Investigation of the rheological and tribological characteristics of human synovial fluid

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Abstract

The rising prevalence of knee arthrosis, driven by aging populations and modern lifestyles, has increased the demand for reliable orthopaedic implants. Metal alloys remain the most widely used implant materials, but their stability and performance are influenced by interactions with biological substances. The present study evaluates the rheological and tribological characteristics of human synovial fluid acquired from different age groups and genders with the future aim of developing a synthetic laboratory-produced lubricant that mimics the characteristics of synovial fluid and other surgical field materials. Rheological and tribological tests were performed on a rheometer to analyse viscosity, flow behaviour, and coefficient of friction. Fourier-Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR) spectroscopy was applied to evaluate the chemical composition of different biological samples. The study revealed that osteoarthritic synovial fluid viscosity was higher in male patients compared to females, while bovine-derived and artificial synovial fluid exhibited significantly higher viscosities than water-based solutions, which demonstrated the lowest viscosity. The tribological measurements revealed no significant difference in the coefficient of friction between samples from the young individual and the patients, whereas artificial synovial fluid and bovine synovial fluid demonstrated slightly higher coefficients of friction compared to the other samples. The findings provide key insights into the consistency and lubricity of human biological fluids and demonstrate the potential of synthetic lubricants for implant stability testing.

Keywords: rheology, tribology, friction, spectroscopy, synovial fluid, lubricity

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Introduction

Musculoskeletal disorders (MSDs) are a major cause of disability on a global scale. They are broadly defined as diseases or injuries of the joints, bones, muscles, nerves, tendons, ligaments, supporting soft tissues, cartilage, and spinal discs, and comprise over 150 diagnoses.[1] Notable examples include arthritis (e.g. rheumatoid arthritis, systemic lupus erythematous, or osteoarthritis), osteoporosis, infection, neoplasms, and neck and lower back pain. The clinical significance of MSDs is increasing due to an aging population and evolving lifestyles, making this diverse group of diseases and injuries a major global contributor to disability.[2]

Osteoarthritis (OA) is a degenerative joint disease, affecting over 200 million people worldwide. It is frequently associated with aging, obesity, and joint trauma. This disorder represents one of the leading causes of activity limitations in adults and impaired mobility in the elderly.[3] Its prevalence is expected to increase as global life expectancy increases in the coming decades.[4] Those affected by OA face the challenge of living with a chronic condition for which there is currently no cure to halt tissue degradation, including wear and the loss of the tissue's protective function.[5]

Joint replacement surgery (arthroplasty) can relieve the symptoms of advanced OA of the knee and restore the function of a joint very effectively in most people.[6] Arthroplasty is defined as the essential surgical replacement of a joint with artificially produced material which is fixated in the bone.[7] Depending on the extent of the osteoarthritis, either the entire knee joint can be replaced, or a partial replacement of the more severely affected part of the joint can be carried out.[8,9]

Despite the accelerated development of diverse implant materials, different metal alloys remain the most popular materials. The most commonly used materials for knee implants are titanium, cobalt-based alloys, and stainless steels.[10] Most implanted metals exhibit a tendency to undergo electron loss when in solution, which consequently imparts a high propensity for corrosion. In this context, our research group has conducted an investigation into the corrosion behaviour and cytotoxicity[11] of a cobalt-chromium-molybdenum (CoCrMo) alloy, providing valuable insights into its performance under physiological conditions.[12]

Occult or chronic infections alongside with wear debris[13] are known to inhibit bone regeneration and cause loosening of orthopaedic implants. Despite the state-of-the-art infrastructure and sterile environment in operating theatres[14], yearly around 5% of cases of infection and inadequate fixation of prosthesis are followed by revision surgery. As knee replacements have become more common, the number of revision operations has risen by more than threefold.[15]

The stability and strength of metal implants within the human body is significantly influenced by chemical and biological interactions with substances encountered during surgical implantation (blood, fat, synovial fluid). In accordance with international standards, conventional in-vitro mechanical testing is typically conducted under controlled conditions, such as in dry environments, simulated body fluid, or physiological saline solutions.[16–18] However, these testing conditions do not fully replicate the complex lubricating properties of native human biological materials, potentially limiting their capacity to accurately simulate in-vivo performance.

For instance, with regard to spinal implants, the American Society for Testing and Materials (ASTM) does not provide definitive guidelines for in-vitro testing of mounted spi-

nal implants with screws and hooks attached to longitudinal rods.[19] However, the analysis indicates that screw loosening plays a key role in the failure of cervical[20] and lumbar[21] spine implants. This finding suggests that lubrication should be incorporated into the in vitro test. Manufacturers have determined the necessary assembly parameters, however, we believe that standards should also specify the examination environment to ensure the comparability of all measurements. In previous studies, the research team has investigated the problem in question. The objective of this study was to design and construct a custom, inexpensive device for the measurement of relative lubricity, of everyday general lubricants, such as motor oil and grease.[22,23] Although ASTM standards recommend the utilisation of bovine serum, or equivalent pseudo-SF for in-vitro knee prosthesis test[24], it is essential to critically evaluate the tribological and rheological properties of these surrogate substances in comparison to human synovial fluid (SF), as well as the difference between healthy, and osteoarthritic SF.[25–27]

This study aimed to evaluate the viscosity and viscosity loss of SF under varying conditions obtained from patients undergoing arthroplasty. Viscosity measurements were performed to characterise the fluid's resistance to flow. Furthermore, tribological measurements were conducted to determine the coefficient of friction (COF) with comparisons made between those obtained from male and female patients. By integrating these analyses, the study sought to enhance the understanding of sex-related differences in SF properties and their implications for joint lubrication and implant performance. In addition, infrared spectroscopy was utilised to investigate the chemical composition of biological samples, with comparisons made based on variables such as age, sex, and clinical condition. Comprehensive analyses were conducted to compare the pH levels of blood and SF with those of a standard water-based solution. These findings are critical for understanding the role of pH in the degradation processes of biomaterials and the performance of implants in-vivo.

The overarching objective of this research is to develop novel, laboratory-produced lubricants that closely replicate the rheological and tribological properties of human biological fluids, thereby enabling the standardisation of measurement protocols. This approach not only facilitates more accurate simulation of the friction-reducing effects characteristic of human materials but also lays the foundation for the future development of synthetic lubricants tailored for implant stability testing. By providing a viable alternative to human-derived substances, laboratory-produced lubricants have the potential to eliminate the ethical challenges and logistical complexities associated with acquiring human samples during surgical procedures.

Materials and Methods

Human samples

Human samples were obtained during the operation of patients undergoing joint replacement surgery in Kaposi Mór Teaching Hospital (Department of Trauma Surgery, Kaposvár, Hungary). The samples were taken with the written consent of the patients and with the permission of the Scientific and Research Ethics Committee of the Health Science Board (ETT TUKEB, Case number: BM/9062-1/2024). In the frame of this study, 21 patients were enrolled (ten male and eleven female) undergoing arthroplasty with SF, blood, and fat tissue collection. The reference sample was a healthy 29-year-old male. The samples were immediately transferred to a sterile specimen container and stored at -25°C until processing. Blood samples were transferred into specialized tubes pre-coated with

a clotting factor inhibitor to prevent coagulation. Anticoagulant in appropriate concentrations did not influence the shear viscosity. This approach ensured the preservation of the blood's natural fluid properties.

Water-based replacement materials

In comparison to human SF, different solutions were tested, including Phosphate Buffered Saline (PBS) for its similarity to the ionic composition and pH (7.4) of bovine blood/SF. Bovine Serum Albumin (BSA) was added to PBS at a concentration of 0.5 g/L as a model protein to simulate protein interactions with metal implants. Additionally, human SF was benchmarked against distilled water, lyophilized bovine SF and artificial SF. The artificial SF, produced in cooperation with the University of Continuing Education Krems, contained the following substances: 3 mg/mL hyaluronic acid (HA), 19 mg/mL bovine serum albumin (BSA), 11 mg/mL immunoglobulin G (IgG), 0.1 mg/mL phospholipids (PL), and 0.1 mg/mL glycerol.

Tribological measurements

Friction measurements were performed using the tribological setup of an Anton Paar MCR 302 rheometer (Anton Paar GmbH, Graz, Austria). This comprises a ball on three pins system configuration (*Figure 1*). The setup consists of 3 pins (ø6×6 mm) that are placed at an angle of 45° and subjected to a constant total normal force from a 12.7 mm diameter ball (1.3505 UNI100Cr6 G28), with a predetermined rotational velocity. The test temperature can be controlled within the range of -20 to 200 °C.

The sliding speed of the ball is measured from $1 \cdot 10^{-8}$ m/s to 0.1 m/s at a constant tribological normal force (F_N) of 5 N. The ball and pins are of the same material, namely hardened and polished 100Cr6 bearing steel (64 HRC hardness). Before starting the measurements, the ball and pins are rinsed with toluene, isopropanol, and petroleum ether. The torque (M) on the rotating shaft is measured and used for calculating the friction force F_f:

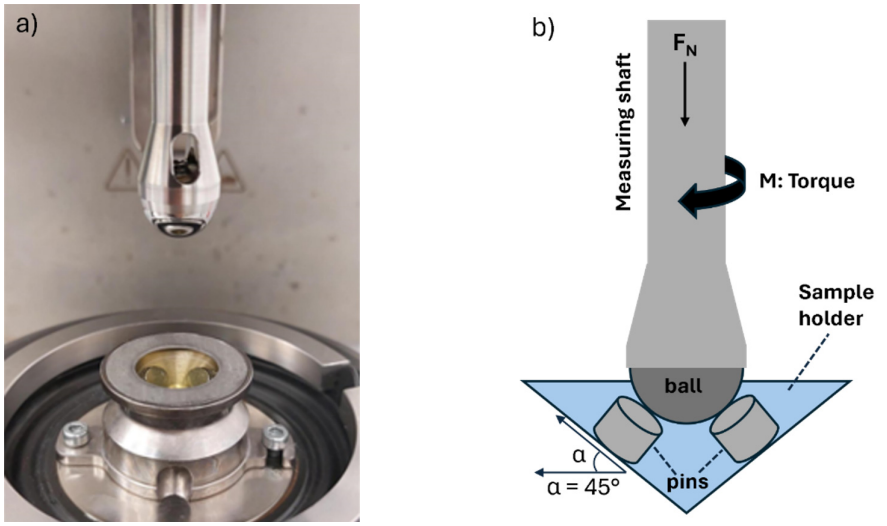

Figure 1. A close-up of the "ball on 3 pins" cell of the MCR 302 rheometer for tribological measurements (a) and a schematic overview of Rheometer tribological cell (b)

$$F_f = \frac{M}{3 \cdot R \cdot \sin \alpha}$$

where R is the ball radius and α is the angle of the pin samples to the normal force (45°). The total normal force, F_N, can be transferred into tribological normal force, $F_{N;tribo}$ normal to the plane of the pins

$$F_{N;tribo} = \frac{F_N}{3 \cdot \sin \alpha} \cdot$$

The resulting coefficient of friction (μ) is the ratio between tribological normal force and the calculated friction force

$$\mu = \frac{F_f}{F_{N;tribo}} \cdot$$

With regard to the tribological aspect of the work presented, the following samples were tested: SF of two male and two female patients, one young reference patient, artificial SF and bovine SF. Amounts between 0.7 and 1 mL were utilized in each test. The tribological setup was cleaned between repetitions and new ball and pins are used for the tests.

Additional tests were performed with a tribological normal force of 1 N, as well as a test in which friction is measured from high to low rotation speed. Tribological tests were performed three times with the same lubricant type, where the first measurement, running-in, is not used for calculation. The entire procedure was repeated two times with a fresh sample, with the exception of the reference sample, where only one fresh sample was utilised due to the lack of sufficient amount of sample. Prior to testing, the samples were defrosted in a water bath set at 35 °C. The tribological measurements were performed at 37 °C in order to simulate body temperature.

Viscosity measurements

Viscosity measurements were performed on the same MCR 302 Anton Paar rheometer, equipped with temperature control system to ensure precise thermal regulation. The method was developed for the purpose of measuring human samples of limited volume. A disposable plate was utilised to prevent the sample from potential leakage from the measuring

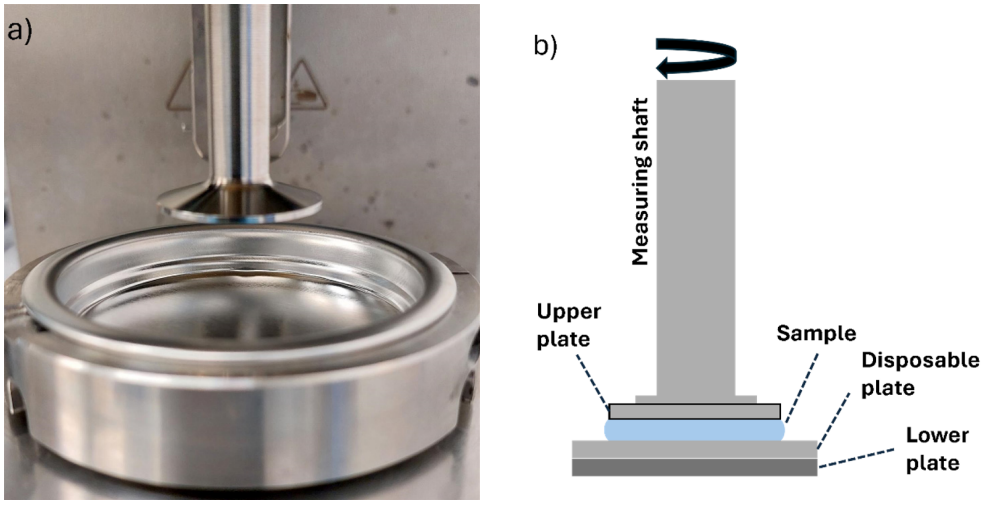

Figure 2. A close-up of the "plate-disposable plate" cell of the MCR 302 rheometer for rheological measurements (a) and schematic overview of Rheometer rheological setup (b)

cell. As in tribological studies, a protective cap and body temperature were employed during testing. The measurements utilized a PP25 parallel plate configuration, featuring an upper plate with a diameter of 25 mm and a lower aluminium disposable plate (*Figure 2*). The distance between the two plates was 1 mm. All tests were performed at a controlled temperature of 37°C to simulate physiological conditions. The rheometer operated in rotational mode across a shear rate range of 0.01-100 s^{-1}, enabling a comprehensive analysis of the viscosity profiles and shear-thinning behaviour of the tested samples.

Samples were collected and analysed from six distinct materials to evaluate their properties: human synovial fluid (four female patients, four male patients and one reference sample), bovine synovial fluid, artificial synovial fluid, phosphate-buffered saline (PBS), PBS supplemented with bovine serum albumin (PBS+BSA), and distilled water. These materials were selected to represent a range of biological, synthetic, and aqueous solutions commonly utilized in the study of lubrication experimental conditions.

Analysis of the chemical structure

Fourier-transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) provides information on the chemical structure and composition through identification and analysis of functional groups. An FTIR-ATR system (Tensor 2 FTIR spectrometer, Bruker Optik GmbH, Ettlingen, Germany and a SensIR DuraSamplIR ATR unit, SensIR technologies, Chapel Hill, North Carolina, USA) was employed to detect and characterize the compositional changes in the synovial fluid, blood and adipose tissue samples. The examination included a total of 10 samples, consisting of 5 male and 5 female participants. Scans were performed in the range of 600 cm^{-1} to 4000 cm^{-1}, with a resolution of 4 cm^{-1}. Both background and samples were measured 32 times, the reported spectra are the corresponding average. Data processing and interpretation was performed in OPUS 8.7.31 (Bruker Optik GmbH, Ettlingen, Germany). In detail, an automatic background correction was performed and the spectral range associated with atmospheric carbon dioxide interference was excluded.

This technique allowed for the identification of molecular functional groups and chemical bonds, providing detailed insights into the biochemical variations within and between the analysed biological materials. The obtained results were compared with those of healthy subjects, facilitating an understanding of the deviations associated with pathological conditions.

pH analysis

The pH of the samples was measured using a microelectrode Metrohm 780 pH meter (Metrohm Deutschland GmbH , Filderstadt, Germany) specifically designed for small-volume and biological fluid applications to ensure precision and accuracy. Measurements were conducted at 37 °C on blood and synovial fluid obtained from patients undergoing medical procedures. The electrode was immersed in the solution until a stable pH value was achieved. For comparative purposes, pH measurements were also performed on reference samples, including synovial fluid and blood obtained from a healthy young male, which served as a baseline representing physiological norms. Additionally, various water-based solutions were included in the analysis, such as distilled water, PBS, and PBS supplemented with BSA, to further evaluate how the pH of these standard solutions compared to biological fluids. All measurements were performed under controlled laboratory conditions to ensure consistency and reliability of the data.

Results and Discussion

Viscosity and viscosity loss analyses

Both groups exhibited a shear-thinning behaviour, with viscosity decreasing as shear rate increased, indicative of non-Newtonian fluid characteristics. The viscosity values for males were consistently higher across all shear rates compared to females, suggesting potential differences in fluid composition or rheological properties between the groups (*Figure 3*).

At lower shear rates (10–20 1/s), the male group displayed an initial viscosity of approximately 18–20 mPa·s, which rapidly declined to stabilize around 6–8 mPa·s as the shear rate increased. In contrast, the female group started with a lower viscosity of 6–7 mPa·s and stabilized at approximately 3–5 mPa·s in the same range of shear rates. The shaded regions surrounding each curve represent the standard deviations, indicating inter-individual variability within the groups. Variability was more pronounced at lower shear rates and gradually diminished as the shear rate increased, with both groups converging towards more stable viscosity values at higher shear rates. This data highlights distinct rheological profiles between genders and age brackets, with the male group exhibiting overall higher viscosities and larger initial declines in response to increasing shear rates. Further analysis is warranted to explore the underlying physiological or biochemical factors contributing to these differences. As demonstrated in the extant literature[28], the testing of synovial fluid viscosity appears to be a promising diagnostic tool for periprosthetic joint infection. The synovial fluid viscosity level was significantly lower in patients with joint infection than in patients with aseptic failure.

The obtained data demonstrates that the viscosity of alternative synovial fluid samples, including bovine synovial fluid and artificial synovial fluid, is significantly higher than that of human synovial fluid samples (*Figure 4*).

The reference sample exhibits the lowest viscosity values across all shear rates, with a clear shear-thinning behaviour. This suggests it retains optimal lubrication properties, aligning with its role in healthy joint function. The osteoarthritis synovial fluid shows higher viscosity values compared to the reference, especially at low shear rates. However, it also displays

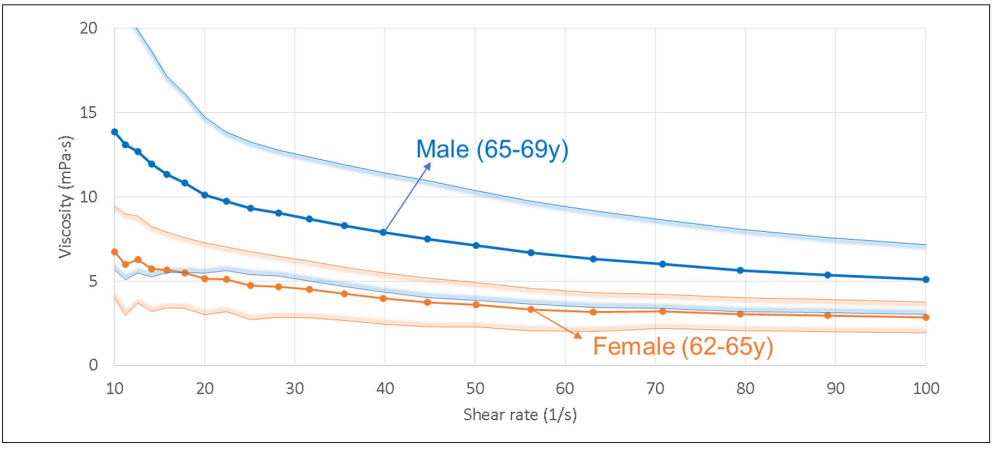

Figure 3. Viscosity profiles of synovial fluid from male patients aged 65–69 years (blue) compared to female patients aged 62-65 years (orange), under varying shear rates, shown by mean values and standard deviation

shear-thinning characteristics. The elevated viscosity highlights the biochemical changes associated with osteoarthritis, potentially impacting joint movement. Bovine synovial fluid demonstrates intermediate viscosity values between the reference and osteoarthritis samples. It shows similar trends in shear-thinning but does not achieve the low viscosity of the reference sample, reflecting partial resemblance to human synovial fluid. The artificial synovial fluid maintains consistently higher viscosity values compared to the other samples across all shear rates. Its limited shear-thinning behaviour and high viscosity suggest a poor mimicry of the natural properties of human synovial fluid.

In addition, water-based solutions exhibit significantly lower viscosity compared to the reference synovial fluid sample (*Figure 5*).

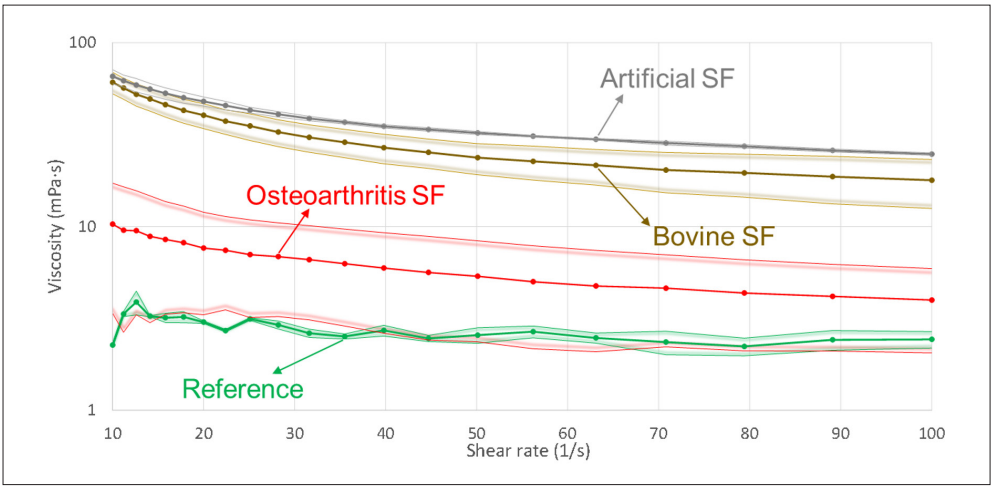

Figure 4. Viscosity variation of different synovial fluid samples as a function of shear rate. The samples include osteoarthritis (red), reference (green), bovine (brown) and artificial (grey) synovial fluid, shown by mean values and standard deviation

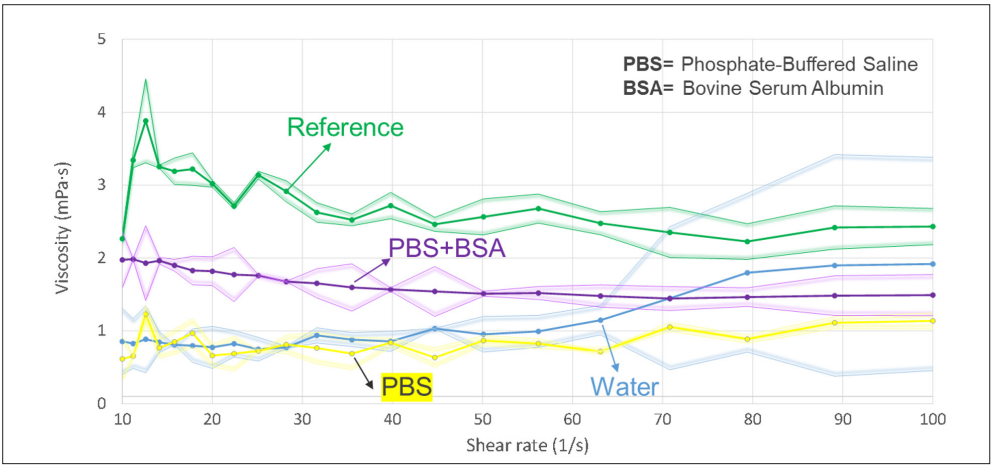

Figure 5. Viscosity measurements of reference synovial fluid (green), PBS+BSA (purple), water (blue), PBS (yellow), presented as mean values with standard deviation

Among the tested solutions, water demonstrates the lowest viscosity across the entire shear rate range, followed by PBS and PBS+BSA, both of which display slightly higher viscosities but remain considerably lower than the reference. These results emphasize the notable rheological differences between water-based solutions and natural synovial fluid. The reduced viscosity of these solutions limits their ability to mimic the lubricative and protective functions of human synovial fluid, particularly under varying shear conditions. This observation further underscores the importance of developing biomimetic alternatives with closer viscosity profiles to human synovial fluid for use in biological or mechanical applications.

This comprehensive analysis demonstrates substantial differences in viscosity between biological and alternative synovial fluid samples, as well as among human samples influenced by age, sex, and pathological conditions (*Figure 6*). The average viscosity was determined at the highest rotational speed, at which point the viscosity attained a constant value.

Male patients (aged 65–69 years) demonstrated significantly higher synovial fluid viscosity across all tested shear rates compared to the male reference sample (29 years old). This indicates age-related or pathological changes in the rheological properties of synovial fluid in male patients. The synovial fluid viscosity in the female group (aged 62–65 years) was lower, suggesting sex-based differences in synovial fluid composition or properties. These variations may reflect differences in joint biomechanics or biochemical composition between males and females. Bovine-derived and artificial synovial fluid samples exhibited significantly higher viscosities compared to human synovial fluid samples. This highlights their limitations as substitutes for mimicking natural human synovial fluid in viscosity-related applications, such as joint lubrication. Water-based solutions, including PBS, PBS+BSA, and water alone, displayed much lower viscosities than the reference human synovial fluid. These results indicate that such solutions lack the necessary rheological properties to effectively simulate natural synovial fluid for mechanical or biological applications.

Figure 7 displays the viscosity loss of human synovial fluid under constant shear conditions,

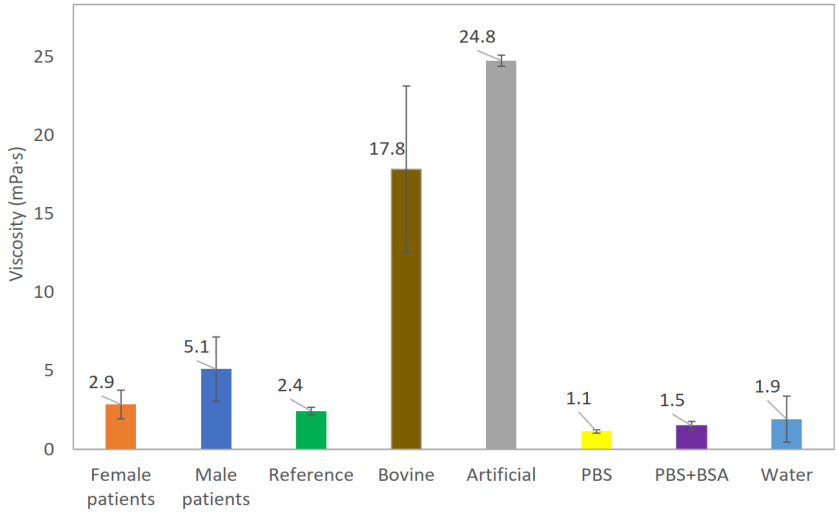

Figure 6. Viscosity comparison for different synovial fluid samples and other solutions, presented as mean values with standard deviation

comparing three sample groups: synovial fluid from male patients (ages 65–69), a younger male reference (age 29), and water to track the evaporation effect during measurements. At the beginning of the test, viscosity for all samples was normalized to 100% to facilitate a direct comparison of viscosity retention over time. As clearly visible in *Figure 7* significant differences in viscosity loss among the sample types are present.

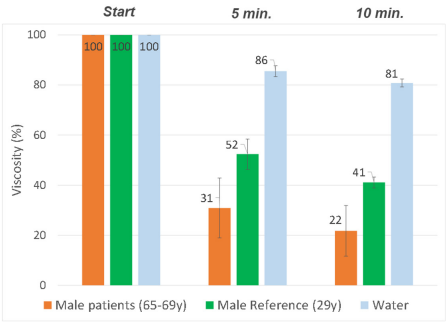

Figure 7. Normalised mean viscosity value with standard deviation for different synovial fluids and water measured at the start of the measurement, after 5 min., and after 10 min

Results after 5 minutes: the viscosity of synovial fluids from the older male patients exhibited a sharp decline, retaining only 31% of its initial viscosity. The synovial fluid from the reference retained 52% of its original viscosity, demonstrating significantly greater resistance to viscosity loss compared to the older male patients. Results after 10 minutes: the viscosity of the older male synovial fluids continued to decrease substantially, retaining only 22% of its initial value. The reference group retained 41% of its viscosity, continuing to show better preservation compared to the older group, although viscosity loss was still evident.

These findings suggest that age-related changes in the composition of synovial fluid may contribute to its reduced ability to maintain viscosity under mechanical stress. A possible explanation is a decline in key lubricating components such as hyaluronic acid or glycoproteins in older individuals, which are critical for maintaining the viscoelastic properties of synovial fluid. This difference highlights the potential impact of aging on joint lubrication and function.

The viscosity loss (%) of synovial fluid under constant shear conditions depends on several factors, including the composition of the fluid, shear rate, duration, and temperature. Under sustained shear, the hyaluronic acid and lubricin molecules, which contribute to viscosity, align in the direction of flow, leading to a reduction in resistance. Continuous shear stress can lead to mechanical degradation of hyaluronic acid and lubricin, resulting in a permanent loss of viscosity over time.[29] Experimental studies indicate that synovial fluid can lose 30–60% of its initial viscosity within a few minutes under high shear rates. In natural synovial fluid, viscosity can partially recover when shear stress is removed, due to the reorganization of hyaluronic acid and lubricin. However, in artificial synovial fluids, recovery is often limited due to the lack of self-repair mechanisms.

Tribological measurements

The Stribeck curves, i.e. coefficient of friction over sliding speed, are given in *Figure 8*. The synovial fluid samples from different patients, categorized by gender (male and female), were compared to the reference sample of the young male. The coefficient of friction was plotted as a function of sliding speed (m/s), with average values represented by thicker lines and circles, while standard deviations are depicted as thinner lines.

The reference sample exhibited the highest coefficient of friction values across most slid-

ing speeds, reaching a peak of approximately 0.30 at intermediate sliding speeds before decreasing at higher speeds. Female synovial fluid samples showed a similar trend but with slightly lower coefficient of friction values compared to the reference. Male synovial fluid samples, however, displayed consistently lower coefficient of friction values than both the reference and female samples across all sliding speeds. The results suggest a noticeable difference in the tribological performance of synovial fluid between genders, with male samples exhibiting reduced frictional behaviour. These differences may have implications for joint

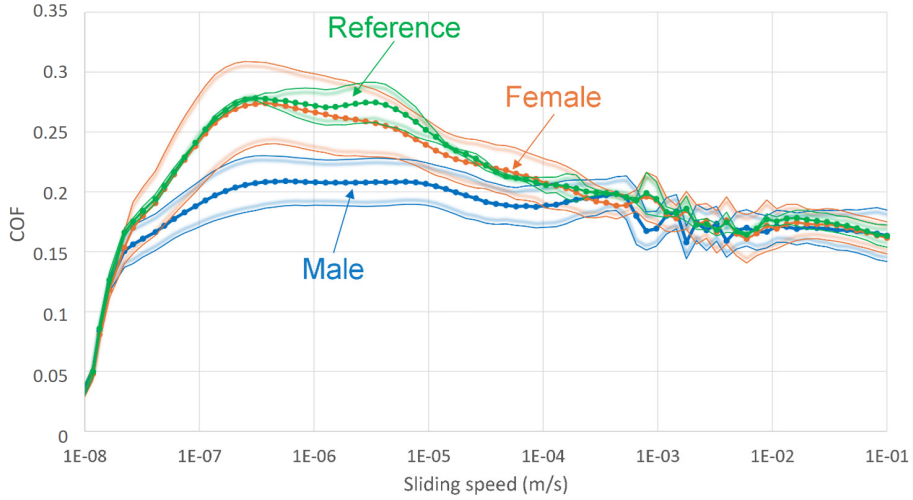

Figure 8. Stribeck curve of different patients' synovial fluid compared to reference sample; Average value of coefficient of friction is displayed with thicker lines and circles signs, standard deviations are shown as thinner lines

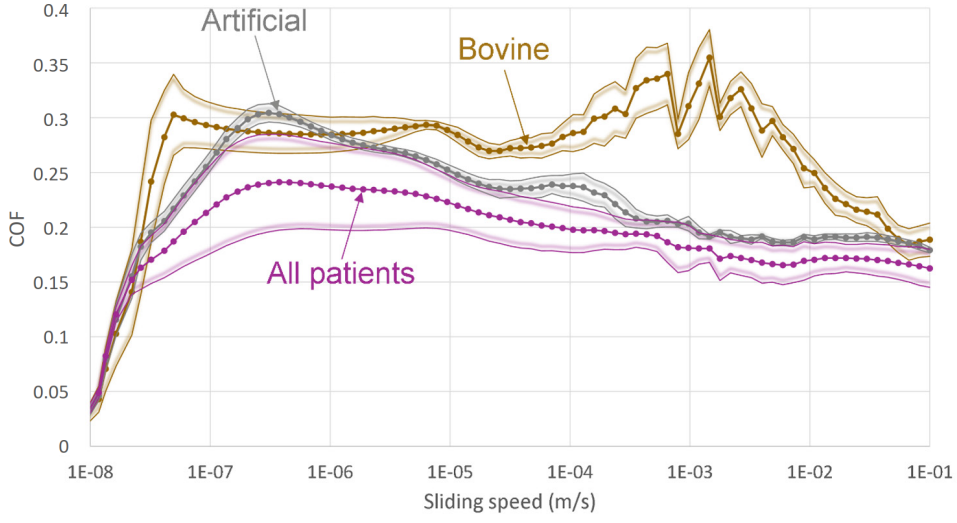

Figure 9. Stribeck curve of patient's synovial fluid compared to artificial and bovine synovial fluid; Average value of coefficient of friction is displayed with thicker lines and circles signs, standard deviations are shown as thinner lines

lubrication and mechanical properties under various physiological conditions. The Stribeck curves of synovial fluid samples from patients were compared to artificial and bovine synovial fluid to evaluate their tribological performance (*Figure 9*).

Bovine and artificial synovial fluid exhibited higher coefficient of friction values across most sliding speeds compared to the patient samples. The bovine synovial fluid curve peaked at approximately 0.35 at intermediate sliding speeds, while the artificial synovial fluid curve displayed a similar peak slightly above 0.30. In contrast, the coefficient of friction values for patient samples were consistently lower, peaking at approximately 0.25 before decreasing with increasing sliding speeds.

These findings highlight the distinct frictional properties of bovine and artificial synovial fluid compared to human synovial fluid samples from patients. The differences may be attributable to variations in biochemical composition and lubrication mechanisms, which could have implications for joint biomechanics and the development of synthetic lubricants.

The average coefficient of friction for synovial fluid samples from different sources, along with their standard deviations, are presented in *Figure 10*. Among the patient samples, male synovial fluid exhibited the lowest coefficient of friction value of 0.18, followed closely by female synovial fluid at 0.20. The reference sample showed a slightly higher coefficient of friction value of 0.21. Bovine synovial fluid demonstrated the highest coefficient of friction value of 0.27, significantly exceeding those of the patient samples and the reference. Artificial synovial fluid exhibited a coefficient of friction value of 0.22, which was marginally higher than the reference but lower than the bovine sample.

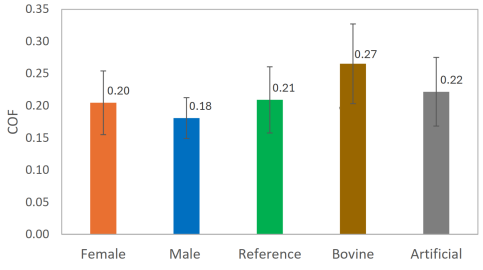

Figure 10. Average coefficient of friction with standard deviation values from different synovial fluid sources

These results highlight that bovine synovial fluid has a higher coefficient of friction compared to both human-derived and artificial synovial fluid, suggesting notable differences in lubrication properties between natural and artificial sources. The lower coefficient of friction in male and female patient samples may reflect specific physiological or biochemical factors influencing frictional behaviour.

The average coefficient of friction of healthy synovial fluid ranges from 0.001 to 0.03, whereas that of osteoarthritic or diseased synovial fluid ranges from 0.05 to 0.2.[30] The coefficient of friction of our reference sample is relatively high compared to healthy synovial fluid, and there are several potential reasons for this observation. Firstly, the control sample is missing key lubricating components such as hyaluronic acid or lubricin. Synovial fluid with a lower molecular weight hyaluronic acid or reduced protein content tends to have a higher COF. The presence of proteins or contamination could contribute to increased friction.

Analysis of the chemical structure

The absorbance spectra for blood samples and synovial fluids show distinct peaks around 3300 cm^{-1} and 1650 cm^{-1} in the infrared (IR) spectrum, due to O-H and N-H stretching vibrations, which are likely associated with water content and protein structures, like amide

groups. C=O stretching vibrations, this band is typically observed around 1650 cm^{-1}.[31] The fat tissues show multiple prominent peaks, particularly around 3000 cm^{-1} and 2800 cm^{-1}, likely corresponding to the asymmetric and symmetric CH_3 stretching vibrations[32], which are indicative of lipid content. Additionally, the CH_2 stretching vibrations typically appear in the 2857–2850 cm^{-1} range, further reflecting the lipid composition of the tissue (*Figure 11*).

The absorbance spectra for blood, fat tissue, and synovial fluid samples revealed key characteristics associated with their molecular compositions. Blood samples: a strong absorbance peak around 3300 cm^{-1} is indicative of O-H stretching vibrations, likely from water and hydrogen-bonded compounds. Peaks in the lower wavenumber region correspond to protein and lipid components. Fat tissue: the spectra show distinct lipid-related peaks, particularly in the 2800–3000 cm^{-1} range (C-H stretching vibrations) and 1700–1800 cm^{-1} region (C=O stretching). synovial fluid samples: similar to blood, the synovial fluid exhibits prominent peaks around 3300 cm^{-1} (O-H stretching) and additional features linked to its proteinaceous and lipid content.

These findings underline the compositional differences and similarities between the analysed biological samples, providing a foundation for further investigations into their functional roles and potential applications in biomedical research.

pH analyses

The pH values of human synovial fluid, blood, and water-based samples were evaluated to assess their acid-base balance (*Figure 12*). The results indicate that the pH of synovial fluid from both male and female samples closely aligns with the reference pH, demonstrating minimal variation between sexes.

Blood pH also exhibits values consistent with the reference sample, remaining near neutral but slightly more alkaline compared to synovial fluid, as expected under physiological conditions. In contrast, artificial synovial fluid showed a markedly acidic pH of 4.7, which is significantly lower than the pH of natural synovial fluid. This deviation highlights a fundamental difference between artificial and physiological synovial fluid in terms of acid-base properties. Water-based solutions, including PBS and PBS supplemented with BSA, maintained a pH conditions. These findings emphasize the relative stability of pH in natural biological fluids (synovial fluid and blood) under physiological conditions, while artificial synovial fluid presents a considerably acidic profile. This distinction is critical when considering the suitability of artificial synovial fluid for experimental applications, because it

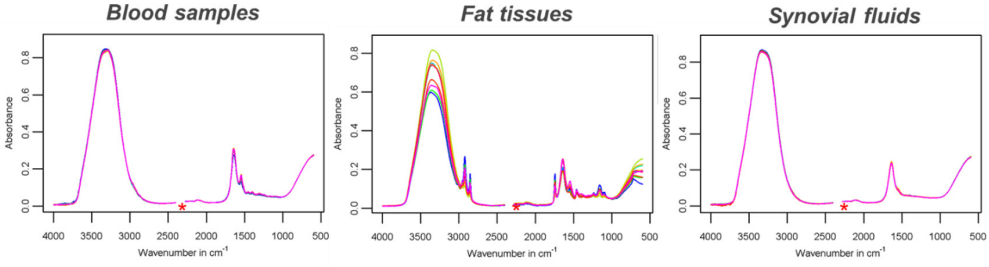

Figure 11. The graphs depict absorbance spectra for blood, fat tissue, and synovial fluid across a wavenumber range of 4000 to 500 cm^{-1}

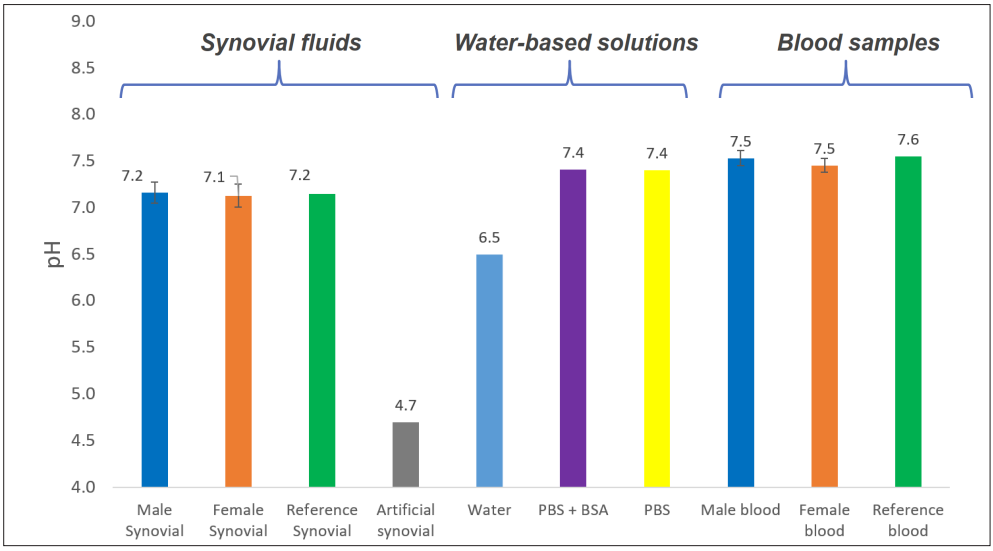

Figure 12. pH values of synovial fluid, blood, and water-based samples, shown as mean values with standard deviation

may not fully replicate the complex rheological and tribological properties of natural synovial fluid, which is essential for joint lubrication and load distribution.

According to the literature, the pH values for human osteoarthritis synovial fluid range from ~7.1 to 7.4 at 37 °C, indicating a mildly acidic condition compared to the pH range of 7.4 to 7.8 observed in healthy synovial fluid. Decreased pH levels are attributed to inflammation, cartilage degradation, and metabolic changes within the joint. Blood pH levels in osteoarthritis patients: ~7.4 – 7.5, but blood pH is not significantly affected unless severe metabolic imbalances occur.[33]

Conclusion

The rheological and frictional properties of human materials, particularly synovial fluid and blood, play a crucial role in the performance and longevity of connected implant components. Our findings highlight significant variations in synovial fluid viscosity based on biological and experimental factors. Male patients exhibited higher synovial fluid viscosity compared to females, suggesting potential gender-based physiological differences. Additionally, artificial and bovine synovial fluid displayed viscosities distinct from human synovial fluid, emphasizing the need for careful consideration when using these substitutes in experimental or clinical settings. Lastly, water-based solutions demonstrated markedly lower viscosities, underlining their limited suitability for replicating the complex properties of natural human synovial fluid. These insights underscore the importance of tailoring implant designs and material compatibility to the specific rheological environment, ensuring optimal function and durability of implant systems.

The study revealed distinct frictional properties of synovial fluid from different sources. Male patient samples consistently exhibited the lowest coefficient of friction, followed by female samples, with both showing superior lubrication compared to artificial and bovine

synovial fluid. The higher coefficient of friction values of bovine and artificial fluids point out their differing lubrication mechanisms and biochemical compositions. These findings highlight gender-based variations in human synovial fluid and emphasize the importance of selecting appropriate lubricants for joint biomechanics and implant designs.

The absorbance spectra of blood, fat tissue, and synovial fluid samples revealed key molecular characteristics associated with water, proteins, and lipids. Blood and synovial fluid exhibited prominent peaks, indicating water content and protein structures, while fat tissue showed distinct peaks, reflecting its lipid content. These spectral differences and similarities emphasize the unique compositions of each sample, providing valuable insights into their molecular properties. This analysis lays the groundwork for further research into the functional roles of these biological materials and their potential applications in biomedical field.

The study showed the stability of pH in natural biological fluids such as synovial fluid and blood, which remain near physiological levels with minimal variation between sexes. In contrast, artificial synovial fluid exhibits a significantly acidic pH, underscoring its divergence from natural synovial fluid acid-base balance. These findings are crucial for evaluating the suitability of artificial synovial fluid in experimental and clinical settings, where maintaining physiological pH is essential for accurate modelling and application.

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