, and inflammatory synovial fluids were 40-100 s, 8-20 s, and 0.02-1 s, respectively. However, another study by Safari et al.[110] reported that the relaxation time ranges for near normal, ligament defected, RA seronegative, and RA seropostitive were 0.65 – 5.82 s, 0.59 – 0.89 s, 0.022 – 0.03 s, and < 0.022 s, respectively. Lai et al. [65] computed the relaxation spectra and stress relaxation functions from experimental data of the dynamic moduli. They reported a significantly shorter relaxation time for osteoarthritic synovial fluid than for normal synovial fluid. The relaxation time for osteoarthritic synovial fluid was in the order of 0.1 s, whereas it was in the order of 10 s for normal synovial fluid. A more recent study by Gomez and Thurston [52] showed that the relaxation times for RA synovial fluids were higher than for mixed connective tissue disease indicating greater elasticity. A higher degree of elasticity found in the degenerative joint disease fluid compared to the RA fluid.

Synovial fluid is elastic at higher concentrations of hyaluronic acid [90]. Anadere at al. [3] found higher concentration of HA in synovial fluids from patients with meniscus defects and OA than from synovial fluids of patients with RA seronegative. HA concentration affected the elasticity of the synovial fluids. Ferguson et al. [45] reported that when osteoarthritic synovial fluid was diluted, relaxation times were lowered and a more Newtonian flow behavior occurred. Concentration of RA synovial fluid by evaporation gave rise to an increase in viscosity, but elastic properties were not restored.

With regard to HA molecular weight, Dahl et al. [36] asserted that normal synovial fluid HA molecular weight ($7x10^6$ Da) is significantly higher than RA synovial fluid HA molecular weight ($4.8x10^6$ Da). Another study by Schurz and Ribitisch [112] reported that the molecular weight of HA in normal synovial fluid is 10^7 Da, while it is reduced to $\leq 10^6$ Da in rheumatoid and osteoarthritic synovial fluid. The deterioration of rheological properties in diseased synovial fluids is likely attributable to the decrease in molecular weight of HA. The decrease in concentration and molecular weight of HA could be responsible for the altered in viscoelastic properties of pathological synovial fluid [129].

Mazzucco et al. [76] have investigated rheological properties of fluid from patients undergoing total knee arthroplasty and revision arthroplasty. The general behavior of joint fluid samples was shear thinning. At low frequencies, loss modulus was found to dominate over shear modulus and both moduli increased at higher frequencies. They also observed that crossover frequency increased in arthroplasty fluids in comparison to normal synovial fluid. Also, storage and loss moduli were found to decrease in patients undergoing arthroplasty.

2.6.3 Rheology of Synovial Fluid in OA with Viscosupplements

The goal of viscosupplementation is to restore the rheological properties of the synovial fluid. Grecomoro et al. [54] studied the rheological changes in synovial fluid of patients due to intraarticular infiltration of HA. Three treatments were administered to the patients i.e. intra articular infiltration of high molecular weight sodium hyaluronate (one 20 mg vial/week for 3 weeks), oral anti-inflammatory agents, and fluid aspiration. One sample per week for 3 treatment weeks followed by a further 3 weeks as control were collected to perform rheologic measurement. The results indicated that intraarticular hyaluronic acid was found to temporarily increase viscosity of synovial fluid in patients.

The rheological behaviors of OA synovial fluid before and after the addition of two commercial viscosupplements (linear and cross-linked) were investigated by Mathieu and colleagues [75]. The results showed that synovial fluid becomes less non-Newtonian when mixed with the linear hyaluronic acid. On the contrary, when mixed with cross-linked hyaluronic acid, the non-Newtonian behavior of the fluid was reinforced. Furthermore, the results suggested that linear and cross-linked viscosupplements induce large differences in OA synovial fluid rheological behavior. They asserted that, compare to the linear one, the cross-linked HA is more efficient in improving the rheological behavior of the OA synovial fluid. The rheology of the fluids was nearly unchanged over 6 weeks.

2.6.4 Summary of Rheology of Synovial Fluid

Non-Newtonian shear thinning behavior is characteristic of normal and traumatic arthritis synovial fluid. Several studies reported that synovial fluid exhibits rheopectic behavior [87, 88, 92]. A study by O'Neill and Stachowiak [92] showed that OA synovial

fluid exhibits rheopectic behavior at temperatures of 20 °C or less. However, it has not been reported whether OA synovial fluid exhibits rheopectic behavior at 37 °C.

The rheological parameters reported in most studies are viscosity at steady shear and dynamic moduli. However, for a thorough elucidation of the rheological properties of synovial fluid, other rheological parameters such as complex viscosity, cross over frequency and relaxation time should also be considered.

One of the treatments for OA that aims to restore the rheological properties of synovial fluid is viscosupplementation. Only one study by Mathieu et al. [75] examined the effect of linear and cross-linked viscosupplements on rheological behaviour of OA synovial fluid. Therefore, further studies are needed to determine the effects of viscosupplements on rheological behavior of synovial fluid.

CHAPTER 3

METHODS

In this chapter, the methodology for this pilot study is provided in detail. Subject's demographic data as well as inclusion and exclusion criteria are presented. The apparatus and experimental procedures are described.

3.1 Subjects

After ethics review and approval by the University of British Columbia Clinical Research Ethics Board and Vancouver Coastal Health Research Institute, twenty six patients volunteered to enroll in the study. Copies of the University of British Columbia Research Ethics Board's Certificates of Approval and Vancouver Coastal Health Authority Clinical Trials Administration Office Approval are included in Appendix A and Appendix B, respectively. However, two patients had "dry" knees and synovial fluid samples from two other patients contained insufficient fluid for rheological testing. Therefore, the synovial fluid samples tested in this study were obtained from 22 subjects (16 females and 6 males) during knee arthroplasty for OA. Subjects ranged from 44 to 85 years old (mean age 64 years) (Table 3.1). Diagnosis of the severity of OA was made according to the Kellgren-Lawrence radiographic grading system [59]. All samples came from orthopedic reconstructive service at Vancouver Acute (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. Subjects were asked to give an informed consent to allow for joint fluid aspiration and access to clinical results.

Inclusion Criteria

- Diagnosed with knee OA and required knee replacement.
- Between 30 and 85 years of age.

Exclusion Criteria

- Diagnosed with other arthritic conditions (e.g. inflammatory arthritis)
- Under the age of 30 or over 85 years of age.
- Had surgery on their study knee within 10 years prior to enrollment in the study.

Table 3.1: Demographic, degree of severity of OA, and synovial fluid appearance

Subject	Age	Gender	Lt/Rt	Degree	SF appearance
SN01	74	F	Lt	4	bright red, slightly cloudy
SN02	71	M	Lt	4	light yellow, clear
SN03	69	F	Rt	4	light yellow, clear
SN04	57	M	Rt	4	light yellow, slightly cloudy
SN05	51	M	Rt	4	orange, slightly cloudy
SN06	76	F	Rt	4	light yellow, clear
SN07	67	F	Lt	4	light yellow, clear
SN08	53	F	Rt	4	red, cloudy
SN09	71	F	Rt	4	light yellow, clear
SN10	65	F	Rt	4	light yellow, fairly clear
SN11	53	F	Rt	4	red, slightly cloudy
SN12	85	F	Lt	4	bright red , clear
SN13	65	M	Rt	4	bright red cloudy
SN14	63	F	Rt	3	yellow, slightly cloudy
SN15	70	F	Rt	4	yellow, clear
SN16	62	F	Rt	4	light yellow, clear
SN17	47	F	Bilateral	4	light yellow, slightly cloudy
SN18	77	M	Rt	4	yellow, clear
SN19	52	F	Rt	2	light yellow, clear
SN20	68	F	Rt	4	orange, clear
SN21	63	M	Lt	4	light yellow, clear
SN22	44	F	Bilateral	4	light yellow, clear

3.2 Apparatus

3.2.1 Bohlin Gemini HR^{nano} Rheometer

The Bohlin Gemini HR^{nano} rheometer is a rotational rheometer. It has a high resolution air bearing design and performance which is sensitive with extremely low torque errors without compromising stiffness and strength. The Gemini provides continuous torque control range from 3 nNm to 200 mNm. The torque resolution is 1 nNm and the position resolution is 50 nrad. The automated zero gap setting and control from PC or on the instrument sets a reproducible zero gap before actual measurement. The gap is closed automatically using this zero gap as the reference, once the sample is loaded on the plate. The 'EasySwap' technology allows the instrument to be configured with a variety of temperature control units attached. The Peltier device is used for an accurate temperature control [23]. The Gemini has undergone a significant number of calibrations and verifications by the manufacturer. The oils used in these calibrations are all traceable to international standards. The Gemini has been approved by the Canadian Standards Association.

3.2.2 Kinexus

The Kinexus is a rotational rheometer. The motor and air bearing system for the Kinexus enables the Kinexus systems to provide the wide continuous torque range (0.05 μNm to 200 mNm). The torque resolution is 0.1 nNm and the position resolution is less than 10 nrad. The gap and normal force system for the Kinexus combines high speed and ultra-fine resolution gap control with high sensitivity and ultra-responsive normal force control. The gap resolution is 0.1 μm. This provides optimal sample loading capabilities for all material types and allows the system to be able to capture transient material

response. The environmental controller incorporates interchangeable lower plate. In addition, the environmental controller is designed for thermal stability, minimized thermal gradients and temperature resolution to 0.01 °C. All measurement geometries and accessories are auto-recognized and auto-configured [24]. The Kinexus has undergone a significant number of calibrations and verifications by the manufacturer. The oils used in these calibrations are all traceable to international standards. The Kinexus has been approved by the Canadian Standards Association.

3.3 Viscosupplements

Viscosupplement is used for reducing pain and improving joint mobility in patients with OA. It aims to restore the homeostasis in the joint. In this study, rheological behaviors of several viscosupplements were examined.

3.3.1 Orthovisc®

Orthovisc® (Anika Therapeutics, Inc.) is derived from rooster combs. It contains 15mg/ml of sodium hyaluronate (NaHA) dissolved in physiological saline.

3.3.2 Suplasyn®

Suplasyn® (Bioniche Pharma Group Ltd.) is produced by biofermentation. It contains 20 mg/ml of hyaluronic acid sodium salt.

3.3.3 Synvisc® Hylan G-F 20

Synvisc® Hylan G-F 20 (Genzyme Biosurgery) is derived from rooster combs. It contains 8 mg/ml of hylan A and hylan B in buffered physiological sodium chloride solution.

3.4 Experimental Procedure

3.4.1 Experiment 1: Rheology of Synovial Fluid

Informed consent was obtained from each subject prior to the surgery. At the time of the surgery, synovial fluid of approximately 2-5 ml. was aspirated from each subjects' knee joint into a test tube by an experienced surgeon under sterile condition, after anesthetic had been achieved and just prior to operation's initial incision. Clinical data including age, gender, radiographs were collected by routine chart review.

Rheological behavior of each synovial fluid sample was evaluated as soon as possible after aspiration (within 2 hours). In case the measurement could not be performed within 2 hours, aspirated synovial fluid was kept in a refrigerator at approximately 4 °C for testing within 2 days after aspiration.

- The rheometer was first calibrated with Cannon Certified Viscosity Standard oil (1 Pa s) (Figure 3.1)
- Rheological characterization of the samples was evaluated by Bohlin Gemini HR^{nano} rheometer with a stainless steel cone and plate geometry (30 mm diameter cone with a 1° cone angle) at 37 °C. The gap was zeroed before loading sample in every measurement. The gap was set at 30 μm.
- In the steady shear test, all samples were presheared at 100 s^{-1} for 2 min. after loading in order to erase any differences in shear history during preparing and loading samples. Then, the shear rates ranged from 0.01 to 1000 s^{-1} were applied to the samples. In addition, constant shear rates at 0.01 s^{-1} and 0.05 s^{-1} were applied to the samples for 450 sec. in order to examine the changes in shear stress over time.

- In the small amplitude oscillatory shear test (SAOS), preliminary strain sweep tests were performed on the samples in order to identify the linear viscoelastic response range of the samples (Figure 3.2). In all SAOS experiments, the amplitude sweeping tests were performed firstly to ensure the frequency sweeping tests in linear viscoelastic regimes. Then, frequency sweep measurements were conducted in the linear region, at 5% strain, over a frequency range of 0.01-10 Hz.
- Two measurements were performed in each steady shear and SAOS test. Note that only two measurements were performed in each test. This is because synovial fluid evaporates quickly at physiological temperature.

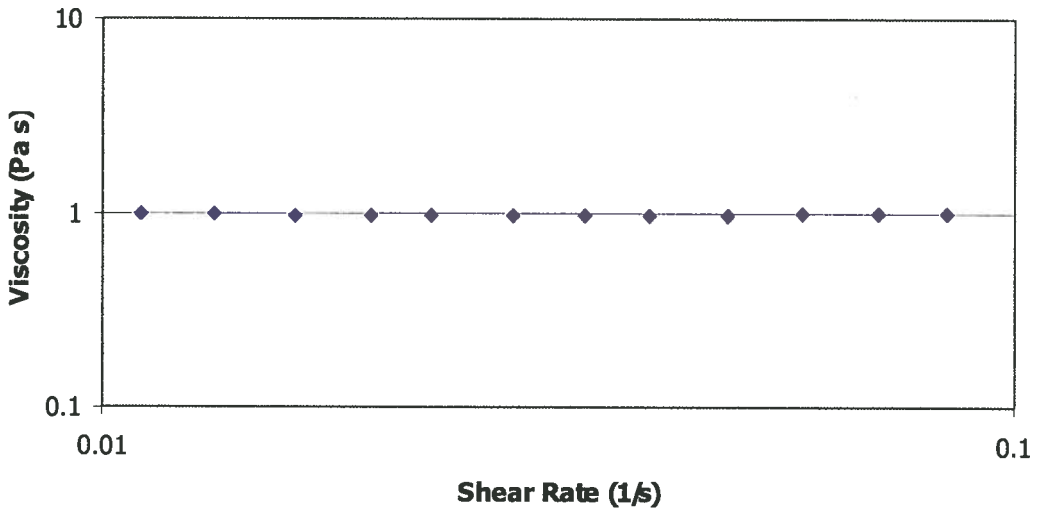

Figure 3.1: Calibration of the Bohlin Gemini HR^{nano} rheometer with Cannon Certified Viscosity Standard oil (1 Pa s)

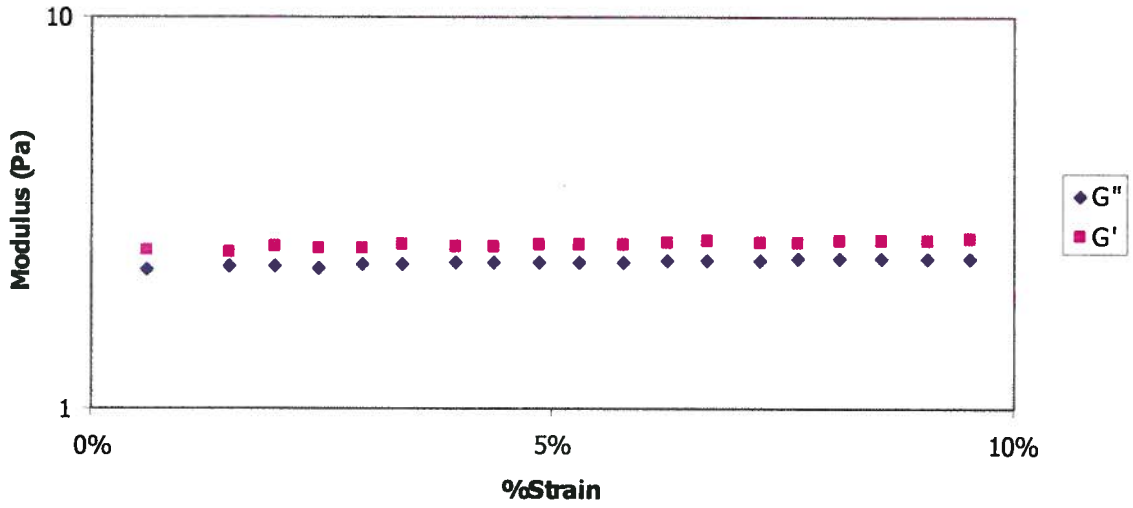

Figure 3.2: Strain sweep test

3.4.2 Experiment 2: Rheology of Viscosupplements

The samples used in this experiment were three different brands of commercial viscosupplements (i.e. Orthovisc®, Suplasyn®, and Synvisc® Hylan G-F 20)

- The rheometer was first calibrated with Cannon Certified Viscosity Standard oil (1 Pa s) (Figure 3.3).
- Rheological behaviors of the samples were evaluated by Kinexus rheometer with a stainless steel plate and plate geometry (20 mm diameter plate) at 25 °C and 37 °C. The gap was zeroed before loading sample in every measurement. The gap was set at 0.5 mm.
- In the steady shear test, all samples were presheared at 100 s^{-1} for 2 min. after loading in order to erase any differences in shear history during preparing and

loading samples. Then, the shear rates ranged from 0.01 to 1000 s^{-1} were applied to the samples.

- In all SAOS experiments, the amplitude sweeping tests were performed firstly to ensure the frequency sweeping tests in linear viscoelastic regimes. Then, frequency sweep measurements were conducted in the linear region, at 5% strain, over a frequency range of 0.1-10 Hz.
- Two measurements were performed in each steady shear and SAOS test.

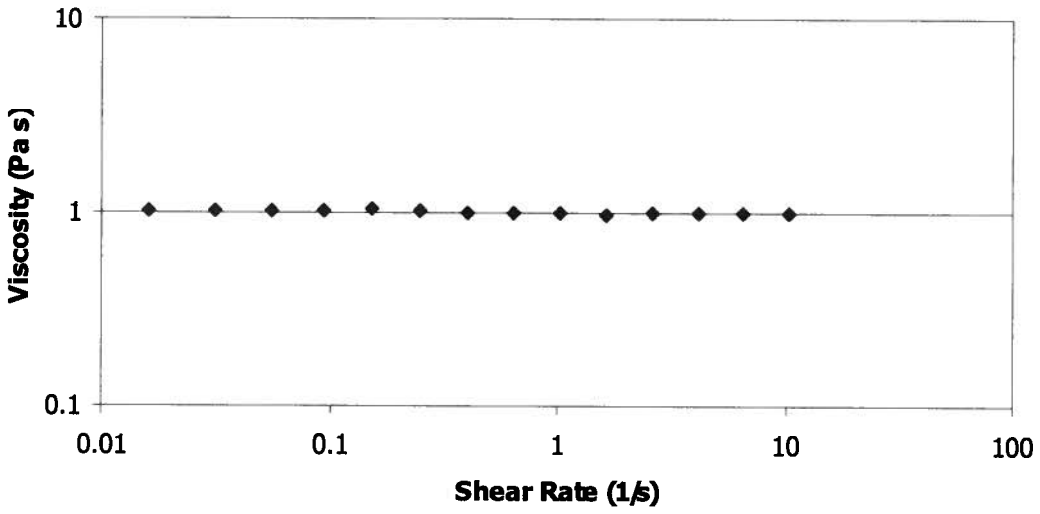

Figure 3.3: Calibration of the Kinexus rheometer with Cannon Certified Viscosity Standard oil (1 Pa s)

3.4.3 Experiment 3: Effects of Viscosupplements on the Rheology of Synovial Fluid

Two of the synovial fluid samples obtained in "Experiment 1" were aliquoted into four tubes. One (0.5 ml) contained synovial fluid alone; in the other three (0.5 ml), viscosupplements (i.e. Orthovisc®, Suplasyn®, and Synvisc® Hylan G-F 20) were added in each of the three tubes in a volume ratio of 1:1 and they were mixed at ambient temperature 30 minutes before rheological measurements.

- The rheometer was first calibrated with Cannon Certified Viscosity Standard oil (1 Pa s).
- Rheological behaviors of the samples were evaluated by Kinexus rheometer with a stainless steel plate and plate geometry (20 mm diameter plate) at 37 °C. The gap was zeroed before loading sample in every measurement. The gap was set at 0.5 mm.
- In the steady shear test, all samples were presheared at 100 s^{-1} for 2 min. after loading in order to erase any differences in shear history during preparing and loading samples. Then, the shear rates ranged from 0.01 to 1000 s^{-1} were applied to the samples.
- In all SAOS experiments, the amplitude sweeping tests were performed firstly to ensure the frequency sweeping tests in linear viscoelastic regimes. Then, frequency sweep measurements were conducted in the linear region, at 5% strain, over a frequency range of 0.1-10 Hz.
- Two measurements were performed in each steady shear and SAOS test.

3.4.4 Experiment 4: Stability of Rheological Properties of Synovial Fluid Mixed with Crossed-Linked Viscosupplement Over Time.

Two of the synovial fluid samples obtained in "Experiment 1" were aliquoted into two tubes. One (1 ml) contained synovial fluid alone; in the other (1 ml), Synvisc® was added in a volume ratio of 1:1 and they were mixed at ambient temperature 30 minutes before rheological measurements at the baseline. Then, mixed samples of synovial fluid and Synvisc® were stored in a refrigerator at approximately 4 °C for rheological measurements at 14 days later. Samples that were stored in a refrigerator were allowed to

come to room temperature 10 min. before loading. Rheological measurements were performed on both synovial fluid and synovial fluid with Synvisc®.

- The rheometer was first calibrated with Cannon Certified Viscosity Standard oil (1 Pa s).
- Rheological characterization of the samples was evaluated by Bohlin Gemini HR^{nano} rheometer with a stainless steel cone and plate geometry (30 mm diameter cone with a 1° cone angle) at 37 °C. The gap was zeroed before loading sample in every measurement. The gap was set at 30 μm.
- In the steady shear test, all samples were presheared at 100 s^{-1} for 2 min. after loading in order to erase any differences in shear history during preparing and loading samples. Then, the shear rates ranged from 0.01 to 1000 s^{-1} were applied to the samples.
- In all SAOS experiments, the amplitude sweeping tests were performed firstly to ensure the frequency sweeping tests in linear viscoelastic regimes. Then, frequency sweep measurements were conducted in the linear region, at 5% strain, over a frequency range of 0.01-10 Hz.
- Two measurements were performed in each steady shear and SAOS test.

CHAPTER 4

RESULTS AND DISCUSSION

In this chapter, the results from this pilot study which are rheological properties of synovial fluid, rheology of viscosupplements, the effects of viscosupplements on the rheology of synovial fluid, and the stability of rheological properties of synovial fluid mixed with cross-linked viscosupplement over time are presented and discussed.

4.1 Rheology of Synovial Fluid

In this experiment, the steady shear and oscillatory shear measurements were performed in OA synovial fluid in order to examine the rheological behaviors of synovial fluid.

4.1.1 Viscometric Property

All 24 synovial fluid samples exhibited non-Newtonian shear thinning behavior; that is viscosity decreases with increasing shear rate (Figure 4.1). This is the characteristic for normal and degenerative arthritis [39, 112]. Results showed that rheological behaviors of synovial fluid varied widely in OA. The variability is demonstrated by the wide range of zero shear viscosity. The zero shear viscosity (η_0) varied from 0.28 to 10.59 Pa s (Table 4.1). However, at high shear rate the viscosities are mostly the same, even though the physiological shear rates are not in that high range. To ensure the repeatability of the data, two measurements were performed for steady shear test and the precision of the data is within 2% (Appendix C.1). Most of the samples (80%) exhibited viscosity plateau at low shear rates.

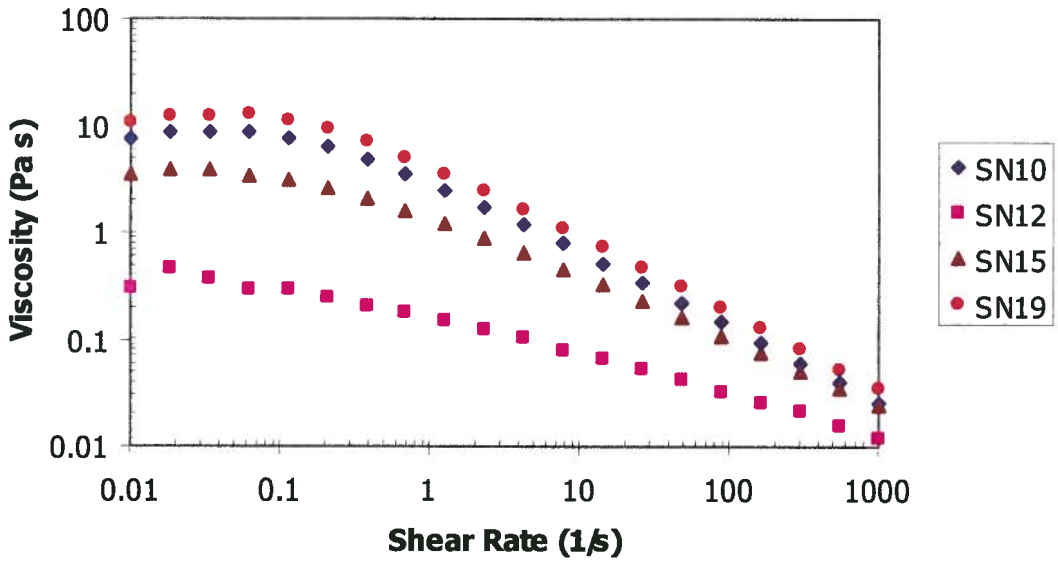

Figure 4.1: Viscosity as a function of shear rate for synovial fluid samples. Synovial fluid samples exhibited non-Newtonian shear thinning behavior. The viscosity decreases with increasing shear rate.

Comparing to another study by Mazzucco et al. [76], in which synovial fluid samples were also obtained from subjects during total knee arthroplastry (TKA), the range of η_0 in the present study was narrower, but the median was higher (Table 4.2). Based on the established range of η_0 from previous work by Schurz [111], 71% of the samples in Mazzucco et al. [76] study fit in the degenerative range, whereas only 17% of the samples in the present study fit in that range (Table 4.3).

Based on the established range of viscous property for normal and degenerative arthritis from previous studies [32, 110-112] (see Table 2.2 in Chapter 2), synovial fluid samples in this study fit in both normal and degenerative range. However, it should be noted that the range of viscosity of synovial fluid for normal joints reported in previous studies [32, 110-112] varied from one study to the others. Moreover, in some studies [111, 112], the viscosities of synovial fluid for normal joints were examined only from post

mortem samples. Therefore, the results reported in these studies might underestimate the viscosity of synovial fluid for normal joints since synovial fluid may become diluted after death [116].

Table 4.1: Zero shear viscosity (η_0) and viscosity at shear rate 1000/s (η_{1000})

Sample	zero shear viscosity	max shear viscosity
SN01	7.62	0.03
SN02	0.93	0.01
SN03	3.46	0.01
SN04	1.53	0.02
SN05	3.98	0.02
SN06	0.71	0.01
SN07	5.22	0.02
SN08	1.14	0.01
SN09	1.80	0.02
SN10	7.33	0.03
SN11	1.75	0.01
SN12	0.30	0.01
SN13	8.19	0.02
SN14	1.27	0.02
SN15	3.50	0.02
SN16	7.87	0.03
SN17 Lt.	2.31	0.02
SN17 Rt.	1.09	0.02
SN18	2.15	0.02
SN19	10.59	0.03
SN20	0.28	0.02
SN21	3.63	0.03
SN22 Lt.	3.16	0.03
SN22 Rt.	1.82	0.02
Range	0.28-10.59	0.01-0.03
Mean±SD	3.40±2.90	0.02±0.01

Table 4.2: Comparison of η_0 between the present study and Mazzucco et al. [76]

η_0 (Pa s)	Present Study	Mazzucco et al. [76]
Range	0.279 – 10.59	0.087 - 25
Median	2.23	1.3

Table 4.3: Percentage of the samples fit in the established range of η_0 from previous work [111] on normal and degenerative synovial fluid.

Parameter	Group	Range	% Samples	
			Present Study	Mazzucco et al. [76]
η_0 (Pa s)	Normal	6- 12	83	29
	Degenerative	0.1 -1	17	71

It should be noted that many patients with OA participated in this study presented an increased volume of synovial fluid. Friction is introduced when excess lubricant is introduced into the bearing. In this case the moving element has to overcome the excess lubricant by pushing it out of the way thereby demanding more energy for the moving element to perform some work. Friction causes wear generated by high viscous stresses. Therefore, a supplementary wear can occur in OA joints, which is not related to the degradation of the cartilage, though to the lubricant friction. In addition, some of the OA synovial fluid in this study presented impurities. One general cause of bearing failure is the dirt in the lubrication system. This will lead to a supplementary wear.

In the present study, two patients had "dry" knees and synovial fluid samples from two other patients contained insufficient fluid for rheological testing. For poorly lubricate bearings, abrasive wear could occur. In this case two surfaces pass over one another close enough for the asperities to "lock and adhere" with one another causing a wear particle to break off. Also in this case a supplementary wear can occur due to the small quantity of synovial fluid.

In general, the viscosity of OA synovial fluid is decreased compare to healthy synovial fluid. For bearings in general, if an application involves high speeds and low loads, then a low viscosity lubricant is adequate. Inversely, if an application involves low speeds and high loads, then high viscosity lubricant should be chosen. In choosing a lubricant we must ensure the viscosity is high enough to provide a continuous fluid film in the contact area, but not too high so as to create friction due to viscous shear. The knee involves low speeds and high loads, so a higher viscosity is recommended.

4.1.2 Rheopectic Property

Constant shear rates of 0.01 and 0.05 s^{-1}, were applied to 19 synovial fluid samples. The results showed that at low shear rates of 0.01 and 0.05 s^{-1}, synovial fluid samples exhibited rheopectic behavior; that is the shear stress increases over time at a constant shear rate. Examples of rheopectic behavior of synovial fluid at the shear rates of 0.01 and 0.05 s^{-1} are shown in Figure 4.2 and 4.3, respectively.

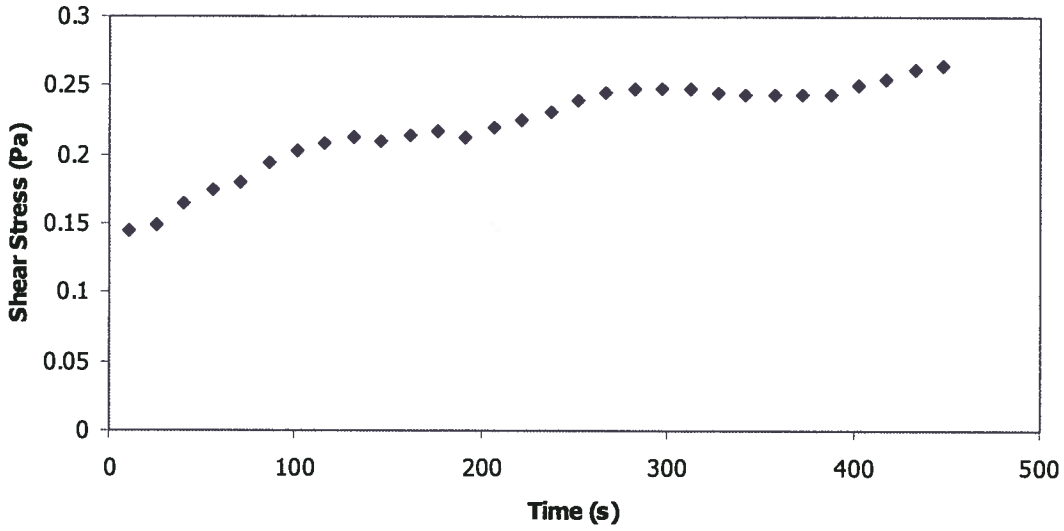

Figure 4.2: Shear stress as a function of time at shear rate 0.01 s^{-1} (sample SN19). Synovial fluid exhibited rheopectic behavior; that is the shear stress increases over time at a constant shear rate of 0.01 s^{-1}.

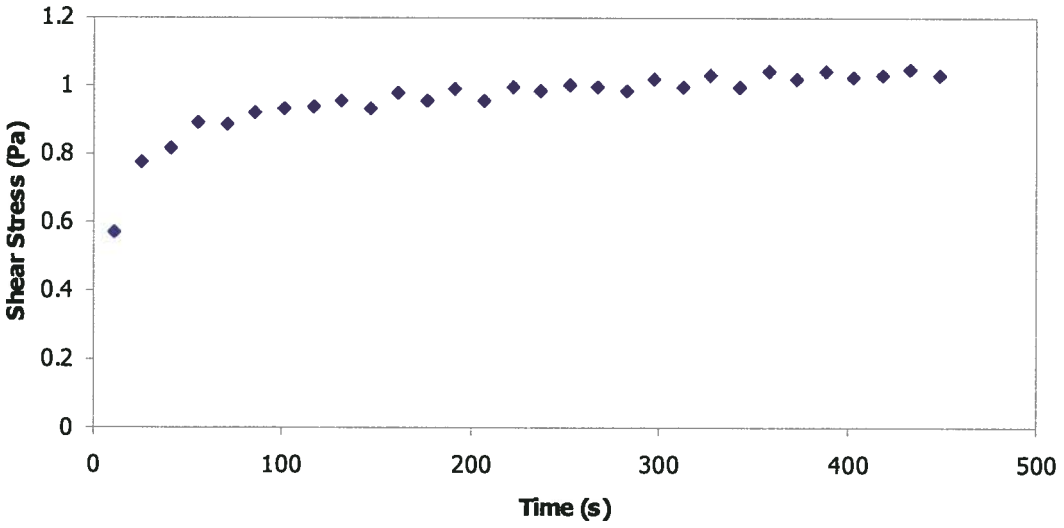

Figure 4.3: Shear stress as a function of time at shear rate 0.05 s^{-1} (sample SN19). Synovial fluid exhibited rheopectic behavior; that is the shear stress increases over time at a constant shear rate of 0.05 s^{-1}.

Table 4.4 shows that the ratio of $\sigma_{t=450s} / \sigma_{t=0}$ at 0.01 s^{-1} ranged from 1.57–6.48 and the ratio of $\sigma_{t=450s} / \sigma_{t=0}$ at 0.05 s^{-1} ranged from 1.23–3.40. In general, the ratios of $\sigma_{t=450s} / \sigma_{t=0}$ at 0.01 s^{-1} in synovial fluid samples were higher than that of $\sigma_{t=450s} / \sigma_{t=0}$ at 0.05 s^{-1}, with the median of 2.12 and 1.59, respectively. These results suggested that the shear stress of OA synovial fluid at the shear rate of 0.01 s^{-1} builds up at higher rate than at the shear rate of 0.05 s^{-1}.

Table 4.4: Ratios of shear stress at 450 s/shear stress at 0 s (at 0.01 s^{-1} and 0.05 s^{-1})

Sample	$\sigma_{t=450s} / \sigma_{t=0}$ at 0.01 s^{-1}	$\sigma_{t=450s} / \sigma_{t=0}$ at 0.05 s^{-1}
SN06	1.89	1.28
SN07	1.83	1.46
SN08	2.10	1.53
SN09	2.10	1.58
SN10	2.24	1.52
SN11	3.55	2.12
SN12	1.71	2.11
SN13	2.96	1.53
SN14	4.63	1.59
SN15	2.22	2.06
SN16	1.72	1.75
SN17 Lt.	5.55	2.46
SN17 Rt.	6.48	3.40
SN18	1.85	1.67
SN19	1.84	1.81
SN20	2.12	1.23
SN21	1.57	1.41
SN22 Lt.	2.37	1.79
SN22 Rt.	2.81	1.52
Range	1.57-6.48	1.23-3.40
Median	2.12	1.59

Rheopexy in synovial fluid has previously been observed in several studies [63, 87, 88, 92]. O'neill and Stachowiak [92] found rheopectic behavior in OA synovial fluid at temperature below 20 °C. Above this temperature, rheopexy was not observed. The ratio $\eta_{t=450s} / \eta_{t=0}$ was approximately 1.15 (note that this value is estimated from published graph). The results from the present study are not consistent with their findings. In the present study, the rheopectic behavior was observed in OA synovial fluid samples at 37 °C. The differences of the findings may be caused by several factors which include the types of instrument and the constant shear rate that was applied to the sample. It should be noted that in O'neill and Stachowiak [92] study the constant shear rate applied to the sample was not reported.

Rheopectic behavior is an important behavior of synovial fluid. Viscosity of synovial fluid increases with duration of shearing. It seems that the longer the duration of shearing, the better the lubricating film which is generated by the body. Oates et al. [88] asserted that rheopectic behavior is attributed to protein aggregation which appears to play an important role in enhancing the viscoelastic properties of synovial fluid. Furthermore, rheopectic behavior indicates the temporary protein network formation which may help explain joint stiffness after inactivity. It has been reported that in degenerative joint diseases, the protein content in synovial fluid increases [12]. The increase of protein content in OA synovial fluid and rheopectic behavior of synovial fluid may be associated with the increase in joint stiffness following prolonged inactivity found in patients with OA. However, the mechanism of rheopectic behavior in relation to joint lubrication is still not well understood.

4.1.3 Viscoelastic Properties

The linear viscoelastic properties were observed in synovial fluid samples. At low frequencies, loss modulus G'' was higher than storage modulus G'. As the frequency increased, both moduli, G'' and G', increased. At higher frequencies, the storage modulus G' exceeded loss modulus G'' in many samples. The cross over frequency, at which the loss and storage moduli are equal, was observed in 18 of the 24 synovial fluid samples obtained (Figure 4.4). In the other 6 samples, even at high frequency, the storage modulus G' was not sufficiently large enough to measure a cross over (Figure 4.5). In the absent of cross over, synovial fluid samples displayed a viscous-like behavior.

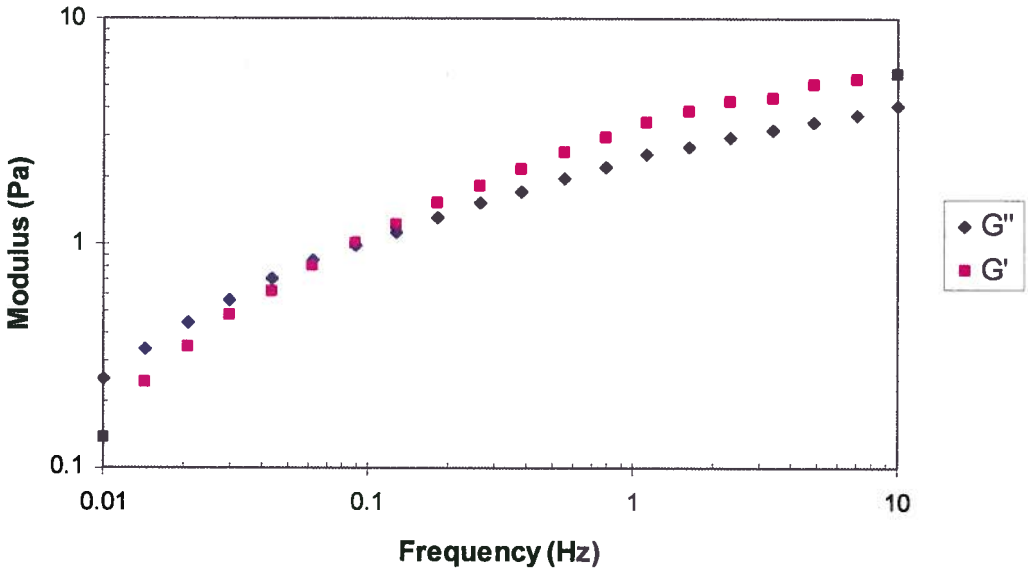

Figure 4.4: Storage and loss moduli as a function of frequency from SAOS measurement (sample SN15). Viscoelastic properties were observed in synovial fluid samples. This graph shows that synovial fluid exhibited viscoelastic behavior. At low frequencies, loss modulus was higher than storage modulus. At higher frequencies, storage modulus exceeded loss modulus. The cross over frequency was observed.

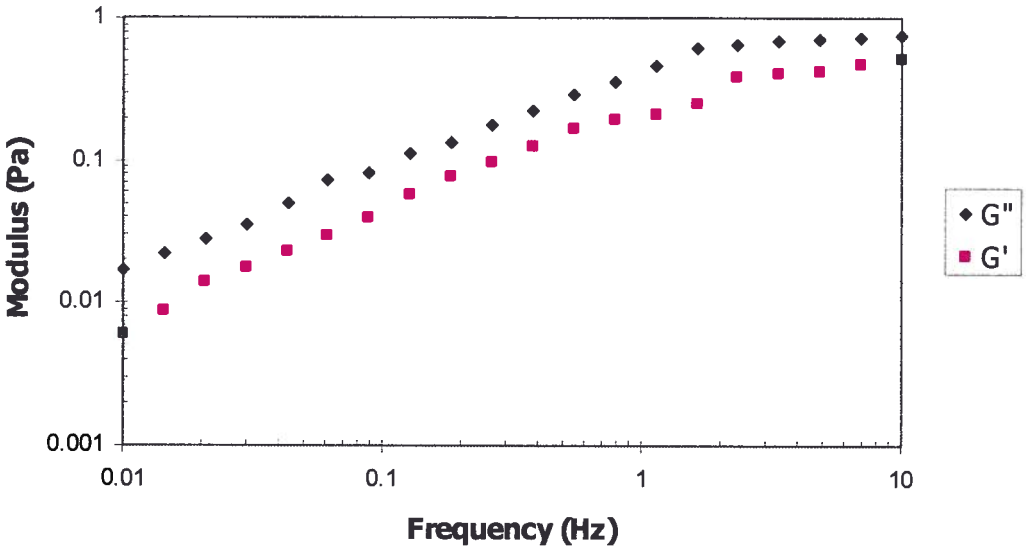

Figure 4.5: Storage and loss moduli as a function of frequency from SAOS measurement (sample SN12). Viscoelastic properties were observed in synovial fluid samples. This graph shows that synovial fluid exhibited viscous-like behavior. Loss modulus was higher than storage modulus over the range of frequency.

Table 4.5 summarizes the storage G' and loss moduli G'' from synovial fluid samples 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 [11]. To ensure the repeatability of the data, two measurements were performed for SAOS test and the precision of the data is within 2% (Appendix C.2-C.3). The results showed that the mean of the storage modulus G' is higher than that of the loss modulus G'' in both frequencies. This suggested that, on average, OA synovial fluids in the present study play a role as elastic shock absorber during both walking and running. The function of shock absorber also is related to viscoelasticity.

Table 4.5: Storage G' and loss moduli G'' at 0.5 and 2.5 Hz. for synovial fluid

Sample	$G'_{0.5\,Hz}$ (Pa)	$G''_{0.5\,Hz}$ (Pa)	$G'_{2.5\,Hz}$ (Pa)	$G''_{2.5\,Hz}$ (Pa)
SN01	4.38	2.99	7.64	4.19
SN02	0.88	0.73	1.22	1.19
SN03	2.16	1.52	2.9	2.17
SN04	1.43	1.67	2.33	3.34
SN05	2.19	1.67	3.78	2.6
SN06	0.46	0.54	0.68	1.02
SN07	2.43	1.8	4.14	2.61
SN08	0.76	0.61	1.19	0.89
SN09	1.38	1.19	2.48	1.93
SN10	5.79	3.29	8.19	4.05
SN11	0.47	0.46	0.6	0.86
SN12	0.26	0.34	0.36	0.65
SN13	3.39	2.07	4.85	2.76
SN14	1.24	1.11	1.99	1.79
SN15	2.78	1.97	4.43	3
SN16	5.12	3.33	8.52	4.41
SN17 Lt.	1.26	1.09	1.86	1.81
SN17 Rt.	1.05	1.01	1.89	1.75
SN18	1.89	1.84	3.51	3.02
SN19	5.67	3.84	9.63	5.09
SN20	0.46	0.76	1.53	1.99
SN21	2.06	1.98	4.67	3.43
SN22 Lt.	3.2	2.74	5.95	4.13
SN22 Rt.	0.54	0.68	0.83	1.66
Range	0.26 - 5.79	0.34 – 3.84	0.36 – 9.63	0.65 – 5.09
Mean±SD	2.14 ± 1.7	1.63 ± 1	3.55 ± 2.72	2.51 ± 1.25

Table 4.6 shows that the average $G'_{2.5Hz}$ and $G''_{2.5\,Hz}$ of synovial fluid samples in this study were less than in normal and osteoarthritis groups reported in previous study [12]. However, when compare to a study by Mazzucco et al.[76], higher averages of $G'_{2.5Hz}$ and $G''_{2.5\,Hz}$ were observed in samples in the present study.

Table 4.6: Storage G' and loss G'' moduli for synovial fluid samples in the present study and in different groups from previous studies.

Group	Frequency (Hz)	G' (Pa)	G'' (Pa)
Normal 52-78 years old * Balazs [12]	2.5	18.9 ± 3.3	10.1 ±1.2
Osteoarthritis * Balaz [12]	NR	8.5±5.4	4.8 ±2.8
TKA Muzzucco et al.[76]	2.5	1.9 ±0.5	1.4 ±0.3
Present Study	**2.5**	**3.55 ± 0.56**	**2.51 ± 0.26**

Data are presented as mean± standard error.
* Data are presented as mean± standard deviation. These results were reported in dynes/sec^{-2} which is assumed to be an editing error for dynes/cm^2 (0.1 Pa).

Viscoelasticity has an important effect on the lubrication system and the lubrication performances. Increasing the viscoelasticity, the load capacity is increased, but at the same time for enough high viscoelasticity the friction is reduced. In OA synovial fluid the viscoelasticity decreases. Therefore, it affects the lubrication performance of the joint.

It should be pointed out that most of the subjects enrolled in this study were diagnosed as "severe" OA (see Table 3.1 in Chapter 3). Diagnosis of the severity of OA in this study was made according to the Kellgren-Lawrence radiographic grading system [59]. The results of viscometric property presented in Table 4.3 showed that based on established range from previous studies [32, 110-112] synovial fluid samples in this study fit in both normal and degenerative range. However, the results of viscoelastic properties presented in Table 4.5 and Table 4.6 showed that all synovial fluid samples in this study fit in osteoarthritis range based on previous study by Balazs [12]. The results from this study suggested that rheological behaviors, both viscosity and viscoelasticity, of synovial fluid could play an important role in diagnosis of the severity of OA.

Table 4.7: Crossover frequency and relaxation time of synovial fluid samples

Sample	**Crossover Frequency** ω_c	**Relaxation Time** λ_r
SN01	0.09	11.24
SN02	0.09	11.24
SN03	0.09	11.24
SN04	No	N/A
SN05	0.13	7.87
SN06	No	N/A
SN07	0.13	7.87
SN08	0.13	7.87
SN09	0.97	1.03
SN10	0.06	16.13
SN11	No	N/A
SN12	No	N/A
SN13	0.06	16.13
SN14	0.18	5.46
SN15	0.09	11.24
SN16	0.06	16.13
SN17 Lt.	0.18	5.46
SN17 Rt.	0.55	1.83
SN18	0.55	1.83
SN19	0.09	11.24
SN20	No	N/A
SN21	0.40	2.51
SN22 Lt.	0.18	5.46
SN22 Rt.	No	N/A
Range	0.06 – 0.97	1.03 – 16.13
Mean±SD	0.22 ± 0.06	8.43 ± 1.17

Table 4.7 shows that the average crossover frequency for the synovial fluid samples in this study was 0.22 Hz., which is much lower than that reported in Mazzucco et al [76]. In their study, the average crossover frequency was at 1.8 Hz. The differences of the results from one study to another may be caused by several factors including the type of the instrument and geometry used in the study, and the way the fluid is handled in each study. In addition, the relaxation time (λ_r), were calculated from crossover frequency ω_c,

$\omega_c = 1/\lambda_r$ [110]. The relaxation time of the synovial fluid samples in the present study varied widely and ranged from 1.03 – 16.13 s. It was observed that most of synovial fluid samples that have high relaxation time also have high viscosity. Synovial fluids with high viscosity tend to take more time for their structures to relax after deformation.

Figure 4.6 shows that from the oscillatory measurement, synovial fluid samples also exhibited non-Newtonian shear thinning behavior, which is consistent with the behavior observed in steady shear test. However, the plateau was observed at high frequency.

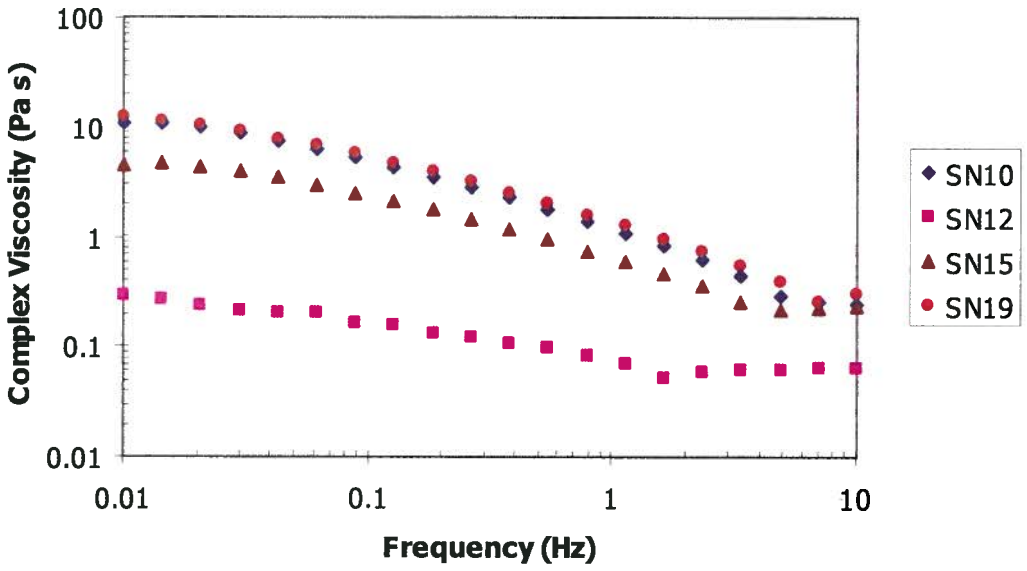

Figure 4.6: Complex viscosity as a function of frequency for synovial fluid samples Synovial fluid samples exhibited non-Newtonian shear thinning behavior. The complex viscosity decreases with increasing frequency.

4.1.4 Bilateral Knee Arthroplasty

In two of the subjects, synovial fluid samples were obtained from both knees during bilateral total knee arthroplasty. The results showed that the viscosity varied substantially from knee to knee in each case. In sample SN17, the viscosity of synovial fluid from the left knee was higher than that from the right knee in lower shear rates (Figure 4.7). However, in sample SN22, the viscosity of synovial fluid from the left knee was greater than the one from the right knee over the range of shear rates (Figure 4.8). These results support the findings from Mazzucco et al. [76]. They suggested that rather than a systemic disorder, local alterations control the properties of the synovial fluid.

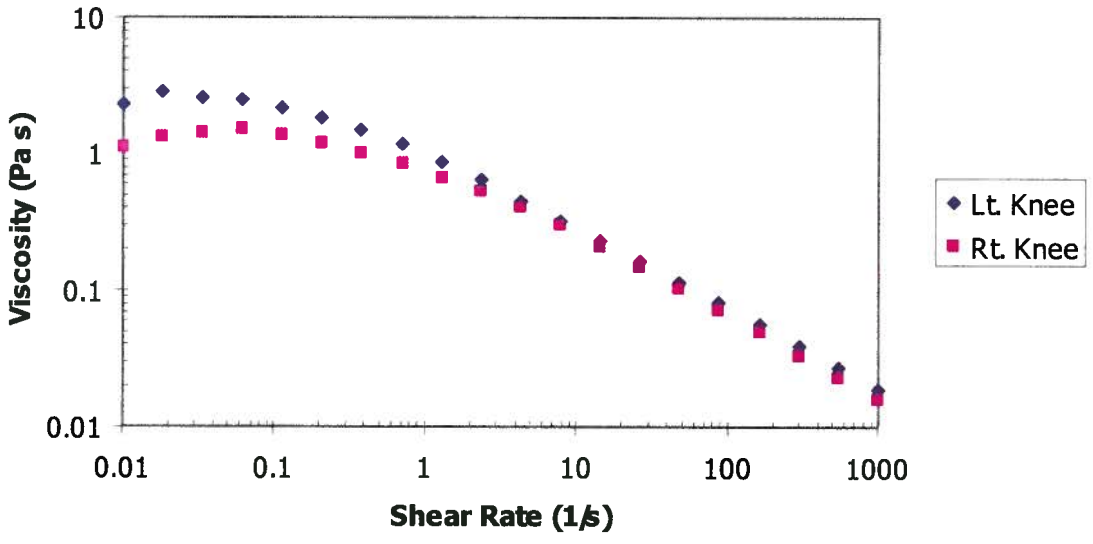

Figure 4.7: Viscosity as a function of shear rate (sample SN 17, left vs. right knees). Synovial fluid from both knees exhibited non-Newtonian shear thinning behavior. The viscosity decreases with increasing shear rate.

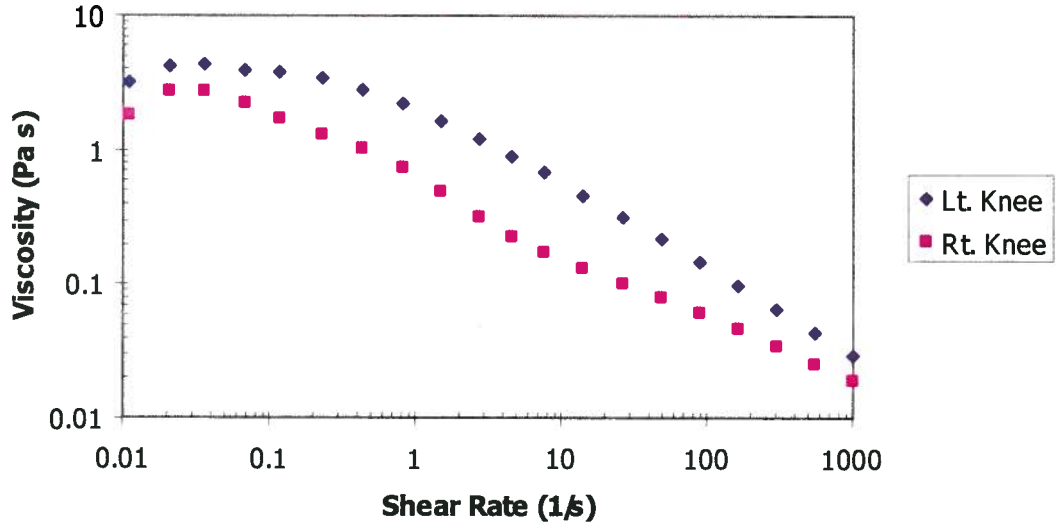

Figure 4.8: Viscosity as a function of shear rate (sample SN 22, left vs. right knees). Synovial fluid from both knees exhibited non-Newtonian shear thinning behavior. The viscosity decreases with increasing shear rate.

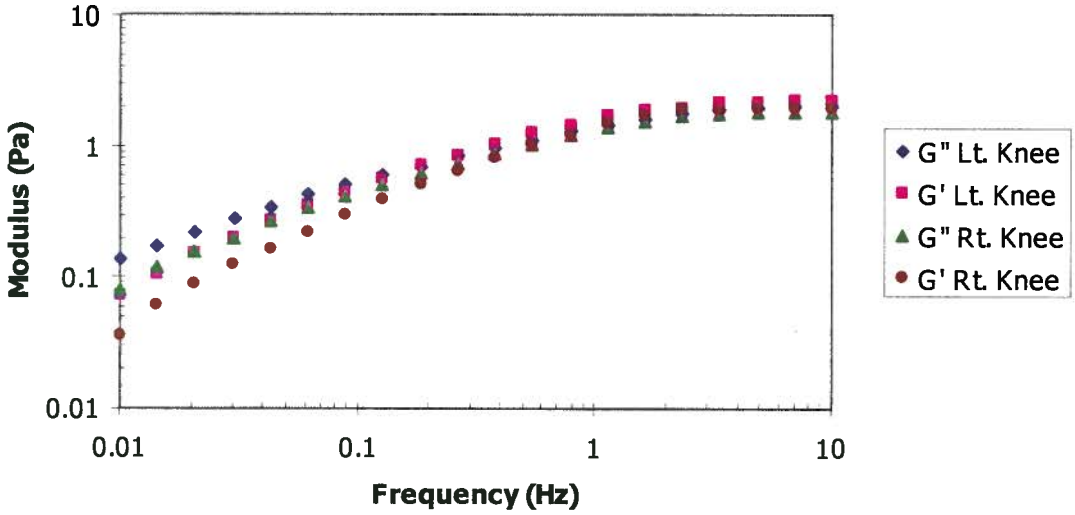

Figure 4.9: Storage and loss moduli as a function of frequency from SAOS measurement (sample SN17, left vs. right knees). Synovial fluid from both knees exhibited viscoelastic behavior. At low frequencies, loss modulus was higher than storage modulus. At higher frequencies, storage modulus exceeded loss modulus. The cross over frequency was observed.

For sample SN17, synovial fluid from both knees exhibited viscoelastic behavior; that is loss modulus G'' was higher than storage modulus G' at low frequencies. At higher frequencies, the storage modulus G' exceeded loss modulus G'', and the cross over frequency were observed (Figure 4.9). The values of viscoelastic parameters were close to each other. However, the cross over for sample from the left knee was observed at lower frequency than the one from the right (Table 4.8).

Table 4.8: Viscoelastic properties of synovial fluid from 2 subjects during bilateral total knee arthroplasty

Subject	Lt/Rt	$G'_{0.5Hz}$	$G''_{0.5Hz}$	$G'_{2.5Hz}$	$G''_{2.5Hz}$	Crossover Frequency
SN17	Lt	1.26	1.09	1.86	1.81	0.264
	Rt	1.05	1.01	1.89	1.75	0.546
SN22	Lt	3.2	2.74	5.95	4.13	0.183
	Rt	0.54	0.68	0.83	1.66	no

For sample SN22, synovial fluid from both knees showed different behaviors. The cross over frequency was observed in synovial fluid sample from the left knee, but not in the other. Synovial fluid from the left knee exhibited viscoelastic behavior. In synovial fluid from the right knee, loss modulus G'' was larger than storage modulus G' over the entire range of oscillation frequency (Figure 4.10). Synovial fluid from the right knee exhibited viscous-like behavior. Moreover, all viscoelastic parameters of synovial fluid from the left knee were considerably higher than the right knee. (Table 4.8)

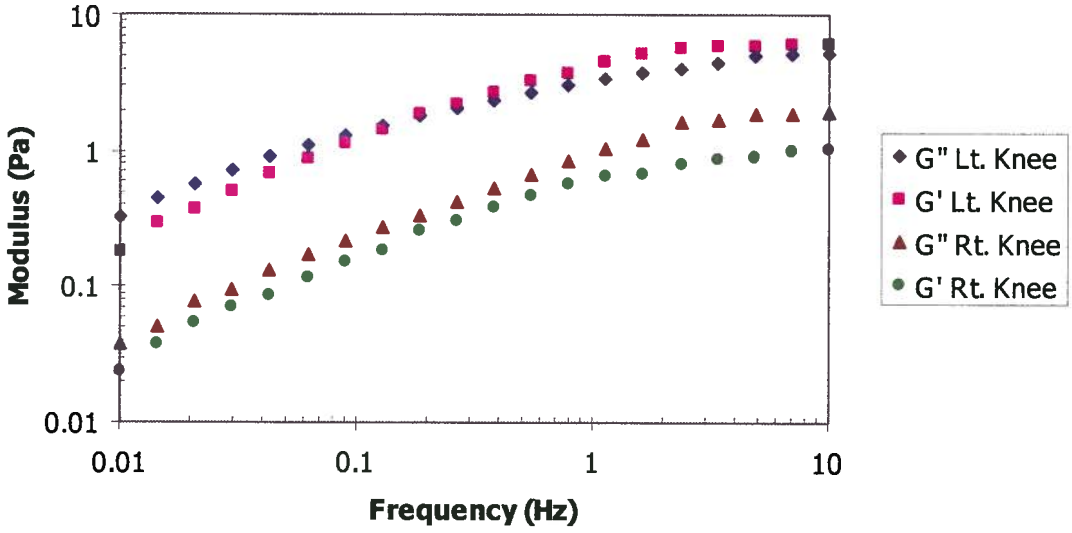

Figure 4.10: Storage and loss moduli as a function of frequency from SAOS measurement (sample SN22, left vs. right knees). Synovial fluid from the left knee exhibited viscoelastic behavior. The cross over frequency was observed. However, synovial fluid from the right knee exhibited viscous-like behavior.

Interestingly, the results from the present study showed that, of the two subjects, a substantial difference in viscoelastic behavior of synovial fluid between the left and right knee only observed in one subject, but not in the other. Since only two cases were examined, no conclusion can be drawn whether deterioration of rheological properties in synovial fluid on one side can predict the deterioration on the contralateral side. There are many factors that may contribute to the variability of the rheological properties of synovial fluid within a single subject, including left- or right-sided dominance, joint geometry, and trauma history.

4.1.5 Model Fitting

The generalized Newtonian constitutive equation can capture the non-Newtonian behavior with sufficient accuracy for inelastic fluids. The fluids can be characterized by fitting

the viscosity experimental data to a model so that a few parameters can be compared among fluids. There are several models that can be used to better fit the material's characteristic.

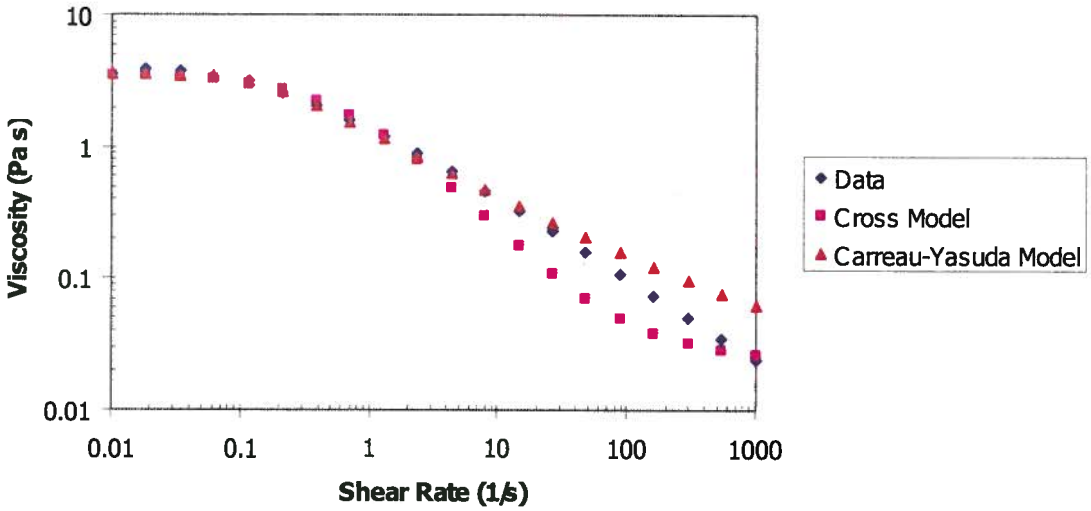

Figure 4.11: Model fitting for sample SN15. At low shear rates, both models fit well with the data. However, within the physiological shear rates range from 0.01-100 s^{-1}, Carreau-Yasuda model appeared to fit the data better than Cross model.

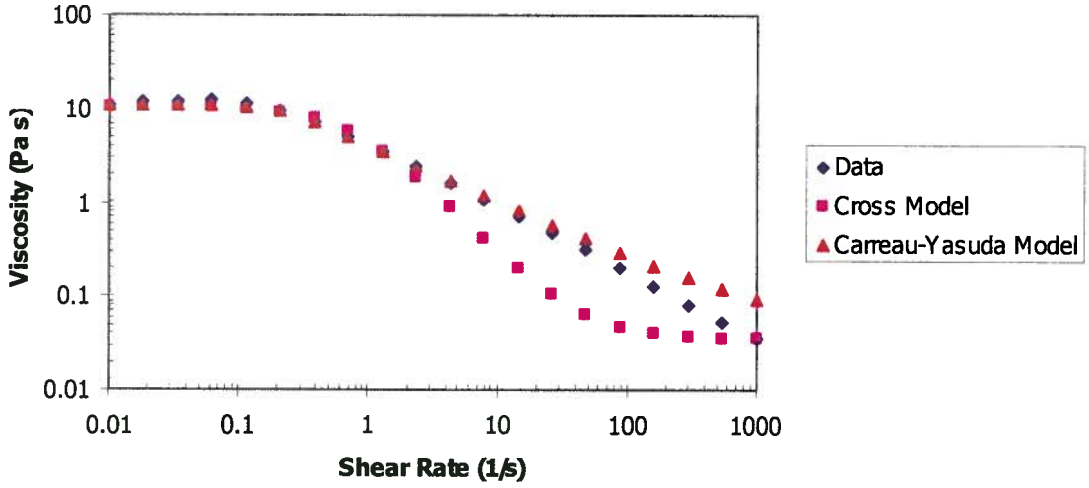

Figure 4.12: Model fitting for sample SN19. At low shear rates, both models fit well with the data. However, within the physiological shear rates range from 0.01-100 s^{-1}, Carreau-Yasuda model appeared to fit the data better than Cross model.

Table 4.9: Rheological indexes of Cross and Carreau-Yasuda models

Sample	Cross		Carreau-Yasuda		
	n	λ	a	n	λ
SN1	1.26	1.19	4.61	0.43	5.3
SN2	1	0.75	2.19	0.49	3.69
SN3	1.32	1.3	6.59	0.44	6.08
SN4	1.31	3.29	82.11	0.53	19.17
SN5	0.9	2.89	2.01	0.51	14.98
SN6	1.25	0.55	8.48	0.52	3.33
SN7	1.05	2.1	2.38	0.44	8.75
SN8	1.27	0.9	5.11	0.48	4.6
SN9	1.33	0.61	6.07	0.46	2.03
SN10	1.32	1.29	4.44	0.42	5.49
SN11	0.71	5.25	0.91	0.47	15.15
SN12	0.83	0.75	3.2	0.65	8.61
SN13	1.41	2.54	101.5	0.45	12.34
SN14	1.19	0.52	4.94	0.5	2.94
SN15	1.01	1.5	2.54	0.5	7.65
SN16	1.4	1.17	5.28	0.41	4.94
SN17 Lt	1.08	1.18	2.83	0.49	6.13
SN17 Rt.	1.23	0.37	4.02	0.49	1.94
SN18	1.34	0.41	5.34	0.45	1.95
SN19	1.42	1.29	5.42	0.39	5.17
SN20	0.89	0.1	2.35	0.6	0.85
SN21	0.79	1.91	1.09	0.46	6.01
SN22 Lt.	1.35	0.5	5.25	0.45	2.38
SN22 Rt.	1.6	1.76	6.07	0.41	7.43
	1.17 ± 0.23 (0.71 – 1.6)	1.185* (0.1 – 5.25)	4.775* (0.91 – 10.15)	0.47 ± 0.39 (0.39 – 0.65)	5.395* (0.85 – 19.17)

Data for n index report in mean ± standard deviation and range.
* Data report in median and range due to high skewness of the data.

Two models were used for fitting the data, Cross model and Carreau-Yasuda model. Figure 4.11 and 4.12 shows that at low shear rates, both models fit well with the data. However, within the physiological shear rates range from 0.01-100 s^{-1}, Carreau-Yasuda model appeared to fit the data better than Cross model. Table 4.9 shows rheological indexes in Cross model and Carreau-Yasuda model. For Cross model, n index ranged from

0.71 – 1.6, while it ranged from 0.39 – 0.65 in Carreau-Yasuda model. These values indicated the non-Newtonian shear thinning behavior.

4.2 Rheology of Viscosupplements

Viscosupplementation is the use of intraarticular injection of hyaluronic acid or hylans to treat OA. There are various commercial products of viscosupplements which differ in origin (animal or bacterial), molecular weight, and resident time in the joint [10, 85]. In this study, rheological characterization was performed on three different viscosupplements (i.e. Orthovisc®, Suplasyn®, and Synvisc®). To ensure the repeatability of the data, two measurements were performed in each test and the precision of the data is within 2% (Appendix C.4-C.6).

4.2.1 Orthovisc®

Orthovisc® exhibited non-Newtonian shear thinning behavior; that is viscosity decreases with increasing shear rate. A plateau at low shear rates was also observed (Figure 4.13). In the oscillatory measurement, it was found that at low frequencies, loss modulus G'' was higher than storage modulus G'. At higher frequencies, the storage modulus G' exceeded loss modulus G''. The cross over frequency was also observed (Figure 4.14). Moreover, there was nearly no change in rheological properties when testing at 25 °C and 37 °C.

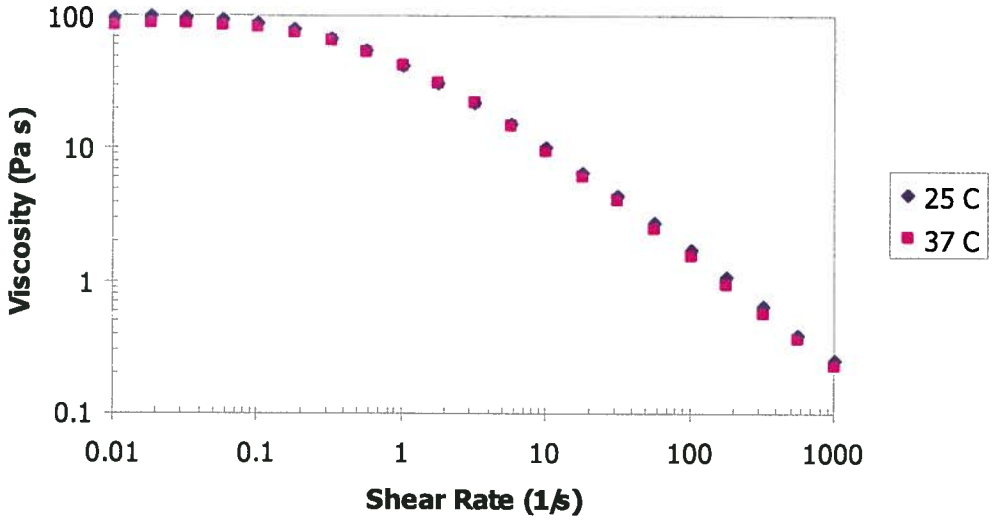

Figure 4.13: Viscosity as a function of shear rate for Orthovisc® at 25 °C and 37 °C. Orthovisc® exhibited non-Newtonian shear thinning behavior; that is viscosity decreases with increasing shear rate. There was nearly no change in rheological property when testing at 25 °C and 37 °C.

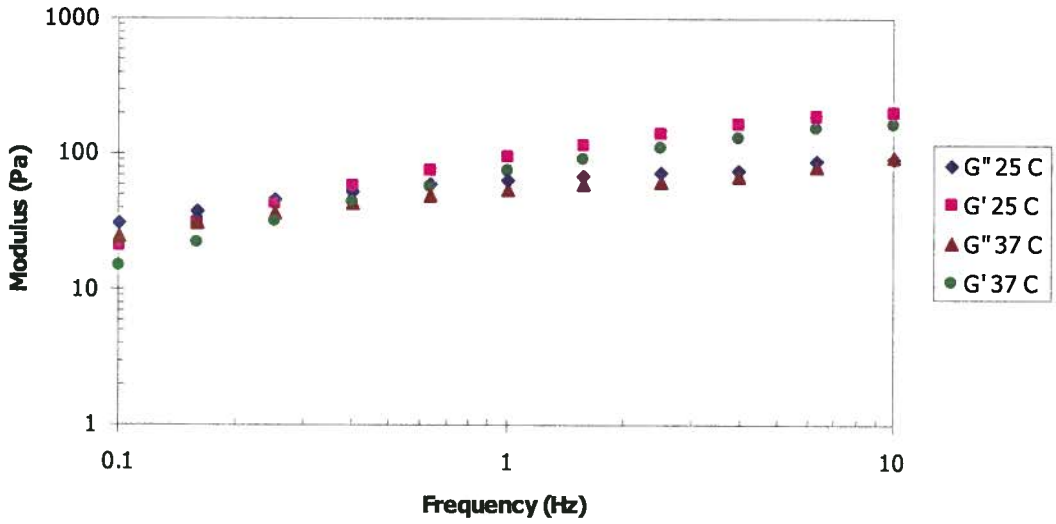

Figure 4.14: Storage and loss moduli as a function of frequency from SAOS measurement for Orthovisc® at 25 °C and 37 °C. Viscoelastic property was observed. Orthovisc® exhibited viscoelastic behavior. There was nearly no change in rheological property when testing at 25 °C and 37 °C.

However, compare to the result from Mazzucco et al. [76], the zero shear viscosity of Orthovisc® in the present study was approximately 2 times higher. The differences of the findings might be due to several factors. First, the result in this study presented the viscosity as a function of shear rate, whereas the result in Mazzucco et al [76] reported the viscosity as function of shear stress. Second, the type of rheometer and the measurement geometry used in this study are different from the ones used in Mazzucco et al. [76]. Finally, Orthovisc® has the molecular weight range from 1-2.9 MDa. This wide range of molecular weight might contribute to the difference of the results.

4.2.2 Suplasyn®

In a steady shear measurement, Suplasyn® showed a shear thinning behavior (Figure 4.15). However, it should be pointed out that there was a sharp decrease in viscosity at lower shear rates. Note that the bump of the data in Figure 4.15 is likely due to the rheometer.

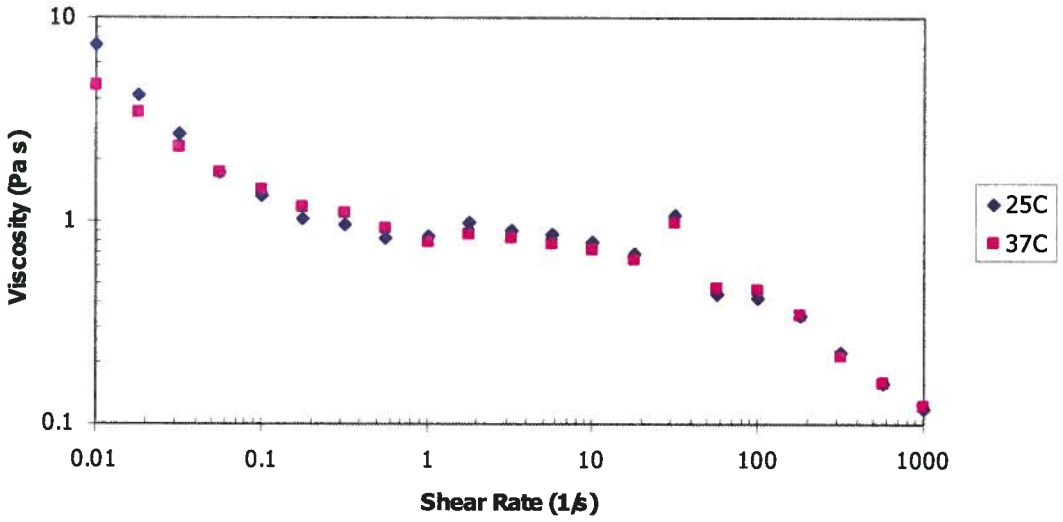

Figure 4.15: Viscosity as a function of shear rate for Suplasyn® at 25 °C and 37 °C. Suplasyn® exhibited non-Newtonian shear thinning behavior; that is viscosity decreases with increasing shear rate. There was only a slight change in rheological property when testing at 25 °C and 37 °C.

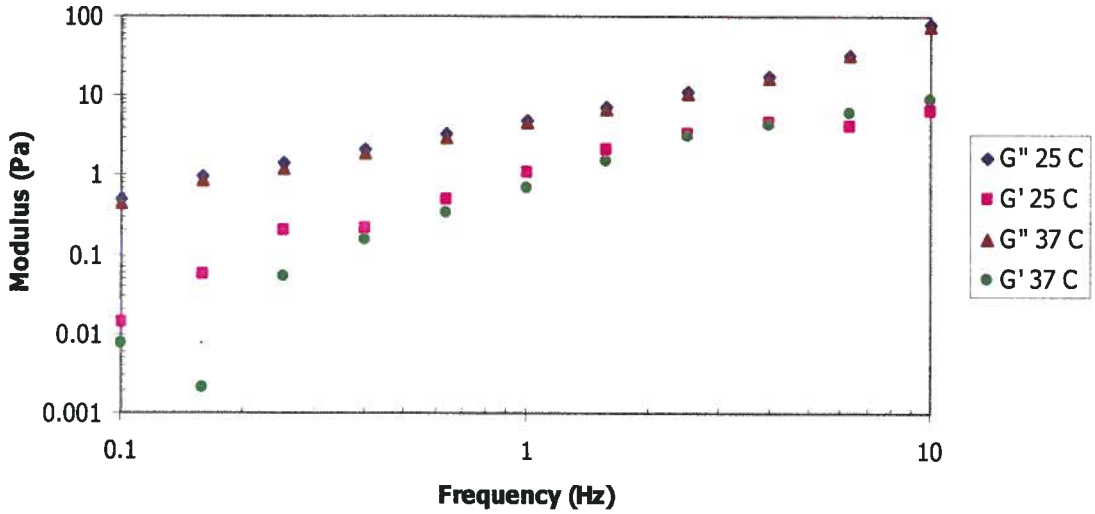

Figure 4.16: Storage and loss moduli as a function of frequency from SAOS measurement for Suplasyn® at 25 °C and 37 °C. Viscoelastic property was observed. Suplasyn® exhibited viscous-like behavior. There was nearly no change in rheological property when testing at 25 °C and 37 °C.

In the oscillation test, Suplasyn® exhibited a viscous-like behavior; that is the loss modulus G'' remained larger than the storage modulus G' over the entire range of oscillation frequency (Figure 4.16). There was only a slight change in rheological properties when testing at 25 °C and 37 °C.

4.2.3 Synvisc®

In a steady shear measurement, Synvisc® showed a shear thinning behavior (Figure 4.17). In the oscillation test, Synvisc® exhibited a gel-like behavior; that is the storage modulus G' remained larger than the loss modulus G'' throughout the range of oscillation frequency (Figure 4.18). This result is consistent with a study by Mathieu et al. [75]. Nearly no change in rheological properties when testing at 25 °C and 37 °C was observed.

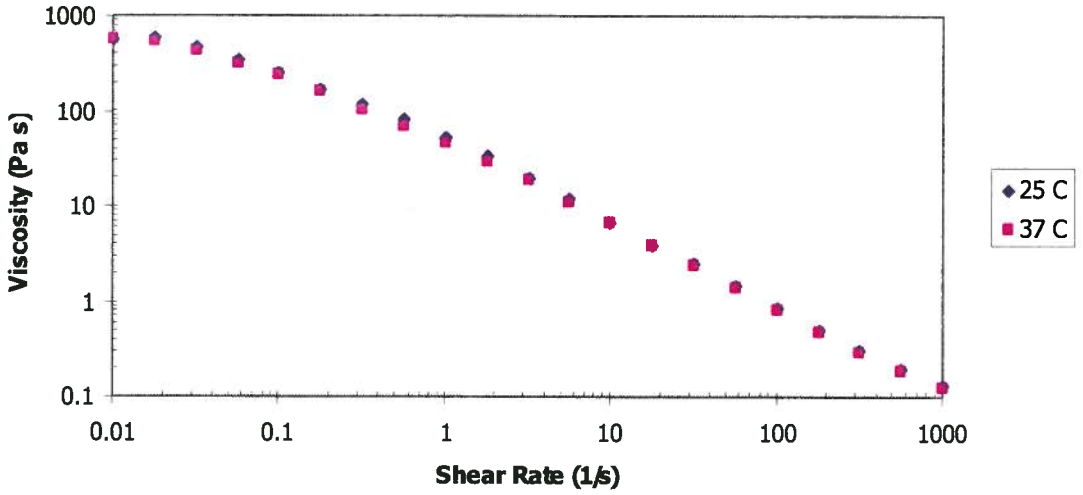

Figure 4.17: Viscosity as a function of shear rate for Synvisc® at 25 °C and 37 °C Synvisc® exhibited non-Newtonian shear thinning behavior; that is viscosity decreases with increasing shear rate. There was nearly no change in rheological property when testing at 25 °C and 37 °C.

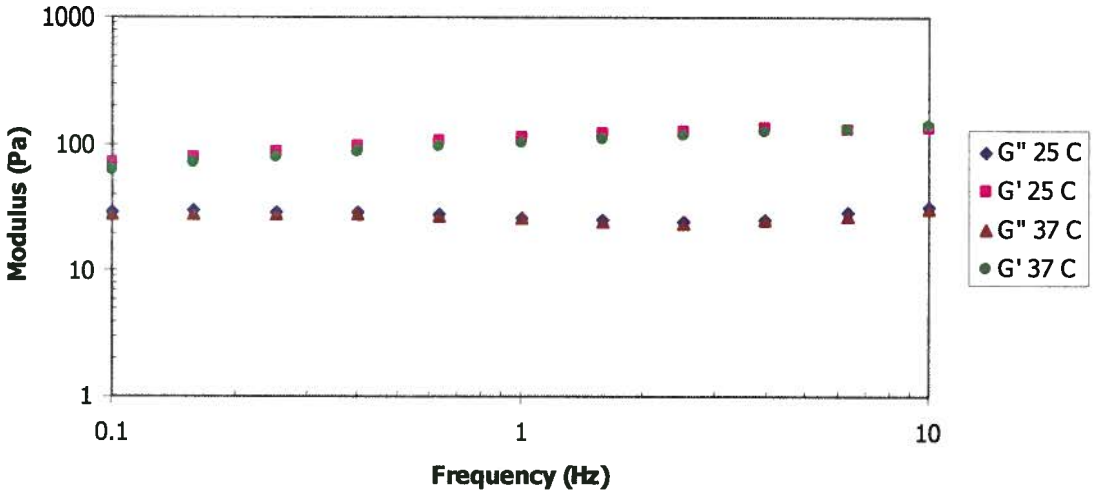

Figure 4.18: Storage and loss moduli as a function of frequency from SAOS measurement for Synvisc® at 25 °C and 37 °C. Viscoelastic property was observed. Synvisc® exhibited gel-like behavior. There was nearly no change in rheological property when testing at 25 °C and 37 °C.

4.2.4 Comparison of the Rheological Properties of Orthovisc®, Suplasyn®, and Synvisc®

Figure 4.19 shows that when compared to Orthovisc® and Synvisc®, Suplasyn® had the lowest viscosity throughout the entire range of shear rates. It was observed that, at lower shear rates (~ 0.01 – 1 s^{-1}), Synvisc® had higher viscosity than Orthovisc®, but had lesser viscosity at higher shear rates. At shear rate 0.01 s^{-1}, the viscosity of Synvisc® was about two orders of magnitude higher than that of Suplasyn®. Note that the bump of the data in Figure 4.19 is likely due to the rheometer.

The results showed that different formulations of viscosupplements used in this study exhibited a consistent trend of differences in rheological properties. All three viscosupplements exhibited a non-Newtonian shear thinning behavior. The viscosity of Synvisc® was the highest, whereas the viscosity of Suplasyn® was the lowest. This finding is consistent with a study by Prieto et al. [100]. The average molecular weight (MW) of Synvisc® is 6 MDa, where as the average MW of Orthovisc® and Suplasyn® are 1-2.9 MDa and 0.5-0.7 MDa, respectively [85]. The results from the present study indicated that viscosity of the viscosupplement highly depends on MW of the hyaluronic acid. In addition, it was observed that, as shear rate increases, the viscosity of Synvisc® showed a more dramatic decrease than that of Orthovisc® and Suplasyn®. This finding is in line with the results from previous study which reported that the decrease in apparent viscosity was more pronounced in high MW hyaluronic acid than low MW hyaluronic acid [80].

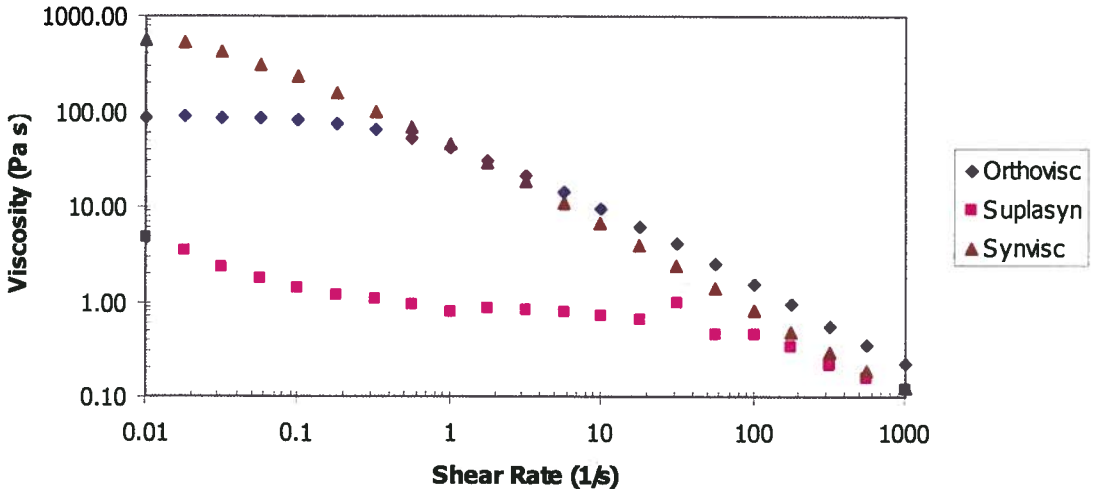

Figure 4.19: Viscosity as a function of shear rate for Orthovisc®, Suplasyn® and Synvisc®. Suplasyn® had the lowest viscosity throughout the entire range of shear rates. At lower shear rates (~ 0.01 – 1 s^{-1}), Synvisc® had higher viscosity than Orthovisc®, but had lesser viscosity at higher shear rates.

Table 4.10 shows that the dynamic moduli, G' and G'', at both 0.5 and 2.5 Hz. were smallest in Suplasyn®. It was also observed that the storage modulus G' of Synvisc® was largest at both frequencies. On the other hand, Orthovisc® was found to have the largest loss modulus G''. It should also be pointed out that, for Orthovisc®, at 37 °C the cross over shifted to a higher frequency than at 25 °C. For Synvisc®, the values of G' and G'', at both 0.5 and 2.5 Hz. were very close to the values reported in a study by Mathieu et al. [75] (estimated from the published graph). However, for Orthovisc®, the values of G' and G'' at 2.5 Hz. in the present study were about 2 times and 1.5 time, respectively higher that that reported in Mazzucco et al.[76]. Several factors, including the type of rheometer, the measurement geometry, the gap used in the measurements, and the wide range of MW of Orthovisc® (MW 1-2.9 MDa) might contribute to these differences.

Table 4.10: Viscoelastic properties of Orthovisc®, Suplasyn®, and Synvisc®

Sample	Temp.	$G'_{0.5Hz}$	$G''_{0.5\,Hz}$	$G'_{2.5Hz}$	$G''_{2.5\,Hz}$	Crossover Frequency
Orthovisc®	25 °C	67.48	56.42	141	72.07	0.251
	37 °C	51.21	45.86	111.2	61.48	0.398
Suplasyn®	25 °C	0.43	2.84	3.67	11.15	no
	37 °C	0.29	2.56	3.36	10.78	no
Synvisc®	25 °C	100.9	27.78	126.1	23.86	no
	37 °C	91.85	26.13	118.1	22.46	no

Regarding the viscoelastic properties, viscosupplements tested in this study behaved differently from each other. Within the frequency ranging from 0.1 to 10 Hz., Synvisc® (MW 6 MDa) exhibited gel-like behavior. For Orthovisc® (MW 1-2.9 MDa), a distinct transition from viscous to elastic behavior occurred as the oscillation frequency increased. However, a viscous-like behavior was observed in a viscosupplement with low MW, Suplasyn® (MW 0.5-0.8 MDa). These findings suggested that the differences in rheological behavior are related to MW of the viscosupplements and its network forming ability. Previous studies reported that at high MW a transient entanglement network is formed, but it was absent for hyaluronic acid at low MW [2, 63]. The network-forming ability of hyaluronic acid in solutions gives rise to the non-Newtonian behavior of hyaluronic acid solutions [64] and affects the viscoelasticity of the solution [80]. In addition, the results from this study showed that there were slight changes in viscosity and viscoelastic behavior of the viscosupplements when the temperature changes from 25 °C to 37 °C.

4.3 Effects of Viscosupplements on the Rheology of Synovial Fluid

A variety of viscosupplement is commercially available and has been used as a treatment for OA for many years. However, its clinical effect is still inconclusive. Therefore, this study aimed to investigate the effect of viscosupplement on rheological behavior of synovial fluid in OA. To ensure the repeatability of the data, two measurements were performed in each test and the precision of the data is within 2 % (Appendix C.4-C6).

4.3.1 Viscometric Properties

In the steady shear measurement, shear thinning behavior was observed in all samples. For sample SN20, the addition of viscosupplements to synovial fluid led to increase in viscosity over the range of shear rates. Among the synovial fluid mixed with viscosupplement samples, the viscosity was lowest in synovial fluid mixed with Suplasyn®, and highest in synovial fluid mixed with Synvisc®. Adding Orthovisc® into synovial fluid increased the viscosity of synovial fluid more than one order of magnitude (Figure 4.20). The result of adding Synvisc® in synovial fluid in the present study is consistent with the finding in Mathieu et al. [75]; that is the viscosity of synovial fluid increased more than two orders of magnitude.

For sample SN21, almost the same behaviors as in sample SN20 were observed. However, in this sample, the viscosity of synovial fluid mixed with Suplasyn® at lower shear rates (~ 0.01 – 10 s^{-1}) was slightly lower than that of synovial fluid alone, but at higher shear rates, the viscosity of synovial fluid mixed with Suplasyn® exceeded that of synovial fluid alone (Figure 4.21). Note that the bump of the data showed in Figure 4.20 and Figure 4.21 is likely due to the rheometer.

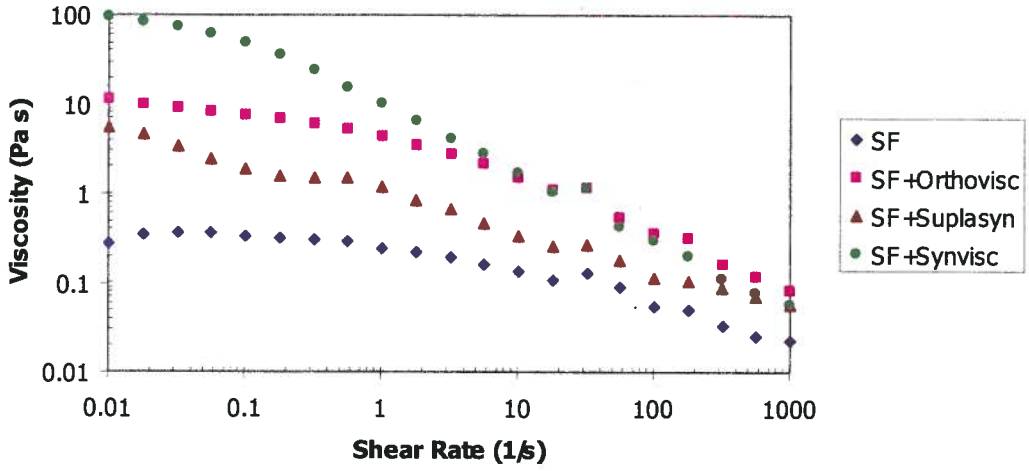

Figure 4.20: Viscosity as a function of shear rate for synovial fluid (SF), SF with Orthovisc®, SF with Suplasyn® and SF with Synvisc® (sample SN20). Non-Newtonian shear thinning behavior was observed in all samples. The addition of viscosupplements to synovial fluid led to increase in viscosity over the range of shear rates. The viscosity was lowest in synovial fluid mixed with Suplasyn®, and highest in synovial fluid mixed with Synvisc®.

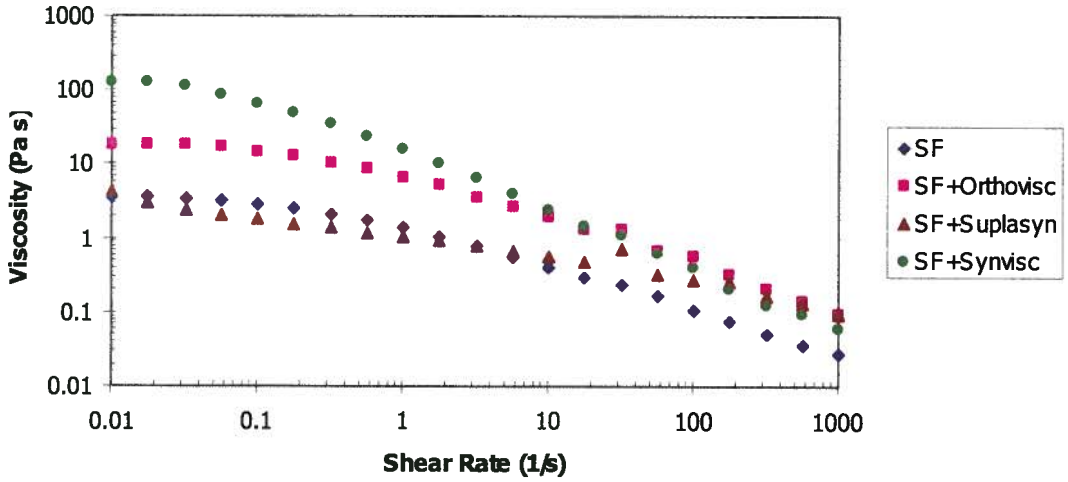

Figure 4.21: Viscosity as a function of shear rate for synovial fluid (SF), SF with Orthovisc®, SF with Suplasyn® and SF with Synvisc® (sample SN21). Non-Newtonian shear thinning behavior was observed in all samples. The viscosity was lowest in synovial fluid mixed with Suplasyn®, and highest in synovial fluid mixed with Synvisc®.

A non-Newtonian shear thinning behavior was observed in all samples of synovial fluid mixed with viscosupplements. In general, the addition of viscosupplements to synovial fluid led to the increase in viscosity. Among the synovial fluid mixed with viscosupplement samples, the viscosity was lowest in synovial fluid mixed with Suplasyn®, and highest in synovial fluid mixed with Synvisc®. The results suggested that viscosupplement with high MW is associated with a greater increase in the viscosity of synovial fluid.

4.3.2 Viscoelastic Properties

Synovial fluid with Orthovisc®

In the oscillatory test, synovial fluid sample SN20 exhibited viscous-like behaviour, that is the loss modulus G'' remained larger than the storage modulus G' throughout the range of oscillation frequency (Figure 4.22). The addition of Orthovisc® in synovial fluid increased both G' and G''. Both dynamic moduli in synovial fluid with Orthovisc® sample were greater than synovial fluid alone about an order of magnitude and they remained greater over the entire range of frequency. Moreover, it was observed that synovial fluid with Orthovisc® exhibited a viscoelastic behavior; that is at low frequencies, loss modulus G'' was higher than storage modulus G'. At higher frequencies, the storage modulus G' exceeded loss modulus G''. The cross over frequency was also observed (Figure 4.22).

In the other synovial fluid sample (SN21), the viscoelastic behavior was observed. By adding Orthovisc® in this synovial fluid sample, both dynamic moduli increased, but it was less pronounced than in synovial fluid sample SN20. Synovial fluid with Orthovisc® also exhibited viscoelastic behavior (Figure 4.23).

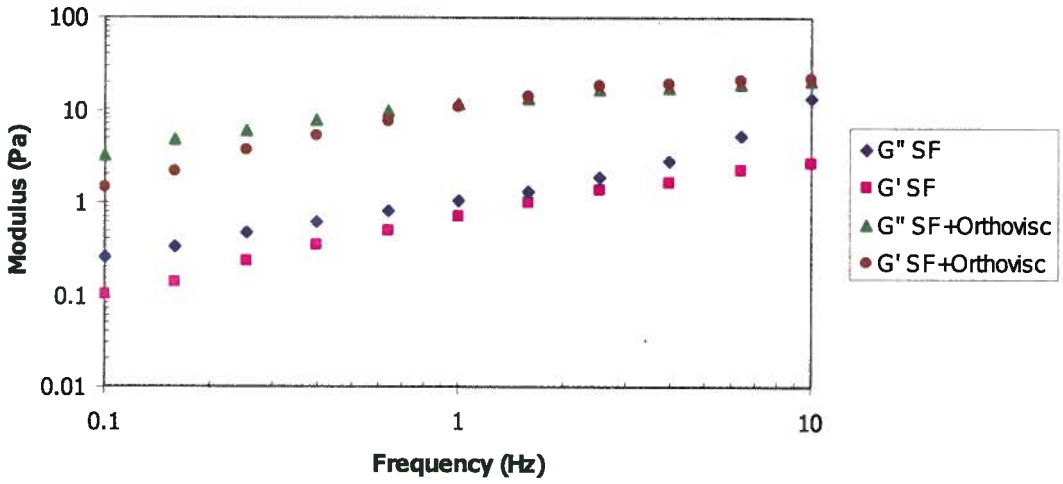

Figure 4.22: Storage and loss moduli as a function of frequency from SAOS measurement for synovial fluid (SF) and SF with Orthovisc® (sample SN20). Synovial fluid sample SN20 exhibited viscous-like behaviour throughout the range of oscillation frequency. Both dynamic moduli in synovial fluid with Orthovisc® sample were greater than synovial fluid alone over the entire range of frequency. Synovial fluid with Orthovisc® exhibited a viscoelastic behavior.

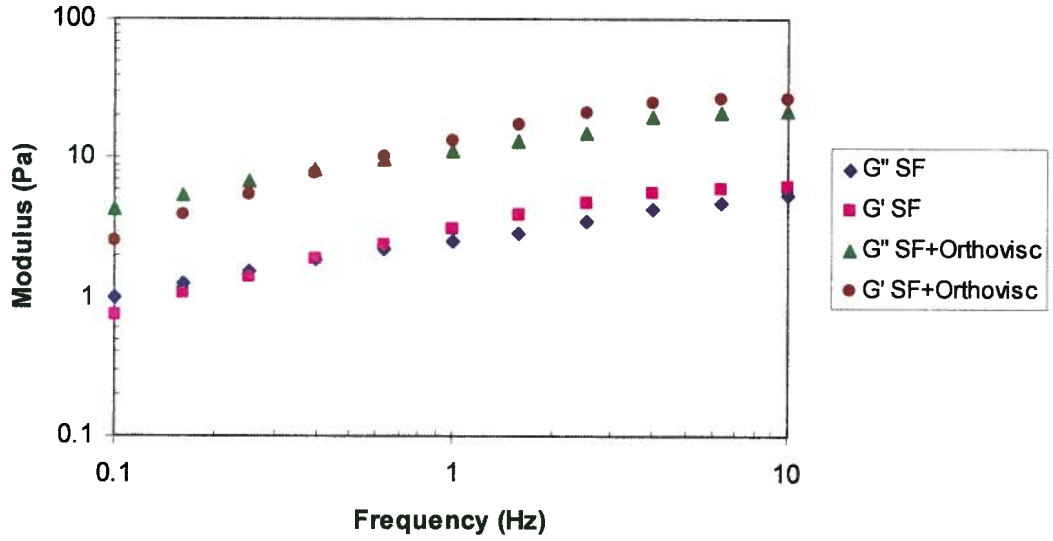

Figure 4.23: Storage and loss moduli as a function of frequency from SAOS measurement for synovial fluid (SF) and SF with Orthovisc® (sample SN21). Synovial fluid sample SN21 exhibited viscoelastic behaviour. Both dynamic moduli in synovial fluid with Orthovisc® sample were greater than synovial fluid alone over the entire range of frequency. Synovial fluid with Orthovisc® exhibited a viscoelastic behavior.

Synovial fluid with Suplasyn®

Figure 4.24 shows that synovial fluid with Suplasyn® exhibited a viscous-like behavior which was also observed in SN20 synovial fluid sample. The addition of Suplasyn® in synovial fluid slightly increased both G' and G''. However, only the loss modulus G'' of synovial fluid with Suplasyn® remained greater than that of synovial fluid alone throughout the range of oscillation frequency.

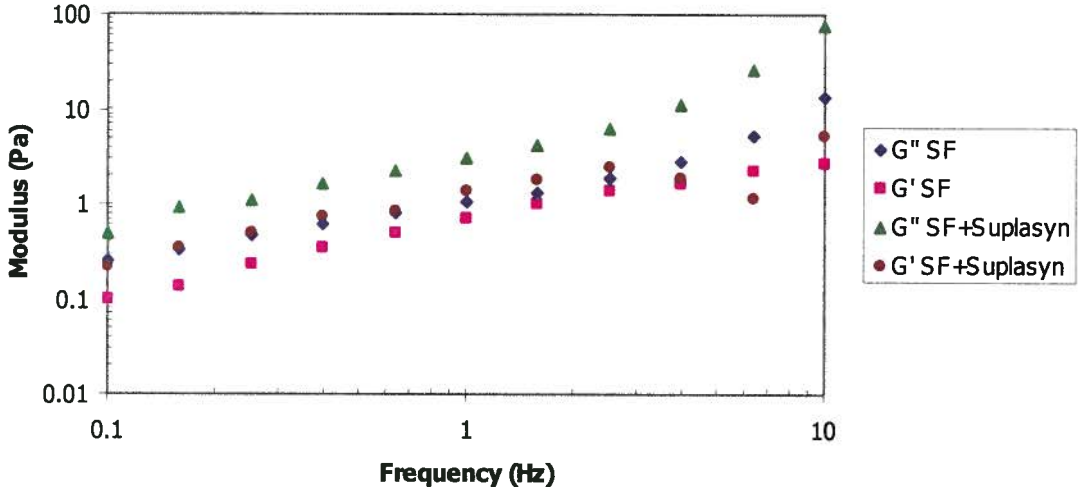

Figure 4.24: Storage and loss moduli as a function of frequency from SAOS measurement for synovial fluid (SF) and SF with Suplasyn® (sample SN20). Synovial fluid sample SN20 exhibited viscous-like behaviour throughout the range of oscillation frequency. The addition of Suplasyn® in synovial fluid slightly increased both dynamic moduli. Synovial fluid with Suplasyn® exhibited viscous-like behavior.

In the other synovial fluid sample (SN21), it was observed that adding Suplasyn® in synovial fluid induced viscous-like behavior to synovial fluid (Figure 4.25). Before the addition of Suplasyn®, SN21 showed a viscoelastic behavior that is at low frequencies, loss modulus G'' was higher than storage modulus G'. At higher frequencies, the storage modulus G' exceeded loss modulus G''. However, in synovial fluid with Suplasyn®, the loss modulus G'' remained larger than the storage modulus G' over the range of

frequency. In addition, it was found that adding Suplasyn® in sample SN21 only increased the loss moduli at higher frequency. However, adding Suplasyn® in synovial fluid did not increase the storage moduli.

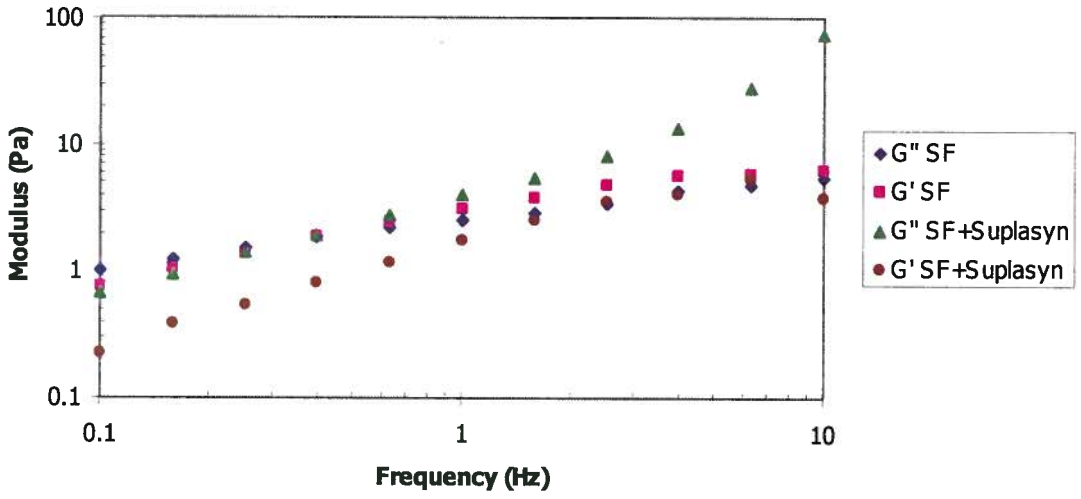

Figure 4.25: Storage and loss moduli as a function of frequency from SAOS measurement for synovial fluid (SF) and SF with Suplasyn® (sample SN21). Synovial fluid sample SN21 exhibited viscoelastic behavior. The addition of Suplasyn® in synovial fluid only slightly increased loss moduli. Synovial fluid with Suplasyn® exhibited viscous-like behavior.

Synovial fluid with Synvisc®

It was observed that the addition of Synvisc® in synovial fluid induced a gel-like behavior in both SN20 and SN21 synovial fluid samples; that is the storage modulus G' remained larger than the loss modulus G'' throughout the range of oscillation frequency. This finding is consistent with a study by Mathieu et al. [75].

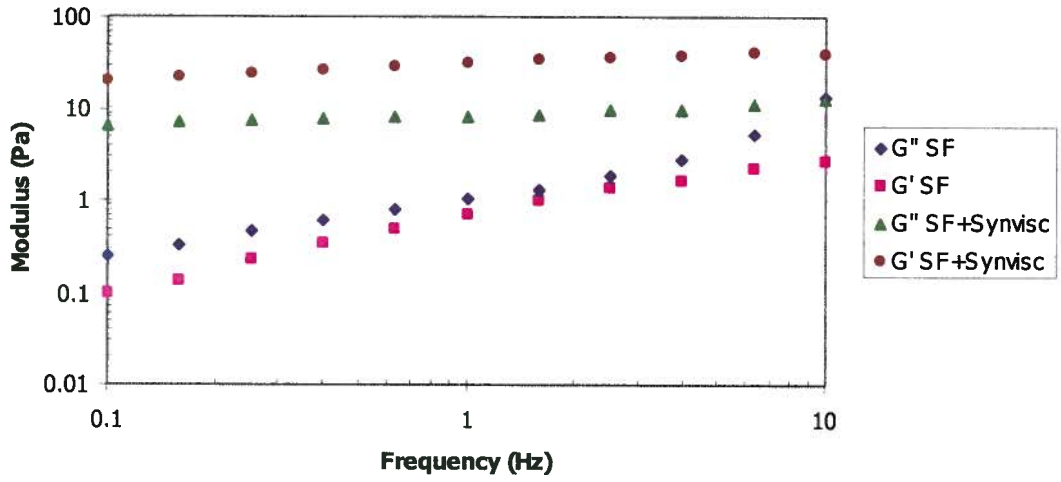

Figure 4.26: Storage and loss moduli as a function of frequency from SAOS measurement for synovial fluid (SF) and SF with Synvisc® (sample SN20). Synovial fluid sample SN20 exhibited viscous-like behaviour throughout the range of oscillation frequency. Both dynamic moduli in synovial fluid with Synvisc® sample were greater than synovial fluid alone over the entire range of frequency. Synovial fluid with Synvisc® exhibited gel-like behavior throughout the range of oscillation frequency.

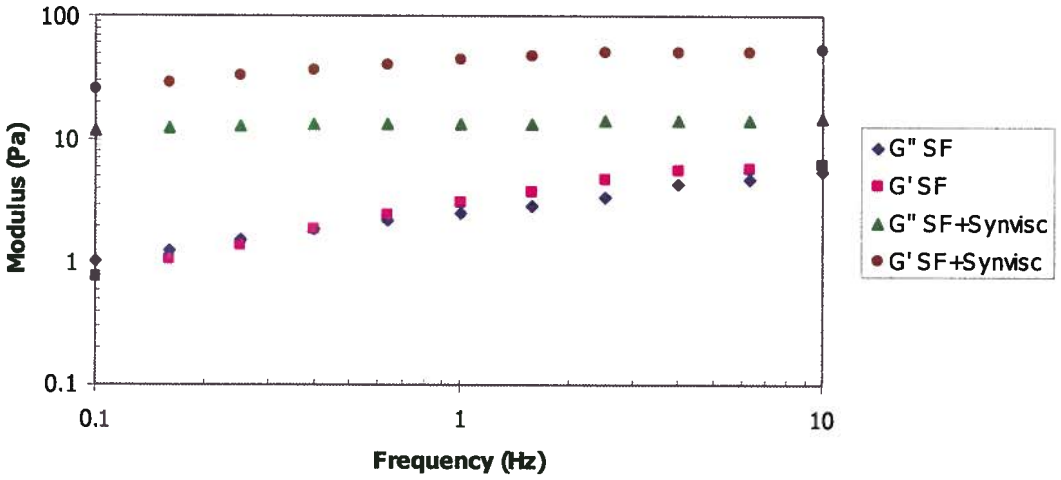

Figure 4.27: Storage and loss moduli as a function of frequency from SAOS measurement for synovial fluid (SF) and SF with Synvisc® (sample SN21). Synovial fluid sample SN21 exhibited viscoelastic behavior. Both dynamic moduli in synovial fluid with Synvisc® sample were greater than synovial fluid alone over the entire range of frequency. Synovial fluid with Synvisc® exhibited gel-like behavior throughout the range of oscillation frequency.

Adding Synvisc® in synovial fluid substantially increased the dynamic moduli in both SN20 and SN21 synovial fluid samples. At frequency 0.1 Hz, the storage modulus G' increased more than two-order of magnitude after added Synvisc® in sample SN20 (Figure 4.26). The less pronounced increase was observed in sample SN21 (Figure 4.27). The loss modulus G'' increased about same magnitude in both SN20 and SN21.

4.3.3 Comparison of the Rheological Properties of Synovial Fluid After Added with Orthovisc®, Suplasyn®, and Synvisc®

Table 4.11 shows that in sample SN20, the addition of viscosupplements improved the rheological properties of the synovial fluid. The dynamic moduli, G' and G'', at both 0.5 and 2.5 Hz. increased in all samples of synovial fluid added with viscosupplements. However, in sample SN21, only the addition of Orthovisc® and Synvisc® increased the dynamic moduli at both frequencies. Suplasyn® only increased the loss modulus at frequency of 2.5.Hz, but not in other parameters. It was also observed that the storage modulus G' of Synvisc® was largest at both frequencies. In general, Orthovisc® seemed to affect both G' and G'' of synovial fluid somewhat the same. However, Suplasyn® affected more on the loss modulus G'' than the storage modulus G', while Synvisc® contributed more to the storage modulus G' than the loss modulus G''.

In addition, the results showed that viscosupplements induced changes in viscoelastic behavior of synovial fluid. Cross-linked high MW viscosupplement (Synvisc®) showed greater effects on the changes in the rheological behavior of synovial fluid than high MW hyaluronic acid (Orthovisc®) and low MW (Suplasyn®) viscosupplement. The viscosupplement with low MW had the least effect on viscoelastic behavior of synovial fluid. Low MW viscosupplement mainly contributed to the increase

in viscous modulus of the synovial fluid. On the other hand, cross-linked high MW viscosupplement led to the overall improvements in rheological behavior of synovial fluid, especially the viscosity and the elasticity of synovial fluid. These findings are in line with a study by Mathieu et al. [75]. In their study, they asserted that, in comparison to linear hyaluronic acid, the cross-linked hyaluronic acid is much more efficient in improving the rheological properties of the OA synovial fluid.

Table 4.11: Viscoelastic properties of synovial fluid (SF), SF with Orthovisc®, SF with Suplasyn®, and SF with Synvisc®.

Sample	Mixed Sample	$G'_{0.5Hz}$	$G''_{0.5\,Hz}$	$G'_{2.5Hz}$	$G''_{2.5\,Hz}$	Crossover Frequency
SN20	SF	0.46	0.76	1.53	1.99	no
	SF+Orthovisc®	6.42	8.61	18.32	16.57	1.585
	SF+Suplasyn®	0.88	2.05	2.89	6.79	no
	SF+Synvisc®	29.35	8.08	37.29	9.71	no
SN21	SF	1.98	2.06	4.67	3.43	0.398
	SF+Orthovisc®	8.95	8.99	21.16	15.21	0.631
	SF+Suplasyn®	0.88	2.05	2.93	6.59	no
	SF+Synvisc®	38.32	13.03	51.51	14.23	no

4.4 Stability of Rheological Properties of Synovial Fluid Mixed with Cross-Linked Viscosupplement Over Time

In this experiment, the effects of Synvisc® on rheological behavior of synovial fluid were determined. To ensure the repeatability of the data, two measurements were performed in each test and the precision of the data is within 2% (Appendix C.4-C.6). Figure 4.28 shows that synovial fluid and synovial fluid mixed with Synvisc® exhibited shear thinning behavior. The addition of Synvisc into synovial fluid samples led to a marked increase in the viscosity throughout the range of shear rates. Furthermore, the

addition of Synvisc® induced a gel-like behavior in the synovial fluid; that is storage modulus G' remained greater than loss modulus G'' throughout the range of oscillation frequency (Figure 4.29). However, there were nearly no changes in rheological behaviors in synovial fluid mixed with Synvisc® on day 1 and 14 days later. Table 4.12 summarizes viscoelastic properties of the samples.

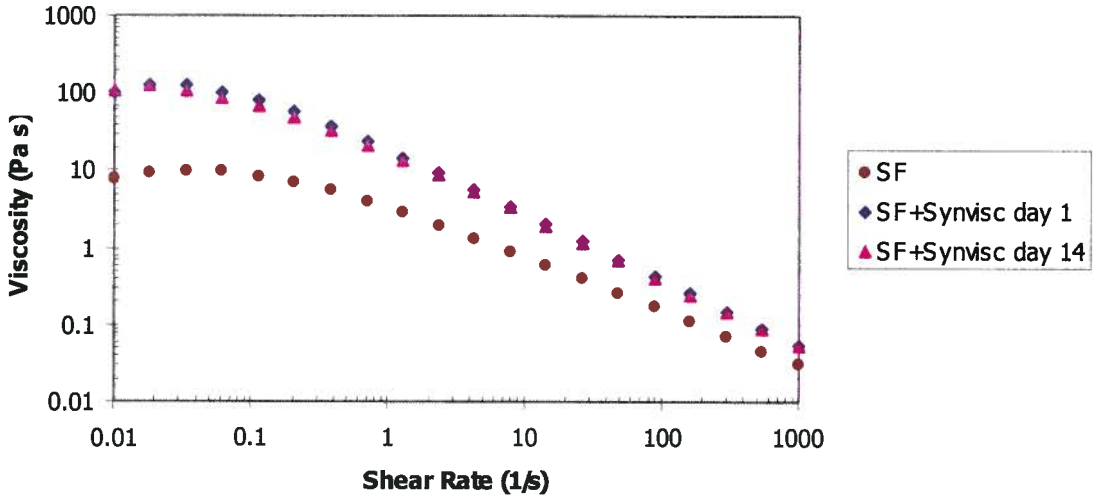

Figure 4.28: Viscosity as a function of shear rate for synovial fluid (SF), SF with Synvisc® day 1 and SF with Synvisc® day 14 (sample SN16). Non-Newtonian shear thinning behavior was observed in all samples. The viscosity increases with increasing shear rate. There were nearly no changes in rheological behaviors in synovial fluid mixed with Synvisc® on day 1 and 14 days later.

The results in the present study suggested that the rheological properties of synovial fluid are nearly unchanged over 2 weeks, which is consistent with previous work [75]. They reported that there were no changes in rheological properties of synovial fluid over 6 weeks which suggested that there is no ongoing hyaluronic acid degradation in isolated synovial fluid. Although the addition of viscosupplements to synovial fluid plays a role in improving the rheological behavior of synovial fluid in OA in this study, it should be pointed out that this is an in-vitro study. Therefore, the results cannot be applied to in vivo

conditions. In order to better understand the mechanisms of viscosupplements in OA joints and its clinical benefits, in vivo studies are needed.

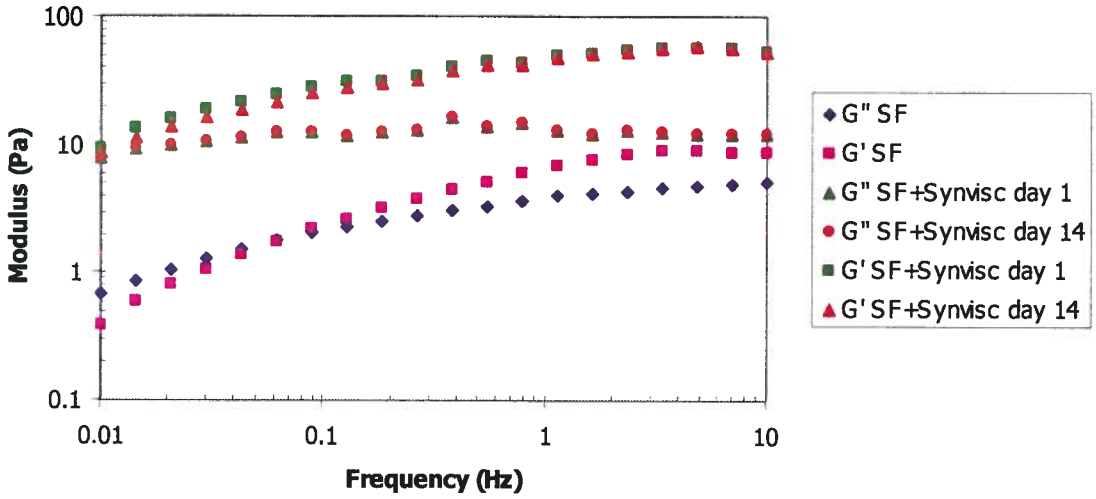

Figure 4.29: Storage and loss moduli as a function of frequency from SAOS measurement for synovial fluid (SF), SF with Synvisc® day 1, and SF with Synvisc® day 14 (sample SN16). Synovial fluid sample SN16 exhibited viscoelastic behavior. Both dynamic moduli in synovial fluid with Synvisc® sample were greater than synovial fluid alone over the entire range of frequency. Synovial fluid with Synvisc® exhibited gel-like behavior throughout the range of oscillation frequency. There were nearly no changes in rheological behaviors in synovial fluid mixed with Synvisc® on day 1 and 14 days later.

Table 4.12: Viscoelastic properties of synovial fluid (SF), SF with Synvisc® day 1, and SF with Synvisc® day 14 (sample SN16 and SN19)

Subject	Sample	$G'_{0.5\,Hz}$	$G''_{0.5\,Hz}$	$G'_{2.5\,Hz}$	$G''_{2.5\,Hz}$	Crossover Frequency
SN16	SF	5.11	3.32	8.46	4.43	0.062
	SF+Synvisc Day1	43.58	14.52	55.81	14.46	no
	SF+Synvisc Day 14	42.27	14.24	55.21	14.18	no
SN19	SF	5.67	3.84	9.62	5.06	0.089
	SF+Synvisc Day1	47.51	13.81	60.03	14.15	no
	SF+Synvisc Day 14	46.61	13.09	59.06	13.22	no

Limitations to study

There are several limitations to the study. Firstly, most of the synovial fluid obtained from subjects diagnosed with degree 4 of OA during the total knee arthroplasty. To better understand the rheological behavior of synovial fluid in OA, synovial fluid from different stages of OA is needed. Secondly, the rheological measurements were performed in a condition that is different from physiological condition of human knee joint. The geometry used in the measurement is not the same as articular cartilage. In addition, the gap used in the measurement is much larger than in the natural knee joint which is estimated at 0.1μm [120]. Finally, this is an in vitro study. Thus, further studies in vivo are warranted in order to better understand the mechanisms of viscosupplement in OA joints and its clinical benefits.

CHAPTER 5

CONCLUSIONS

In this pilot study, rheological characterizations of synovial fluid in OA were performed. In addition, rheological properties of different viscosupplements, the effects of viscosupplements on rheological behavior of synovial fluid in OA, and the stability of rheological behavior of synovial fluid mixed with cross-linked viscosupplement over time were determined. The findings from this pilot study are summarized as follows:

1) Rheological behaviors of synovial fluid varied widely in OA. Synovial fluid in OA exhibited a non-Newtonian shear thinning behavior and viscoelastic property. Moreover, rheopectic behavior (i.e. shear stress increases over time at a constant shear rate) was observed in OA synovial fluid at physiological temperature 37 °C. In addition, the results from this study suggested that the shear stress of OA synovial fluid at the shear rate of 0.01 s^{-1} builds up at higher rate than at the shear rate of 0.05 s^{-1}. The results also indicated that, within an individual, rheological properties of synovial fluid from the left knee differed substantially from the right knee when testing at physiological temperature 37 °C.

2) Apparent differences in rheological properties of different viscosupplements were observed. The viscosity of Synvisc® was the highest, whereas the viscosity of Suplasyn® was the lowest. Within the range of frequency from 0.1 to 10 Hz., Orthovisc® exhibited a linear viscoelastic behavior, whereas Synvisc® and Suplasyn® exhibited a gel-like behavior and a viscous-like behavior,

respectively. There were slight changes in viscosity and viscoelastic properties when the temperature changes from 25 °C to 37 °C.

3) The results suggested that the addition of viscosupplements to synovial fluid led to the increase in viscosity. The viscosity was highest in synovial fluid mixed with Synvisc®, and was lowest in synovial fluid mixed with Suplasyn®. The results also indicated potential trends that, within the range of frequency from 0.1 to 10 Hz., synovial fluid with Orthovisc® exhibited a linear viscoelastic behavior, whereas synovial fluid with Synvisc® and synovial fluid with Suplasyn® exhibited a gel-like behavior and a viscous-like behavior, respectively. The findings suggested that cross-linked viscosupplement (Synvisc®) was more efficient than the non-cross-linked ones (Orthovisc®, Suplasyn®) in improving the overall rheological behavior of synovial fluid.

4) The results indicated that rheological properties of synovial fluid mixed with cross-linked viscosupplement were nearly unchanged over 2 weeks.

Recommendations for future work

- A more complete rheological characterization of synovial fluid in healthy joint and disease joint is still needed in order to better understand its role in joint lubrication. Future studies need to include a larger sample of people with knee OA and over a broader span of OA severity.

- There are many factors that relate to OA, such as the age, gender, body mass index (BMI), and trauma history. Therefore, it is of interest to examine whether there are any associations between these factors and the rheology of synovial fluid.

- Since there is a product using an electrical stimulation as a non-invasive treatment for OA, it is of interest to study the effect of electrical stimulation on the rheological behaviour of synovial fluid. Therefore, further investigation can be done using an electro-rheological cell.

- Varieties of viscosupplements are commercially available. However, its mechanism in improving the rheological behaviour of the synovial fluid in OA is not well understood. Therefore, in vitro and in vivo studies on the effects of various formulations of viscosupplement on OA synovial fluid are warranted.

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APPENDIX A

The University of British Columbia

Research Ethics Board's Certificates of Approval

The University of British Columbia
Office of Research Services
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V5Z 1L8

ETHICS CERTIFICATE OF FULL BOARD APPROVAL

PRINCIPAL INVESTIGATOR:	INSTITUTION / DEPARTMENT:	UBC CREB NUMBER:
Dana Grecov	UBC/Applied Science/Mechanical Engineering	H08-02272

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

Institution	Site
Vancouver Coastal Health (VCHRI/VCHA)	Vancouver General Hospital
UBC	Vancouver (excludes UBC Hospital)

Other locations where the research will be conducted:
N/A

CO-INVESTIGATOR(S):
Ezra Kwok
Petcharatana Bhuanantanondh
Pierre Guy

SPONSORING AGENCIES:
N/A

PROJECT TITLE:
Rheological Characterization of Synovial Fluid in Patients with Osteoarthritis: A Pilot Study

THE CURRENT UBC CREB APPROVAL FOR THIS STUDY EXPIRES: October 21, 2009

The full UBC Clinical Research Ethics Board has reviewed the above described research project, including associated documentation noted below, and finds the research project acceptable on ethical grounds for research involving human subjects and hereby grants approval.

REB FULL BOARD MEETING REVIEW DATE: October 21, 2008	

DOCUMENTS INCLUDED IN THIS APPROVAL:			DATE DOCUMENTS APPROVED:
Document Name	Version	Date	
Protocol:			
Rheological Characterization of Synovial Fluid in Patients with Osteoarthritis (Protocol)	2	January 16, 2009	
Consent Forms:			
Rheological Characterization of Synovial Fluid in Patients with Osteoarthritis (Consent Form)	2	January 16, 2009	**January 27, 2009**
Letter of Initial Contact:			
Letter of Initial Contact: Rheological of SF in OA	2	January 16, 2009	

CERTIFICATION:
In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The documentation included for the above-named project has been reviewed by the UBC CREB, and the research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved by the UBC CREB.

Approval of the Clinical Research Ethics Board by:

Dr. Gail Bellward, Chair

The University of British Columbia
Office of Research Services
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V5Z 1L8

ETHICS CERTIFICATE OF EXPEDITED APPROVAL: AMENDMENT

PRINCIPAL INVESTIGATOR:	DEPARTMENT:	UBC CREB NUMBER:
Dana Grecov	UBC/Applied Science/Mechanical Engineering	H08-02272

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

Institution	Site
Vancouver Coastal Health (VCHRI/VCHA)	Vancouver General Hospital
UBC	Vancouver (excludes UBC Hospital)
Vancouver Coastal Health (VCHRI/VCHA)	UBC Hospital

Other locations where the research will be conducted:
N/A

CO-INVESTIGATOR(S):
Ezra Kwok
Don Garbuz
Petcharatana Bhuarantanondh
Pierre Guy
Bassam A. Masri

SPONSORING AGENCIES:
N/A

PROJECT TITLE:
Rheological Characterization of Synovial Fluid in Patients with Osteoarthritis: A Pilot Study

REMINDER: The current UBC CREB approval for this study expires: October 21, 2009

AMENDMENT(S):

Document Name	Version	Date
Protocol:		
Rheological Characterization of Synovial Fluid in Patients with Osteoarthritis (Protocol)	4	July 16, 2009
Consent Forms:		
Rheological Characterization of Synovial Fluid in Patients with Osteoarthritis (Consent Form)	5	July 16, 2009

AMENDMENT APPROVAL DATE:
July 28, 2009

CERTIFICATION:
In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The amendment(s) for the above-named project has been reviewed by the Chair of the University of British Columbia Clinical Research Ethics Board and the accompanying documentation was found to be acceptable on ethical grounds for research involving human subjects.

Approval of the Clinical Research Ethics Board by one of:

Dr. Peter Loewen, Chair
Dr. James McCormack, Associate Chair

Appendix B

Vancouver Coastal Health Authority

Clinical Trials Administration Office Approval

Vancouver CoastalHealth
Research Institute
Healthier lives through discovery

Robert McMaster, D. Phil.
Interim
Vice President, Research, Vancouver Coastal Health
Executive Director, Vancouver Coastal Health Research Institute

Associate Dean Research, Faculty of Medicine
The University of British Columbia

June 4, 2009

Dr. B.A. Masri
Department of Orthopaedics
Lower Limb Reconstruction and
Oncology
Room 3114, 910 West 10th Avenue
Vancouver, B.C.

Vancouver Coastal Health Authority Research Study #V09-0154

FINAL CERTIFICATE OF APPROVAL

TITLE: Rheological Characterization of Synovial Fluid in Patients with Osteoarthritis: A Pilot Study

Sponsor: Unfunded Research

This is to inform you that your project has been approved. Approval has been granted until October 21, 2009

1. UBC Ethics Committee Certificate of Approval #H08-02272
2. VCHA Clinical Trials Administration Office Approval

Yours truly.

for:
Dr. Robert McMaster
Interim Vice-President Research

APPENDIX C

Data

Appendix C.1: Zero shear stress and maximum shear stress from test 1 and test 2 for synovial fluid samples

Sample	zero shear stress Test 1	zero shear stress Test 2	max shear stress Test 1	max shear stress Test 2
SN1	0.08	0.09	33.70	33.71
SN2	0.01	0.01	12.30	12.31
SN3	0.03	0.03	13.67	13.66
SN4	0.01	0.01	19.78	19.76
SN5	0.04	0.04	19.80	19.82
SN6	0.01	0.01	12.73	12.72
SN7	0.05	0.04	20.98	20.97
SN8	0.01	0.01	11.09	11.11
SN9	0.02	0.02	18.00	18.01
SN10	0.07	0.08	25.67	25.68
SN11	0.02	0.03	11.75	11.77
SN12	0.003	0.002	11.85	11.84
SN13	0.08	0.09	19.09	19.10
SN14	0.01	0.01	18.44	18.44
SN15	0.03	0.04	23.88	23.89
SN16	0.08	0.08	30.37	30.37
SN17 Lt.	0.02	0.02	18.64	18.66
SN17 Rt.	0.01	0.01	16.04	16.02
SN18	0.03	0.03	24.31	24.30
SN19	0.11	0.10	34.67	34.67
SN20	0.003	0.002	21.72	21.71
SN21	0.04	0.04	27.10	27.08
SN22 Lt.	0.03	0.04	28.89	28.91
SN22 Rt.	0.02	0.02	18.87	18.87

Appendix C.2: Dynamic moduli at 0.5 Hz. of synovial fluid (SF) from test 1 and test 2

Sample	$G'_{0.5\,Hz}$ (Pa) Test 1	$G'_{0.5\,Hz}$ (Pa) Test 2	$G''_{0.5\,Hz}$ (Pa) Test 1	$G''_{0.5\,Hz}$ (Pa) Test 2
SN01	4.38	4.38	2.99	2.99
SN02	0.88	0.88	0.73	0.73
SN03	2.16	2.15	1.52	1.53
SN04	1.43	1.44	1.67	1.68
SN05	2.19	2.19	1.67	1.67
SN06	0.46	0.46	0.54	0.54
SN07	2.43	2.44	1.80	1.79
SN08	0.76	0.76	0.61	0.62
SN09	1.38	1.38	1.19	1.19
SN10	5.79	5.78	3.29	3.30
SN11	0.47	0.46	0.46	0.45
SN12	0.26	0.26	0.34	0.33
SN13	3.39	3.38	2.07	2.07
SN14	1.24	1.23	1.11	1.11
SN15	2.78	2.77	1.97	1.96
SN16	5.12	5.12	3.33	3.33
SN17 Lt.	1.26	1.25	1.09	1.09
SN17 Rt.	1.05	1.04	1.01	1.01
SN18	1.89	1.88	1.84	1.84
SN19	5.67	5.66	3.84	3.83
SN20	0.46	0.46	0.76	0.77
SN21	2.06	2.07	1.98	1.99
SN22 Lt.	3.20	3.21	2.74	2.75
SN22 Rt.	0.54	0.53	0.68	0.68

Appendix C.3: Dynamic moduli at 2.5 Hz of synovial fluid (SF) for test 1 and test 2

Sample	$G'_{2.5Hz}$ (Pa) Test 1	$G'_{2.5Hz}$ (Pa) Test 2	$G''_{2.5Hz}$ (Pa) Test 1	$G''_{2.5Hz}$ (Pa) Test 2
SN01	7.64	7.63	4.19	4.19
SN02	1.22	1.23	1.19	1.19
SN03	2.90	2.89	2.17	2.16
SN04	2.33	2.33	3.34	3.33
SN05	3.78	3.78	2.60	2.59
SN06	0.68	0.67	1.02	1.02
SN07	4.14	4.15	2.61	2.61
SN08	1.19	1.19	0.89	0.89
SN09	2.48	2.48	1.93	1.92
SN10	8.19	8.19	4.05	4.04
SN11	0.60	0.59	0.86	0.87
SN12	0.36	0.36	0.65	0.66
SN13	4.85	4.85	2.76	2.77
SN14	1.99	1.99	1.79	1.80
SN15	4.43	4.43	3.00	2.99
SN16	8.52	8.51	4.41	4.41
SN17 Lt.	1.86	1.85	1.81	1.80
SN17 Rt.	1.89	1.90	1.75	1.75
SN18	3.51	3.51	3.02	3.01
SN19	9.63	9.62	5.09	5.09
SN20	1.53	1.53	1.99	1.98
SN21	4.67	4.67	3.43	3.43
SN22 Lt.	5.95	5.94	4.13	4.13
SN22 Rt.	0.83	0.83	1.66	1.65

Appendix C.4: Zero shear stress and maximum shear stress from test 1 and test 2 for viscosupplements and synovial fluid with viscosupplements samples

Sample	zero shear stress Test 1	zero shear stress Test 2	max shear stress Test 1	max shear stress Test 2
Orthovisc	0.85	0.84	224.10	224.08
SN20+Orthovisc	0.11	0.11	79.77	79.79
SN21+Orthovisc	0.18	0.17	96.40	96.38
Suplasyn	0.04	0.04	120.70	120.69
SN20+Suplasyn	0.05	0.05	56.47	56.46
SN21+Suplasyn	0.04	0.04	96.29	96.28
Synvisc	5.57	5.56	131.80	131.78
SN20+Synvisc	0.95	0.96	57.31	57.30
SN21+Synvisc	1.26	1.25	60.73	60.71
SN16+Synvisc Day 1	0.98	0.99	54.47	54.48
SN16+Synvisc Day 14	1.07	1.07	54.47	54.47
SN19+Synvisc Day 1	1.62	1.61	61.92	61.90
SN19+Synvisc Day 14	1.57	1.58	58.71	58.71

Appendix C.5: Dynamic moduli at 0.5 Hz. from test 1 and test 2 for viscosupplements and synovial fluid with viscosupplements samples

Sample	$G'_{0.5\,Hz}$ (Pa) Test 1	$G'_{0.5\,Hz}$ (Pa) Test 2	$G''_{0.5\,Hz}$ (Pa) Test 1	$G''_{0.5\,Hz}$ (Pa) Test 2
Orthovisc	51.21	51.22	56.42	56.41
SN20+Orthovisc	6.42	6.41	8.61	8.60
SN21+Orthovisc	8.95	8.96	8.99	9.00
Suplasyn	0.29	0.30	2.56	2.55
SN20+Suplasyn	0.88	0.88	2.05	2.04
SN21+Suplasyn	0.88	0.89	2.05	2.05
Synvisc	91.85	91.85	26.13	26.14
SN20+Synvisc	29.35	29.36	8.08	8.09
SN21+Synvisc	38.32	38.31	13.03	13.05
SN16+Synvisc Day 1	43.58	43.59	14.52	14.51
SN16+Synvisc Day 14	42.27	42.26	14.24	14.23
SN19+Synvisc Day 1	47.51	47.52	13.81	13.82
SN19+Synvisc Day 14	46.61	46.61	13.09	13.10

Appendix C.6: Dynamic moduli at 2.5 Hz. from test 1 and test 2 for viscosupplements and synovial fluid with viscosupplements samples

Sample	$G'_{2.5Hz}$ (Pa) Test 1	$G'_{2.5Hz}$ (Pa) Test 2	$G''_{2.5Hz}$ (Pa) Test 1	$G''_{2.5Hz}$ (Pa) Test 2
Orthovisc	111.20	111.19	61.48	61.48
SN20+Orthovisc	18.32	18.31	16.57	16.56
SN21+Orthovisc	21.16	21.15	15.21	15.20
Suplasyn	3.36	3.37	10.78	10.79
SN20+Suplasyn	2.89	2.88	6.79	6.77
SN21+Suplasyn	2.93	2.93	6.59	6.60
Synvisc	118.10	118.09	22.46	22.45
SN20+Synvisc	37.29	37.30	9.71	9.72
SN21+Synvisc	51.51	51.50	14.23	14.23
SN16+Synvisc Day 1	55.81	55.82	14.46	14.46
SN16+Synvisc Day 14	55.21	55.20	14.18	14.17
SN19+Synvisc Day 1	60.03	60.02	14.15	14.14
SN19+Synvisc Day 14	59.06	59.07	13.22	13.23