Rheology of Joint Fluid in Total Knee Arthroplasty

by

Daniel Clarke Mazzucco

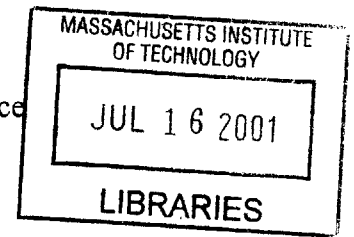B.A. Engineering Sciences (1998)
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ABSTRACT

Polyethylene wear is a significant clinical problem in total knee arthroplasty (TKA). Although the tribology of joint replacement has consequently become an area of significant research, the effect of joint fluid on lubrication has been largely overlooked in the replaced joint. One factor that affects the tribology of metal on polyethylene articulation in joint prostheses is mechanical properties of the joint fluid. In particular, viscosity and linear viscoelasticity are flow properties that likely contribute to fluid film lubrication in TKA, as they do in the natural knee. The primary objective of this study was to evaluate the flow properties of joint fluids in the context of TKA.

Viscosity and linear viscoelasticity were evaluated in joint fluid from patients undergoing TKA, revision of a TKA, and aspiration of joint effusion. The flow properties of two commercially available hyaluronic acid joint supplements were also evaluated. Viscosity was measured over a range of strain rates and fitted to a common model of viscous behavior. Storage and loss moduli were measured for small amplitude oscillatory motion as a function of frequency.

Each joint fluid demonstrated a characteristic shear-thinning, but viscosity varied over three orders of magnitude among samples. Both viscoelasticity and viscosity were compromised in many joint fluid samples. None of the samples obtained at revision were as viscous as healthy synovial fluid. In each effusion case, viscosity was at least a factor of six below the lower limit of the normal range. The joint supplements were more viscous than most joint fluid samples, likely based on higher concentration of hyaluronic acid. Normal viscosity correlated to high viscoelasticity (p=0.013). The fluid lubricating replacement knees and joints with effusion differed from healthy joint fluid in flow properties.

Further examination of the connection between viscosity and tribology of joint replacement prostheses is warranted. Understanding the nature and performance of joint fluid as a prosthesis lubricant, particularly with respect to variability among patients, can lead to more meaningful prognoses and better therapies.

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CHAPTER 1:   INTRODUCTION

1.1   The Motivation Behind the Research

Prosthetic wear, leading to fracture of the bone at the device interface and aseptic prosthesis loosening, has been a serious problem in total knee arthroplasty (O'Connor 1996; Jones 1992; Saikko 1998; Rose 1984; Kovacik 2000). As patients opt for joint prostheses earlier in life, demanding both longer life and more athletic activity of them (Healy 2000), the problem of prosthesis wear will only be magnified. In the effort to solve the wear problem, substantial effort has been put into finding synthetic materials that mimic the natural joint in combining low friction with low wear rates (Costa 1998; Muratoglu 1999; Patel 1997). Less effort, however, has gone toward examining how the fluid in the joint affects the tribology of the joint replacement. My research is intended to examine the effects of the joint fluid on wear in the replaced knee joint, with the long-term goal of finding ways to improve the performance and life span of total knee replacements. As a first step, the objective of this thesis was to examine flow properties that may be relevant in the tribology of joint prostheses.

1.2   Background

1.2.1   Osteoarthritis

Osteoarthritis occurs as the result of a long process of progressive articular cartilage degeneration in the joints, causing pain, stiffness, and instability. It can originate from trauma to the joint, bone misalignment, and/or prolonged misuse, but often takes decades to become symptomatic. Consequently, osteoarthritis typically presents in older patients, and can be present in one or many joints. Some evidence of osteoarthritis can be found in 80% to 90% of people over the age of 65 (Cotran 1999).

1.2.2   Total Knee Arthroplasty

Total knee arthroplasty is indicated in patients who have a particularly painful or dysfunctional knee joint with extensive loss of the natural articulating surface due to osteoarthritis or other joint disease. More than 300,000 people undergo total knee arthroplasty in the U.S. annually (National Center for Health Statistics, 1997). These surgeries are generally considered very successful, in that patients have a 90% chance of keeping the prosthesis for more than ten years (Healy W 2000). Patients report better mobility, less joint pain, and higher quality of life after arthroplasty. Consequently, an increasing number of younger patients opt for knee replacement every year.

In knee replacement surgery, the surgeon removes a portion of the distal femur, replacing it with a metal component, usually composed of a cobalt-chromium alloy. The surgeon also removes the proximal portion of the tibia, replacing it with a polyethylene plateau, which fits into a metal casing mounted to the tibia. Finally, the patella is shaved down, and a polyethylene button fixed onto its remains. During the surgery, the menisci are sacrificed, along with the collateral ligaments and anterior cruciate ligament, the synovial sac and fluid, and the articular cartilage. Depending on the implant and the condition of the patient, the posterior cruciate ligament may or may not be saved. The replacement knee can be cemented in place with self-curing polymethylmethacrylate or press-fit into the remaining portions of the femur and tibia.

1.3   Wear in TKA

One of the limiting factors in joint replacement durability is wear. When metal rubs against polyethylene, particles of polyethylene are removed from the surface. Synovial macrophages (Type A synoviocytes) respond to these foreign bodies in an effort to digest and remove them. Synovial macrophages release a variety of regulators, including interleukin-1β, which is linked both to inflammation and to bone resorption by osteoclasts. (Kovacik 2000) Bone resorption leads to prosthesis loosening (Spector 1996), instability, and pain. Consequently, wear particle generation can lead to prosthesis failure even if the worn surface continues to provide appropriate articulation.

The magnitude of the problem of wear in TKA cannot be ascertained from prosthesis failure rates, which are actually remarkably low. The American Academy of Orthopedic Surgeons discourages knee replacement patients from contact sports, skiing, tennis, and "vigorous" walking because they can lead to excessive wear. Some researchers more liberally allow activity because of the numerous overall health benefits associated with an active lifestyle. (Healy 2000) Nonetheless, patients undergoing TKA must curtail some of their more strenuous behaviors to avoid the threat of wear-related complications. The trend toward younger, more active patients only serves to heighten this problem. Consequently, much research in the area of knee replacement has been devoted to reducing wear of the polyethylene component.

1.3.1   Wear Mechanisms

There are three main mechanisms of wear common to joint prostheses. (Spector 1993; Schmalzried and Callaghan 1999) The first, adhesive wear, affects hip replacements more than knees. In adhesive wear, the asperities on the polyethylene and metal become chemically bonded when pressed together at high pressure. The metal-polyethylene adhesive strength exceeds the cohesive strength of polyethylene. As the surfaces move relative to each other, the polyethylene-metal bond holds and the polyethylene-polyethylene bond fails, thus producing a wear particle.

The second wear mechanism, abrasive wear, is common to hip and knee replacements. In adhesive wear, an asperity in the metal plows a path through the polyethylene when the surfaces are brought into contact. Sometimes, a particle, such as bone cement, becomes lodged between the polyethylene and the metal, and acts as the plowing body. In this case, the wear mechanism is known as three-body wear.

Finally, in fatigue wear (or delamination wear), cyclic loading initiates cracks under the polyethylene surface. When the cracks become large enough, they produce large wear particles. This type of wear abounds in the knee replacement, and can cause wear to the point of large-scale fracture.

The roles that joint fluid plays in these wear mechanisms have yet to be fully explored.

1.3.2   Lubrication Mechanisms

There are two types of lubrication mechanisms relevant to joint replacement: fluid-film lubrication and boundary layer lubrication. In the most basic kind of fluid-film lubrication, hydrodynamic lubrication, a wedge of fluid forms such that surface movement squeezes fluid from the base of the wedge to its apex. When the articulating surfaces are pliable, as they are in the knee, they will deform to provide a larger surface

over which the fluid can be squeezed. In this case, the lubrication mechanism is called elastohydrodynamic lubrication.

In any type of fluid-film lubrication, surface roughness, velocity of motion, and the quantity and viscosity of the lubricant play fundamental roles in determining the efficacy of lubrication. Surface properties do not directly affect fluid film lubrication.. The fluid film thickness (and fluid quantity) must exceed the roughness of the counterface to prevent asperity contact. The load borne by the fluid and the thickness of the film depend on the viscosity of the lubricant and the velocity of relative motion. Fluid films can produce coefficients of kinetic friction as low as 0.001, but require a velocity of about 0.1 m/sec (Hills 2000).

In contrast, to fluid-film lubrication, boundary lubrication does not rely on motion for load support. In boundary lubrication, a component of the lubricant adheres to the articulating surfaces, forming two molecular monolayers. The two monolayers repel each other, providing the normal force to support a load. Given a sufficiently smooth surface, high loads can be supported with a coefficient of kinetic friction of 0.1 even in the presence of little or no motion.

1.3.3 Synovial Fluid

The body prevents wear in the natural joint by a remarkable lubricating system consisting of articular cartilage and synovial fluid. Synovial fluid, an aqueous solution, resides in a sac between the articular cartilage of the femur and the tibia. The synovial membrane extends from the edge of the articular cartilage on the tibia and femur, and consists of macrophages (Type A synoviocytes) and fibroblasts (Type B synoviocytes). Synovial fluid consists of proteins derived from blood serum, hyaluronic acid (HA), glycoproteins, and phospholipids (Swann, 1978). In addition to lubricating the synovial membrane and articular cartilage, synovial fluid serves to nourish the avascular soft tissues of the knee joint.

With the help of synovial fluid, diarthrodial joints produce motion with a coefficient of friction as low as 0.002 (Charnley 1959). These joints can function *in vivo* for 70 years with cartilage turnover outweighing wear. Traditional engineering models have difficulty explaining such low friction and wear between opposing articular cartilage. Fluid-film lubrication should not bear loads while standing motionless, and boundary lubrication predicts a coefficient of friction 100 times higher than is actually observed. Consequently, no less than 12 mechanisms have been proposed for lubrication of synovial joints, many of which were invented solely to describe these joints (McCutchen 1978). In recent years, some level of consensus has been reached, and it is believed that a number of mechanisms contribute to the incredible tribological properties of the knee joint. These mechanisms include elastohydrodynamic, boundary, and weeping lubrication. Weeping lubrication involves the continuous secretion of water by cartilage to maintain fluid-film load bearing without motion.

1.4   Components of Synovial Fluid

1.4.1   Hyaluronic Acid (HA)

HA is a long chain polymer, reaching molecular weights of 10^6 to 10^7 Da in synovial fluid (Bjelle 1982). It has a density of about 1.65 g/cm^3 (Radin 1970). HA is

produced by fibroblasts (Type B synoviocytes) and secreted into the synovial sac. The porous lining of the synovium allows water and small proteins such as albumin to diffuse in and out of the synovial fluid, but prevents large proteins from entering or HA from leaving the sac.

At low molecular weight ($< 10^6$ Da) and concentration (< 1 mg/ml), HA molecules form random coils, and do not interlock with each other (Swann 1978). Random coiling leads to a lower viscosity that does not depend on strain rate. At physiological molecular weight ($\sim 10^7$ Da) and concentration (2 mg/ml) (Balazs, Watson et al. 1967), HA chains interlock to form an entanglement network that has a much higher viscosity than the dilute solution (De Smedt 1993). Furthermore, this heightened viscosity depends highly on strain rate. At low strain rate, the interlocked network does not separate easily, and contributes to a higher viscosity. At higher strain rate, the chains become quickly disentangled, and provide less resistance to flow. This shear behavior based on molecular weight is common for polymers in solution.

In addition to high viscosity, the entanglement network exhibits considerable elasticity. When subjected to small amplitude oscillatory shear, as in normal joint movement, the network does not become disentangled. Rather, the chains are stretched and compressed, and store energy as well as dissipating it. During high frequency movement, such as running or jumping, energy storage figures more prominently than it does at walking frequencies, where viscosity is more dominant. It is believed that the viscoelastic properties of HA serve to support and protect joint tissue under these conditions, and to stabilize the extra cellular matrix (Balazs 1993).

Because HA is by far the largest abundant molecule in synovial fluid, it determines the viscosity of synovial fluid and therefore its ability to perform fluid-film lubrication. Inviscid lubricating fractions of synovial fluid lacking HA have been isolated (Swann D 1974; Radin 1970), however, indicating that a fluid-film does not lubricate the articular surface by itself. Such findings led to the search for a boundary lubricant in synovial fluid.

There are other soft tissues that articulate in the knee. For example, the synovial membrane contains folds that move when the joint bends. These folds rub against each other, and so require lubrication. Loads in this interaction are low enough, however, to permit hydrodynamic lubrication, and HA provides sufficient lubrication. (Swann 1978). These soft tissues are often more highly innervated than articular cartilage, however, and could cause more joint pain than articular cartilage when not lubricated properly.

1.4.2 Lubricin and Surface-Active Phospholipid

There are two strong candidates for the role of boundary lubricant in synovial fluid. Lubricin is a protein similar to "surface zone protein" expressed in articular cartilage. Surface active phospholipid is known to lubricate the lung and other articulating surfaces in the body. Both have been found in lubricating fractions of synovial fluid, but researchers disagree on the mechanisms and role of the two components. Some suggest that lubricin lubricates the joint (Jay 1992; Jay, Haberstroh et al. 1998; Jay and Cha 1999). while others suggest that phospholipid performs the lubrication function, and lubricin enables the phospholipid to exist in solution (Williams, Powell et al. 1993; Hills 1996; Williams, Iwasaki et al. 1997; Hills and Monds 1998; Schwarz and Hills 1998; Foy, Williams et al. 1999; Hills 2000).

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1.5   Tribology of the Replacement Knee

The tribology of the replacement knee bears several important distinctions from that of the natural knee, and each mode of natural lubrication is compromised in the replacement joint. First, the polyethylene and metal surfaces used in knee replacement are not nearly as flexible as the natural knee, and will not support elastohydrodynamic lubrication as well. Second, the replacement joint does not continually secrete and absorb water at the same rate as cartilage, and so does not support weeping lubrication. Third, boundary lubrication requires a specific chemical interaction between specific molecules and articular cartilage. Although hydrophobic polyethylene may interact similarly with these molecules, the metal surface is not likely to provide the same bonding atmosphere. Finally, the synovial fluid and sac are removed during replacement surgery, as is the articular cartilage. A synovial-like membrane regenerates and fills with fluid. It is not clear whether the replacement joint fluid and periprosthetic tissue have the same makeup as their original counterparts, and the lubricating characteristics of the fluid are not known.

1.6   Previous Work in the Field

Understanding the role of the lubricant requires assessing both its bulk fluid properties and fluid composition. In particular, viscosity and linear viscoelasticity are flow properties that likely contribute to fluid film lubrication in total knee arthroplasty, as they do in the natural knee. These flow parameters have been examined previously in both normal and diseased knees (Schurz, 1987; Schurz, 1996; Cooke, 1978), but not in knees of patients undergoing total knee arthroplasty. The range of viscosities found in these prior studies in normal and diseased knees will be examined in further detail in Chapter 2. The composition of synovial fluid, similarly, has been examined in the context of physiologic as well as pathologic conditions, but not in the context of TKA. Composition of joint fluid in TKA, particularly as it relates to boundary lubrication, is the subject of future work.

Recent evidence in an animal model suggests that HA exists in different concentrations in synovial fluids of normal and prosthetic joints (Delecrin, 1994). If substantial differences in HA molecular weight or concentration occur in humans after TKA, fluid film lubrication could be compromised in the replacement joint.

1.7   Specific Aims and Hypotheses

This thesis was specifically designed to evaluate the flow properties of several fluids relevant to total knee arthroplasty. Viscosity and linear viscoelasticity were evaluated in joint fluid from patients undergoing total knee arthroplasty and patients undergoing revision of total knee arthroplasty. These flow properties were compared to those previously reported in normal and diseased patients. As a third comparative group, joint fluid from patients undergoing aspiration for joint effusion was evaluated. Fourth, the flow properties of lubricants currently used in knee simulators and wear tests were evaluated and compared with the properties of periprosthetic fluid. Finally, the flow properties of two commercially-available hyaluronic acid preparations were evaluated as potential wear test lubricant additives.

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It was hypothesized in this thesis that:

1) viscosity of joint fluid obtained at arthroplasty would vary widely;
2) joint fluid obtained at revision would be substantially less viscous than that obtained at TKA procedure;
3) joint fluid aspirated from patients presenting with effusion would be less viscous than healthy synovial fluid;
4) lubricants used currently in laboratory wear tests would not mimic the viscous properties of joint fluid;
5) hyaluronic acid supplements would approximate the viscosity of joint fluid more closely than bovine serum does.

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CHAPTER 2:   MATERIALS AND METHODS

2.1   Experimental Groups

The study was originally designed to evaluate 25 samples each of joint fluid at TKA and joint fluid at revision surgery. This design was based on demonstrating with 95% confidence ($\alpha = 0.05$, $\beta = 0.2$) whether a difference of 20% or more existed between the groups of certain flow properties, assuming a standard deviation in each group of 25% of the mean. The flow properties of the samples were not, however, normally distributed, so the Gaussian statistical analyses used to choose the sample sizes could not be employed to analyze the results. Information regarding the samples obtained for the study can be found in Appendix A.

2.1.1   Synovial Fluid from TKA

For comparison with normal and diseased synovial fluid, fluid was obtained from 52 joints of patients undergoing TKA for osteoarthritis. In two cases, the sample contained blood, and it was not possible to separate a portion not visibly contaminated. In six cases, there was not sufficient fluid to perform a test on the sample. In eleven cases, the rheometry failed due to technical difficulties. In the remaining 33 cases, evaluation of viscosity was successfully performed. In two of these cases, there was slight contamination with blood, but it was possible to obtain an aliquot of joint fluid without visible contamination by blood.

2.1.2   Joint Fluid from Revision of TKA

Ten prosthetic joint fluid samples were obtained during revision of total knee arthroplasty. These were obtained from a different group of patients than the other samples. Two samples lacked sufficient fluid for testing. In one case, the experiment failed due to technical difficulties. In the remaining seven cases, evaluation of flow parameters was performed.

2.1.3   Joint Fluid from Patients with Joint Effusion

Joint fluid was aspirated by percutaneous syringe from patients presenting with joint effusion in natural knees. In one case, insufficient fluid was obtained, and in one case, the experiment failed, leaving five cases in which fluid properties were measured.

2.1.4   Effusion Subsequent to TKA

One sample was aspirated from a patient presenting with effusion subsequent to TKA. This fluid was evaluated, and was not included with the other groups in the study. Fluid was also obtained from the same patient at subsequent revision surgery, but not enough was obtained to evaluate its properties.

2.1.5   Bilateral TKA

In two cases, fluid from each knee was obtained at bilateral TKA. These samples were included in the first group, but also considered in comparison to each other.

2.1.6 Bovine Serum

The standard lubricant employed for laboratory wear testing, bovine calf serum, was also tested in this study. The concentration of bovine serum used varies between laboratories.(Ahlroos 1997) All bovine serum samples evaluated in this study came from Life Technologies calf serum lot # 1023609, 73 mg/ml, and were diluted to 40% by volume in distilled water. Because the flow properties of bovine serum fell consistently within ten percent of the mean, the average curve of three samples was used to characterize the fluid.

2.1.7 Hyaluronic Acid Preparations

Flow properties were measured for two HA preparations, Supartz (Smith & Nephew, Memphis, TN) and Orthovisc (Anika Therapeutics, Woburn, MA). Both are sold commercially as injectable agents for the treatment of patients with osteoarthritis. All Supartz samples came from Artz lot #9Z683A 2002.11, and contained 10 mg/ml hyaluronic acid at molecular weight 620,000 to 1,170,000. All Orthovisc samples came from Anika Therapeutics lot #60382000, and contained 13.6 mg/ml hyaluronic acid at average molecular weight 1,390,000. Because the flow properties within each lot fell consistently within ten percent of the mean, the average curve of three samples was used to characterize the fluid.

2.2   Procurement of Joint Fluid

2.2.1 Institutional Review Board Approval

Approval was obtained from the Institutional Review Board of Partners HealthCare, the Institutional Review Board at New England Baptist Hospital, and the MIT Committee on the Use of Humans as Experimental Subjects. Samples were obtained from patients with their written informed consent, as proscribed by the above governing bodies. An Exposure Control Plan associated with working with biological materials is on file with the MIT Biosafety office.

2.2.2 Surgical Removal

Joint fluid was obtained during surgeries and outpatient aspirations at Brigham and Women's Hospital, New England Baptist Hospital, and Massachusetts General Hospital. After the knee had been opened and the synovium exposed, the surgeon cut open the synovium, and withdrew as much synovial fluid as possible using a syringe with no needle attached. Since hemorrhage accompanies the incision, the blood was often inadvertently obtained along with joint fluid at this time. The fluid was then refrigerated at 5°C in a stopped 15 ml glass test tube until evaluated (typically zero to three days).

2.2.3 Storage

The first samples were centrifuged at 1500 rpm at 18°C for ten minutes to separate red blood cells and particles. After obtaining a few samples, however, it became clear that the viscosity of the samples would make it difficult to separate particles quickly without the risk of separating viscous components from inviscid ones. At first, samples were stored in a -5°C or -70°C freezer between aspiration and evaluation. When thawed, the samples were placed in a 5°C refrigerator for one hour, then thawed to room

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temperature in a 37°C water bath. Later, however, it was determined that the effects of freezing and thawing introduced variables in the system that could be avoided by keeping the samples in a 5°C refrigerator for the entire time before testing, both at the hospital and at MIT. Between thawing and rheometry, and during transport, the samples were kept at 5°C.

2.2.4 Transportation

Joint fluid samples were brought from the hospital to MIT for evaluation according to the protocol laid out in the Exposure Control Plan. This necessarily entailed exposing the samples, with limited insulation, to outside air temperature for ten to fifteen minutes. This exposure had no observable detrimental effects on the samples or their flow properties.

2.2.5 Obtaining Patient Information

The volume obtained was recorded when its properties were evaluated. All other pertinent information was obtained from medical records. These included: surgeon; date of surgery; patient gender; knee, patient's age at surgery; occasion of fluid withdrawal – TKA, revision, or aspiration; and indication for surgery or aspiration.

2.3   Experimental Setup

2.3.1 Devices Used

All joint fluid and bovine serum experiments were performed on a CSL 500 controlled stress rheometer (TA Instruments, New Castle, DE) using Rheology Advantage Software (TA Instruments, New Castle, DE), and a double cylinder Couette flow geometry. All HA experiments were performed on an AR1000 controlled stress rheometer using the same software and 6cm 1° cone and plate geometry.

When using cone and plate geometry, 1.7 ml of fluid was poured onto the lower plate of the rheometer, which was then raised to within 28 μm of the upper plate. When using double cylinder Couette geometry (for fluids with low viscosity), 3.0 ml of fluid was pipetted into the space between the double cylinders, which were then raised to within 380 μm of the upper cylinder. In either of these geometries, the rheometer applied a fixed torque to the movable cylinder or cone. This torque was proportional to the shear stress applied to the fluid. The rheometer measured the steady-state angular velocity of the movable cylinder or cone. This angular velocity was proportional to strain rate. The software performed these computations, and output the shear stress and strain rate.

2.3.2 Calibration

The rheometer was calibrated daily to ensure accurate viscosity measurement. Calibration was performed by comparing the measured viscosity of Cannon Certified Viscosity Standard Mineral Oil to its actual value through the range of 12 Pa to 5 Pa, correcting for temperature variation. The ratio of the given value to the measured value was multiplied by all viscosity results obtained until the next calibration. The calibration typically fell within 20% of unity.

2.3.3 Finding the Relationship between Viscosity and Strain Rate

Before each test, the shear stress required to rotate the rotor at a constant strain rate of 500 s^{-1}, which corresponded to half the maximum strain rate for the given geometry, was measured. This shear stress was used for the first calculation of viscosity. Strain rate as a function of shear stress was determined using a stepped ramp sweep decreasing logarithmically over two decades of shear stress from the initial point. Because the rheometer inputs shear stress, not strain rate, a stress-dependent test was deemed appropriate despite the eventual analysis of viscosity as a function of strain rate.

For each of ten steps in the first decade, the mean strain rate was measured over intervals of twenty seconds until the measured mean strain rates two consecutive intervals agreed to within one percent. For each step in the second decade, the mean strain rate was measured over forty seconds until two consecutive intervals agreed to within three percent. The measurements continued in this fashion until reaching the minimum strain rate measurable on the rheometer. Typically, it was possible to evaluate strain rate over 1.5 to 2 decades of shear stress for each joint fluid sample. The range for which viscosity could be measured in hyaluronic acid was much larger.

2.3.4 Measurement of Storage and Loss Moduli

In order to measure linear viscoelasticity, storage and loss moduli were measured for 24 synovial fluid samples obtained at the time of total knee arthroplasty, four samples at revision, and three of each of the hyaluronic acid samples. These tests were performed on joint fluid whenever sufficient fluid could be collected to run both flow and oscillation tests. During each test, the strain response to a small, sinusoidal shear stress was measured for twenty-five frequencies between 25 Hz and 0.25 Hz. For sufficiently small strains, the output was a sine wave of different phase and amplitude than the input. The portion of the strain in phase with the stress input related to the elastic character of the fluid sample, and was converted into G' [Pa], the storage modulus. The portion of the strain out of phase with stress related to the viscous character of the fluid sample, and was converted into G" [Pa], the loss modulus. These parameters described the relative importance of elasticity and viscosity in small amplitude, high velocity or oscillatory motion. These viscoelastic properties were measured for four different torque (shear stress) inputs: 25, 50, 100, and 200 μNm. From these curves, single plots of the storage and loss moduli as functions of frequency were compiled when possible. To compare samples, the viscoelastic crossover frequency and modulus at crossover were calculated when possible. The protocol for determining a single graph of moduli versus frequency given four curves is given in Appendix C.

2.3.5 The Relationship between Temperature and Viscosity

Because water evaporates at higher temperatures, leaving more concentrated (and more viscous) solution behind, it was not possible to run these tests at body temperature, 37°C. Instead, the above experiments were performed at 25°C. The dependence of viscosity on temperature was measured for five synovial fluid samples. Viscosity was measured continuously at a constant stress of 1 Pa, while the temperature rose from 25°C to 40°C at a rate of 1°C every four seconds.

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2.5 Methods of Analysis

It was expected that fluids containing HA would be non-Newtonian; that is, viscosity would vary with strain rate. From previous work on synovial fluid, shear-thinning behavior was expected. Within the range measured in this study, it was expected that a region would be found at low shear in which the viscosity did not vary with strain rate. Parameters would have to be chosen to consider this study's results in the context of previous work.

2.5.1 The Cross Model

Parameters were chosen using the Cross model of shear thinning to represent each viscosity versus strain rate curve for comparative purposes. In the Cross model, strain rate ($\dot{\gamma}$) and viscosity (η) are related by Equation 1, shown below.

$$\frac{\eta - \eta_\infty}{\eta_0 - \eta_\infty} = \frac{1}{1 + (c \cdot \dot{\gamma})^d}$$ (Equation 1)

where η_0 is viscosity at low strain rate;

η is viscosity at high strain rate;

c is the consistency, which is related to the longest relaxation time; and

d is the rate index, a dimensionless variable that is the negative slope on a logarithmic scale of the shear-thinning region.

This model can be used to characterize fluid samples. η_0 is a particularly useful parameter because it can be used to compare with samples in other work on synovial fluid that did not use the same model. In particular, several authors have measured viscosity as a function of strain rate in synovial fluid characterized as "normal," "degenerative," or "chronically inflamed" (Schurz, 1996; Swann, 1978; Cooke, 1978). The ranges for η_0 established for these categories are shown in Table 1. These ranges are not fixed, however, and do not represent pathognomonic or diagnostic tools.

2.5.2 η_{1Pa} as a Means of Comparison

A second method of comparison, the viscosity at 1 Pa shear stress (η_{1Pa}), was also used as a comparative tool among samples. This value of shear stress, while lower than the peak shear stresses generated in the prosthetic joint, correspond to a shear stress at which fluid-film lubrication is likely to occur *in vivo*, and thus a shear stress at which viscosity is relevant. Furthermore, it was possible to measure η_{1Pa} for all samples, making it an easy parameter to use to compare samples. The ranges for η_{1Pa} have also been established (Schurz, 1996; Swann, 1978; Cooke, 1978), and are shown in Table 1.

Table 1: Viscous parameters for physiologic and pathologic synovial fluid in [Pa·s]. Most fluids obtained from joints categorized as "normal," "osteoarthritic," or "chronically inflamed" exhibit viscous parameters in the given range.

Parameter	Normal	Osteoarthritic	Chronically Inflamed
η_{1Pa}	2-10	0.05-2	0.003-0.02
η_0	6-12	0.1-1	0.005-0.05

2.5.3 Non-parametric comparisons

Although the sample sizes were chosen based upon the assumption of a Gaussian distribution of results, it quickly became evident that no such distribution was forthcoming. Consequently, median and range, rather than mean and standard deviation, were used to compare these fluids. In order to determine statistically significant difference, the Mann-Whitney test was used. Simple regression was used to try to correlate calculated parameters. Finally, Fisher's exact test was used to demonstrate differences between groups.

CHAPTER 3:   RESULTS

3.1   Description of Samples

Between one and twenty-two milliliters of joint fluid were obtained from the knees. Although these quantities may not have been representative of the amount of fluid in the joint capsule, surgeons were instructed to obtain at least six milliliters when possible. Consequently, the range of quantities obtained likely represents the variability of fluid quantity within the joint capsule.

All samples were transparent, with slight to strong yellow tint. Seven of the 70 samples had a pink tint. This coloration was different from the opaque red layer or crimson color characteristic of blood contamination, which occurred in five samples. Some samples contained up to a few tenths of a milliliter of white particles, no larger than a hundred microns long, which appeared to have precipitated from solution after surgery. In some cases, a small amount of precipitation occurred over time during storage. When measuring the properties of joint fluid samples, care was taken to exclude particles from the fraction evaluated because inclusion of particles could affect the measured viscosity. These particles were not analyzed quantitatively.

Each sample's relative viscosity at low strain rate was evident immediately upon physical examination. By tilting the test tube slowly, the flow properties of a sample could be quantitatively evaluated. This physical finding was used as a means of checking the measurement of the rheometer.

All information, observations, and measurements obtained for these samples are given in Appendices A and B.

3.2   Viscosity Parameters

3.2.1   Joint Fluids

The relationship between shear stress and strain rate was described using viscosity as a function of strain rate. Figure 1 shows three viscosity-strain rate curves obtained from patients at TKA. These curves are typical of all joint fluid samples, in that, at low strain rate, they exhibit a plateau viscosity (η_0), but at high strain rate, the samples exhibit shear-thinning. Although each joint fluid curve exhibited the same characteristic shape, the viscosity varied over three orders of magnitude. Fifteen percent of the samples fit the normal range of viscosity for healthy synovial fluid. The other 85% exhibited degenerated viscosity in the range established for disease synovial fluid at least at some strain rates. Table 2 shows more specifically in what category each joint fluid sample was categorized, using the parameters η_0 and η_{1Pa}.

Figure 1: Viscosity-strain rate curve for three selected joint fluid sample obtained at TKA. Note the region of zero-shear plateau at low strain rates, accompanied by a region of shear-thinning at higher strain rates. These three samples were representative of the samples obtained at TKA. The most viscous sample of the three (squares) was close to the most viscous of those obtained at TKA. The least viscous of the three (triangles) was near the least viscous sample obtained at TKA. The third sample (diamonds) had close to the median viscosity of the samples obtained at TKA.
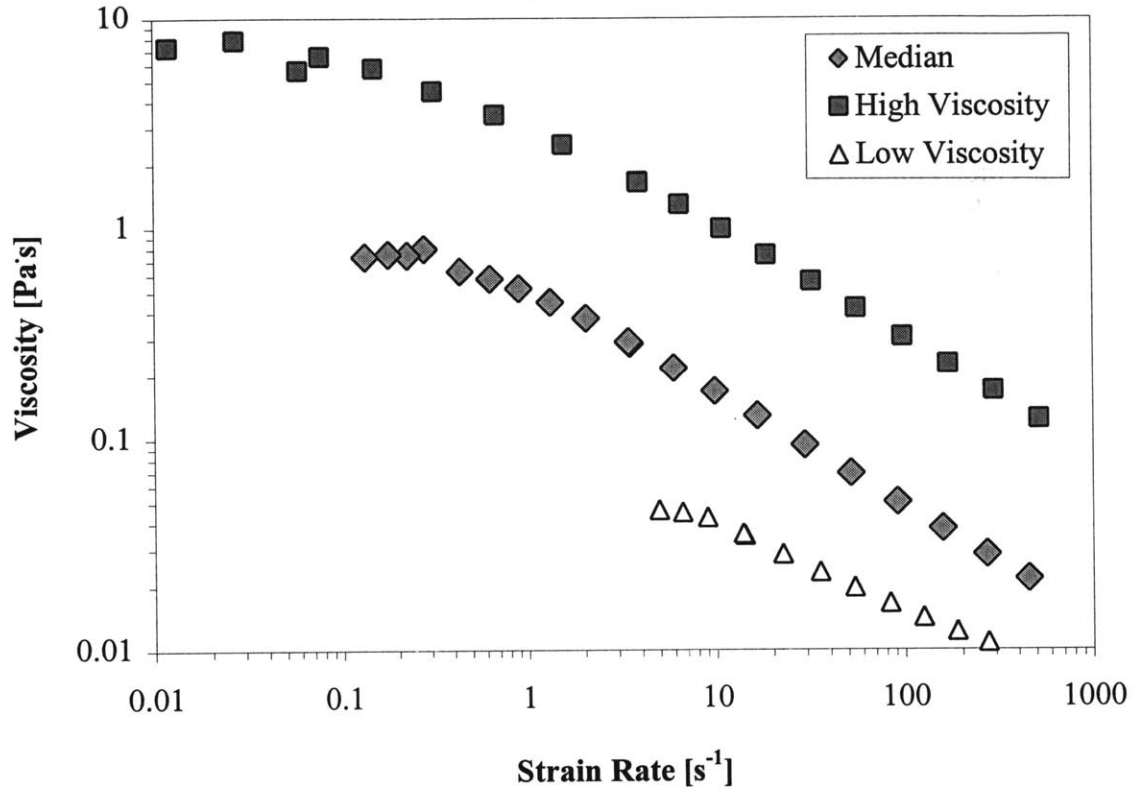
The Cross model was chosen to fit the curves despite the absence of a high shear plateau because it was expected that, at high strain rates, joint fluid viscosity would not drop below that of water. Furthermore, recent work examining the viscosity of synovial fluid at high shear rates has shown a plateau at high strain rates. (Yao, 2001) The Cross model described the viscosity-strain rate curve for all joint fluid and hyaluronic acid samples ($R^2 > 0.87$ in all cases) despite the absence of a high shear plateau. Since all curves exhibited a zero shear plateau and a region of shear-thinning, the parameters η_0, consistency, and rate index were considered useful for comparing results. The parameter η was not included in the analysis because the range of strain rates did not extend high enough to reach a high shear plateau and because fluid-film lubrication is not likely to occur at high strain rates, where η becomes relevant.

As was the case with the viscosity-stress curves, η_{1Pa} varied over a wide range for the joint fluids. A histogram showing the spread of these data is shown in Figure 2. These data do not form a Gaussian distribution, since most data points fall within the lowest part of the range. Therefore, any comparison involving the entire group of samples obtained at TKA cannot employ mean and standard deviation as comparative parameters. Instead, the median and range for each group are calculated in Table 2.

Figure 2: Histogram showing distribution of values of η_{1Pa} within the group of fluids obtained at TKA. Note that 76% of samples fell in the lowest part of the range. These data do not form a Gaussian distribution.
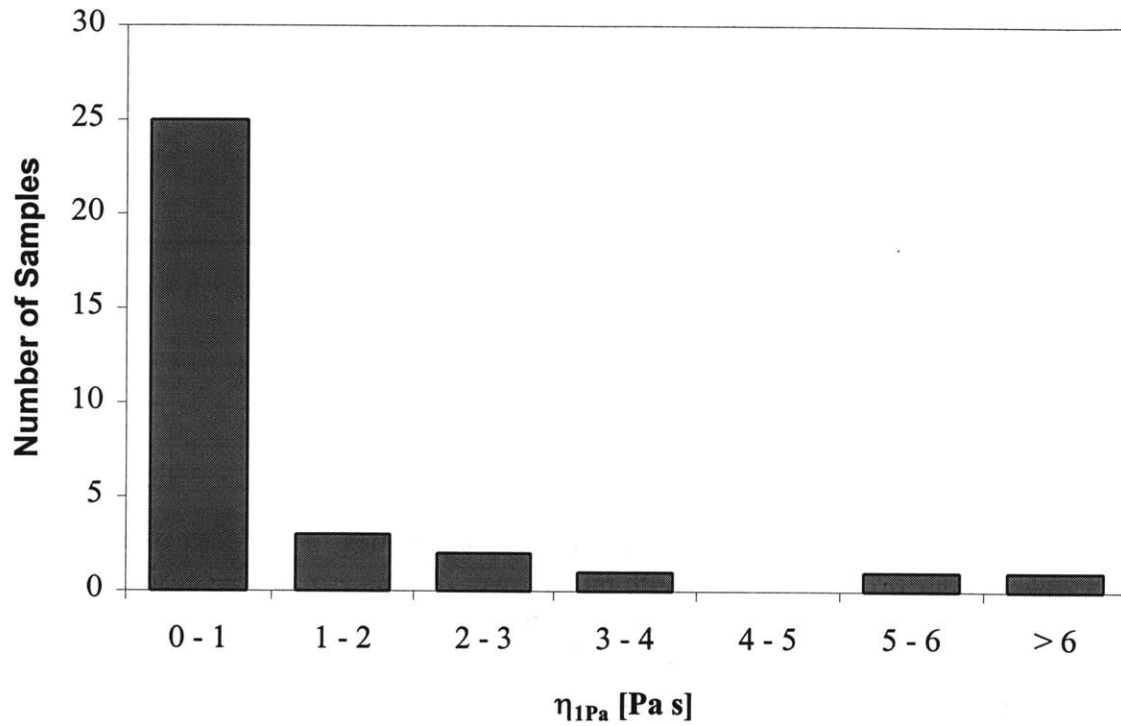
Table 2: Median and range of viscous parameters from several sample groups.

Group	η_{1Pa} [Pa·s]	η_0 [Pa·s]	consistency [s]	rate index
TKA median (n=33)	0.26	1.2	2.9	0.60
TKA range	0.009-11	0.08-24	0.05-23	0.36-0.76
Revision median (n=7)	0.17	0.59	2.5	0.59
Revision range	0.02-0.77	0.14-3.6	0.32-7.5	0.53-0.62
Effusion median (n=5)	0.11	0.61	1.5	0.55
Effusion Range	0.04-0.18	0.26-0.63	0.53-5.0	0.44-0.58
Effusion after TKA (n=1)	0.18	2.7	38	0.48
Supartz	3.0	3.1	0.055	0.80
Orthovisc	37	39	1.0	0.71
Bovine Serum	0.0015	N/A	N/A	N/A

Joint fluid samples obtained from revision surgery exhibited the same characteristic shear-thinning curve as those samples obtained at primary TKA. Although these samples exhibited a wide range of viscosity, like those obtained at primary TKA, this range did not extend as high as the bottom of the normal range established for healthy synovial fluid. (One sample from this group was placed in with joint fluids of normal η_0 because its viscosity at low strain rate more closely approached the normal group than the diseased group, but at 1 Pa, its viscosity fit the degenerative range.)

Comparison of η_{1Pa} and η_0 for each group (primary TKA, revision of TKA, and effusion) to normal, degenerative, and chronically inflamed knees is given in Table 3.

Table 3: Grouping of samples into three categories for two viscous parameters. All values are given in Pa·s.

Group	Parameter	Normal	Degenerative	Chronic, Inflamed	Total
Established Range	η_{1Pa}	2-10	0.05-2	0.003-0.02	
	η_0	4-20	0.1-1	0.005-0.05	
TKA	η_{1Pa}	5	24	4	33
	η_0	10	23	0	
Revision	η_{1Pa}	0	6	1	7
	η_0	1	6	0	
Effusion	η_{1Pa}	0	5	0	5
	η_0	0	5	0	

As a consequence of the smaller range of viscosities in the case of revision of TKA, the median η_{1Pa} and η_0 were both much lower for this group. Because of the wide range found in both groups, however, the Mann-Whitney test could not demonstrate a statistically significant difference between them ($p = 0.56$ for η_{1Pa}, $p = 0.33$ for η_0).

Joint fluid obtained at aspiration from patients with effusion exhibited the same characteristic shear-thinning as the other groups of joint fluid. Like the fluid obtained at revision surgery, the range of viscosities was smaller than that found in joint fluid at TKA, and did not extend to the established normal range. Tables 2 and 3 summarize the results from these joint fluids. All fluids obtained from natural knees during aspiration subsequent to effusion fit in the degenerative range established previously.

Very little connection was found between parameters that could have been relevant indicators of joint viscosity. For example, no correlation could be found between any viscous parameter and age, gender, or involved leg. Moreover, there was no correlation between the viscosity and the volume of joint fluid. Using regression analysis, it was not possible to correlate η_0 to either consistency of rate index in any group.

In the case of effusion subsequent to TKA, the viscous parameters could not be fit easily into any of the other groups. Its zero-shear viscosity was normal for synovial fluid, but shear-thinning began at much lower shear stress, as evidenced by its high consistency. The onset of shear-thinning at low strain rate made η_{1Pa} relatively low. This sample was not included in any of the three groups for analysis.

In two cases, fluid was taken from each knee during bilateral TKA. For these two cases, right and left knees were compared. In both cases, the two knees had very different viscous properties. These properties are shown in Table 4.

Table 4: Two examples comparing joint fluid between knees in the same patient. Viscosities are given in Pa.s. Consistency is given in seconds.

	Leg	η_{1Pa}	η_0	consistency	rate index
52 y.o.	Right	0.443	1.570	1.817	0.672
female	Left	1.694	5.555	5.602	0.710
68 y.o.	Right	0.052	0.114	0.080	0.674
female	Left	0.407	1.585	2.127	0.606

3.2.2 Hyaluronic Acid Joint Supplements

Both HA preparations demonstrated shear-thinning within the range of the tests. Like those of joint fluid samples, these viscosity-strain rate curves could be described using the Cross model. The mean viscous parameters for both HA preparations at 25°C, as determined by the Cross model, are given in Table 2. Supartz was found to be among the more viscous fluids studied, and fit in the normal range found for synovial fluids at low strain rate. Supartz was more viscous than most joint fluids studied. Orthovisc was twelve times as viscous as Supartz at zero shear and at 1 Pa, and was more viscous than any joint fluid sample studied.

Supartz was found to decrease in viscosity at higher temperature. Figure 3 shows the viscosity-strain rate curve for Supartz at 25°C and 37°C.

Figure 3: Viscosity-strain rate curve for Supartz joint supplement at two temperatures. The supplement was much more viscous at the lower temperature than the higher temperature. This behavior was found in all fluids tested.

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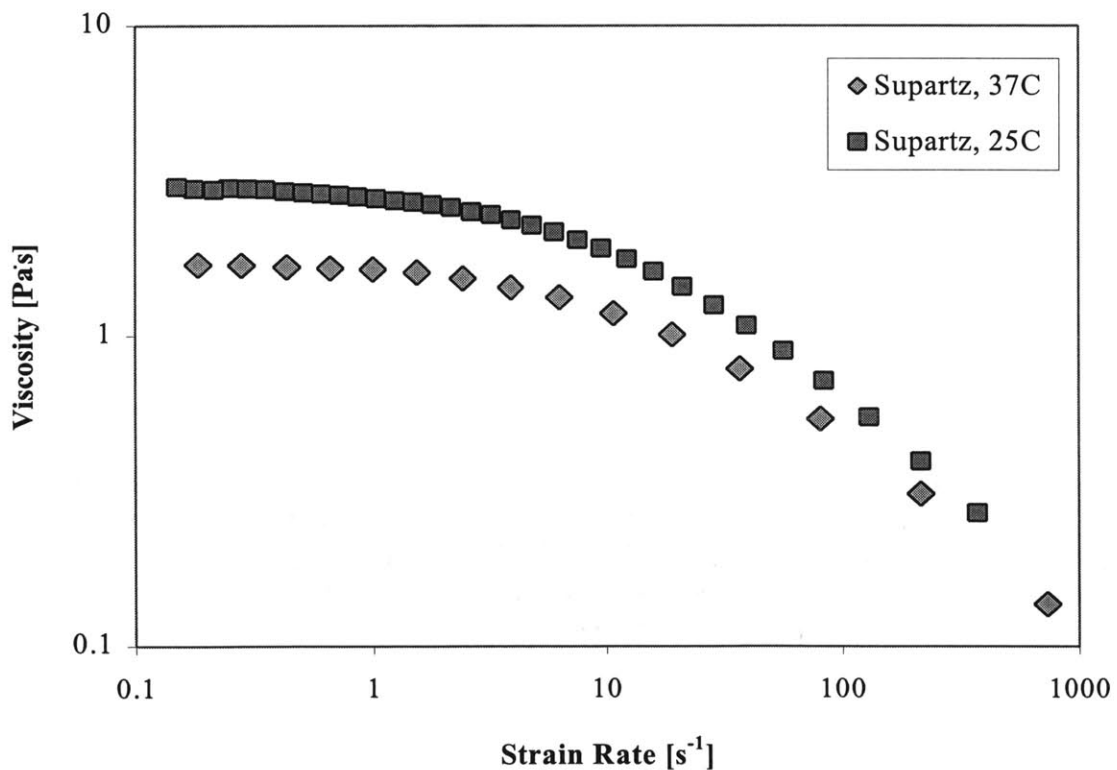
3.2.3 Bovine Serum

The viscosity-strain rate relationship for bovine serum did not fit the Cross model in the range of strain rates studied. Therefore, the parameters used to compare other fluids could not be used for bovine serum. As demonstrated in Figure 4, bovine serum did exhibit shear-thinning in the test range, but it was not possible to reach the low-shear plateau region using the available experimental setup. Consequently, η_{1Pa} has been used to compare bovine serum to other fluids in this study. Bovine serum was a factor of seven less viscous than any joint fluid sample tested in this study, and would fit in the low viscosity end of the chronically inflamed range for joint fluids. Bovine serum is 1000 times less viscous than the low viscosity end of normal for joint fluids.

Figure 4: Viscosity-strain rate curve for bovine serum. Although some shear-thinning was evident, no plateau could be measured at low strain rate. Bovine serum was one thousand times less viscous than healthy joint fluid.
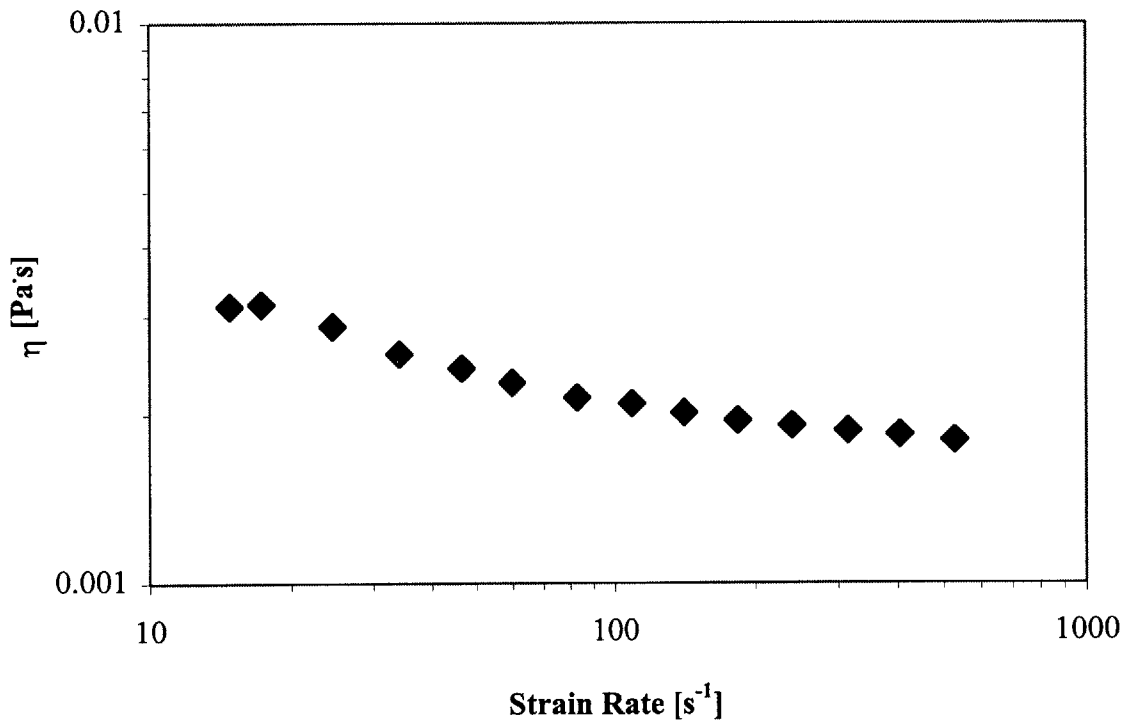
3.3 Viscoelastic Parameters

The viscoelastic curves for joint fluid samples for which storage and loss moduli could be measured had a characteristic shape, as shown in Figure 5. At low frequency, loss modulus exceeded storage modulus. As the frequency increased, the storage modulus increased more than loss modulus, so that at high frequency, storage modulus was more significant. The crossover frequency, that frequency at which the storage and loss moduli were equal, has been used to characterize the relative importance of elastic and viscous effects in a particular fluid. It was possible to measure crossover in 10 of 24 joint fluid samples obtained at arthroplasty and three of four joint fluid samples obtained

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at revision. In the other fifteen samples, the storage modulus was not sufficient, even at high frequency, to measure a crossover.

Both HA joint supplements exhibited viscoelasticity. Because it was possible to repeatably obtain the same moduli within 5% of the mean for these fluids, only the mean is given. Bovine serum did not exhibit sufficient elasticity to measure storage and loss moduli.

Figure 5: Storage and loss moduli as functions of frequency for one joint fluid sample. Crossover occured between the frequency of walking and the frequency of running.
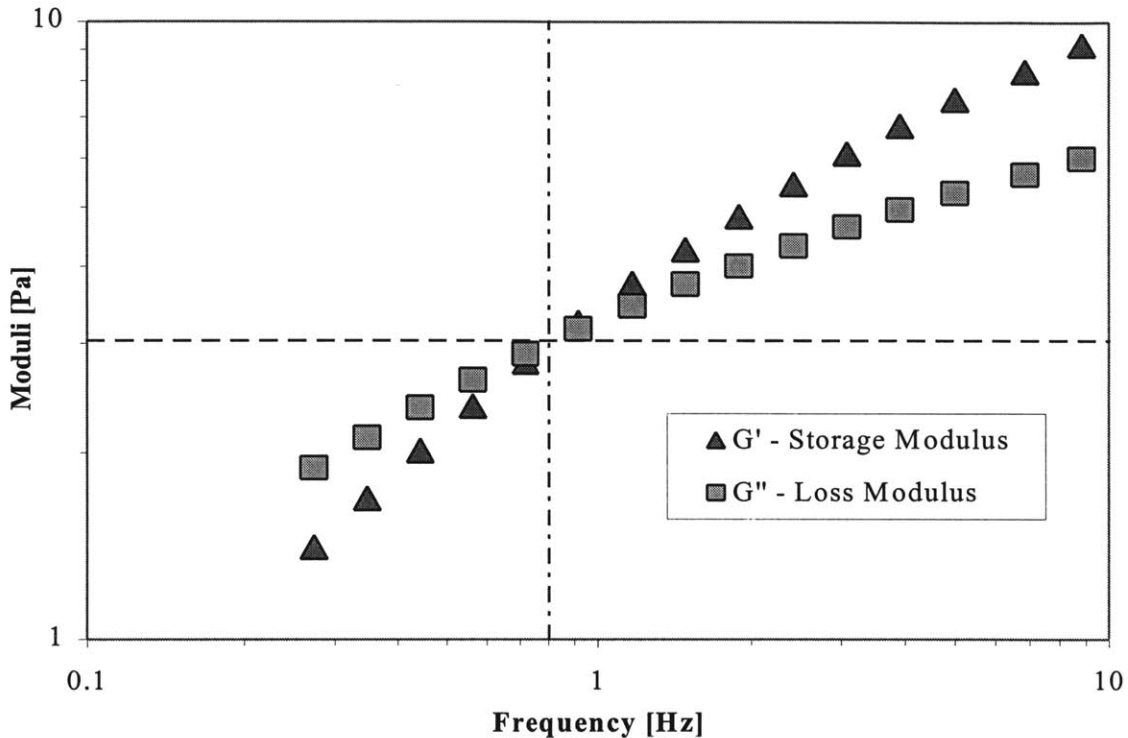
Table 5: Crossover frequency and modulus at crossover for joint fluid samples and joint supplements. Crossover occurred within or near the range of frequencies encountered in normal knee movement for all joint fluids.

Group		Crossover Frequency [Hz]	Modulus at Crossover [Pa]
TKA	Median	0.80	1.35
n=10	Range	0.19-5.54	0.85-2.54
Revision	Median	3.96	1.58
n=3	Range	2.83-6.23	1.56-2.14
Supartz	Mean	10.59	39.04
Orthovisc	Mean	0.83	37.93

3.4 The Relationship Between Temperature and Viscosity

Difficulties were encountered in measuring the viscosity of joint fluid at body temperature. Consequently, all fluids were evaluated at 25°C. Six synovial fluid samples obtained at TKA were evaluated at 1 Pa shear stress continuously as temperature was

increased from 25°C to 40°C. Viscosity related to temperature via the Arrhenius equation, which is given below as Equation 2. In this equation, viscosity increases exponentially with inverse temperature. Figure 6 shows a representative experiment performed on joint fluid obtained at TKA. Values obtained for the constants for various samples of joint fluid can be found in Appendix D.

$$\eta = Ae^{-B/T}$$ (Equation 2)

Figure 6: Graph of natural log of viscosity versus inverse temperature for one joint fluid sample. A linear relationship between the two, as described by Equation 2, is evident.
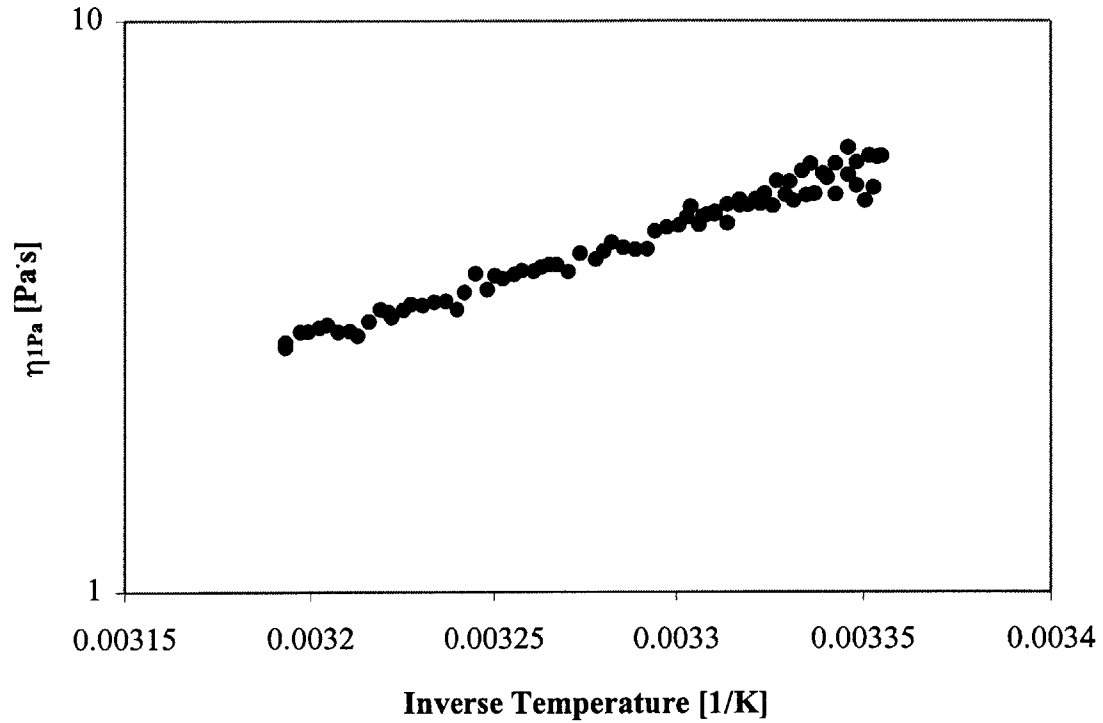
CHAPTER 4:   DISCUSSION

4.1   Properties of Joint Fluid at the Time of TKA

4.1.1   Viscous Parameters

All flow data confirmed the same families of curves found in studies of joint fluid from other types of patients. All joint fluid samples exhibited shear-thinning, as did all HA joint supplements. Furthermore, these groups all exhibited a plateau region in which viscosity was independent of shear rate. In joint fluid, this type of curve indicates a solution containing a network of large HA molecules sufficiently concentrated to interlock and resist relative motion, especially at low strain rates. At higher strain rates, the network becomes less entangled, and loses its structure. Others have shown previously that HA must exist in concentrations greater than 1 mg/ml (Schurz 1987) and molecular weights greater than 10^5 Da (Ambrosio 1999) to create such a network. This finding supports published HA concentrations (Gomez 1993) and molecular weights (Bjelle 1982) in degenerative knees, and further connects low viscosity with degenerative joint disease. Moreover, the least viscous joint fluid samples exhibited less shear-thinning than other joint fluid samples, a finding consistent with the supposition of low molecular weight and concentration in these joints.

The viscous parameters of synovial fluid taken at primary total knee arthroplasty spanned a remarkably wide range, spanning three orders of magnitude. Five specimens (15%) matched best the normal range established for both η_{1Pa} and η_0, but twenty-three samples (70%) fit in the diseased or inflamed range for both η_0 and η_{1Pa}. This finding suggests that alteration of the properties of joint fluid is frequently associated with the pathology of patients undergoing total knee arthroplasty. Furthermore, the variability in synovial fluid viscosity, coupled with variation in prosthetic wear rates observed *in vivo* (Schmalzried 1999), raises the question of the importance of fluid film lubrication in the tribology of prosthetic joints and, in particular, the connection between viscosity and wear. This issue warrants the study of wear test lubricants with different flow properties to determine the effect of viscosity on wear rates in total knee arthroplasty.

Although many of the joint fluid specimens obtained at arthroplasty were shown to have abnormal properties, no correlation could be drawn among specific flow parameters (*i.e.*, η_0, consistency, and rate index). Since these parameters relate to average hyaluronic acid molecular weight, relative distribution of hyaluronic acid molecular weight, and hyaluronic acid concentration, this result implies that these parameters can vary independently.

4.1.2   Quantity of Fluid in the Joint

Notably, in nine of fifty-thee of the patients undergoing TKA, less than three milliliters of joint fluid could be removed for evaluation. This raises the question of the role of joint fluid volume in the wear of TKA prostheses. Even though all fluid present in the knee could not be removed, it is unlikely that the amount remaining in the capsule exceeded one milliliter. No work has yet been conducted to correlate fluid volume to the tribology of total knee arthroplasty, though a strong connection is recognized in other (non-medical) articulations.

4.1.3 Bilateral TKA

Even in the same patient, the viscosity of synovial fluid differed between the left and right legs. This result suggests local disease, rather than some systemic disorder, has altered the properties of the joint fluid. It would be worthwhile to examine patients longitudinally, to examine how synovial fluid changes over time within a patient, particularly in the course of the disease.

4.1.4 Linear Viscoelastic Parameters

Viscoelastic properties of joint fluids, as well as viscosity, could play a role in the tribology of total knee arthroplasty. Consequently, viscoelastic parameters were also evaluated in conjunction with viscosity, as has been done previously (Gomez 1993; Anadere 1969). Among those samples for which crossover could be measured, crossover occurred within the range of frequencies encountered by the knee *in vivo*. The frequency of knee motion is approximately 0.5 Hz in walking and 2.5 Hz in running. Although crossover did not occur within this range for all samples, both storage and loss moduli were of the same order of magnitude throughout this range. Consequently, both storage and loss are important parameters within the *in vivo* range. In one study, crossover for joint fluid from a young adult patient's knee joint with HA concentration 2.38 mg/ml was reported at 0.1 Hz, with elastic modulus substantially higher at *in vivo* frequencies (Weiss 1999). The finding that crossover frequency varies inversely with molecular weight (Kobayashi 1994) suggests that the fluids evaluated here are of lower molecular weight than those previously reported. The lower modulus at crossover found in these samples further supports the notion that joint fluid at arthroplasty may contain HA in lower concentrations than that in the healthy knee. The lower storage modulus at frequencies encountered *in vivo* implies that energy storage as a method of load bearing may be compromised in these knees undergoing TKA.

In fifteen of twenty-eight cases (54%), storage modulus was too low to measure crossover. In each of these cases, η_0 and η_{1Pa} placed the sample in the diseased (n = 12) or diseased and inflamed (n = 3) range. In contrast, all five fluids for which η_0 fell within the normal range for synovial fluid were sufficiently elastic to measure crossover. The other eight samples in which crossover could be measured had viscosities within the degenerative range. Using Fisher's exact test, normal η_0 correlated to high viscoelasticity (p = 0.013). Because of this strong correlation between these parameters, it is not necessary to examine the effects of viscosity and viscoelasticity separately in wear tests even though storage and loss may both be important *in vivo*.

4.2 Properties of Joint Fluid after Total Knee Arthroplasty

All seven samples of joint fluid obtained at revision surgery had viscosities within or just outside the range of diseased synovial fluid found by others. Unlike joint fluid at total knee arthroplasty, however, none of the samples obtained at revision arthroplasty were as viscous as healthy synovial fluid. This finding suggests that joint fluid in TKA differs from normal synovial fluid, and supports the finding in an animal model that hyaluronic acid concentration did not return to normal values after arthroplasty (Delecrin 1994). This finding further supports a study conducted in hip replacements that found fluid in the replacement joint to be different in composition from that of the natural joint. In particular, hyaluronic acid concentration in the replacement hip was different from that

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found in the natural osteoarthritic hip (Saari 1993). Additional studies of joint fluid in patients after total knee arthroplasty are needed to quantify the difference in joint fluid between natural and prosthetic knees.

Consistency and rate index, which give an indication of concentration and molecular weight distribution of HA in joint fluid, could not be well correlated in any of the groups. Although a larger range existed for joint fluid at TKA than for joint fluid at revision, the disparity may be due to the greater number of samples obtained at primary arthroplasty. The median for both consistency and rate index was very close in the two groups, suggesting that both groups are likely to have the same range of molecular weights.

4.3   Effused Synovial Fluid

The effusion samples spanned a smaller viscous range than either of the other two joint fluid groups. In each case, viscosity was at least a factor of six below the lower limit of the normal range. This suggests that patients with effusion may be at increased risk of altered joint tribology and possibly greater wear of articular cartilage. Others have shown that lubrication of natural joints depends on synovial fluid components independent of viscosity (Radin 1970), so other alterations in synovial fluid may be more significant.

The fluid aspirated for effusion had a lower consistency than either of the TKA groups, but the range of each group prevented the difference from being statistically significant. There was no difference in rate index for either of these groups. Consequently, it seems that HA concentration, which affects η_0, and not molecular weight distribution of HA, which affects consistency and rate index as well, is deficient in the case of effusion.

In the single case of effusion subsequent to total knee arthroplasty, the viscous parameters could not be fit easily into any of the other groups. Its zero-shear viscosity was normal for synovial fluid, but shear-thinning began at much lower shear stress (high consistency), making η_{1Pa} relatively low. These abnormalities suggest differences between the composition and possibly development of effusion in synovial fluid and effusion in this particular case of knee arthroplasty.

4.4   Synthetic Hyaluronic Acid Preparations

As expected, the hyaluronic acid preparations were substantially more viscous than the joint fluid samples. *Orthovisc* was ten times more viscous than *Supartz* primarily due to its higher molecular weight and concentration. Furthermore, the molecular weight of *Orthovisc*, which also exceeded that of *Supartz*, explains its higher consistency. That the consistency of *Supartz* was less than that of normal joint fluid samples correlates with its low mean molecular weight. *Orthovisc*, having a mean molecular weight of 1.3×10^6 Da, had a consistency greater than that of many joint fluids. This, too, confirms the range of molecular weights of HA in joint fluid at TKA. Finally, rate index was closer to unity in both preparations than in most joint fluid samples. This result implies that the molecular weight of HA encountered in these joints is distributed over a larger range than is produced in the joint supplements.

4.5   Bovine Serum

Bovine serum was close to an order of magnitude less viscous than the least viscous joint fluids, and over 1000 times less viscous than the normal range of synovial fluid. If viscosity affects wear at the strain rates encountered in the replacement joint, then bovine serum cannot mimic the *in vivo* environment in lubricating metal on polyethylene. A lubricant should be used that has all relevant tribological properties and components in common with joint fluid. This finding warrants further study into the relative importance of fluid film lubrication on tribology of these components, and specifically the effect of viscosity on wear.

Bovine serum did not fit the model of shear-thinning with a low-shear plateau found for other fluids. Instead, a high shear plateau occurred. It is likely that, for bovine serum, any molecular interaction occurs between protein molecules thousands of times smaller than HA. Consequently, shear-thinning occurs at much lower shear rates. It is not clear how high bovine serum's viscosity becomes at low shear rate. Furthermore, it is not clear whether these shear rates are encountered in laboratory wear tests.

Since the joint fluid supplements tended to be more viscous than the joint fluid samples, the addition of hyaluronic acid to bovine serum could provide a mixture whose bulk flow properties more closely mimic the *in vivo* environment. Since endogenous hyaluronic acid imparts to joint fluid its viscosity (Swann 1974), these supplements would mimic the *in vivo* environment chemically as well as rheologically, and could therefore be a more appropriate mixture for use in wear tests. To truly mimic *in vivo* flow properties over a large range of shear rates, however, one would have to match not only HA concentration, but molecular weight.

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CHAPTER 5:   CONCLUSIONS

This study examined the five hypotheses outlined in Chapter 1. With regard to those hypotheses, we have found that:

1) viscosity of joint fluid obtained at arthroplasty varies widely;

2) the difference between viscosity of joint fluid obtained at revision and viscosity of joint fluid obtained at primary TKA is overwhelmed by the variability within the groups;

3) the difference between viscosity of joint fluid obtained at aspiration subsequent to effusion and viscosity of joint fluid obtained at primary TKA is overwhelmed by the variability within the groups

4) lubricants used currently in laboratory wear tests do not mimic the viscous properties of joint fluid.;

5) HA of appropriate concentration and molecular weight does appear to closely approximate the viscosity encountered in joint fluid.

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APPENDIX A: PATIENT INFORMATION

#	Study ID #	Notebook page	Gender	Age	Left or Right	Indication	Date Aspirated
1	143	140-80	Female	89	Right	Osteoarthritis	10/18/2000
2	145	140-82	Female	88	Left	Osteoarthritis	10/18/2000
3	033	140-62, N35	Female	88	Right	Osteoarthritis	9/22/2000
4	142	140-80	Female	88	Left	Osteoarthritis	10/24/2000
5	006	127-94,96,99	Female	86	Right	Osteoarthritis	6/20/2000
6	032	140-60	Female	85	Left	Osteoarthritis	9/25/2000
7	044	140-63, N32	Female	83	Right	Osteoarthritis	10/11/2000
8	007	140-05,8, N9	Female	83	Left	Osteoarthritis	6/30/2000
9	028	140-55	Female	79	Left	Osteoarthritis	9/19/2000
10	035	140-62, N34	Male	78	Right	Osteoarthritis	10/2/2000
11	042	140-63, N33	Female	78	Right	Osteoarthritis	10/10/2000
12	009	140-06,8,N11	Male	76	Left	Osteoarthritis	7/11/2000
13	146	140-83	Female	73	Left	Osteoarthritis	10/18/2000
14	159	140-88	Female	71	Right	Osteoarthritis	11/6/2000
15	011	140-10, N15	Female	71	Left	Osteoarthritis	7/18/2000
16	010	140-10, N19	Female	70	Left	Osteoarthritis	7/17/2000
17	040	140-63	Female	70	Right	Osteoarthritis	10/2/2000
18	025	140-52, N23-5	Female	70	Right	Osteoarthritis	9/11/2000
19	018	140-14, N25,29	Male	68	Right	Osteoarthritis	4/7/2000
20	156	140-87	Female	68	Left	Osteoarthritis	10/31/2000
21	155	140-87	Female	68	Right	Osteoarthritis	10/31/2000
22	151	140-84	Male	68	Left	Osteoarthritis	11/7/2000
23	147	140-84	Female	68	Right	Osteoarthritis	10/24/2000
24	149	140-84	Female	66	Right	Osteoarthritis	11/7/2000
25	031	140-60,79, N36	Female	65	Left	Osteoarthritis	9/25/2000
26	034	140-62, N31	Male	64	Right	Osteoarthritis	10/3/2000
27	045	140-63, N32	Male	63	Left	Osteoarthritis	10/11/2000
28	150	140-84	Female	62	Left	Osteoarthritis	10/17/2000
29	144	140-81	Female	58	Right	Osteoarthritis	10/30/2000
30	158	140-88	Male	54	Right	Osteoarthritis	11/6/2000
31	152	140-85	Female	52	Right	Osteoarthritis	11/1/2000
32	153	140-85	Female	52	Left	Osteoarthritis	11/1/2000
33	037	140-62,80	Female	50	Right	Osteoarthritis	10/3/2000
34	163	153-05	Female	76	Left	Unstable TKR	12/20/2000

APPENDIX A: PATIENT INFORMATION

#	Study ID #	Notebook page	Gender	Age	Left or Right	Indication	Date Aspirated
35	019	140-21, N21,27	Male	72	Left	Osteoartritis after TKR	7/31/2000
36	047	N33	Female	70	Left	Unstable TKR	10/25/2000
37	161	140-87	Female	69	Right	Synovitis, wear after TKR	11/28/2000
38	165	153-14	Female	67	Left		2/6/2001
39	003	127-94	Female	66	Left	Failed Left TKA	6/26/2000
40	160	140-87	Female	61	Right	Wear, Osteolysis after TKR	11/29/2000
41	030	140-57	Male	69	Left	Effusion Subsequent to TKR	9/19/2000
42	013	140-11, N17	Unknown	0	Unknown	Effusion	12/29/1999
43	012	140-11, N17	Unknown	0	Unknown	Effusion	12/29/1999
44	015	140-11, N20	Unknown	0	Unknown	Effusion	12/29/1999
45	048	N20	Unknown	0	Unknown	Effusion	12/29/1999
46	016	140-11	Unknown	0	Unknown	Effusion	12/29/1999
47	005	N6-8	Male	81	Right	Osteoarthritis	6/30/2000
48	027	140-55	Male	79	Left	Osteoarthritis	9/18/2000
49	141	140-80	Female	78	Left	Osteoarthritis	10/24/2000
50	157	N/A	Female	77	Left	Osteoarthritis	11/6/2000
51	026	140-55	Female	74	Left	Osteoarthritis	9/15/2000
52	162	140-88	Male	73	Left	Wear, Osteolysis after TKR	12/4/2000
53	029	140-57, N27-8	Female	73		Osteoarthritis	9/19/2000
54	038	140-63	Female	73	Right	Osteoarthritis	9/29/2000
55	043	140-63	Male	72	Right	Osteoarthritis	10/10/2000
56	023	140-53	Male	72	Right	Failed TKR	9/6/2000
57	024	140-53, N29	Female	72	Right	Osteoarthritis	9/11/2000
58	039	140-63	Female	72	Right	Osteoarthritis	10/2/2000
59	041	140-63	Female	72	Right	Osteoarthritis	10/4/2000
60	164	153-05	Male	69	Left	Polyethylene Wear	12/19/2000
61	008	140-05	Female	68	Right	?	7/10/2000
62	036	140-62	Female	68	Left	Osteoarthritis	10/4/2000
63	154	140-86	Male	64	Right	Osteoarthritis	10/26/2000
64	021	?	Female	64	Left	Osteoarthritis	11/18/1999
65	020	140-33	Male	63	Right	Osteoarthritis	8/14/2000
66	148	140-84	Female	63	Right	Osteoarthritis	10/18/2000
67	022	140-37	Female	59	Right	Osteoarthritis	8/18/2000
68	014	140-11	Unknown	0	Unknown	Effusion	12/29/1999
69	017	140-21	Unknown	0	Unknown	Effusion	12/29/1999
70	046	N36	Unknown	0	Unknown		

APPENDIX A: PATIENT INFORMATION

#	Occasion	Surgeon	Rheologist	Date	Calibration	Quantity	Description
1	TKR	Scott	DCM	11/30/2000	0.861	4.0 mL	Normal
2	TKR	Scott	DCM	11/30/2000	0.861	7.0 mL	Normal
3	TKR	Brick	NER	10/29/2000	0.860		Orange-yellow, with precipitates
4	TKR	Scott	DCM	11/29/2000	0.826	6.0 mL	Very Viscous
5	TKR	Wright	DCM	6/30/2000	1.000	7.0 mL	slightly red, stringy
6	TKR	Minor	NER	10/26/2000	0.860		Yellow, clear, chalky
7	TKR	Scott	NER	10/14/2000	0.860	3.0 mL	Lots of precipitate
8	TKR	Wright	DCM	7/12/2000	1.000	8.5 mL	-
9	TKR	Scott	NER	9/22/2000	1.000		Pinkish, aggregations of RBC's present
10	TKR	Thornhill	NER	10/25/2000	0.860	3.0 mL	Yellow, clear, some precipitates
11	TKR	Scott	NER	10/14/2000	0.860	3.0 mL	Some precipitate
12	TKR	Rubash	NER	7/13/2000	1.000	7.1 mL	-
13	TKR	Scott	DCM	12/1/2000	0.827	6.0 mL	Not so viscous
14	TKR	Scott	DCM	12/5/2000	1.040	5.5 mL	Light yellow with little precipitate
15	TKR	Brick	NER	7/18/2000	1.000	9.0 mL	Clear, yellow, normal consistency
16	TKR	Scott	NER	7/17/2000	1.000	6.0 mL	-
17	TKR	Scott	DCM	11/30/2000	0.861	9.0 mL	Some Precipitate
18	TKR	Estok	DCM	9/15/2000	0.860	11.5 mL	yellow, with white particles
19	TKR	Wright	NER	9/19/2000	1.000	19.0 mL	Yellow, with no particles
20	TKR	Scott	DCM	12/5/2000	1.040	9.0 mL	Same - bilateral with 155
21	TKR	Scott	DCM	12/5/2000	1.040	7.5 mL	Some precipitate, not very viscous
22	TKR	Scott	DCM	12/4/2000	0.984	2.5 mL	-
23	TKR	Scott	DCM	12/1/2000	0.827	6.5 mL	Very Viscous
24	TKR	Scott	DCM	12/1/2000	0.827	3.5 mL	-
25	TKR	Thornhill	DCM	11/29/2000	0.826	14.0 mL	Some precipitate
26	TKR	Scott	NER	10/12/2000	0.860		Yellow, no precipitate
27	TKR	Scott	NER	10/14/2000	0.860	6.5 mL	Thick, but normal consistency
28	TKR	Scott	DCM	12/1/2000	0.827	5.5 mL	Very Very Viscous
29	TKR	Scott	DCM	11/30/2000	0.861	15.0 mL	Not so viscous
30	TKR	Scott	DCM	12/5/2000	1.040	15.0 mL	Deep yellow with some precipitate
31	TKR	Scott	DCM	12/4/2000	0.984	5.5 mL	Bilateral - see 153
32	TKR	Scott	DCM	12/4/2000	0.984	7.5 mL	Bilateral - see 152
33	TKR	Scott	DCM	11/28/2000	0.853	5.0 mL	Some precipitate
34	Revision	Scott	DCM	2/7/2001	1.095	9.0 mL	-

APPENDIX A: PATIENT INFORMATION

#	Occasion	Surgeon	Rheologist	Date	Calibration	Quantity	Description
35	Revision	Scott	NER	9/26/2000	0.860	22.4 mL	Contained Blood
36	Revision	Scott	NER	10/25/2000	0.860	2.0 mL	Watery, pink, and clear
37	Revision	Scott	DCM	12/6/2000	0.857	3.5 mL	Yellow
38	Revision	Scott	DCM	2/28/2001	0.803	4.0 mL	Yellow, viscous
39	Revision	Estok	NER	9/19/2000	1.000	22.0 mL	Ran test twice - 6/30 and 9/19
40	Revision	Scott	DCM	12/6/2000	0.857	4.0 mL	Pink, very viscous, some RBC's
41	Effusion	Scott	DCM	9/22/2000	1.000	4.5 mL	-
42	Effusion	Fitz	NER	7/21/2000	1.000	6.5 mL	pink
43	Effusion	Fitz	NER	10/29/2000	0.860	7.0 mL	Switched w/ 016 - not sure.
44	Effusion	Fitz	NER	7/21/2000	1.000	1.5 mL	pink
45	Effusion	Fitz	NER	8/8/2000	1.000	2.0 mL	Clear, yellow, normal consistency
46	Effusion	Fitz	DCM	7/20/2000	1.000	7.0 mL	Watery, yellowish, some precipitates
47	TKR	Brick	NER	6/30/2000	1.000	?	Too much stress
48	TKR	Scott	N/A	N/A	N/A	4.0 mL	Blood layer above yellow, viscous layer
49	TKR	Scott	N/A	N/A	N/A	N/A	N/A
50	TKR	Scott	N/A	N/A	N/A	3.5 mL	Orange
51	TKR	Scott	N/A	N/A	N/A	1.5 mL	Insufficient for viscometry
52	Revision	Scott	N/A	N/A	N/A	1.5 mL	Yellow, clear
53	TKR	Scott	NER	9/26/2000	0.860	6.0 mL	N/A
54	TKR	Wright	N/A	N/A	N/A	N/A	-
55	TKR	Scott	N/A	N/A	N/A	N/A	-
56	Revision	Brick	N/A	N/A	N/A	N/A	Unknown
57	TKR	Estok	NER	9/29/2000	0.860	2.5 mL	Some precipitate, yeloow
58	TKR	Scott	N/A	N/A	N/A	N/A	-
59	TKR	Scott	N/A	N/A	N/A	N/A	-
60	Revision	Scott	N/A	N/A	N/A	2.0 mL	Not enough fluid
61	TKR	Scott	N/A	N/A	1.000	2.0 mL	bloody - no good
62	TKR	Scott	N/A	N/A	N/A	N/A	-
63	TKR	Poss	N/A	N/A	N/A	1.5 mL	Yellow, clear
64	TKR	Wright	N/A	N/A	1.000	2.0 mL	Insufficient for viscometry
65	TKR	Miegel	N/A	N/A	1.000	1.5 mL	clear, but insufficient for viscometry
66	TKR	Scott	DCM	12/1/2000	0.827	4.5 mL	Chunky
67	TKR	Estok	N/A	N/A	1.000	1.0 mL	Insufficient for viscometry
68	Effusion	Fitz	N/A	N/A	1.000	< 1 mL	-
69	Effusion	Fitz	NER	8/7/2000	1.000		N/A
70	TKR		NER	10/30/2000	0.860	?	-

APPENDIX B: EXPERIMENTAL DATA

#	Test Geometry	Flow test	Stress [Pa]	η (1Pa) Actual	η (0) Actual	c [s]	d	Fit
1	Couette	043.01F	12-0.12	0.618	1.995	2.380	0.682	0.968
2	Couette	045.01F	10-0.1	0.307	1.193	1.788	0.666	0.968
3	Couette	33.01F	3-0.1	0.009	0.275	37.740	0.412	0.963
4	Couette	42.01F	20-0.2	2.556	6.207	5.224	0.665	0.990
5	1 deg, 6 cm ss	006.01F	12-0.2	0.317	11.900	232.800	0.543	0.985
6	Couette	32.01F	4-0.02	0.020	0.182	2.925	0.429	0.993
7	Couette	044.01F	4-0.6	0.026	0.592	9.531	0.550	0.986
8	1 deg, 6 cm ss	071200	20-0.2	2.602	6.881	5.524	0.646	0.987
9	Couette	028.01F	7-0.6	0.049	0.714	7.871	0.534	0.950
10	Couette	35.01F	6-0.06	0.049	0.619	4.719	0.568	0.983
11	Couette	042.01F	7-0.2	0.066	0.595	4.758	0.510	0.928
12	1 deg, 6 cm ss	071300	10-1.0	11.400	24.270	13.380	0.743	0.869
13	Couette	046.01F	10-0.1	0.322	0.299	2.388	0.531	0.978
14	Couette	159.01F	20-0.1	1.624	4.992	4.716	0.668	0.978
15	1 deg, 6 cm ss	071800	10-0.1	0.294	1.378	2.716	0.603	0.992
16	1 deg, 6 cm ss	010.01F	70-0.07	5.461	8.726	2.899	0.597	0.937
17	Couette	040.01F	12-0.12	0.256	0.861	1.278	0.612	0.959
18	Couette	025.01F	5-0.2	0.017	0.077	0.118	0.756	0.935
19	Couette	018.01F	11-0.1	0.375	3.794	17.700	0.579	0.989
20	Couette	156.01F	20-0.1	0.407	1.585	2.127	0.634	0.971
21	Couette	155.01F	10-0.1	0.052	0.114	0.080	0.606	0.975
22	Couette	151.01F	10-0.1	0.263	2.434	9.651	0.597	0.980
23	Couette	047.01F	20-0.1	3.152	8.923	8.996	0.674	0.917
24	Couette	049.01F	10-0.1	0.075	0.478	1.850	0.574	0.972
25	Couette	031.01F	12-0.12	0.181	0.653	1.162	0.591	0.974
26	Couette	034.01F	7-0.25	0.154	1.645	6.428	0.641	0.916
27	Couette	045.01F	6-0.1	0.045	0.363	3.111	0.483	0.975
28	Couette	050.02F	25-0.25	1.651	3.411	2.005	0.656	0.987
29	Couette	044.01F	10-0.1	0.040	0.185	1.991	0.362	0.956
30	Couette	158.01F	10-0.2	0.036	0.186	0.918	0.456	0.872
31	Couette	152.01F	12-0.1	0.443	1.570	1.817	0.672	0.982
32	Couette	153.01F	14-0.1	1.694	5.555	5.602	0.710	0.968
33	Couette	037.01F	10-0.1	0.042	0.080	0.047	0.546	0.949
34	Couette	163.01F	20-0.06	0.306	1.344	3.092	0.528	0.995

APPENDIX B: EXPERIMENTAL DATA

#	Test Geometry	Flow test	Stress [Pa]	η (1Pa) Actual	η (0) Actual	c [s]	d	Fit
35	Couette	019.01F	8-0.1	0.070	0.271	0.598	0.551	0.872
36	Couette	revision	4-0.1	0.023	0.523	4.151	0.612	0.972
37	Couette	161.01F	20-0.1	0.173	0.586	0.883	0.601	0.995
38	Couette	165.15F	15-0.1	0.416	1.447	2.497	0.591	0.871
39	Couette	003.01F	10-0.3	0.033	0.135	0.322	0.557	0.970
40	Couette	160.01F	20-0.1	0.767	3.611	7.450	0.623	0.987
41	Couette	030.04F	12-0.2	0.183	2.700	37.590	0.483	0.924
42	1 deg, 6 cm ss	072100a	8-0.38	0.123	0.625	1.484	0.553	0.960
43	Couette	014B.01F	7-0.05	0.070	0.607	5.016	0.499	0.992
44	1 deg, 6 cm ss	072100	6-0.4	0.039	0.267	2.259	0.436	0.957
45	1 deg, 6 cm ss	SF013B	9-0.09	0.111	0.369	0.532	0.549	0.953
46	Couette	072000	10-0.1	0.180	0.626	0.897	0.579	0.960
47	1 deg, 6 cm ss	No	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
48	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
49	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
50	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
51	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
52	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
53	Couette	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
54	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
55	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
56	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
57	Couette	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
58	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
59	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
60	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
61	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
62	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
63	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
64	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
65	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
66	Couette	048.01F	10-0.1	#VALUE!	#VALUE!	N/A	N/A	N/A
67	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
68	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
69	N/A	N/A	N/A	#VALUE!	#VALUE!	N/A	N/A	N/A
70	Couette	MAREOZA	7-0.05	#VALUE!	#VALUE!	N/A	N/A	0.959

APPENDIX B: EXPERIMENTAL DATA

#	X-over Freq. [rad/sec]	G" @ X-over [Pa]	G" Actual	Osc. Test	Range η(1Pa)	Range η(0)
1	#VALUE!	#VALUE!	#VALUE!	No	Degenerative	Degenerative
2	5.52	0.99	0.85	Yes	Degenerative	Degenerative
3	#VALUE!	#VALUE!	#VALUE!	Yes	Inflamed	Degenerative
4	1.79	2.06	1.70	Yes	Normal	Normal
5	#VALUE!	#VALUE!	#VALUE!	No	Degenerative	Normal
6	#VALUE!	#VALUE!	#VALUE!	Yes	Inflamed	Degenerative
7	#VALUE!	#VALUE!	#VALUE!	No	Inflamed	Degenerative
8	4.80	2.00	2.00	Yes	Normal	Normal
9	#VALUE!	#VALUE!	#VALUE!	Yes	Degenerative	Degenerative
10	#VALUE!	#VALUE!	#VALUE!	No	Degenerative	Degenerative
11	#VALUE!	#VALUE!	#VALUE!	No	Degenerative	Degenerative
12	#VALUE!	#VALUE!	#VALUE!	No	Normal	Normal
13	#VALUE!	#VALUE!	#VALUE!	Yes	Degenerative	Degenerative
14	#VALUE!	#VALUE!	#VALUE!	No	Degenerative	Normal
15	#VALUE!	#VALUE!	#VALUE!	No	Degenerative	Degenerative
16	#VALUE!	#VALUE!	#VALUE!	No	Normal	Normal
17	14.68	1.37	1.18	Yes	Degenerative	Degenerative
18	#VALUE!	#VALUE!	#VALUE!	Yes	Inflamed	Degenerative
19	#VALUE!	#VALUE!	#VALUE!	No	Degenerative	Normal
20	7.16	1.18	1.23	Yes	Degenerative	Degenerative
21	#VALUE!	#VALUE!	#VALUE!	Yes	Degenerative	Degenerative
22	#VALUE!	#VALUE!	#VALUE!	Yes	Degenerative	Degenerative
23	1.46	1.94	1.61	Yes	Normal	Normal
24	#VALUE!	#VALUE!	#VALUE!	Yes	Degenerative	Degenerative
25	34.79	1.78	1.47	Yes	Degenerative	Degenerative
26	#VALUE!	#VALUE!	#VALUE!	Yes	Degenerative	Degenerative
27	#VALUE!	#VALUE!	#VALUE!	Yes	Degenerative	Degenerative
28	5.22	3.07	2.54	Yes	Degenerative	Normal
29	#VALUE!	#VALUE!	#VALUE!	Yes	Degenerative	Degenerative
30	#VALUE!	#VALUE!	#VALUE!	Yes	Degenerative	Degenerative
31	4.53	1.01	1.00	Yes	Degenerative	Degenerative
32	1.22	1.13	1.11	Yes	Degenerative	Normal
33	#VALUE!	#VALUE!	#VALUE!	Yes	Degenerative	Degenerative
34	17.83	1.95	2.14	Yes	Degenerative	Degenerative

APPENDIX B: EXPERIMENTAL DATA

#	X-over Freq. [rad/sec]	G" @ X-over [Pa]	G" Actual	Osc. Test	Range η(1Pa)	Range η(0)
35	#VALUE!	#VALUE!	#VALUE!	Yes	Degenerative	Degenerative
36	#VALUE!	#VALUE!	#VALUE!	No	Inflamed	Degenerative
37	39.14	1.82	1.56	Yes	Degenerative	Degenerative
38	24.84	1.96	1.58	Yes	Degenerative	Degenerative
39	#VALUE!	#VALUE!	#VALUE!	No	Degenerative	Degenerative
40	#VALUE!	#VALUE!	#VALUE!	No	Degenerative	Normal
41	#VALUE!	#VALUE!	#VALUE!	No	Degenerative	Degenerative
42	#VALUE!	#VALUE!	#VALUE!	No	Degenerative	Degenerative
43	#VALUE!	#VALUE!	#VALUE!	No	Degenerative	Degenerative
44	#VALUE!	#VALUE!	#VALUE!	No	Degenerative	Degenerative
45	#VALUE!	#VALUE!	#VALUE!	No	Degenerative	Degenerative
46	70.90	2.31	2.31	No	Degenerative	Degenerative
47	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
48	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
49	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
50	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
51	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
52	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
53	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
54	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
55	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
56	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
57	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
58	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
59	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
60	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
61	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
62	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
63	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
64	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
65	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
66	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
67	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
68	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
69	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!
70	#VALUE!	#VALUE!	#VALUE!	No	#VALUE!	#VALUE!

APPENDIX C: Determining the Linear Viscoelastic Range

When measuring viscoelastic parameters using the methods described above, four curves of G' and G" were obtained for different torque inputs. These curves coincide only when all four inputs result in motion within the linear viscoelastic range, a result that occurred only in experiments using HA preparations. Due to the high concentration of HA in each sample, much greater viscoelasticity and a much larger linear viscoelastic range was measured in these samples. For the joint fluid samples, however, it was necessary to piece together the curve from each of the four runs.

There are two reasons why a given measurement might not reflect the true linear moduli. If the torque input is too small, it does not cause measurable motion in the sample. The output graph, which should be a sine wave, exhibits irregular peaks and valleys representative of measurement noise. The software calculates erroneous moduli by decomposing the irregular wave into a series of harmonic waves. Consequently, one can eliminate such data points by observing the output curves.

If the torque input is too large, motion exceeds the linear range for the fluid, but this cannot necessarily be observed from the output curves. Experience has demonstrated that strain less than 0.6 tended to measure true linear viscoelastic moduli in many samples, and strains greater than that value tended to exceed the linear range. When tests with different inputs output different sinusoidal strains with amplitude less than 0.6, it was assumed that the smaller torque elicited the linear response.

APPENDIX D: Temperature Dependence of Viscosity in Joint Fluid

As discussed above (see 3.4), viscosity was found to depend on temperature through the range of 25°C to 40°C by the Arrhenius relationship, given below.

$$\eta = Ae^{-B/T} \qquad\qquad \text{(Equation 2)}$$

These parameters were determined using the method of least squares for six samples of joint fluid. The parameters A and b, as well as correlation coefficient R^2, are given below in Table D. Other patient information can be found in Appendices A and B.

Table D: Constants obtained for the exponential relationship between inverse temperature and viscosity for six joint fluids, as found in Equation 2. Patient information can be found in Appendix A.

Patient #	A [Pa·s]	B [K]	R^2
5	6×10^{-7}	4.0×10^3	0.98
8	3×10^{-8}	5.5×10^3	0.77
12	6×10^{-5}	3.6×10^3	0.64
15	1×10^{-6}	3.7×10^3	0.94
16	1×10^{-6}	4.5×10^3	0.98
39	2×10^{-6}	2.8×10^3	0.98

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APPENDIX E: STATISTICAL METHODS

E.1 Power Calculation

A power calculation was performed in order to determine the sample size that would be necessary to detect a significant difference between experimental groups. The sample size could be calculated as follows:

$$n = 2(\frac{\sigma}{\delta})^2 (t_{\alpha,v} + t_{2\beta,v})$$

where: n = sample size

σ = standard deviation, which was assumed to be 25% of the mean

δ = desired difference to detect

α = desired significance level (probability of obtaining a false positive result)

β = desired statistical power (probability of obtaining a false negative result)

$t_{\alpha,v}$ = t statistic corresponding to a significance level α and v degrees of freedom

$t_{2\beta,v}$ = t statistic corresponding to significance level 2β and v degrees of freedom

The solutions of this equation have been tabulated for various values of σ, δ, α, and β.

The difference (δ) between groups that would be meaningful was assumed to be a 20% difference between means. The standard deviation for each group was assumed to be 25% of the mean. Using these values and setting the criteria for significance to be α=0.05 and β=0.2, the samples size should be twenty-five for each group.

This analysis tests the hypothesis that there is no difference in the mean values between two groups. It assumes that each group is normally distributed with the same variance. After evaluating the samples, it was found that these assumptions were not true, and such an analysis could not be used.

E.2 The Mann-Whitney Test

The Mann-Whitney test was used to demonstrate a significant difference between two groups that are not normally distributed. This test compares the ranks of the two groups, rather than their actual values. The existence of a difference between two groups was calculated as follows:

$$Z = \frac{U - \frac{n_1 n_2}{2}}{\sqrt{\frac{n_1 n_2 (n_1 + n_2 + 1)}{12}}}$$

where: n_1 = number of samples in the first group

n_2 = number of samples in the second group

U equals *either* the sum, over each sample in the first group, of the number of members of the second group preceding it in rank *or* the sum, over each sample in the second group, of the number of members of the first group preceding it in rank, *whichever is less*

Z = the z value determining p value for a two-tailed test, and therefore the probability of a false positive result

This analysis tests the hypothesis that there is no difference in the range of values between two groups. It does not assume that each group is normally distributed.

E.2 Fisher Exact Test

A two-tailed Fisher Exact test was also used to determine significant differences using a two by two matrix as follows:

	Group A: X-over Measured	Group B: No X-over Measured	Row Total
$\eta < a$	w	x	$R_1 = w + x$
$\eta > a$	y	y	$R_2 = y + z$
Column Total	$C_1 = w + y$	$C_2 = x + z$	$N = w + x + y + x$

$$P_{crit} = \frac{(R_1!R_2!)(C_1!C_2!)}{N!(w!x!y!z!)} \text{ and}$$

$$\text{p-value} = \sum (P - values \leq P_{crit})$$

This test was used to demonstrate a correlation between viscosity and viscoelasticity, using a = 1 Pa·s or a = 0.5 Pa·s.