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The Effects of Increasing Positively Charged Ions Within Synovial Fluid: Relation to Osteoarthritis and Overall Performance of a Joint

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Abstract

Osteoarthritis is a degenerative joint disease that affects 10% of men and 13% of women over age of 60. It is the degradation of the cartilage between two bones; obesity, age, overuse, or injury are major contributors to the development of this disease. The joint is incapsulated by the synovial sac filled with a viscous solution that aids in lubrication referred to as synovial fluid. If the synovial sac is ruptured due to injury, positive ions (K^+, Na^+, Ca^{2+}, and Fe^{3+}) may affect viscoelastic properties within the sac. The purpose of this study was to understand how positive metal ions affect the synovial matrix and its rheological and tribological properties across two different age groups to better the understand the development of osteoarthritis. The hypothesis was that positively charged metal ions from blood or ruptured cells weaken the repulsive forces in the meshwork causing destructive changes in the rheological and tribological properties of the synovial fluid. The results for NaCl and KCl supported the hypothesis by disrupting the rheological properties of old synovial fluid, but other data suggested a significant increase in the viscoelastic behavior of young synovial fluid when NaCl and KCl are introduced. The data also suggested that $FeCl_3$ and $CaCl_2$ significantly increase the elastic potential in old synovial fluid.

Table of Contents

Introduction

Osteoarthritis is a degenerative joint disease in which the cartilage between the bones degrades and is characterized by swelling, pain, and limited movement. It can be hereditary, due to injury, or caused by obesity, high mechanical stress (overuse) of the joint, and age; however, in elderly subjects it can also be caused by minimal use or movement [1]. In joints, synovial fluid surrounds the cartilage to aid in lubrication that decreases soft tissue wear and assists in long term protection of the joint. It also acts as a shock absorbent. In the case of an injury, the synovial sac can be ruptured and allow ions found in the blood to disrupt the synovial matrix [2]. This could potentially increase the risk of chronic pain and illness, such as osteoarthritis, in the joint later in life.

Synovial fluid is a viscoelastic liquid which is comprised of a fibrillar[1] matrix of hyaluronic acid (HA) chains [3]. HA chains have a structure of two disaccharides (N-acetyl-β-D-glucosamine and β-D-glucuronic acid) arranged in a low-energy $^4C_1$ conformation with a glycosidic linkage [4]. The structure has a negatively charged hydrophilic backbone which promotes hydrogen bonding and thus, strong chain interaction. The strong chain interaction and negative charge perpetuates the rigidity of HA's molecular structure and therefore, elastic and gel-like properties [3]. The length of the chains also contributes to their stability and ability to act as a shock absorbent. Under normal circumstances, SF behaves as a nonnewtonian[2] liquid, in which it displays more elastic or viscous properties at certain levels of stress. However, when positively charged ions, such as K^+, Na^+, Ca^{2+}, and Fe^{3+}, are introduced to this framework, they may

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[1] Fibrillar- Fibrous; having characteristic of a fiber

[2] Nonnewtonian liquid- a nonnewtonian material does not follow Newton's law of viscosity in which a material has a constant viscosity independent of the stress applied to it.

weaken and disrupt the repulsive forces within it. Thus, the gel-like structure and lubrication effects may be hindered, as well as the nonnewtonian behavior.

The objective of this study was to understand how positive metal ions such as K^+, Na^+, Ca^{2+} and Fe^{3+}, affect the synovial matrix and its rheological and tribological properties across two different age groups. The samples were tested with a rheometer, which measured the way liquids or suspensions flow when a force is applied to them. It was expected that the newfound data would give an improved explanation on the mechanical stress aids in cartilage degeneration and the development of osteoarthritis, thus enabling the development of new techniques for the prevention or treatment of osteoarthritis.

Methods

Synovial Fluid

Bovine Synovial Fluid was originally to be obtained from Summa Health Services, but due to COVID, the partnership and funding was cancelled. Instead, SF from Lampire Biological Laboratories of two different manufacturing lots, or sample groups, were tested (#8600853, Lampire Biological Laboratories, Pipersville, Pennsylvania). The first lot was the “old” SF (OSF) was collected from bovine specimens over the age 30 months. The second lot was the “young” SF (YSF), and it was collected from bovine specimens under the age of 4 months.

Positive ions and solutions

Positive ions (K^+, Ca^{2+}, Fe^{3+}, and Na^+) were isolated by creating aqueous NaCl, KCl, $CaCl_2$, and $FeCl_3$ base solutions from Sigma-Aldrich chemicals (#7647-14-5, #7447-40-7, #10043-52-4, #10025-77-1, Sigma-Aldrich, Milwaukee, Wisconsin). Final concentrations of ionic solutions were equivalent to the number of ions within one liter of human blood plasma. This was to simulate a scenario where the synovial sac could be ruptured, and blood would seep in. According to Covington and Robinson [5], the concentration of K^+ is 4mM/L, Ca^{2+} is 2mM/L, Na^+ is 100-200mM/L, and Fe^+ is 3mM/L in human blood. This was converted to g/L where there is 0.298g/L of KCl, 0.222g/L of $CaCl_2$, 8.78g/L of NaCl, and 0.487g/L of $FeCl_3$.

250-500mL stock solutions were made by dissolving the salts in deionized water and then further diluted to achieve the final ionic concentration. To avoid the dilution of SF with water, 1mL of salt solution was pipetted on a spot plate and the water was evaporated. Then, 1mL of SF was pipetted into the spot plates to dissolve the salt residue. Eight solutions were made of OSF+KCl, OSF+$CaCl_2$, OSF+NaCl, OSF+$FeCl_3$, and YSF+KCl, YSF+$CaCl_2$, YSF+NaCl, YSF+$FeCl_3$.

Rheology

The rheological procedure was obtained from a combination of Dr. Paul Shiller's prior research on this project and Bhuanantanondh et al. research [6]. Rheological tests were completed using a TA Instruments AR32 rheometer with a 2° angle, 40mm cone and plate. The gap was set at 30µm and temperature at 37°C. A solvent trap was used to prevent evaporation of SF at physiological temperatures. For the conditioning step, a pre-shear was performed at 0.5968Pa for 10s and equilibration was done for 2min. An oscillation frequency sweep test, which measures the phase angle[3] of a fluid at increasing shear stress and shear rate, was executed at an angular frequency of 0.3142-314.2rad/s. The rheometer was set to 25°C post-experiment.

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[3] Phase angle- a measurement of the viscoelasticity of a fluid. ≤0° indicates deformation as a solid ≥90° indicates deformation as a liquid.

Results

Statistics

Please see Discussion- Project Limitations

Data

Figures (Fig.) 1 and 2 display the logarithmic graphs of angular frequency vs. delta of the OSF and YSF solutions when 1 mL each was tested. Delta is the phase angle of a sample and represents the ratio of viscous to elastic capacity of a viscoelastic material, or the energy dispersion potential of that material [7]. The flow point, or period in which the sample makes the phase change from elastic to viscous, for healthy SF can be found where the material surpasses the 45.00° threshold in relation to the phase angle [8]. YSF reaches the threshold at 29.19rad/s (radians per second), whereas NaCl+YSF and KCl+YSF reach the threshold at 67.79rad/s and 60.95rad/s. $FeCl_3$+YSF and $CaCl_2$+YSF's flow point are at 33.78rad/s and 38.90rad/s. The flow point for plain OSF occurs at 19.80rad/s, while $FeCl_3$+OSF and $CaCl_2$+OSF increased to 66.56rad/s and 77.35rad/s. The starting phase angle for both NaCl+OSF and KCl+OSF was above 45.00° so while there may have been a logarithmic increase, there was not a phase change from elastic to viscous because the starting material did not have stable elastic properties.

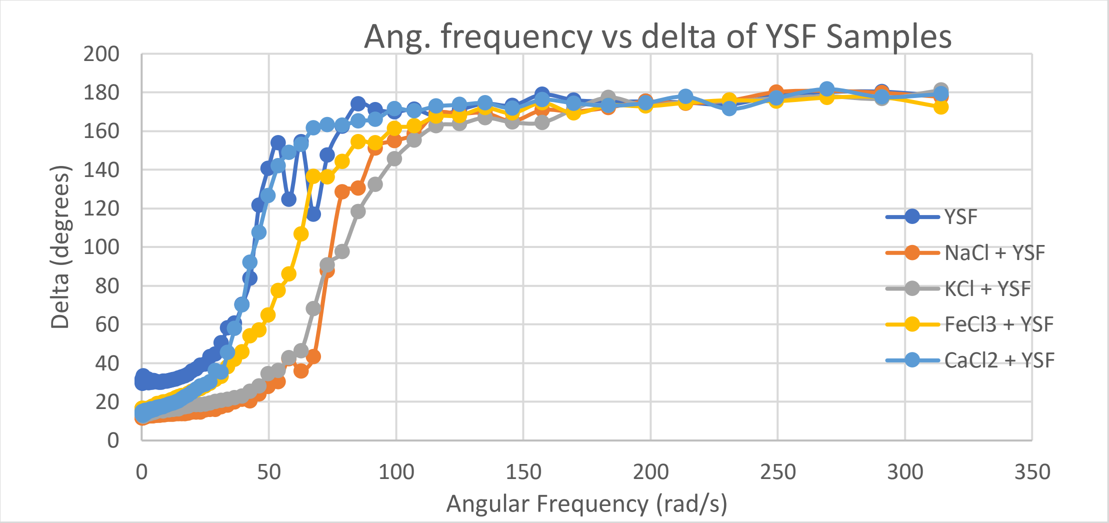

Figure 1. Comparing the Logarithmic Data of YSF Solutions + additives. The starting phase angle for these samples was less than 40° which suggests an elastic structure present. The ending phase angle was ~180° so samples deformed as liquids.

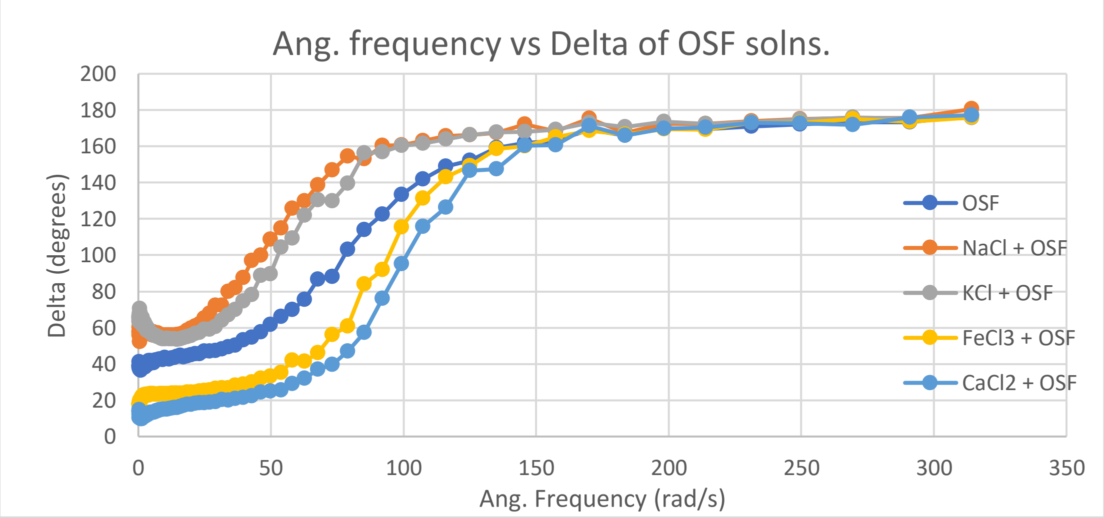

Figure 2. Comparing the logarithmic data of OSF solutions + additives. Starting phase angle for NaCl+OSF and KCl+OSF is above 45° which suggests there is not a stable elastic structure for the material. The ending phase angle for all samples was ~180°. They deformed as liquids.

Fig. 3 Compares the exact flow point in rad/s for each synovial composition, differentiated by the age group. Missing data denotes that no phase change was observed for the sample.

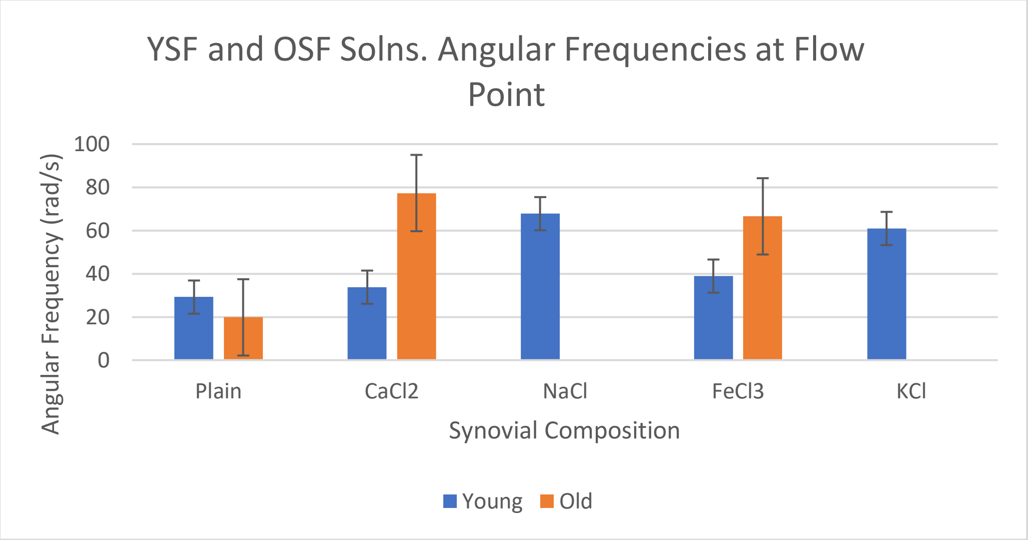

Figure 3. Comparing the flow point between Young and Old SF solutions in rad/s.

In Fig. 6, the graph compares the shear stress (Pa) of the samples at their flow point between the groups of young and old SF solutions. It is measuring the storage modulus of the viscoelastic solutions at the change from elastic to viscous as angular frequency increases. Missing data denotes that a phase change was not observed.

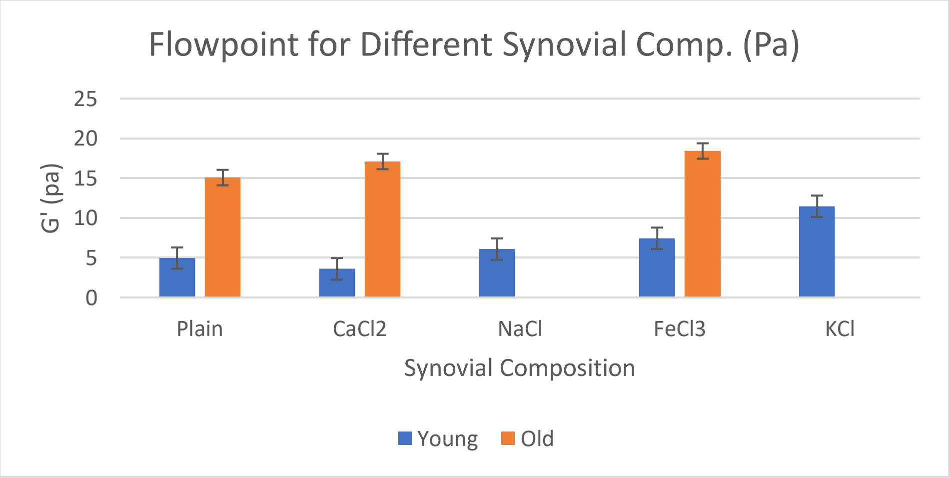

Figure 4. Comparing the shear stress of YSF and OSF at their flow points.

Figures 5-9 are images of the plain (undoped), KCl, NaCl, $CaCl_2$, and $FeCl_3$ doped YSF oscillating frequency test results. Figures 12-16 are the images of OSF oscillating frequency test results. The images display the logarithmic graph of the phase angle (delta) at increasing angular frequency (rad/s). The red line (G’) is the elastic modulus and the blue line (G”) is the viscous modulus. The point in which the two moduli intersect is indicative of the flow point.

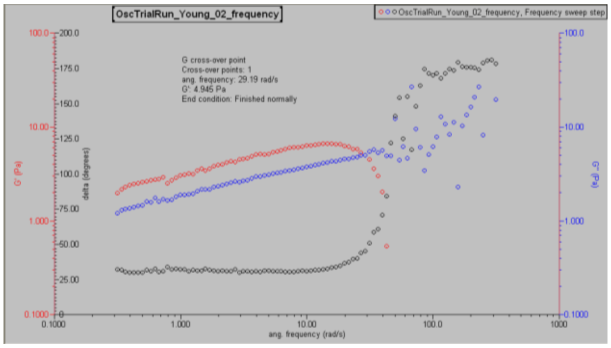

Figure 5. Oscillating Frequency Test of Plain YSF

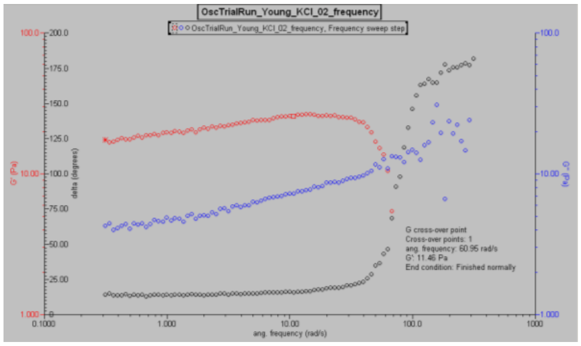

Figure 6. Oscillating Frequency Test of YSF+KCl

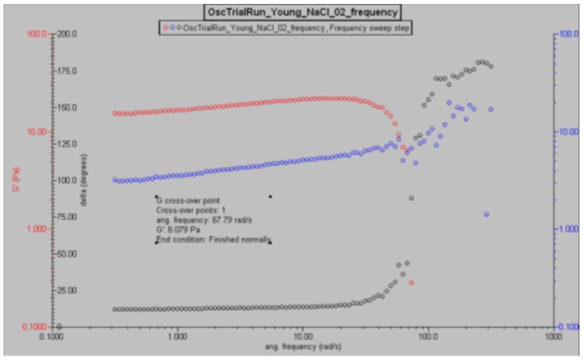

Figure 7. Oscillating Frequency Test of YSF+NaCl

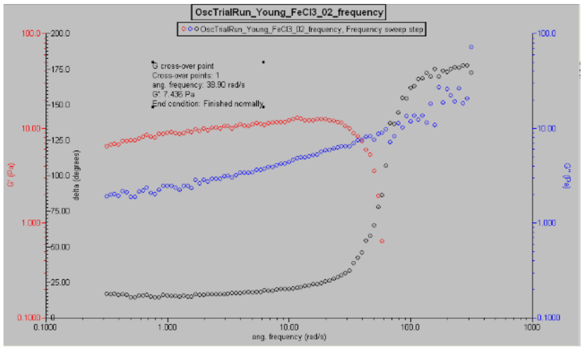

Figure 9. Oscillating Frequency test of YSF+FeCl3

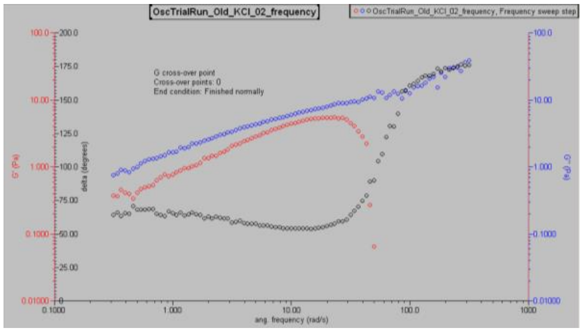

Figure 11. Oscillating Frequency Test of OSF+KCl

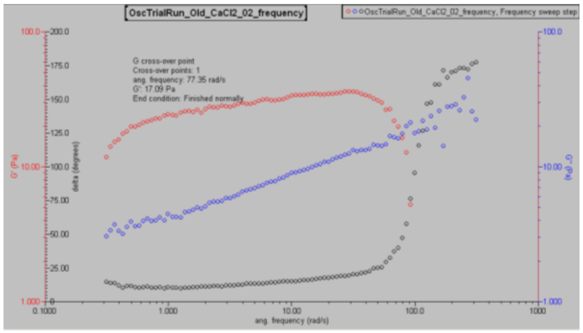

Figure 13. Oscillating Frequency Test of OSF+CaCl2

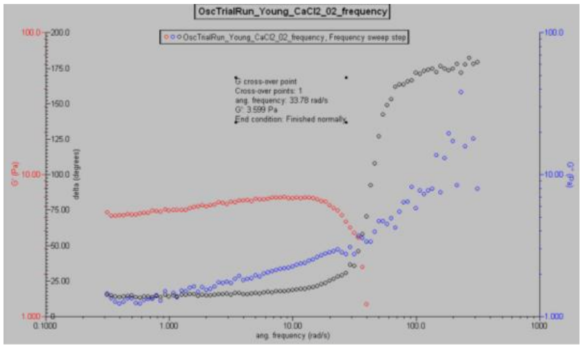

Figure 8. Oscillating Frequency test of YSF+CaCl2

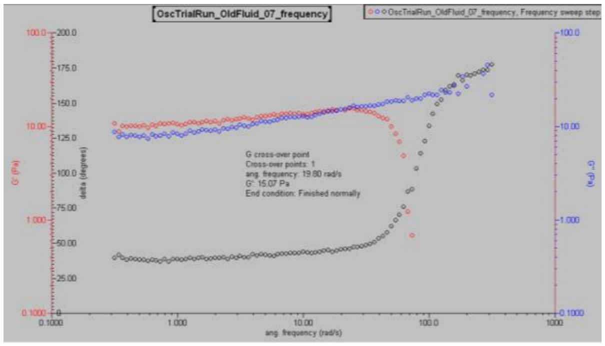

Fig 10. Oscillating Frequency Test of Plain OSF

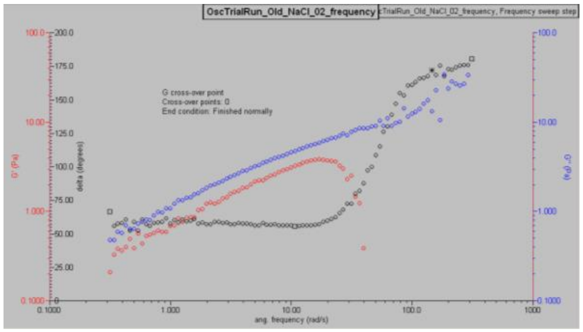

Figure 12. Oscillating Frequency Test of OSF+NaCl

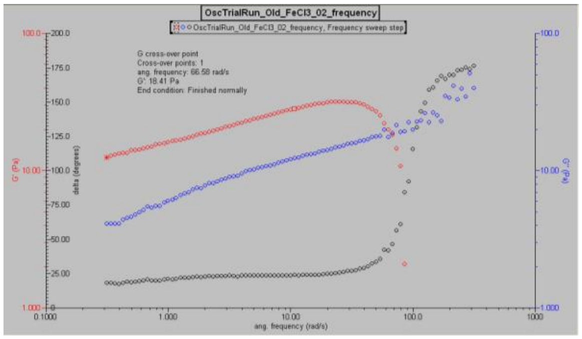

Figure 16. Oscillating Frequency test of

OSF+FeCl3

Discussion

Analysis

In oscillating frequency sweep tests (Figs. 7-16) one can compare delta at increasing angular frequencies while simultaneously noting shear stress and its relation to the storage and loss moduli (G' and G") of different synovial fluid compositions. Typically, when testing more elastic materials, G' will be larger than G", and when they intersect this signifies the change from elastic to viscous [6]. As previously stated, delta represents the energy dissipation of a material, so an increasing delta denotes an increasing energy dissipation potential and decreasing energy storage potential (less elasticity) [8]. A delta closer to 90.00° indicates that the material deforms as a liquid and a delta closer to 0° signifies that the material deforms as a solid.

YSF reached its flow point at 29.19rad/s whereas NaCl+YSF and KCl+YSF undergo a significant increase in frequency of 67.79rad/s and 60.95rad/s to reach the threshold. OSF surpassed the phase angle threshold at 19.80rad/s, but $FeCl_3$+OSF and $CaCl_2$+OSF required a rate of 66.56rad/s and 77.35rad/s to meet the flow point. In comparison to plain SF, these samples can experience a higher shear rate before making the phase change from elastic to viscous. However, when NaCl and KCl were introduced to OSF, the starting phase angle was above 45.00° at low stress. This means that the elastic structure within SF was no longer intact and a phase change from an elastic to viscous material was not observed.

Synovial fluid SF is a meshwork composed of HA is a long-chain polysaccharide with a negatively charged, hydrophilic backbone, and its molecular structure forms a meshwork of rigid hydrogen-bonded chains which gives it viscoelastic properties [4]. Its stiff structure causes SF to behave as a nonnewtonian, gelatinous material, but positive ions may inhibit the molecular interactions and the viscoelastic properties.

When $CaCl_2$ and NaCl are introduced to SF, calcium and sodium weaken the hydrogen bonds in the HA backbone and cause the structure to become more flexible which decreases the persistence length[4] of the hyaluronic polymer [4]. This leads to the strong coil contraction of HA chains into helical structures. A similar effect was observed when KCl interacted with HA; the $^4C_1$ conformation is arranged into an antiparallel, double helix [9]. This conformation disrupts the ability of HA's backbone to bind with itself and decreases the chain rigidity. $FeCl_3$ also causes the structure of HA to rearrange into a tridimensional structure [10]. These rearrangements were thought to reduce the viscoelastic properties of HA, but further analysis is necessary to

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[4] Persistence length- Basic mechanical property measuring bending stiffness of a polymer.

disentangle the mechanisms. NaCl and KCl increased the shear rate and stress necessary for YSF to deform as a viscous liquid. The reverse effect occurred when introduced to older specimens because both ions deteriorated the elastic structure of the starting material. However, $FeCl_3$ and $CaCl_2$ significantly increased the shear rate and stress for OSF flow point.

Project Limitations

Due to COVID-19, collaboration with our synovial fluid provider, Summa Health was terminated. This resulted in a lack of materials necessary to repeat tests so many of the results are uncorroborated and therefore, theoretical. Statistics were unable to be completed for this project because for many of these tests, there were not enough trials to compare data. Also, in order to reduce costs, the OSF samples were collected from lots in 2015 which may have led to invalid results. There was no access to the lab for an entire semester due to COVID lockdown, therefore time constraints were increased for this project.

Conclusion

Synovial Fluid is necessary for the lubrication and reduction of wear in joints. Its matrix composed of stiff, interconnected chains of HA yields viscoelastic properties that fluctuate at varying levels of stress. In order to better aid those suffering from osteoarthritis or other joint injuries, it is crucial to understand how ions found in blood could affect the matrix after penetrating the synovial sac. The objective of this study was to understand how K^+, Ca^{2+}, Fe^{3+}, and Na^+ affect the synovial matrix and its rheological and tribological properties across two different age groups. The hypothesis was that positively charged metal ions from blood or ruptured cells weaken the repulsive forces in the meshwork causing destructive changes in the rheological and tribological properties of the synovial fluid. NaCl and KCl displayed a significant increase in shear rate before reaching the phase angle threshold for YSF, but it exhibited significant adverse effects in OSF. Also, $FeCl_3$ and $CaCl_2$ showed a significant increase for shear rate in OSF. In the future, more research should be conducted to further analyze the rheological and tribological properties of SF and better comprehend the effects of positive ions on the synovial matrix.

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