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Faculty of Mechanical Engineering
Institute of Machine and Industrial Design

EFFECT OF VISCOSUPPLEMENTATION ON FRICTION OF ARTICULAR CARTILAGE

Ing. David Rebenda
Autor práce
Author

doc. Ing. Martin Vrbka, Ph.D.
Vedoucí práce
Supervisor

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ABSTRAKT

Disertační práce se zabývá experimentálním studiem viskosuplementů na bázi kyseliny hyaluronové, které se aplikují do synoviálních kloubů postižených osteoartrózou. Hlavní pozornost byla věnována objasnění vlivu koncentrace a molekulové hmotnosti kyseliny hyaluronové na tření v kontaktu kloubí chrupavky resp. změnám tření v kontaktu po smíchání osteoartritické synoviální kapaliny s exogenní kyselinou hyaluronovou. Důležitou součástí experimentů bylo rovněž studium reologických vlastností synoviální kapaliny a kyseliny hyaluronové. Výsledky ukázaly, že molekulová hmotnost kyseliny hyaluronové významně ovlivňuje viskozitu a viskoelastické vlastnosti roztoku. Výrazná závislost mezi reologickými vlastnostmi kyseliny hyaluronové a třením v kontaktu však nebyla pozorována. Přimíchání kyseliny hyaluronové do synoviální kapaliny způsobí výrazný pokles součinitele tření v kontaktu. Rozdíly mezi viskosuplementy obsahující kyselinu hyaluronovou s různou molekulovou hmotností ale nijak výrazné nejsou. Nicméně, výsledky poukazují na možné ovlivnění režimu mazání v důsledku vysoké molekulové hmotnosti kyseliny hyaluronové. Tyto původní výsledky rozšiřují pochopení mechanizmů, ke kterým dochází v kloubu bezprostředně do vstříknutí kyseliny hyaluronové a mohou být použity při dalším vývoji viskosuplementů či v klinické praxi.

KLÍČOVÁ SLOVA

kloubní chrupavka, viskosuplementace, kyselina hyaluronová, tření, reologie

ABSTRACT

This dissertation thesis deals with the experimental analysis of hyaluronic acid-based viscosupplements which have been applied into the synovial joints in order to slow down the osteoarthritis progression. The main attention was paid to the effect of hyaluronic acid concentration and molecular weight on the articular cartilage friction as well as to the frictional changes after mixing of osteoarthritic synovial fluid with exogenous hyaluronic acid. An important part of the experiments was also an analysis of synovial fluid and hyaluronic acid rheological properties. The results showed that the hyaluronic acid molecular weight can significantly affect the viscosity and viscoelastic properties of the solution. However, no dependency between the hyaluronic acid rheological properties and friction in the articular cartilage contact was observed. The admixture of hyaluronic acid into the synovial fluid caused a significant decrease in the coefficient of friction within the contact but the differences between individual viscosupplements were not so significant. Nevertheless, the results indicate a possible change in the lubrication regime due to the high molecular weight of hyaluronic acid. These original results deepen the understanding of the mechanisms that occur in the synovial joint immediately after the injection of hyaluronic acid and can be further used in the future development of viscosupplements or in clinical practice.

KEYWORDS

Articular cartilage, viscosupplementation, hyaluronic acid, friction, rheology

BIBLIOGRAPHICAL REFERENCE

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STATEMENT

I hereby declare that I have written the PhD thesis *Effect of Viscosupplementation on Friction of Articular Cartilage* on my own according to advice of my supervisor doc. Ing. Martin Vrbka, Ph.D., and using the sources listed in references.

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Author's signature

CONTENT

1 INTRODUCTION

OA is one of the most common diseases of the musculoskeletal system among older people in developed countries. According to the OARSI (Figure 1-1), approximately 55 million adults were diagnosed with arthritis in the United States in 2015 [1]. However, the number of patients still increases mainly due to the aging population or the obesity of people. OARSI assumes a twenty percent increase in patients over the next ten years. This brings a lot of attention to the development of new treatment methods or to the improvement of existing treatment methods and thus the reduction of treatment costs.

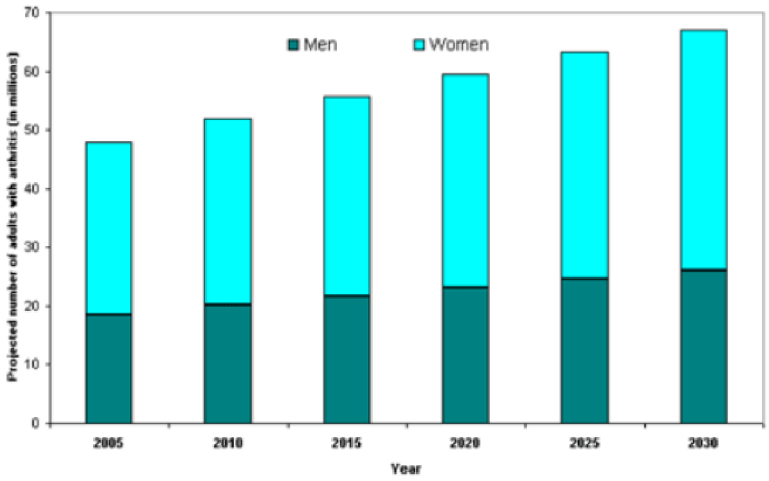

Figure 1-1 Numbers of Patients with OA [1]

Viscosupplementation is one of the non-invasive methods for the treatment of OA for more than 30 years. It consists in HA-based injection into the joint capsule. The original idea of viscosupplementation was the resumption of healthy SF rheological properties but long-term results of medical studies also pointed out to some physiological effects inside the affected joint. However, there are still many debates about the effectiveness of this treatment method, which also leads to the conflicting recommendations of medical associations. This is mainly due to the unexplained phenomena which occur in the synovial joint after viscosupplementation.

Most of the experimental studies, which are connected with viscosupplementation effectiveness, focus on the rheological analysis of osteoarthritic SF, HA or VSs. However, the connection between SF rheological changes after viscosupplementation and the friction in the osteoarthritic joint are still unclear. Interaction of HA with cartilage structure and other SF constituents plays an important role in the lubrication of articular cartilage. However, changes in the articular cartilage friction and lubrication, caused by increased concentration and molecular weight of HA in SF after viscosupplementation, are uncleaned.

Therefore, the aim of this PhD thesis is to provide an experimental analysis of frictional changes in the osteoarthritic synovial joint after viscosupplementation. The main attention is paid to the effect of HA concentration and molecular weight on the rheology of SF and the friction within the articular cartilage contact. So far, there is no such a complex study combining rheological measurements of SFs and HA solutions with an analysis of their frictional response within the articular cartilage contact.

2 STATE OF THE ART

Articular cartilage is a white gloss porous tissue which covers sliding surfaces in big synovial joints like hip, knee or shoulder. Under physiological conditions, cartilage-on-cartilage motion is characterized by extremely low friction and minimal wear [2]. These excellent tribological properties are achieved by the biphasic structure of articular cartilage, i.e., by the interaction between its solid and fluid phase [3].

The solid phase makes up 20 – 30 % of the cartilage mass. It is composed of an extracellular matrix from collagen II fibers, proteoglycans and chondrocytes. Chondrocytes synthesize and repair the extracellular matrix [4]. Proteoglycans, mainly glycoprotein PRG4 [5], are mostly contained in the middle layer of cartilage and less in the upper layer of cartilage (Figure 2-1). These macromolecules are able to bind water to each other. As a result, most of the interstitial fluid is close to the surface. Proteoglycans are also able to bind HA [6] and thus improve cartilage friction by forming a boundary lubricant layer on the cartilage surface.

According to the orientation of collagen fibers and chondrocyte shape, cartilage structure can be divided into several layers (Figure 2-1) [7]. In the superficial zone, collagen fibers are parallel to the surface, which allows the transmission of high shear loads [3]. Contrary to this, collagen fibers are randomly organized in the intermediate zone, which results in better resistance against compressive load.

Cartilage extracellular matrix is filled with SF. Water make up for 65 - 80 % of cartilage structure and is the main constituent of SF. Besides that, proteins, HA, phospholipids, PRG4, etc. are dissolved in SF. These constituents play an important role during articular cartilage lubrication. The effect of individual synovial constituents on the friction of articular cartilage will be discussed in detail later.

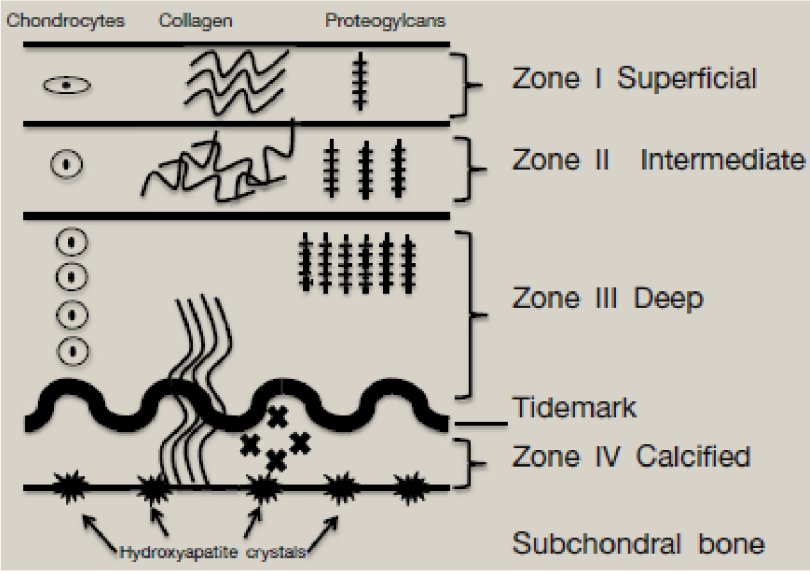

Figure 2-1 Scheme of articular cartilage structure [3]

2.1 Rheology of SF and HA

The progression of OA is connected with changes in SF composition. Among other things, the concentration and molecular weight of HA are decreased. As commonly seen in the literature, the rheology of SF is strongly connected with HA characteristics. Proteins, even at high concentrations, contribute minimally to solution viscosity. Little or no effect of proteins on SF rheology was reported by Zhang et al. [8]. Authors utilized several techniques of viscosity measurements (different geometries – single gap, double gap, and parallel plate) to examine the role of bovine serum albumin and γ-globulin on model SF rheology. Measurements were realized on TA Instruments AR-G2 rheometer. The tested solutions were subjected to steady and oscillatory shear tests for different ranges of shear rate and oscillation frequencies. Model solutions composed of albumin (10 mg/ml), γ-globulin (0.5 mg/ml) and HA (3.4 mg/ml) with a molecular weight of 1.6 MDa were dissolved in PBS to simulate SF. Results of steady shear tests with model SF and HA were very similar (Figure 2-2). Therefore, the concentration of albumin and γ-globulin did not affect SF viscosity. The authors conclude that proteoglycan PRG4, which is also part of the synovial fluid, could affect the rheology. However, this SF constituent was not tested in the study.

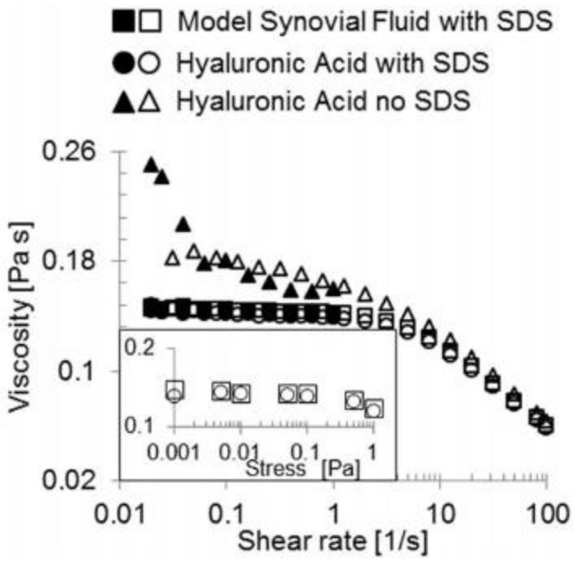

Figure 2-2 Result of viscosity measurements with HA and model SF [8]

Changes in SF composition and rheology due to the progression of OA were analyzed by Tyrnenopoulou et al. [9]. Sixteen samples of equine SFs were extracted from osteoarthritic fetlock joints. Results were compared with 6 samples obtained from normal joints. Refractometer was used to analyze the total amount of proteins in SFs. Total protein concentration (Figure 2-3) has increased from 1.37 ± 0.15 g/dl to 1.74 ± 0.14 g/dl whereas the concentration of HA decreased from 864.4 ± 260.42 µg/ml to 402.67 ± 131.3 µg/ml

in the osteoarthritic SF. The reduced HA concentration affected the results of subsequent analysis of viscoelastic properties (Figure 2-3). Viscoelastic properties of osteoarthritic SFs were significantly lower compared to the ones obtained from normal joints. The intersection of storage and loss modulus curves has also changed. Crossover frequency moved from 2 Hz to 8 Hz.

Variable	Joint status	Number of Joints (N)	Mean (M)	Standard Deviation SD)
TP (g/dl)	Normal	6	1.37	0.15
	OA	16	1.74	0.14
TNC	Normal	6	130.00	54.41
	OA	16	243.13	41.10
HA (µg/ml)	Normal	5	864.40	260.42
	OA	9	402.67	131.30

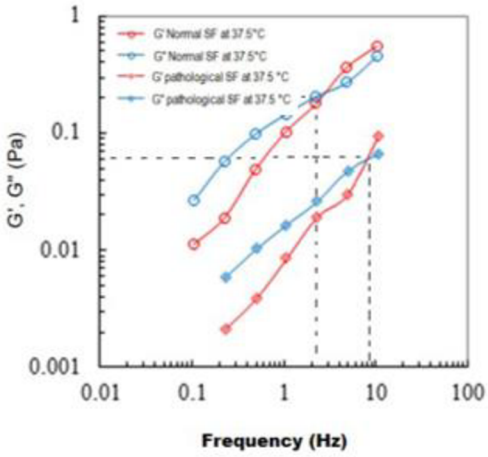

Figure 2-3 Left: Total protein (TP) and HA concentration in normal and osteoarthritic SF. Right: Frequency sweeps of SFs [9]

Differences in equine SF rheology due to OA were also analyzed by Borzachiello et al. [10]. However, rather different results of the oscillatory shear tests were observed. Lower values of storage and loss modulus were also measured for diseased SF. Nevertheless, an increased value of crossover frequency caused by lower concentration and molecular weight of HA was not reported. Contrary to the results of the previous study, a slight decrease in crossover frequency was reported.

Differences in viscosity and viscoelastic properties of human SFs in patients undergoing primary and revision knee arthroplasty were analyzed by Mazzucco et al. [11]. The results were compared with bovine serum, which is commonly used as a lubricant during tribological tests of human joints and joint replacement. In total, 58 samples of SFs from patients undergoing primary arthroplasty and 19 samples from patients undergoing revision arthroplasty were tested. SFs and bovine serum were tested on a TA Instruments CSL 500 rheometer in a concentric cylinder configuration.

Pseudoplastic behavior was observed in all samples of SFs. Zero shear viscosity of individual samples varied by three orders of magnitude (Figure 2-4). SFs from patients with primary arthroplasty reported higher viscosity than samples from revision arthroplasty patients. The measured viscosities of SFs were approximately two orders of magnitude higher compared to the bovine serum. From the analysis of viscoelastic properties, most of the samples exhibited viscoelastic behavior. Crossover frequency of 1.8 Hz was measured for primary arthroplasty patients, whereas a relatively high crossover point of 3.1 Hz was measured for revision arthroplasty patients.

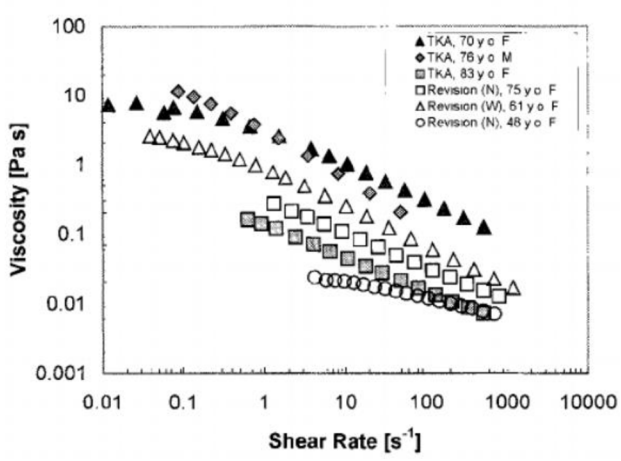

Figure 2-4 Viscosity of SF from patients who undergoes knee arthroplasty [11]

Viscosity and viscoelastic properties of 13 SF samples aspirated from osteoarthritic joints were analyzed by Mathieu [12]. The rheological properties of pure SF, as well as SF mixed with native HA or commercial VS based on cross-linked HA, were discussed. The SF was mixed with HA or VS in a 1:1 ratio. This ratio corresponds to the in vivo viscosupplementation in clinical practice. HA had a molecular weight of 1.14 MDa. As a VS, Synvisc® based on a cross-linked Hylan G-F 20 was used. Measurements were conducted at 25 ° C. This time, relatively large differences in viscosity between individual SF samples were also observed. The zero shear viscosity varied between 0.1 and 10 Pa·s (Figure 2-5a). For HA-based solutions, the results showed a significant difference between linear and cross-linked HA. Moreover, Synvisc® exhibited gel-like behavior during oscillatory shear tests whereas linear HA exhibited viscous-like behavior. The addition of linear HA into the SF caused only a slight increase of viscosity (Figure 2-5b) whereas the addition of Synvisc® to the SF led to a significant increase in viscosity across the whole range of shear rate. The viscosity of Synvisc® mixture was approximately 2 orders of magnitude higher than the viscosity of the linear HA mixture.

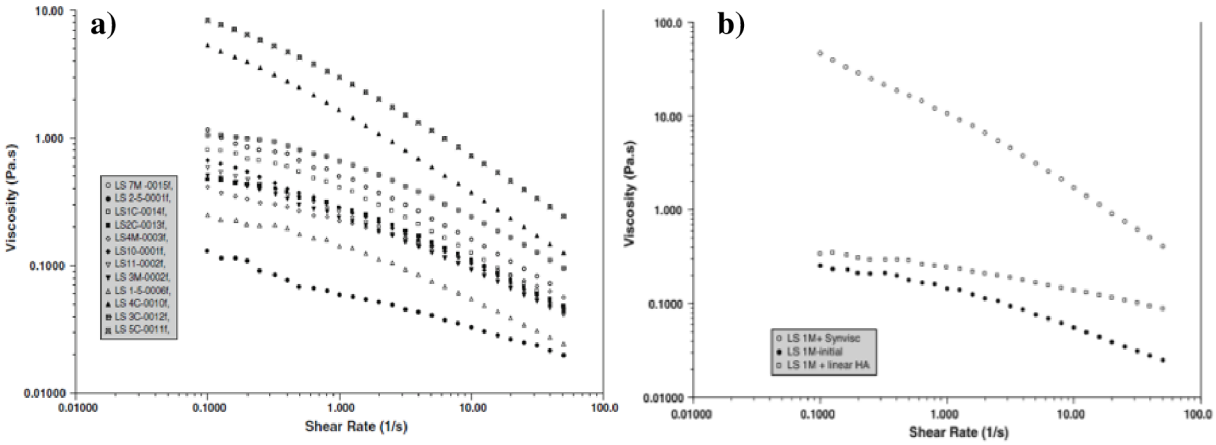

Figure 2-5 a) Viscosity of aspirated SF s, b) Viscosity of pure SF and mixtures with HA solutions [12]

Viscosity of different SFs was also investigated by Bingöl et al. [13]. In total, seven SF samples were gathered post mortem from human knee joints. The age of patients was between 62 and 89 years. No clinical evidence of OA was mentioned. Therefore, a healthy or osteoarthritic SF could be tested. For comparison, the viscosity of HA from fermentative production was analyzed. Powder with a molecular weight of 1.7 x 10^6 g/mol was dissolved in PBS. The measurements were performed using TA Instruments AR G2 rheometer in cone-plate and plate-plate (SF samples due to their small amount) geometry. The highest measured zero shear viscosity of gathered SF was 445 Pa·s. This value is even outside of the commonly reported range of normal SF [14] and is in accordance with the reported gel-like character of the sample. On the contrary, 1.2 Pa·s and 2.5 Pa·s were the lowest measured zero shear viscosities. These low values were attributed to the progression of OA. All viscosity curves of gathered SFs are displayed in Figure 2-6a. The HA concentration dependence on the shear rate is shown in Figure 2-6b. The results showed a strong dependency between HA concentration and viscosity. With increasing concentration, the viscosity increased by four orders of magnitude. For high concentration solutions, the transition between Newtonian and non-Newtonian behavior occurred at lower values of shear rate.

Another rheological characterization of SFs from 22 patients undergoing total knee arthroplasty and three commercially available VSs was performed by Bhuanatanondh [15]. The commercial VSs were represented by Orthovisc® and Suplasyn®, which are based on linear HA, and Synvisc®. Rheology measurements were performed in a cone-plate configuration (40 mm diameter cone with a 1° angle) at 37 °C. Viscosity dependence on shear rate was measured for a shear rate between 0.01 s^{-1} and 1 000 s^{-1}. Consequently, storage and loss modulus dependency on the frequency of oscillating motion was measured at 5 % strain over the frequency range of 0.1 – 10 Hz. The average zero shear viscosity for SFs was 3.4 ± 2.9 Pa·s. At a frequency of 0.5 Hz, a storage modulus of 2.14 ± 1.7 Pa and

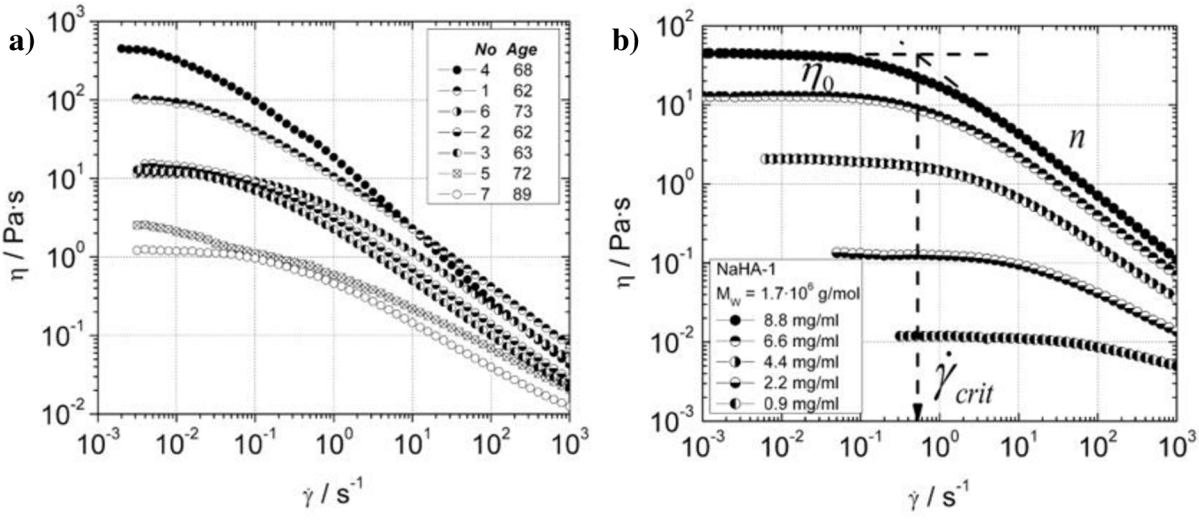

Figure 2-6 a) Shear viscosities of human SFs, b) Influence of concentration on the viscosity of sodium hyaluronate [13]

a loss modulus of 1.63 ± 1 Pa were measured. At a frequency of 2.5 Hz, the storage modulus increased to 3.55 ± 2.72 Pa and the loss modulus has changed to 2.51 ± 1.25 Pa. For commercial VSs (Figure 2-7a), the lowest viscosity across the entire range of shear rate was measured for VS with the lowest molecular weight HA - Suplasyn®. Cross-linked HA had the highest zero shear viscosity. Compared to low molecular weight HA, the viscosity at low shear rates was more than two orders of magnitude higher.Cross-linked HA also reported gel-like behavior during the measurement of viscoelastic properties (Figure 2-7b). Low molecular weight HA reported viscous-like behavior. To sum it up, the viscosity of VSs depends on molecular weight and HA cross-linking.

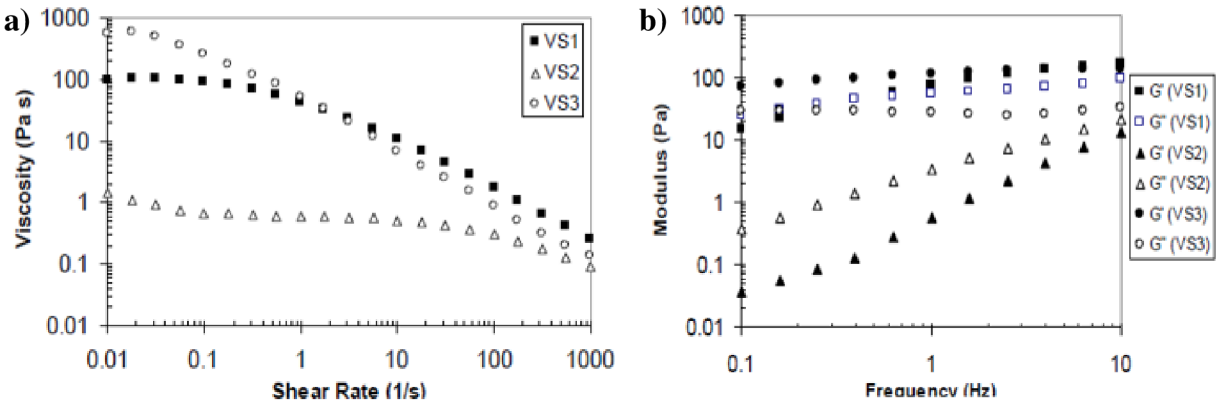

Figure 2-7 a) Viscosity of tested VSs, b) Viscoelastic properties of tested VSs (VS1 = Orthovisc®, VS2 = Suplasyn®, VS3 = Synvisc®) [15]

In a consequent study by Bhuanatanondh et al. [16], rheological changes after mixing of osteoarthritic SF with the same VSs were analyzed. SF was mixed in a 1:1 ratio with VSs while the experimental conditions have not been changed. Mixing of the VS with SF led to an increase in viscosity over the whole range of shear rate (Figure 2-8a). High molecular weight linear HA caused an increase in viscosity by one order of magnitude, cross-linked HA by two orders of magnitude. On the other hand, Suplasyn® did not perform quite well. Only a slight viscosity increase at higher shear rates was observed. Higher molecular weight of VS also led to a more pronounced shear-thinning behavior. Figure 2-8b shows the results of viscoelastic properties measurements. The admixture of low molecular weight HA caused an increase in the loss modulus and the solution exhibited liquid-like behavior. The addition of high molecular weight HA increased the values of storage and loss modulus by one order of magnitude and a crossover point at 1 Hz was measured. The addition of cross-linked HA caused an increase of both modules by more than two orders of magnitude and the solution exhibited gel-like behavior over the whole range of oscillating frequency.

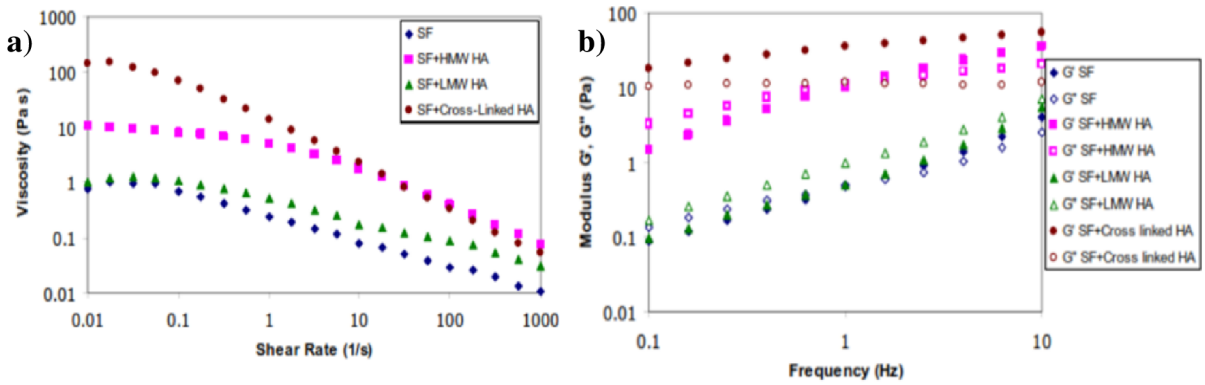

Figure 2-8 a) Viscosity of SF and mixtures with VSs, b) Viscoelastic properties of SF and mixtures [16]

Several commercial VSs in their pure form and newly developed VS Hymovis® were analyzed by Finelli et al. [17]. Viscosity and viscoelastic properties were analyzed with a stress-controlled rheometer by TA Instruments in a 20 mm parallel plate geometry. Samples were heated to 25 °C by a Peltier plate. Firstly, to determine the region of linear response of the VSs, an initial strain sweep was applied to the tested samples. All products exhibited the independence of storage and loss modulus from strain in a region between 0.1 and 50 %. Therefore, subsequent frequency sweeps were conducted at a 5 % strain.

Results in Figure 2-9b showed that Hymovis®, Durolane® and Synvisc® exhibited gel-like behavior, whereas the crossover frequency lay below the range of knee motion frequency. The Durolane® crossover frequency was not even measurable. Gel-like behavior is typical for chemically cross-linked VSs with relatively high viscosity. However, Hymovis® was a hexadecyl derivate based on the non-chemically cross-linked HA with a relatively low molecular weight of 700 kDa. Very similar results of Hymovis® and cross-linked HA products were also reported during steady shear measurements (Figure 2-9a). Zero shear viscosity (measured at a shear rate of $7 \cdot 10^{-2}$ s^{-1}) was approximately 2 000 Pa·s for Hymovis® and 1 300 Pa·s for Synvisc®.

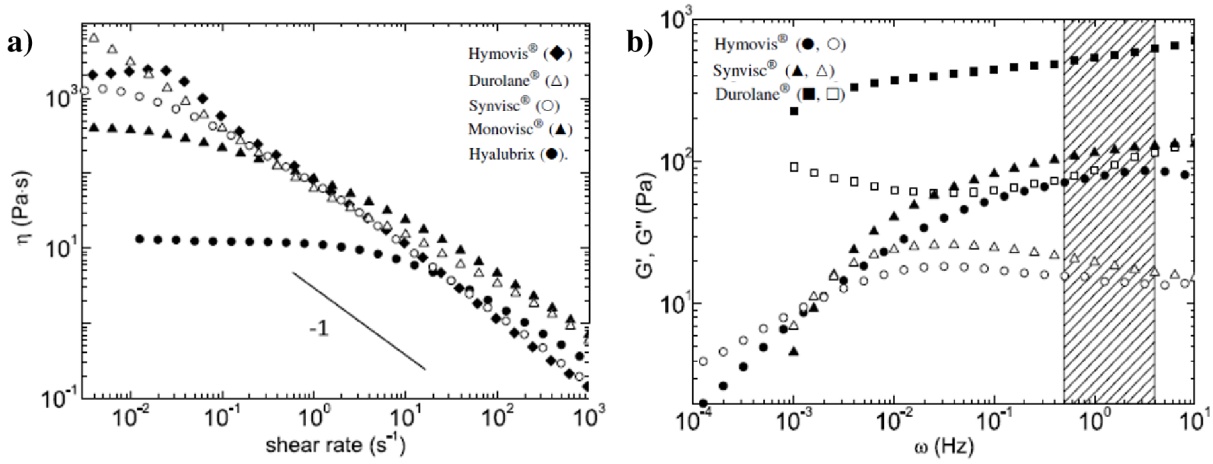

Figure 2-9 a) Viscosity curves of VSs, b) Viscoelastic properties of VSs [17]

Viscosity and viscoelastic properties of pure commercial HA-based VSs were also analyzed by Nicholls et al. [18]. Table 2-1 summarizes the values of all measured and calculated quantities – zero shear viscosity, shear-thinning ratio (ratio of zero shear viscosity and viscosity at 250 s^{-1}) and crossover frequency. Differences between all tested products were seen in all of the investigated parameters. Several solutions had zero shear viscosity that fell outside the healthy SF viscosity range. Shear-thinning ratios of Euflexxa®, Gel-One® and Orthovisc® were the most similar to a healthy knee SF. The crossover frequencies of Orthovisc® (0.16 Hz) and Euflexxa® (0.1 Hz) were the closest to the values of a healthy knee SF too. Crossover frequencies of Synvisc® and Synvisc One® were below 0.01 Hz, whereas Hyalgan® reported a crossover frequency of 10 Hz. This shows that cross-linked products report gel-like behavior and Hyalgan® reported liquid-like behavior under kinematic conditions of the knee. To sum it up, Orthovisc® and Euflexxa® are the most similar to a healthy knee SF in terms of viscosity and viscoelasticity.

Table 2-1 Rheological properties of tested HA-based VSs [18]

Product	Mw (kDa)	Cross-linking	Zero shear rate viscosity ($\eta_{0.1}$ (Pa s))	Shear thinning ratio ($\eta_{0.1}/\eta_{250}$)	Crossover frequency (Hz)
Hyalgan®	500–730	No	0.27	2.33	> 10[c]
Supartz®	620–1170	No	3.07	10.9	3.98
Monovisc®	1000–2900[a]	Yes	56.4	51.5	2.51
Orthovisc®	1000–2900	No	120.8	170.4	0.16
Euflexxa®	2400–3600	No	91.2	237.2	0.10
Gel-One®	N/A[c]	Yes	190.2	243.0	[b]
Synvisc®	6000[d]	Yes	191.7	740.7	< 0.01[c]
Synvisc-One®	6000[d]	Yes	184.4	651.2	< 0.01[c]

2.2 Effect of SF composition on cartilage friction

In 2006, Forsey et al. [19] analyzed the frictional behavior of cartilage-on-cartilage contact lubricated by HA, DPPC or their combinations. The frictional measurements were performed on a pin-on-plate tribometer. The pin was loaded with a force of 25 N, which corresponded to a contact pressure of 1.3 MPa. The speed was set to 4 mm/s. The contact was lubricated by HA solution with a molecular weight of 3 MDa and a concentration of 5 mg/ml or 10 mg/ml. The concentration of DPPC solutions was 100 mg/ml or 200 mg/ml.

From the results in Figure 2-10, it can be concluded that the concentration of HA has no significant effect on friction. According to the authors, under boundary lubrication regime, the friction mainly depends on the biphasic structure of cartilage and the boundary lubricating layer which is mostly composed of proteins and phospholipids. On the other

hand, the DPPC concentration significantly affected the values of CoF. The highest decrease in friction was observed for the solution containing HA at a concentration of 10 mg/ml and DPPC at a concentration of 200 mg/ml. All measurements were conducted with fluorescently labeled HA. The consequent analysis showed that HA penetrates through the cartilage superficial layer into the intermediate layer. HA was concentrated around chondrocytes. It probably interacts with them via CD44 receptors and stimulates chondrocytes to synthesize new HA.

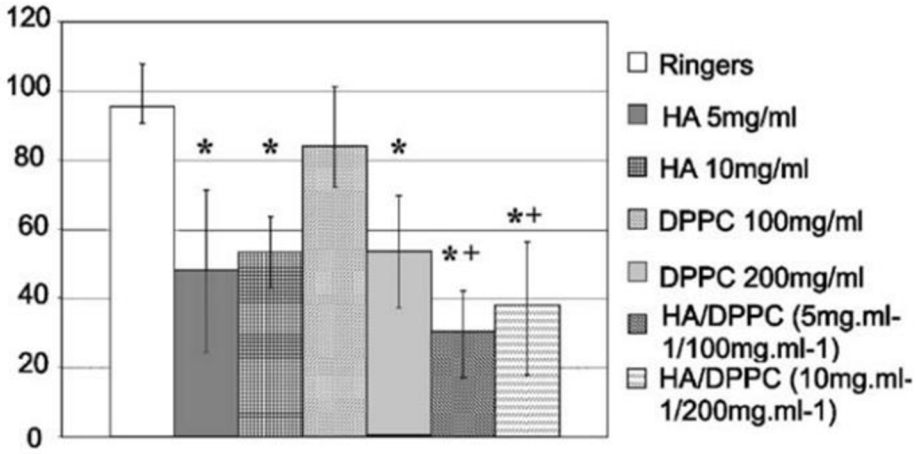

Figure 2-10 Percentage difference in CoF for various lubricants [19]

In the same year, Bell et al. [20] analyzed the frictional behavior of cartilage-on-cartilage contact lubricated by HA. CoF under static and dynamic loading conditions was measured for healthy and mechanically damaged articular cartilage. Mechanically damaged cartilage exhibited higher values of the CoF during all measurements. HA proved to be a very effective lubricant. During static loading tests, the values of the CoF were significantly lower compared to the PBS. In the dynamic loading tests (Figure 2-11), the difference became apparent in the later stages of the measurement due to the lubricant discharge from the articular cartilage structure.

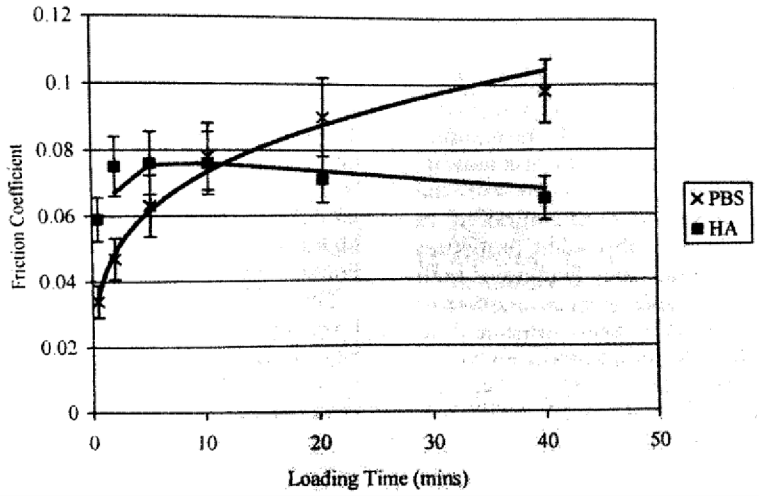

Figure 2-11 CoF for damaged articular cartilage under dynamic loading conditions [20]

The effectiveness of interactions between HA, PRG4 and SAPL within the boundary lubrication of cartilage-on-cartilage contact was investigated by Schmidt et al. [21]. Results were also compared with bovine SF. HA solutions with a concentration of 110 µg/ml, 1 100 µg/ml and 3 300 µg/ml, PRG4 solutions with a concentration of 4.5 µg/ml, 45µg/ml and 450 µg/ml and SAPL solutions with a concentration of 200 µg/ml were tested. The highest value of the CoF was measured for PBS and the lowest value was measured for SF (Figure 2-12). Solutions containing individual SF constituents always reduced friction in comparison with PBS, whereas a higher concentration of the constituents led to a more pronounced decrease. Mixing of HA with PRG4 caused a more significant decrease of friction in comparison with simple solutions. The addition of SAPL did not cause any changes in friction. Based on the results of SF and HA + PRG4 + SAPL solution, some other constituents of the SF, such as proteins, contribute to the reduction of friction.

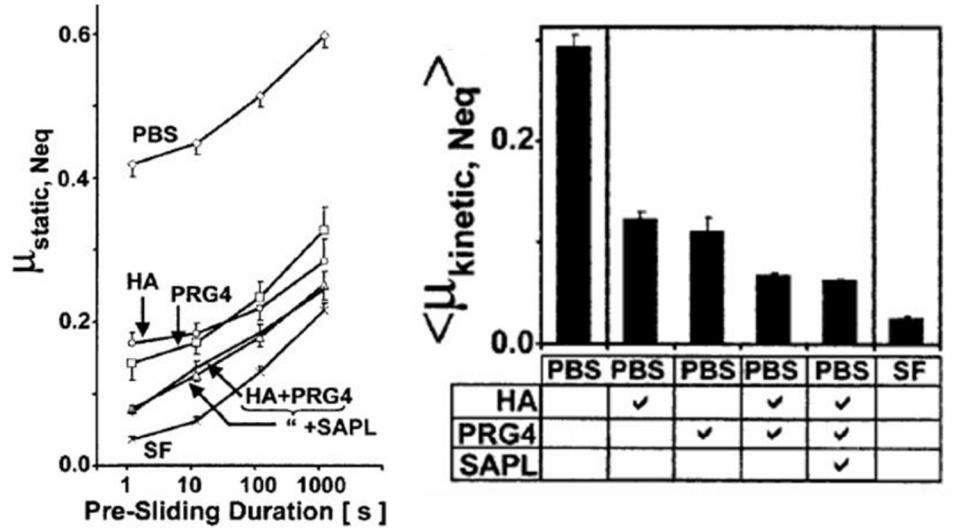

Figure 2-12 Effect of HA, PRG4 and SAPL within articular cartilage friction [21]

The formation of an adsorbed layer on the articular cartilage surface plays an important role within articular cartilage boundary lubrication. The PRG4 gel layer on the cartilage surface plays an important role during hydration lubrication. Murakami et al. [22] focused on the synergistic reactions between these two layers. By a series of reciprocating tests in a cartilage-on-glass configuration, they investigated the CoF development as well as the role of adsorbed film within the hydration lubrication of cartilage. The frictional measurements were conducted at a contact pressure of 0.47 MPa, whereas the speed of movement was 20 mm/s and the stroke length was 35 mm. Saline solutions containing 0.7 % albumin or γ-globulin were used as lubricants.

The values of the CoF (Figure 2-13) gradually increased during tests while the highest values were measured for γ-globulin. γ-globulin forms a boundary lubricating layer that protects the cartilage surface against wear. However, during the contact of surface irregularities, it has adhesive properties which resulted in higher friction. The addition of proteins into the saline solution also caused a reduction of friction after rehydration. This decrease was

attributed to the adsorption of proteins on the cartilage surface. Removal of the PRG4 layer caused an increase of friction after rehydration. However, the friction at the end of measurement was lower. This type of behavior was attributed to the insufficient adsorption of γ-globulin on the cartilage surface.

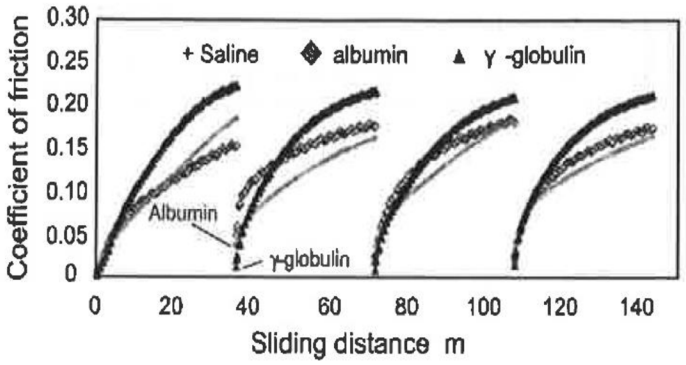

Figure 2-13 Influence of proteins on articular cartilage friction [22]

The role of HA within articular cartilage friction was further investigated by Kwiecinski et al. [23]. The main attention was paid to the HA molecular weight. Frictional measurements were performed in a cartilage-on-cartilage configuration while the contact was lubricated by HA solutions with a concentration of 3.33 mg/ml and a molecular weight of 20 kDa, 132 kDa, 780 kDa, 1 500 kDa and 5 000 kDa. HA solutions were tested in their pure form as well as mixtures with PRG4 (concentration 450 µg/ml).

Results showed that the reduction of friction caused by HA strongly depends on the HA molecular weight. For 5 000 kDa HA, the measured values of static and dynamic CoF were significantly lower compared to the 10 kDa HA. Based on the results in Figure 2-14a, a linear dependence between the CoF and molecular weight was observed. The addition of PRG4 caused a decrease in friction. However, the decrease in the CoF was independent of the HA molecular weight (Figure 2-14b). Similar CoF values were measured for all PRG4 and HA mixtures. Thus, even low molecular weight HA can be effective within the formation of a protective boundary layer on the cartilage surface. However, the presence of PRG4 in the solution is essential for its formation.

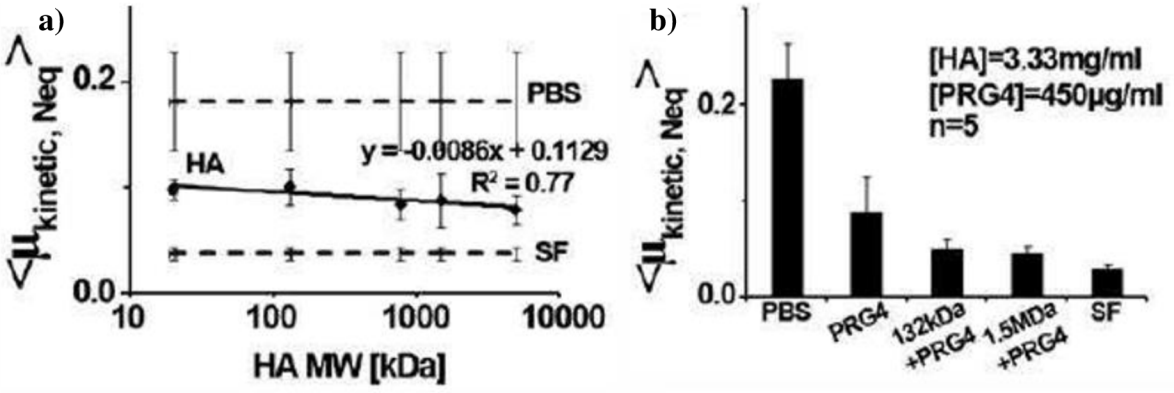

Figure 2-14 Dependence of the CoF on the HA molecular weight for: a) pure solutions, b) mixtures with PRG4 [23]

In another study by Murakami et al. [24], differences in friction between healthy and osteoarthritic cartilage lubricated by HA solutions were investigated. Reciprocating sliding tests were performed on a pin-on-plate tribometer in a cartilage-on-glass contact while the experimental conditions were the same as in the previous article [22]. Cartilage samples with underlying subchondral bone were extracted from porcine femoral condyles. The contact was lubricated with a 0.5 % HA solution (molecular weight of 950 kDa). HA solution was also mixed with albumin and γ-globulin solutions with a concentration of 7 mg/ml. HA solution reported lower friction than the saline solution (Figure 2-15). The lowest values of CoF were measured for a mixture of HA and γ-globulin. For a HA + albumin solution, the friction was very similar to pure saline. This indicates a synergistic reaction between HA and γ-globulin and the adverse interaction between HA and albumin. Albumin, due to repulsive forces between negatively charged HA and albumin molecules, worsened HA adsorption. Thereby, only a negligible reduction of friction was observed. Similar results were obtained for damaged cartilage. The synergistic reaction between γ-globulin and HA was also demonstrated during in situ observation of the contact area by the fluorescence microscope. γ-globulin formed a thick adsorbed layer over the whole contact area. Contrary to this, albumin adsorbates on the cartilage surface were distributed locally.

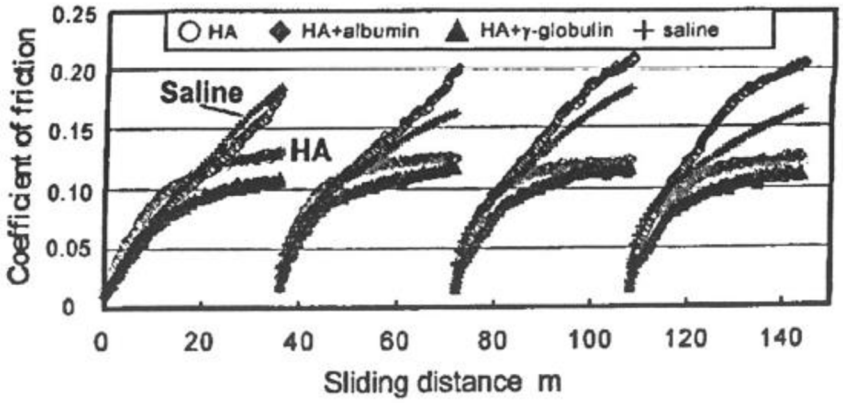

Figure 2-15 CoF for a HA solution mixed with proteins [24]

Long term complex study about the effect of SF constituents within the articular cartilage friction was completed by another study by Murakami et al. [25]. This time, the roles of albumin, γ-globulin, HA and phospholipids were investigated. Saline solutions containing various combinations of albumin (0.7 or 1.4 wt%), γ-globulin (0.7 wt%), HA (0.5 wt%, MW 920 kDa) and phospholipids (0.01 wt%) were used as lubricants.

From the one-component solutions (Figure 2-16a), γ-globulin exhibited the highest friction at the end of the measurement, whereas the lowest values of CoF were reported for phospholipids. Pure γ-globulin exhibited even higher friction than pure saline. Differences in albumin and γ-globulin behavior were attributed to the stronger adsorption properties of γ-globulin. Due to the adsorbed film, the initial values of the CoF are lower. However, the binding of the protein molecules to the second contact body caused higher friction at the end of the measurement. The reactions of both proteins with phospholipids led

to a reduction of friction. Mixing of phospholipids and HA into one solution led to a significant decrease in friction (Figure 2-16b), which indicates the formation of complex structures formed by these two components. Admixing both proteins into the solution caused an even more significant decrease in friction. However, if proteins were added to this solution separately, an increase in friction was observed.

Each component of the SF has a role in cartilage lubrication. However, their interactions are also very important. Mixing of individual components into complex solutions can lead to increased friction. However, the complex model SF exhibited the lowest values of the coefficient of friction. γ-globulin forms, thanks to its strong adsorption ability, a protective layer on the articular cartilage surface. HA increases the viscosity of the SF and forms a protective gel layer on the cartilage surface.

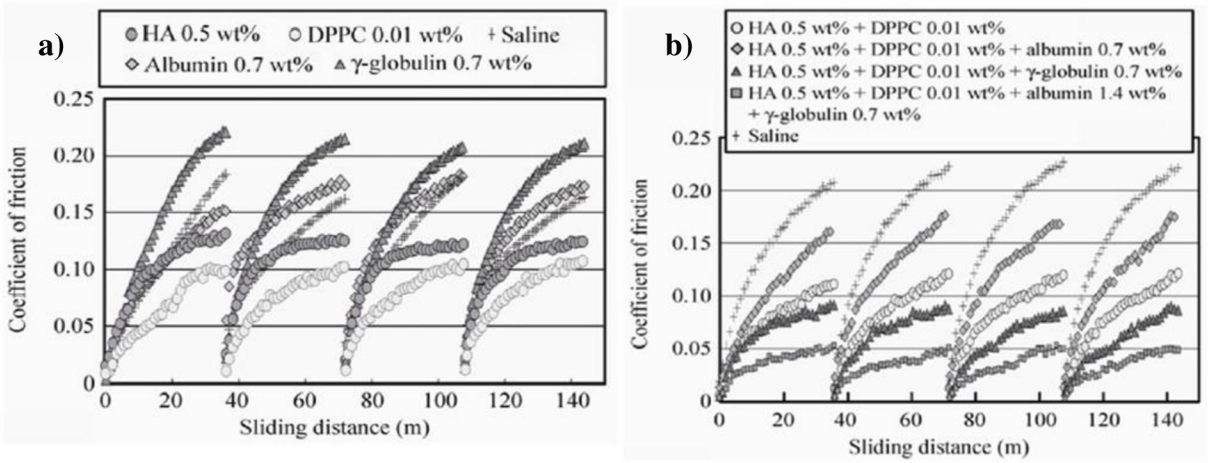

Figure 2-16 Frictional behavior of: a) one-component solutions, b) complex solution of HA, phospholipids and proteins [25]

The concentration-dependent frictional behavior of γ-globulin and HA within the boundary lubrication regime was also investigated by Park et al. [26]. Frictional measurements were performed with PBS solutions containing γ-globulin at concentrations of 0.5 and 2 mg/ml and HA with a molecular weight of 1.63 MDa and concentrations of 1 mg/ml, 3 mg/ml and 5 mg/ml. Pure PBS was used as a reference. The effect of frictional changes due to the OA was also analyzed. Cartilage samples were extracted from human hip joints at various stages of OA. The progression of OA was also monitored by surface topography measurements.

The results of surface topography measurements showed a significant increase in osteoarthritic cartilage surface roughness. The surface roughness for healthy cartilage was 137 ± 25 nm, while the surface roughness of 533 ± 196 nm was measured for the osteoarthritic cartilage. Results of all fictional measurements are summarized in Table 2-2. In healthy cartilage, no improvement in friction was observed for HA and γ-globulin over PBS. Contrary to these results, the solution of γ-globulin and HA at a concentration of 5 mg/ml caused an increase in the value of CoF. In the early-stage osteoarthritic cartilage, similar behavior was observed. In the severely damaged

osteoarthritic cartilage, solutions of both substances caused a significant decrease in friction. For γ-globulin, the decrease depended on the protein concentration, whereas this dependence was not observed for HA. These differences between cartilage samples were attributed to a higher content of PRG4 in healthy cartilage. The results also showed no correlation between the surface topography measurements and friction.

Table 2-2 CoF values for human cartilage [26]

Lubricant	Concentration	$\mu \pm$ SD ($R^2 \pm$ SD, n=measurements number)		
		Normal	Early OA	Advanced OA
PBS		0.119±0.036 (0.973±0.031, n=16)	0.151±0.039 (0.976±0.040, n=16)	0.409±0.119 (0.976±0.056, n=16)
HA	1.0 mg/ml	0.126±0.038 (0.928±0.059, n=16)	0.160±0.059 (0.907±0.056, n=16)	0.262±0.083 (0.959±0.060, n=16)
	3.0 mg/ml	0.119±0.027 (0.976±0.017, n=16)	0.152±0.059 (0.941±0.038, n=16)	0.269±0.119 (0.981±0.017, n=16)
	5.0 mg/ml	0.181±0.039 (0.971±0.017, n=16)	0.143±0.041 (0.940±0.038, n=16)	0.221±0.067 (0.914±0.074, n=16)
γ–globulin	0.5 mg/ml	0.207±0.042 (0.962±0.022, n=10)	0.203±0.050 (0.979±0.011, n=10)	0.266±0.089 (0.962±0.038, n=10)
	2.0 mg/ml	0.182±0.055 (0.932±0.031, n=10)	0.213±0.053 (0.898±0.073, n=10)	0.126±0.039 (0.915±0.069, n=10)

The findings from the previous study by Schmidt et al. [21] were further investigated by Ludwig et al. [27]. This time, the effect of HA and PRG4 structure on friction within cartilage-on-cartilage contact was also investigated. Therefore, various solutions containing linear HA with a molecular weight of 1.5 MDa, Hylan G-F 20, PRG4 and reduced/alkylated PRG4 (potentially occur in osteoarthritic SF) were used as lubricants. Results were also compared with PBS and bovine SF.

Results (Figure 2-17) showed that the concentration of PRG4 and high molecular weight HA can affect the reduction of friction during the boundary lubrication. Most of the HA + PRG4 solutions exhibited nearly the same friction as bovine SF. However, solutions with a low concentration of one of these constituents were an exception. These results demonstrated that PRG4 and high molecular weight HA concertation are crucial for cartilage-on-cartilage friction within the boundary lubrication regime. Furthermore, the addition of alkylated PRG4 to HA was inefficient within HA - PRG4 synergism. This indicated that PRG4's tertiary and quaternary protein structure is important during the formation of a boundary lubricating layer on the cartilage surface. Finally, PRG4 + Hylan G-F 20 solution reported lower values of CoF compared to the pure Hylan G-F 20. This indicates that synergy between HA and PRG4 could be achieved even with cross-linked HA. The results also indicated that HA-based VSs could be inefficient during the improvement of osteoarthritic joint friction due to the damaged structure of PRG4 contained in osteoarthritic SF. Therefore, combined PRG4 + HA VSs could be more effective within cartilage boundary friction while linear or cross-linked HA structure is applicable.

In another study, Bonnevie et al. [28] investigated the role of HA and PRG4 within articular cartilage lubrication. CoF dependency on Sommerfeld number was analyzed. Frictional measurements were realized on pin-on-plate tribometer while the contact couple consisted

of bovine cartilage and glass. PBS, HA (500 – 730 kDa, 10mg/ml), hexadecyl derivate of HA (HYADD4, 8mg/ml) and rh-Lubricin (20 µg/ml) were used as lubricants.

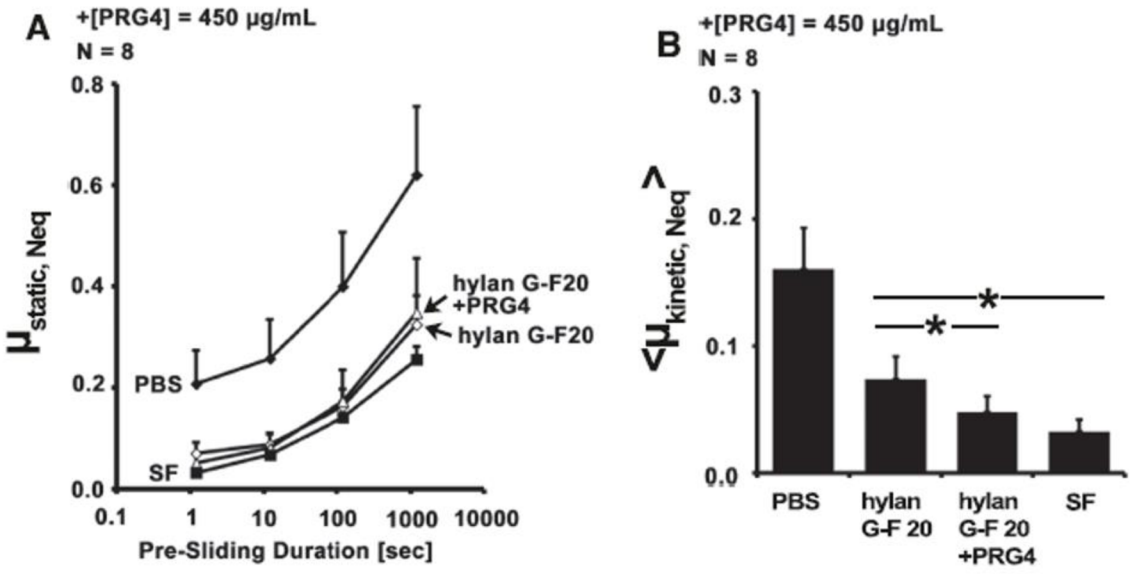

Figure 2-17 Effect of Hylan G-F 20 on: a) Static CoF, b) Kinetic CoF [27]

Based on the results, interaction between PRG4 and HA that enhanced articular cartilage lubrication was identified. This synergy is based on the binding of PRG4 on the articulating surface. Figure 2-18 shows the results of frictional measurements for intact cartilage and cartilage with removed PRG4 layer. HA binds to the PRG4 layer and creates a highly viscous layer. This interaction should be due to the entanglement of the molecules or should be dictated by the hydrophobic or hydrophilic nature of HA and PRG4 molecules. The gel layer could be up to 4 times more viscous than the surrounding lubricant and should significantly reduce the articular cartilage friction. The formation of this layer was also confirmed by experiments with fluorescently labeled HA.

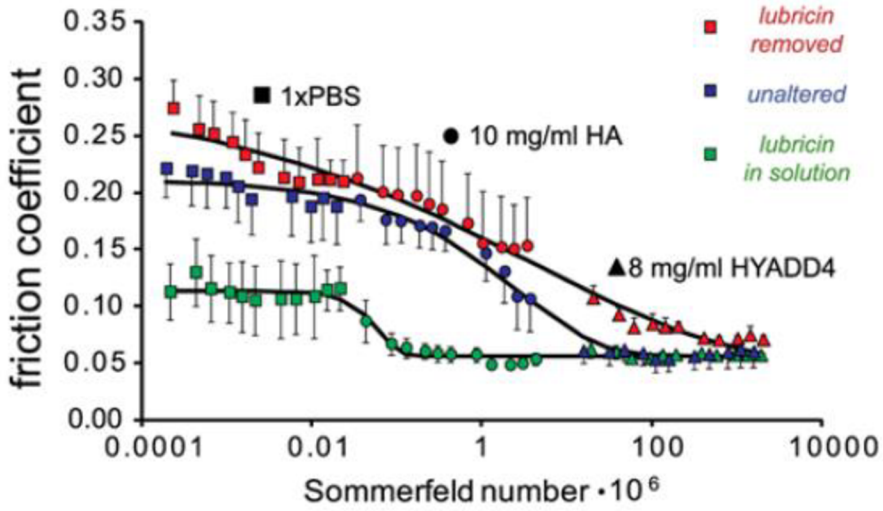

Figure 2-18 CoF dependency on Sommerfeld number for cartilage with/without PRG4 and PRG4 dissolved in lubricant [28]

Another mechanism of synergy, this time between HA and phospholipids, was introduced by Seror et al. [29]. The surface force balance technique was involved to analyze the friction forces between mica surfaces which were coated by avidin and biotinylated HA. HA and DPPC solutions were used as lubricants. Results for uncoated mica surfaces were similar for simple DPPC solution as well as for mixture of HA and DPPC. Measured values of CoF were approximately 0.3. However, the results of measurements with mica coated by HA were significantly lower. Values of CoF ranged in the thousands. The liposome structure of the phospholipids was disrupted and transformed into a lipid layer on mica. Due to this transformation, the hydrophilic headgroups of the DPPC were in contact. During movement, they exchange water molecules by diffusion. This synergy was called hydration lubrication. For effective boundary lubrication of cartilage, the formation of a robust boundary layer (Figure 2-19) composed of HA and DPPC is an essential thing. To bound HA on the articulating surface, avidin-biotin interaction was used. However, in the natural synovial joint, the attachment of the HA at the cartilage surface may be due to the entanglement of HA with the collagen network in the superficial layer. Another possibility is the previously described HA- PRG4 interaction [28].

Figure 2-19 Scheme of HA-DPPC synergy [29]

This research was further investigated by Zhu et al. [30]. Repeatedly, the surface force balance technique was involved to analyze the friction forces between mica surfaces. This time, the role of different phosphatidylcholine lipids in the form of liposomes was investigated. To be more specific, HSPC, DMPC and POPC were studied. Under high pressure (up to 15 MPa), HA-HSPC complex provided very efficient lubrication. The values of CoF were as low as 0.001. On the other hand, HA-DMPC and HA-POPC complexes were effective only under low pressure up to 2 MPa. The extremely low values of the CoF were attributed to the previously described hydration lubrication [29]. The formation of the boundary lubricating layer was also confirmed by SEM images of the mica surface after the experiments (Figure 2-20). Better results of the HSPC under higher pressure were attributed to the stronger van der Waals attraction between the HSPC acyl tails.

A significant reduction of friction due to the HA-lipid synergy was previously investigated only on the mica surface. HA-lipid layer, together with PRG4, has been proposed as a boundary layer that stands behind the extremely low friction in synovial joints. In another study, Lin et al. [31] investigated the HA-lipid synergy in contact of chicken tendon

and digit. The contact was lubricated by pure PBS, HA with a molecular weight of 1.5 MDa, HSPC and dopamine. The contact was loaded with a force of 0.4 N or 0.8 N and the speed of sliding motion was 0.5 mm/s or 2 mm/s.

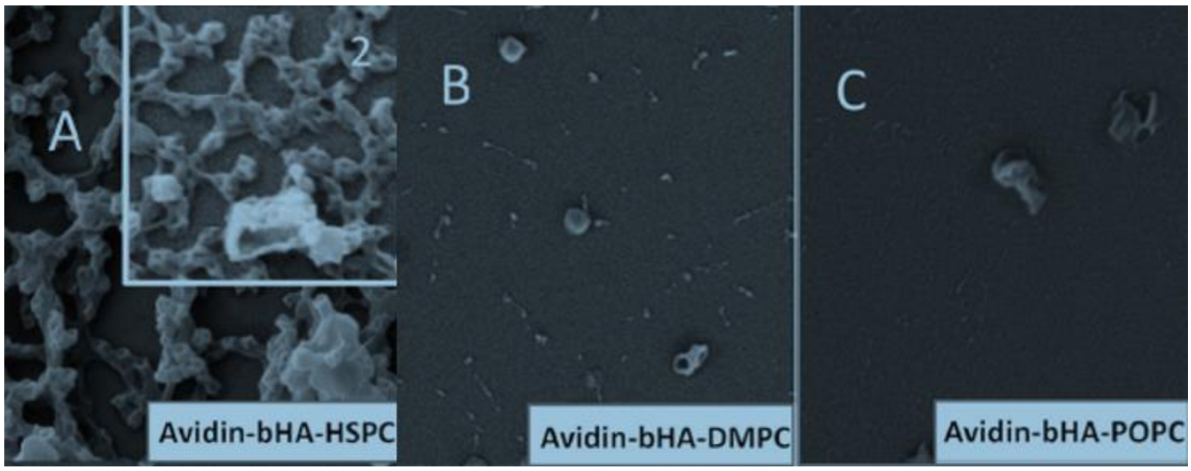

Figure 2-20 SEM images of mica coated with Avidin-bHA and PCs: a) HSPC, b) DMPC, c) POPC [30]

Based on the result in Figure 2-21, pure HA or HSPC decreased friction within the contact as the sparsely-attached liposomes provided some lubrication by their phosphocholine headgroups. However, a more pronounced decrease of friction was observed for the solution containing both of these constituents. A two-fold decrease of friction against simple solutions was attributed to a synergy between the HA and the PC-lipid forming a dense complex exposing the highly hydrated phosphocholine groups. Even better results were measured for the lubricant with dopamine. Dopamine groups were bounded to collagen or GAGs at the tendon surface and increased HA coverage at the tendon surface. This study was the first evidence that HA/PC-lipid complexes strongly influence friction within biological surfaces.

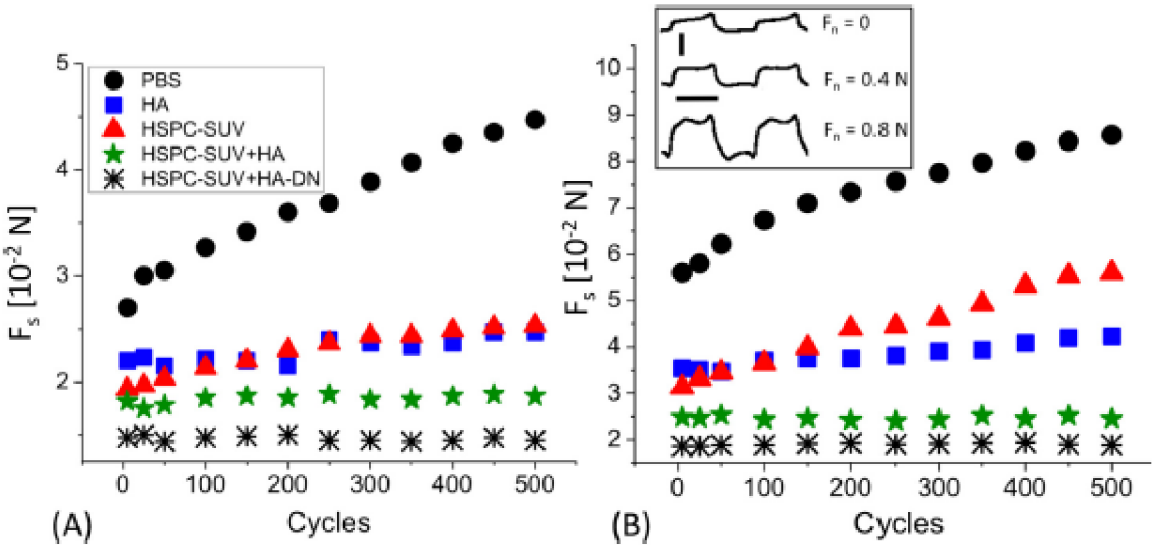

Figure 2-21 Friction force as a function of cycles number under a normal load of: a) 0.4 N; b) 0.8 N [31]

Liu et al. [32] also used the previously mentioned surface force balance technique to assess the role of HA molecular weight within the boundary lubrication of articular cartilage. For this purpose, friction between mica surfaces coated with HSPC and low (35 kDa), medium (240 kDa) or high (1.8 MDa) molecular weight HA were analyzed. Results (Figure 2-22) showed that the boundary lubricating layer composed of high molecular weight HA provides very efficient lubrication. Values of the CoF were as low as 10^{-3} - 10^{-4}. Moreover, the boundary lubricating layer was stable at contact pressures up to 12 MPa. However, for low and medium molecular weight HA, the initial low friction ($\mu \approx 10^{-2}$ - 10^{-3}) increases at much lower pressures. This higher friction of HA with shorter chains was due to the lower adhesion energy to the mica surface coated with gelatin layer. Thus, they are easily removed by shear stress during sliding motion, especially at higher values of contact pressure. Authors suggest this as a cause of higher friction within the osteoarthritic synovial joint.

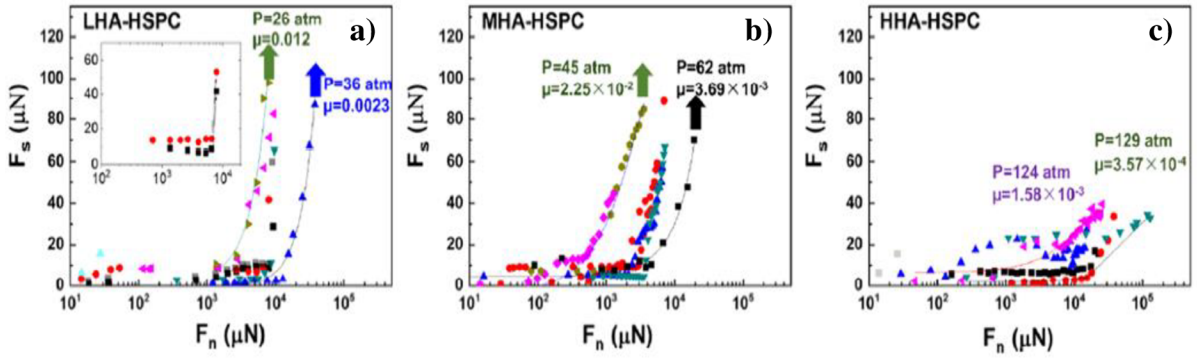

Figure 2-22 Friction force as a function of applied load between different surfaces: a) mica-gelatin-LHA-HSPC, b) mica-gelatin-MHA-HSPC c) mica-gelatin-HHA-HSPC [32]

2.3 Role of viscosupplementation in articular cartilage friction

Quite extensive research has already been conducted in the field of articular cartilage friction and lubrication. However, the viscosupplementation issue remains neglected. Only a limited number of articles that deal with the problematic of VSs frictional analysis have been published. One of the first articles which dealth with the friction of VSs was published by Cherniakova et al. [33]. The authors investigated the CoF differences within UHMWPE-on-steel contact lubricated by various commercially available preparations that are injected into the joint cavity during the treatment of synovitis. They investigated the differences between various groups of drugs with different mechanisms of action (antibacterial, anti-inflammatory, immunomodulatory, VSs), as well as differences in products belonging to the same group. The results were compared with the values obtained for a healthy SF.

The results of the CoF measurements are shown in Figure 2-23. The healthy SF exhibited the lowest CoF values. From the commercially available VSs, Diprospan® and Hyalgan®

exhibited the lowest CoF values. The differences in VSs behavior were attributed to their different viscosity. For example, Synvisc®, with its relatively high viscosity and gel-like structure, was not able to form a continuous lubricating layer on the polyethylene surface during the sliding motion. On the other hand, Hyalgan®'s much lower viscosity made it easier to form a continuous layer. The increase in friction during later stages of the measurements was attributed to the degradation of HA. The shortening of HA chains led to a decrease in viscosity and deterioration of the lubrication ability.

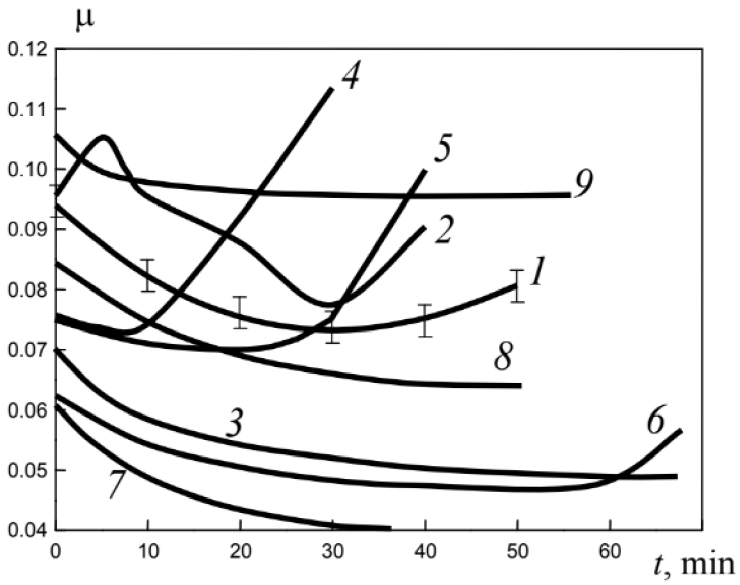

Figure 2-23 CoF of lubricating drugs: 1 – hydrocortisone, 2 – Kenalog®-40, 3 – Diprospan®, 4 – lincomycin, 5 – Synvisc®, 6 – Hyalgan®, 7 – SF + blood serum, 8 – hydrocortisone + blood serum, 9 - chondrosamine [33]

Results of a study combining rheological and tribological analysis of the commercial VSs were published by Bonnevie et al. [34]. In total, six commercial VSs were tested. Measurements of viscosity and viscoelastic properties were conducted on a rotational rheometer TA Instruments DHR3 in cone-plate configuration (40 mm cone with 2° angle and 50 μm gap) whereas CoF was measured on a custom-built tribometer in the bovine cartilage-on-glass configuration.

The main conclusion was that the widely varying rheological properties of the tested VSs (Figure 2-24a) did not predict their frictional behavior within articular cartilage contact (Figure 2-24b). Adsorption of HA on the cartilage surface may cause a previously reported local increase in viscosity [28]. However, this is probably not possible for the HA-steel interface. Therefore, the measured viscosities may significantly vary from the HA effective viscosities within cartilage-on-cartilage contact during boundary lubrication. Interestingly, data from frictional measurements were significantly more predictive of the clinical outcomes. A strong correlation between the CoF and the reduction of pain reported by WOMAC scores was found.

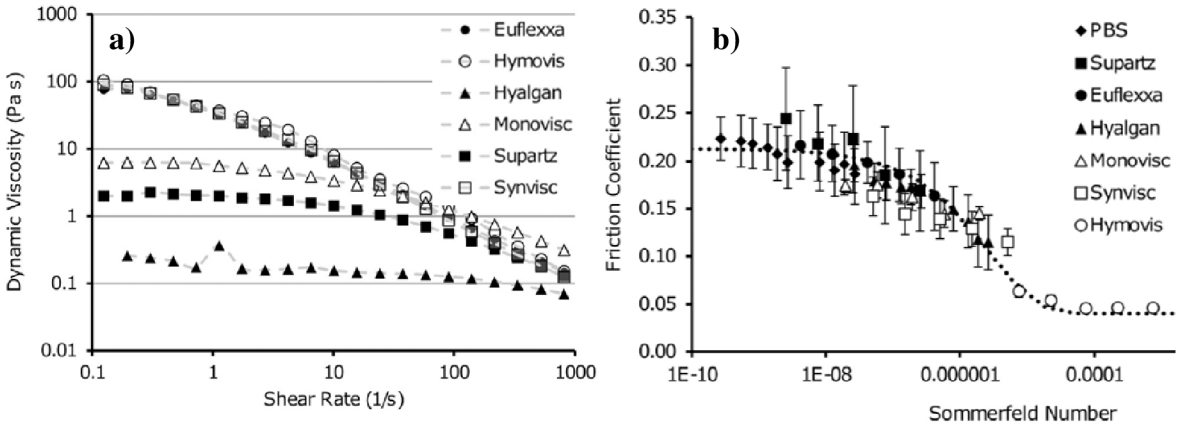

Figure 2-24 a) Viscosity curves of tested VSs, b) CoF as a function of the Sommerfeld number [34]

Prekasan et al. [35] also combined rheological and tribological measurements for the assessment of VSs effectiveness. The effect of the SF:VS ratio on the viscosity as well as on the friction and wear of bovine cartilage were analyzed. Results showed that SF viscosity can be increased by viscosupplementation in a concentration-dependent manner. However, in accordance with previous studies, viscosity-wear dependency was not observed. The lowest wear of bovine cartilage was observed for the lowest concentration of VS in SF. In this case, wear was approximately the same as during measurement with pure inflammated SF. Higher concentrations of VSs led to higher values of wear. For SF:VS ratio, it should be noted that the 1:1 ratio is commonly used in clinical practice. The lowest wear was reported for pure HA. Therefore, the authors recommended to aspirate the inflammated SF from the osteoarthritic joint and replace it with pure HA.

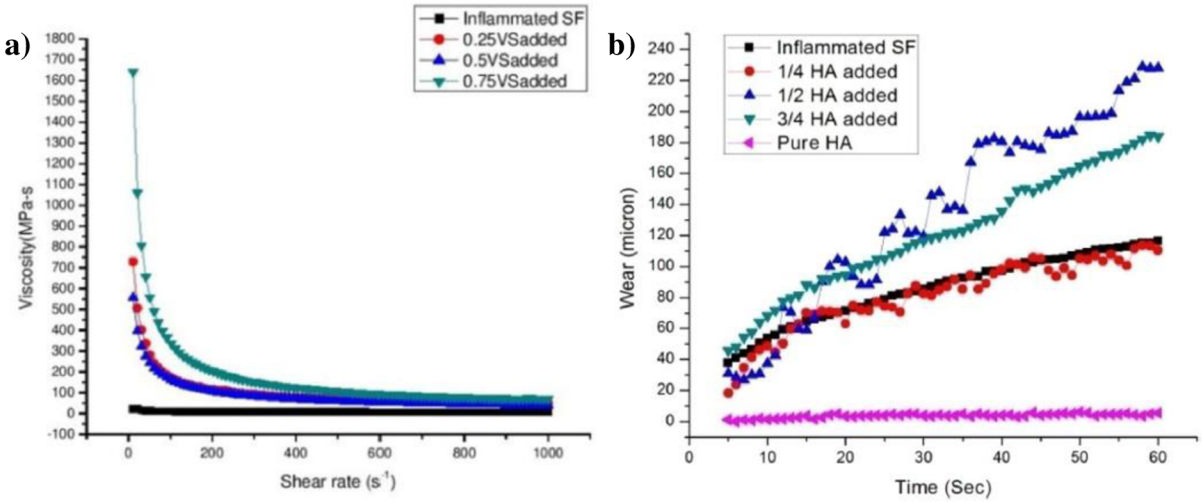

Figure 2-25 a) Viscosity of different SF:VS ratio solutions, b) Wear of bovine cartilage lubricated by various SF:VS ratio solutions [35]

2.4 Artificial articular cartilage

For articular cartilage friction and lubrication research, natural articular cartilage is occasionally replaced by artificial materials. PVA hydrogels are one of the considered materials for natural articular cartilage replacement due to their similar structure - porous structure with high water content and low elastic modulus. In this chapter, studies focused on the friction and lubrication of PVA hydrogels as well as their tribological comparison with natural articular cartilage are presented.

One of the first studies about PVA hydrogel as artificial cartilage was published by Nakashima et al. [36]. Reciprocating sliding tests in PVA hydrogel-on-glass configuration were performed to identify the role of proteins albumin and γ-globulin during boundary lubrication of the PVA hydrogel. The roles of proteins during the formation of the boundary lubricating layer were directly monitored by in situ observation of the contact area by a fluorescent microscope. Based on these results, a protein adsorption model was introduced (Figure 2-26). The role of albumin layer is to maintain a low shear layer on the hydrogel surface and adsorbed γ-globulin protects the surface from wear. An appropriate ratio of these proteins is also important for the protein boundary layer. Only a little wear reduction was observed for lubricants with only one protein or with an excessive concentration of one of them.

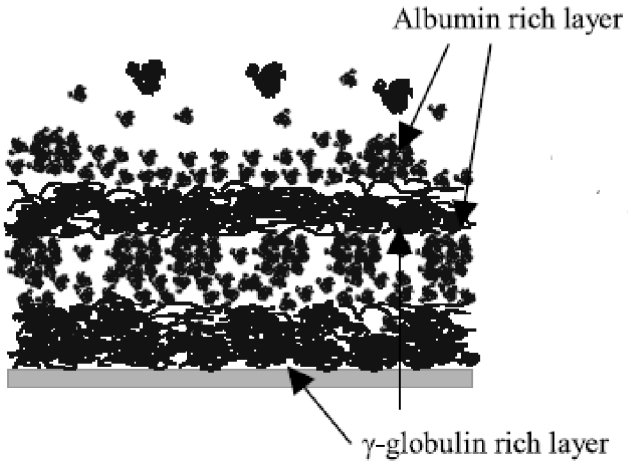

Figure 2-26 Adsorbed protein layer on PVA hydrogel surface [36]

Further in situ observation and frictional measurements of PVA hydrogel-on-glass contact were performed in a study by Murakami et al. [37]. The main attention was paid to the role of albumin:γ-globulin ratio within the formation of a boundary lubricating layer. The protein adsorption was analyzed on a pin-on-plate tribometer. The spherical PVA hydrogel sample made a reciprocating sliding motion against the glass plate. The contact pressure was 0.1 MPa. The speed of sliding motion was set at 0.2 mm/s and the stroke length was 4mm. The contact was flooded with saline solutions containing fluorescently labeled albumin and γ-globulin.

Results of in-situ observation showed relatively weak adsorption of albumin. Molecules were distinguishable but the adsorbed film was very thin. On the other hand, γ-globulin formed a smooth and uniform boundary layer. For a mixture of these two proteins, the amount of albumin in the adsorbed layer increased and the overall stability of the layer depended on the albumin:γ-globulin ratio. Frictional measurements (Figure 2-27) showed that albumin worsened the adsorption ability of γ-globulin, which led to a reduction of friction compared to the pure γ-globulin solution. These results confirmed the previously presented protein adsorption model.

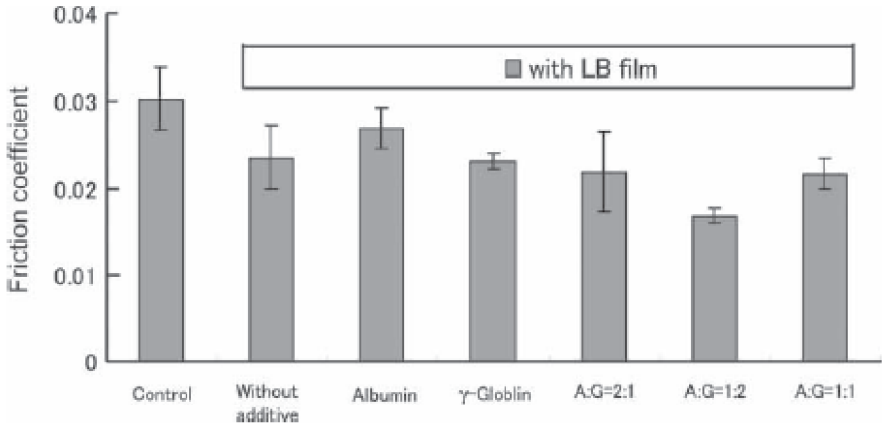

Figure 2-27 CoF values for protein solutions [37]

Previous studies were further expanded by Yarimitsu et al. [38]. This time, HA was also involved as one of the lubricant constituents. The adsorption of HA from the albumin solution was significantly lower than from the HA and γ-globulin solution. Due to their opposite electric charges, HA and albumin repel each other. Therefore, the adsorption of HA on the hydrogel surface is likely to be prevented by already adsorbed albumin. On the other hand, γ-globulin and HA form complex structures. Thus, the interaction between γ-globulin and HA contributes to the role of HA within boundary film formation and friction reduction. HA should also contribute to the formation of a lubricating film by increasing the viscosity of the solution.

The role of phospholipid DPPC within the lubrication of hydrogel-on-hydrogel contact was also investigated by Yarimitsu et al. [39]. Based on the results in Figure 2-28, CoF was reduced in a concentration-dependent manner for simple DPPC solutions. Wear of PVA hydrogel was also suppressed by high concentration DPPC solution and the wear mechanism shifted from adhesive to abrasive wear. Wear was reduced by an increase of liposomes in the contact area but the additional effect of friction reduction was not obtained due to the suppression of multi-lamellar phospholipid film formation. This was probably due to the stabilization of liposomes. For DPPC and protein solutions, the results indicated that not only single constituent concentration but also relative concentration of protein and DPPC plays an important role within the formation of a boundary lubricating layer with very low friction.

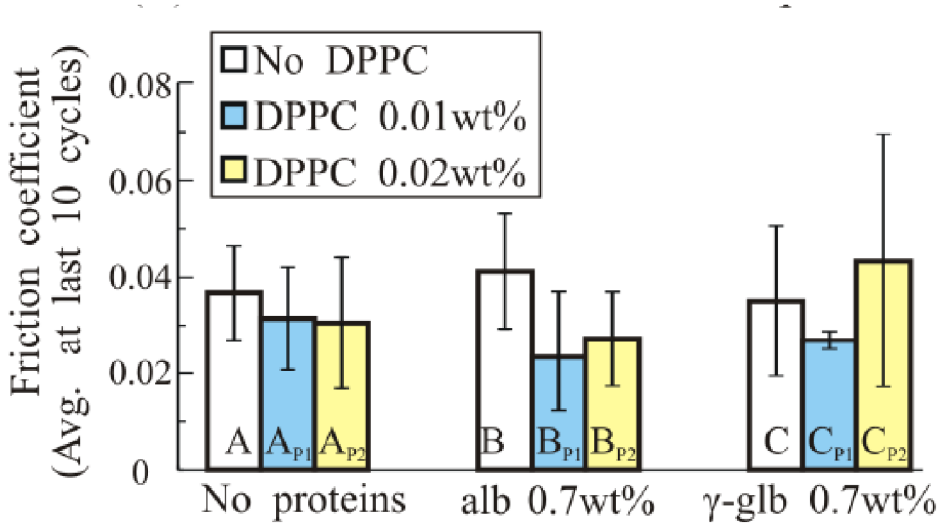

Figure 2-28 CoF for protein and DPPC solutions [39]

Previously mentioned results were expanded by another study by Murakami et al. [40]. The roles of individual SF constituents and their mixtures within PVA hydrogel friction and wear were investigated. Two types of PVA hydrogels prepared by FT and CD methods were also compared with porcine articular cartilage. Measurements were conducted on a pin-on-plate tribometer, whereas the contact pair consisted of PVA hydrogel ellipsoid/porcine femoral condyle and a glass plate. The sliding speed was 20 mm/s and the stroke length was 35 mm. Applied load was 2.94 or 9.8 N.

As shown in Figure 2-29a, the articular cartilage and FT PVA hydrogel exhibited low initial friction with a subsequent gradual increase while FT hydrogel exhibited higher values of CoF. On the other hand, CD hydrogel exhibited lower friction with only a slight increase during the experiment. The differences in frictional behavior of PVA hydrogels were attributed to their different permeability and elastic modulus. Thus, permeability controls the biphasic fluid flow behavior and therefore the rate of fluid load support. The material properties strongly depend on the formation of hydrogen bonds and microcrytallities. The transparency of CD hydrogel corresponds to the uniform network structure, whereas milky freeze-thawing hydrogel corresponds more to the heterogeneous structure.

For FT hydrogel, the addition of proteins into saline worsened the friction within PVA hydrogel-on-glass contact. On the other hand, HA or DPPC significantly reduced friction within the contact. Combination of HA and DPPC (Figure 2-29b) can maintain low friction with or without the addition of proteins albumin and γ-globulin.

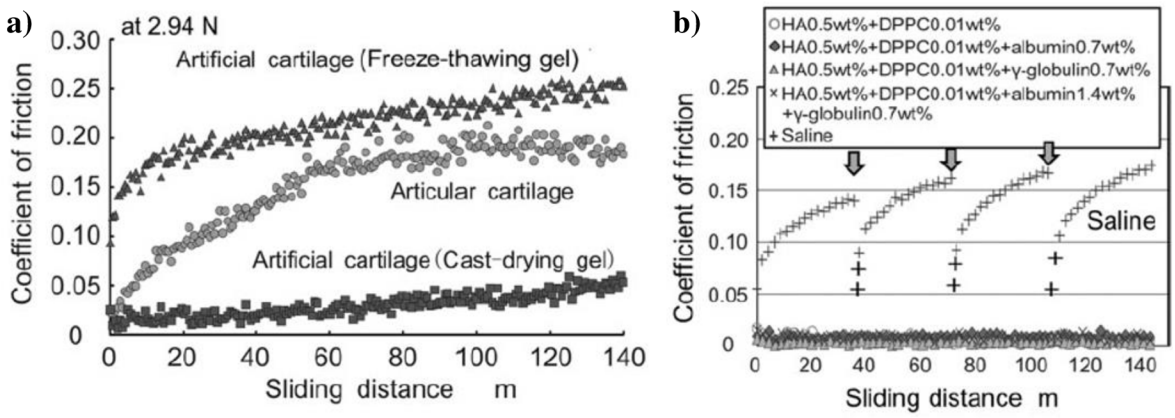

Figure 2-29 a) Friction of articular and artificial cartilage in saline, b) Influence of synovia constituents combinations on the friction of FT PVA hydrogel [40]

The role of protein albumin within FT PVA hydrogel friction was also investigated by Li et al. [41]. For this purpose, CoF between steel ball and artificial cartilage plate was measured during the reciprocating motion. Results in Figure 2-30a showed a similar growth trend of the friction with increasing load for deionized water as well as for albumin solution. No significant differences were observed for these solutions. However, as the frequency of reciprocating motion increased to 2 Hz, different results were observed. CoF for deionized water was twice as high as that for albumin solution. This indicates a reduction of friction by albumin under high-frequency motion.

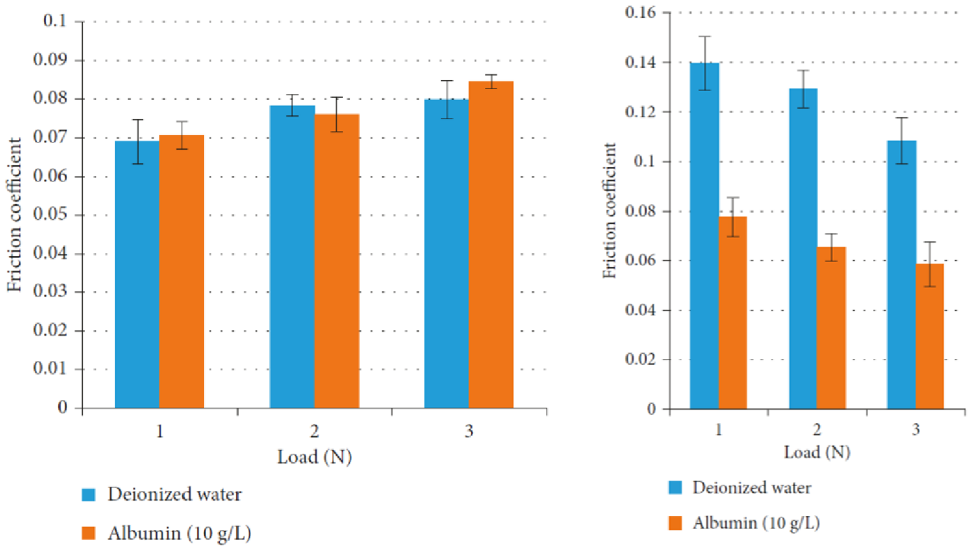

Figure 2-30 Effect of lubricant on CoF at a oscillating frequency of: a) 1 Hz, b) 2 Hz [41]

A frictional comparison of CD and FT hydrogels with human cartilage was also conducted by Oliveira et al. [42]. The CoF was investigated using a pin-on-disc tribometer while the contact pair consisted of PVA hydrogel/cartilage and steel ball. The contact was lubricated by PBS or a simulated SF composed of bovine serum albumin (4 mg/ml) and HA (3 mg/ml). Based on Figure 2-31a, the hydrogel samples exhibited CoF values between 0.062 and 0.115.

Under the same experimental conditions, the measurement with human cartilage exhibited a higher value of CoF - 0.192. Due to the higher wear resistance, only CD hydrogel samples were tested under higher loads. Higher values of CoF were reported from the measurements with SF (Figure 2-31b). The higher values of CoF were attributed to the interaction between HA or albumin and matrix of PVA hydrogel as well as to the conformation of the adsorbed molecules and their structural changes. Overall, CD hydrogels presented more similarities with natural articular cartilage in terms of morphology and mechanical behavior.

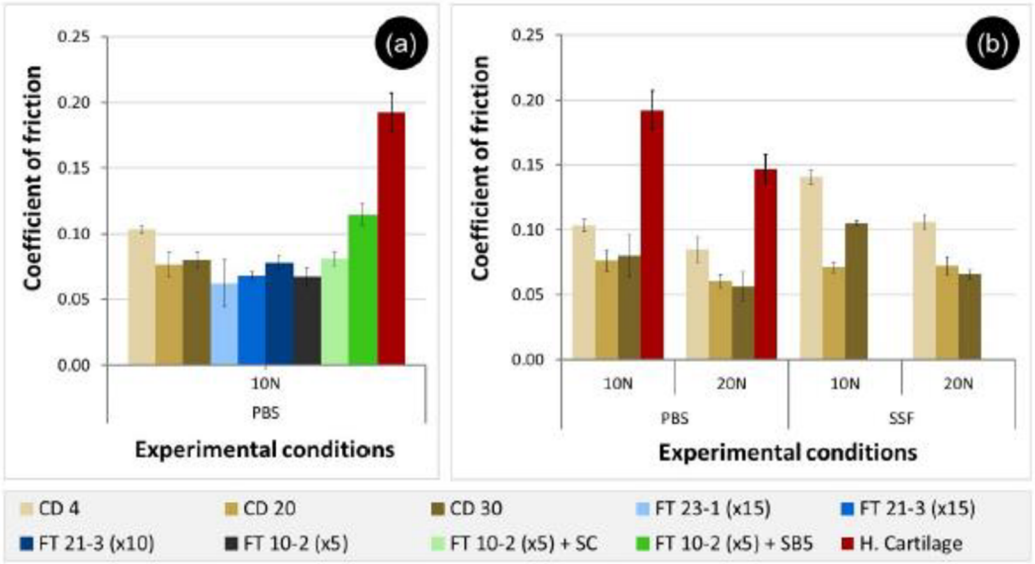

Figure 2-31 a) CoF for various CD and FT hydrogels b) Frictional comparison of CD hydrogels and human cartilage under different experimental conditions [42]

3 ANALYSIS AND CONCLUSION OF LITERATURE REVIEW

SF is a complex solution composed of proteins, HA, phospholipids, PRG4, etc. These constituents separately and in combination with other SF constituents play an important role within the articular cartilage friction. The SF behaves like a non-Newtonian fluid. The authors of the article [8] confirmed that the proteins albumin and γ-globulin do not affect SF rheology. The primary component influencing the SF rheological properties appears to be HA. Therefore, the pseudoplastic behavior of SF is caused by the straightening of the HA linear chains and their orientation in the fluid flow direction. The authors of the article assumed that PRG4 can also affect the SF rheology. However, this phospholipid was not part of the experiments.

During the progression of OA, the SF rheological properties are deteriorated due to a decrease in HA concentration [9] and molecular weight. In response to these changes, one of the viscosupplementation effects is to restore a healthy SF rheology by mixing SF with endogenous HA. However, based on the literature, it is hard to define healthy SF rheology. In the most frequently mentioned article by Rainer et al. [14], zero shear viscosities of a healthy SF in a range between 1 Pa·s and 175 Pa·s were measured. Bingöl et al. [13] reported an even higher zero shear viscosity of 445 Pa·s for a SF gathered post mortem from the human knee of a 68 years old man. The rheological measurements of osteoarthritic SF also reported a large dispersion of data. The zero shear viscosities of the analyzed osteoarthritic SFs in a study by Mathieu et al. [12] varied by 3 orders of magnitude between 0.1 Pa·s and 10 Pa·s. It seems that the exact composition and thus SF rheological properties strongly depend on the specific situation in the joint, such as the stage of OA. In the same article, significant differences between SF from the right and left knees of patients with bilateral OA were observed. As a result of lower HA concentration and molecular weight, SF viscoelastic properties are also aggravated. For example, Balazs [43] reported a crossover frequency of 0.4 Hz for a healthy synovial fluid. This frequency represents the frequency of oscillating motion at which the elastic and viscous moduli cross and the transition from viscous-like to gel-like behavior occurs. Mazzucco et al. reported a crossover frequency of 1.8 Hz for patients undergoing primary arthroplasty. The higher crossover frequency of osteoarthritic SF was also reported by Tyrnenopoulou et al. [9]. A crossover frequency of 8 Hz was measured for equine osteoarthritic SFs, whereas 2 Hz were measured for healthy equine SFs.

The rheological properties of HA are mainly influenced by concentration, molecular weight and cross-linking. In general, VSs and HA solutions with higher molecular weight reported higher zero shear viscosities [11; 15; 17; 18; 34]. Significant differences in viscosity between linear and cross-linked HA are also evident [15; 17; 18]. Results of VSs viscoelastic properties usually report gel-like behavior of cross-linked VSs, viscous-like behavior

for VSs with low molecular weight HA and viscoelastic behavior with a crossover point for VSs with high molecular weight HA [15; 17]. The mixing of SF with VS causes an increase in viscosity and an improvement of the viscoelastic properties, while the rate of improvement significantly depends on the type of HA contained in the VS. For VSs with low molecular weight HA, almost no increase in viscosity was observed [16]. The addition of low molecular weight HA into osteoarthritic SF increased only the values of loss modulus. Therefore, this type of VSs is not very suitable for the improvement of osteoarthritic SF viscoelastic properties. Thanks to the higher storage modulus, the SF is able to carry a higher proportion of the load which is applied to the cartilage during motion. It also guarantees better shock absorption ability. VSs based on high molecular weight linear HA caused an increase in viscosity by one order of magnitude [12; 16]. Values of storage and loss modulus were also increased and a crossover point for a mixed solution was measured. In comparison with pure VS, a higher value of crossover frequency was observed. Cross-linked HA increased viscosity, storage and loss modulus by approximately two orders of magnitude, whereas the values of storage modulus were higher. Thus, SF mixed with cross-linked HA exhibited gel-like behavior.

Contrary to the SF rheology, all SF constituents can significantly affect the articular cartilage friction. Due to its hydrophilic nature, protein albumin is very difficult to adsorb on the cartilage surface. The adsorbed film is relatively thin and only locally distributed [22; 37]. On the other hand, γ-globulin exhibited a highly hydrophobic nature. It forms an even and stable lubricating film on the cartilage surface [22; 37]. During boundary lubrication, this protein layer protects the articular cartilage surfaces against direct contact of rubbing surfaces. Contrary to the saline or albumin solution, γ-globulin friction is lower after rehydration but higher at the end of measurements. During boundary lubrication, adsorbed γ-globulin tends to bind to the counterface articular cartilage. Breaking of the bonds leads to an increase in friction. When proteins are mixed, the presence of albumin in solution reduces the adsorption properties of γ-globulin [38]. Albumin is able to adsorb to the γ-globulin layer significantly better than to the cartilage surface. At high concentrations, it also tends to replace γ-globulin molecules. However, the albumin layer has low shear resistance and desorption may occur. The formation of a basic γ-globulin layer is crucial to the stability of the adsorbed film. The stability of the layer also depends on the albumin:γ-globulin ratio in the protein solution.

The addition of HA or phospholipids into PBS or saline will also cause a decrease in friction. Schmidt et al. [21] reported a HA concentration-dependent decrease in articular cartilage friction. On the other hand, Forsey et al. [19] did not observe any dependence between HA concentration and friction. The reduction in cartilage friction lubricated by HA should also depend on HA molecular weight. In a study by Kwiecinski et al. [23], CoF values were significantly lower for 5 MDa HA compared to 10 kDa HA. An approximately linear dependence between HA molecular weight and CoF was observed. Consequent observation of cartilage structure by fluorescence microscopy showed that HA penetrates through

the cartilage structure and concentrates around chondrocytes. It probably stimulates chondrocytes to synthesize new HA. In a study by Liu et al. [32], HA molecular weight affected friction within mica contact. CoF for high molecular weight HA was lower and boundary lubricating layer was more stable under higher pressures. The phenomenon was attributed to the higher adhesion energy between HA and coated mica surface.

For phospholipids, concentration dependence on friction reduction was also observed [19; 21]. When phospholipids were mixed with HA, the reaction was synergistic within cartilage friction. However, the CoF values were higher compared to the SF and the effect of HA molecular weight was significantly reduced [23].

When HA and proteins were mixed, a significant difference between albumin and γ-globulin was reported [24]. Due to the negatively charged molecules of albumin and HA, albumin worsened HA adsorption against simple HA solution. On the other hand, the reaction of HA with γ-globulin is synergistic for cartilage friction. Due to the formation of complex structures, a gel layer on the cartilage surface caused a significant reduction in COF compared to the HA + albumin solution. The admixture of albumin or γ-globulin and phospholipids also reduces friction [25]. The friction is further reduced when HA is added to the solution and all four basic components of the SF are mixed. Complex SF showed the lowest values of CoF in all studies that worked with this solution [21; 23; 25; 27; 44].

Bonnevie et al. [26] also reported the binding of HA to PRG4 on cartilage surface due to the entanglement of the molecules or due to the hydrophobic/hydrophilic nature of HA and PRG4 molecules. HA created a highly viscous layer which significantly contributed to the articular cartilage friction. The formation of HA layer is an essential thing for a hydration lubrication mechanism presented by Seror et al. [29]. Due to the disruption of DPPC liposome structure, a lipid layer on HA is created. During the movement, hydrophilic headgroups of DPPC exchange water by diffusion. Due to this lubrication mechanism, CoF within the mica contact ranged in the thousands. This lubrication mechanism was later demonstrated in a chicken tendon-digit contact [31]. This study was the first direct evidence that HA/phospholipid interaction strongly influences friction within biological surfaces.

Not much an effort was previously dedicated to the tribological measurements with commercial VSs. Cherniakova et al. [33] reported CoF values of three commercial VSs within UHMWPE-on-steel contact. Differences in their frictional behavior were attributed to their different viscosity. Bonnevie et al. [34] performed a rheological and frictional analysis of six commercial VSs. No dependency between viscosity and CoF within cartilage-on-cartilage contact was observed. Nevertheless, a strong correlation between COF and WOMAC scores was found. This pointed out the importance of frictional measurements within the assessment of VSs effectiveness. On the other hand, no dependency between VSs viscosity and wear of bovine cartilage was reported by Prekasan et al. [35].

PVA hydrogels represent a possible substitute for articular cartilage within tribological measurements due to the similar mechanical properties with articular cartilage and biphasic porous structure. Murakami et al. [40] compared CD hydrogel and FT hydrogel with porcine articular cartilage. Biphasic lubrication was reported for both PVA hydrogels, whereas CD hydrogel exhibited lower CoF values than natural cartilage. Oliveira et al. [42] also compared FT and CD hydrogel with natural articular cartilage, whereas CD hydrogels presented more similarities with articular cartilage. The lubrication of PVA hydrogels was intensively studied by Murakami et al. [37; 40] and Yarimitsu et al. [38; 39]. In addition to the previously mentioned results, a simple DPPC solution reduced friction in a concentration-dependent manner within PVA hydrogel-on-glass contact. For DPPC mixed with proteins, the relative concentration of these two constituents plays an important role within a formation of a boundary lubricating layer. The combination of HA and DPPC exhibited very low friction with or without the addition of proteins. Therefore, the formation of a boundary lubricating layer composed of HA and phospholipids plays an important role during the lubrication of PVA hydrogel as well as natural articular cartilage.

Based on the current state of art, studies focused on the frictional behavior of complex SFs with different compositions have not been published so far. There are no studies that would map the effect of the individual SF constituents' concentration within the frictional behavior of complex SF. Frictional differences between healthy and osteoarthritic SF within cartilage contact have not been analyzed yet. Most of the previously mentioned articles are focused on measurements with solutions containing only individual SF constituents or their mixtures. Tribological characterization of commercial VSs is also a relatively unexplored area of research. Studies focused on the CoF measurements with clear commercial VSs were recently published. However, the changes in frictional behavior of cartilage contact after mixing of osteoarthritic SF and VS were not analyzed, even though studies that dealt with changes in osteoarthritic SF rheology after mixing with VSs were published. Moreover, the literature reported a better correlation between CoF and WOMAC scores than between viscosity and WOMAC scores. Therefore, tribological measurements with complex SFs mixed with VSs could represent a better approach for viscosupplementation clarification.

4 AIMS OF THE THESIS

The aim of this dissertation thesis is to clarify the changes in friction of a synovial joint model after viscosupplementation. The main emphasis is on the effect of HA concentration and molecular weight on the friction within the articular cartilage contact. For this purpose, rheological measurements of HA solutions and commercial VSs will be conducted as well as the measurements of CoF within the model of a synovial joint. To achieve the main goal of this thesis, the solution of following sub-goals will be necessary:

- Development of a methodology for the extraction and storage of articular cartilage samples.
- Selection of suitable HA solutions and VSs.
- Preparation of protein solutions and model SFs.
- Design of the experiments and approaches for an evaluation of VSs effectiveness.
- Rheological analysis of selected HA solutions and VSs.
- Series of experiments focused on the effect of SF composition on the friction of articular cartilage.
- A series of experiments focused on the effectiveness of VSs within the articular cartilage friction.
- Data analysis.
- Results discussion and publication.

4.1 Scientific questions

- What is the effect of changes in SF composition due to OA on the friction of articular cartilage?
- How are the viscosity and viscoelastic properties of HA solutions connected with the friction of articular cartilage?
- How is the friction of articular cartilage influenced by the molecular weight of HA contained in the VSs?

4.2 Hypotheses

- *A lower concentration of HA in osteoarthritic SF fluid will increase the friction within the articular cartilage model.*
  The formation of a boundary lubricating layer on articular cartilage is mostly affected by the interaction between HA and PRG4 [23; 28] or phospholipids [25; 29]. Due to the decrease of HA concentration, formation of this layer worsens. This will lead to a higher CoF within contact.

- *The higher viscosity of HA and VSs will cause a more pronounced decrease of friction within a synovial joint model.*
  The higher molecular weight and concentration of HA solutions or VSs leads to a higher value of zero shear viscosity [15; 17; 18]. Kwiecinski et al. [23] reported an approximately linear dependence between HA molecular weight and CoF within cartilage-on-cartilage contact lubricated by a simple HA solution. Therefore, it is expected that the higher viscosity of HA or VS will cause a more pronounced decrease of friction within a synovial joint model.
- *Large molecules of high molecular weight/cross-linked HA will perform better in the reduction of friction within the articular cartilage model.*
  Kwiecinscki et al. [23] reported linear dependency between HA molecular weight and CoF within cartilage-on-cartilage contact. The question is how the HA molecular weight influences the reactions between HA and other SF constituents or cartilage structure. Liu et al. [32] reported better stability of boundary lubricating film composed of high molecular weight HA. Due to this, boundary lubricating layer composed of high molecular weight/cross-linked HA will exhibit lower values of CoF.

4.3 Thesis layout

This dissertation thesis is composed of three papers published in journals with impact factor. The first article is focused on the effect of kinematic and loading conditions on the friction within a cartilage-on-glass contact under reciprocating sliding motion. Several lubricants composed of individual SF constituents and their mixtures were employed as lubricants. Differences between healthy and osteoarthritic SF were also examined. The second article is focused on the HA molecular weight. Viscosity and viscoelastic properties of four HA solutions with a molecular weight between 77 and 2 010 kDa were analyzed as well as their frictional behavior in cartilage-on-glass contact. In the last article, we focused on the effectiveness of five commercially available VSs. Repeatedly, the viscosity and viscoelastic properties of VSs were examined. This time, changes in VSs rheology after mixing with osteoarthritic SF were also analyzed. Restoration of a healthy SF rheology after mixing of osteoarthritic SF with individual VSs was discussed. Changes in the friction of articular cartilage after viscosupplementation and differences between individual VSs were analyzed and discussed too.

- FURMANN, D., D. NEČAS, D. REBENDA, P. ČÍPEK, M. VRBKA, I. KŘUPKA and M. HARTL. 2020. The Effect of Synovial Fluid Composition, Speed and Load on Frictional Behaviour of Articular Cartilage. *Materials*. **13**(6).
  Author's contribution: 10%

Journal impact factor = 3.057, Quartile Q2, CiteScore = 3.26

- REBENDA, D., M. VRBKA, P. ČÍPEK, E. TOROPITSYN, D. NEČAS, M. PRAVDA and M. HARTL. 2020. On the Dependence of Rheology of Hyaluronic Acid Solutions and Frictional Behavior of Articular Cartilage. *Materials*. **13**(11). *Author's contribution: 50%*

Journal impact factor = 3.057, Quartile Q2, CiteScore = 3.26

- REBENDA, D., M. VRBKA, D. NEČAS, E. TOROPITSYN, S. YARIMITSU, P. ČÍPEK, M. PRAVDA and M. HARTL. 2021. Rheological and frictional Analysis of Viscosupplements Towards Improved Lubrication of Human Joints. *Tribology International.* **(UNDER REVIEW)** *Author's contribution: 50%*

Journal impact factor = 4.271, Quartile Q1, CiteScore = 7.9

5 MATERIALS AND METHODS

5.1 Experimental devices

5.1.1 Rotational rheometers

To analyze the flow properties of the tested lubricants, rheological measurements were conducted on two rotational controlled stress rheometers by TA Instruments (Figure 5-1) – Discovery HR-3 and AR-G2. In this kind of rheometers, the tested sample is placed between two surfaces – a stationary bottom surface and a rotating top surface which is also called as geometry. Typical configurations or rotational rheometers are parallel plates, cone-plate or concentric cylinders. Parallel plates are usually used for gels, pastes or solids; cone-plate for low to high viscosity liquids and concentric cylinders for very low to medium viscosity fluids. For the rheological analysis of HA solutions, cone-plate and parallel plate configurations were used. A 60 mm diameter cone-plate set up with a 1° cone angle was used for the viscosity measurements on the HR-3 rheometer and a 20 mm plate-plate configuration was used for the analysis of HA solution viscoelastic properties on the AR-G2 rheometer. The lower part of the geometry always consisted of a Peltier plate that uses the Peltier principle to control the temperature of tested solution. Rotating or oscillating geometry is usually attached to a shaft which is mounted in air or magnetic bearing and connected with an optical encoder. From the values of torque and angular displacement, rheological parameters like applied stress are counted by built-in-software.

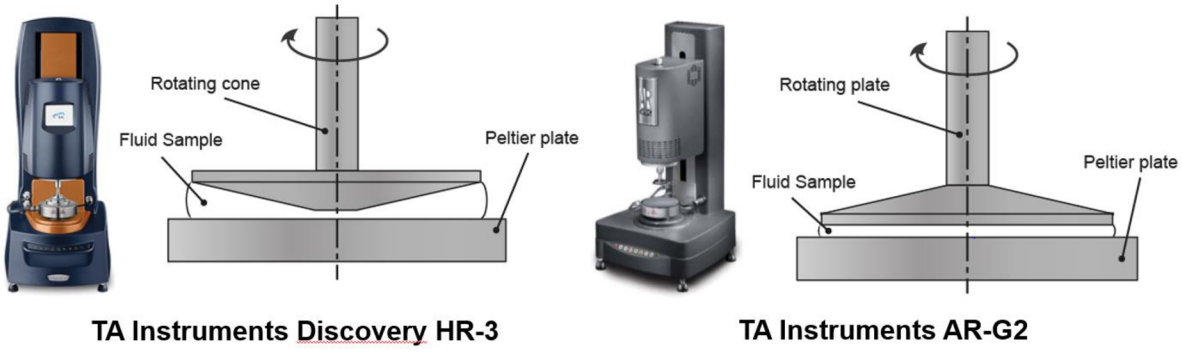

Figure 5-1 Rotational rheometers and their geometry configurations

5.1.2 Pin-on-plate tribometer

For the frictional measurements, commercially available tribometer UMT TriboLab (Bruker, Billerica, MA, USA) was used. UMT Tribolab is a modular tribometer that enables tribological experiments in a wide range of conditions. Due to its interchangeable lower drive (Figure 5-2), experiments under linear, rotational or oscillating types of motion can

be performed. The lower drive and loading mechanism of the tribometer offers big variability in the shapes of tested samples. Therefore, tribological measurements in pin-on-plate, pin-on-disc, ball-on-disc, block-on-ring and many other configurations are possible. Interchangeable biaxial load sensors are also a big advantage of this tribometer. Loading forces ranging between 1 mN and 2 000 N can be applied to the tested samples.

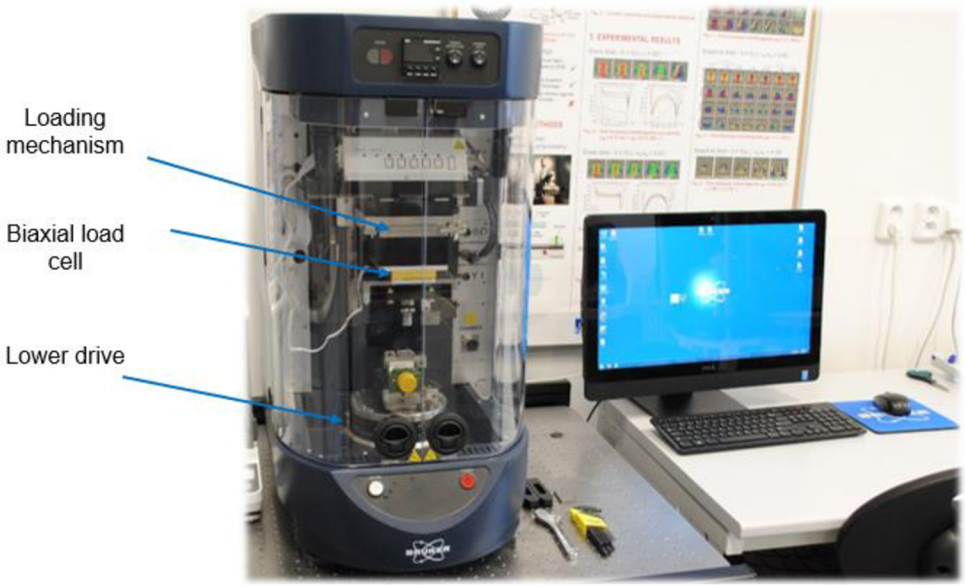

Figure 5-2 Bruker UMT Tribolab

All frictional measurements were conducted in a pin-on-plate configuration (Figure 5-3). The CoF was measured as a function of a time or sliding distance for the sliding pair of a stationary glass plate made from optical glass B270 (E = 71 GPa, μ = 0.22) and moving cartilage. Glass plate was mounted in a stainless steel chamber on a lower drive and heated via heating cartridges. Heating cartridges were controlled by the external temperature controller Hotcontrol c448 (Hotset ČR, Pilsen, Czech Republic). Cartilage samples were mounted in a loading mechanism of the tribometer. Two types of cartilage were used during the experiments (chapter 5.2.2) - natural porcine articular cartilage and artificial cartilage based on PVA hydrogel. Two different types of sample holders were manufactured due to the different shapes of cartilage samples. Porcine cartilage samples had a cylindrical shape due to the methodology of extraction, whereas PVA hydrogel was prepared as a 2 mm thick plate. During the experiments, the porcine cartilage pin was mounted directly in a pin holder but the PVA hydrogel plate was deployed on the AISI 5200 steel ball with a diameter of 19 mm. Pin holder was connected to the loading mechanism of the tribometer and was doing a reciprocating sliding motion against the glass plate with defined load, speed and stroke length. Loading and frictional forces were continuously monitored by a biaxial load cell with a maximum capacity of 50 N which is mounted in the loading mechanism of the tribometer. From these data, the values of CoF were calculated.

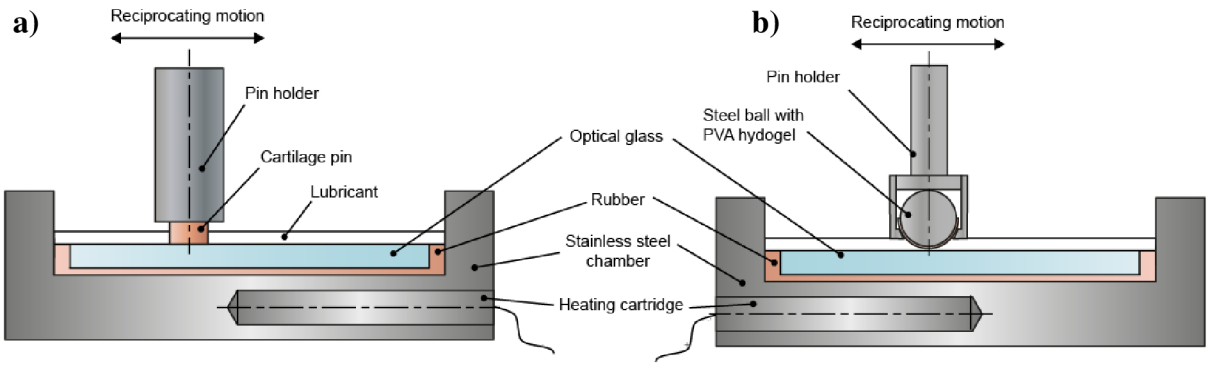

Figure 5-3 Schemes of frictional measurements: a) cartilage-on-glass, b) PVA hydrogel-on-glass

5.2 Test samples

5.2.1 Lubricants

Among other things, the first paper was focused on the effect of SF composition on friction within articular cartilage contact. We tried to investigate the role of individual SF constituents as well as changes in SF composition due to the OA on friction within the contact. Therefore, two types of model SFs and many other solutions which contained individual SF constituents or their mixtures were prepared. The composition of model SFs should correspond to healthy people and orthopedic patients who suffer from OA. PBS was used as a basic solution to which albumin (24.9 mg/ml), γ-globulin (6.1 mg/ml), HA (1.49 mg/ml) and phospholipids (0.34 mg/ml) were added. The following products were used for preparation – bovine serum albumin (powder, ≥ 96 %; A2153, Sigma-Aldrich, St. Louis, MO, USA), γ-globulin from bovine blood (powder, ≥ 99 %; G5009, Sigma-Aldrich, St. Louis, MO, USA), sodium hyaluronate HySilk (powder, quality class – cosmetic; molecular weight = 820 – 1 020 kDa, Contipro, Dolní Dobrouč, Czech Republic) and L-α-Phosphatidylcholine (powder, Type XVI-E, lyophilized powder; ≥ 99 %; vesicles form; P3556, Sigma-Aldrich, St- Louis, MO, USA). Table 5-1 displays the concentration of individual constituents in model SFs.

Table 5-1 Composition of model SFs

Constituents	Healthy SF	Osteoarthritic SF
Albumin	20 mg/ml	24.9 mg/ml
γ-globulin	3.6 mg/ml	6.1 mg/ml
HA	2.5 mg/ml	1.49 mg/ml
Phospholipids	0.15 mg/ml	0.34 mg/ml

During the solutions preparation, individual constituents were dissolved in PBS overnight at 4 °C using a rocker-shaker (MR-12, Biosan, Riga, Latvia). Consequently, the solutions were mixed together and deeply frozen at -22 °C. Before the experiments, test tubes with solutions were thawed at laboratory temperature.

Besides complex SFs, simple protein solutions and their mixtures with HA or phospholipids were prepared to examine their role during the friction of articular cartilage. The combinations of chosen lubricant constituents can be seen in Table 5-2. Lubricants with both concentrations, i.e., healthy and osteoarthritic were prepared and used for the frictional experiments.

Table 5-2 The combinations of lubricant constituents

	Model fluid Constituents				
1	PBS	———	———	—	———
2	PBS	Albumin	———	—	———
3	PBS	———	γ-globulin	—	———
4	PBS	Albumin	γ-globulin	—	———
5	PBS	Albumin	———	HA	———
6	PBS	———	γ-globulin	HA	———
7	PBS	Albumin	γ-globulin	HA	———
8	PBS	Albumin	———	—	Phospholipids
9	PBS	———	γ-globulin	—	Phospholipids
10	PBS	Albumin	γ-globulin	—	Phospholipids
11	PBS	Albumin	———	HA	Phospholipids
12	PBS	———	γ-globulin	HA	Phospholipids
13	PBS	Albumin	γ-globulin	HA	Phospholipids

One of the key parameters which affect HA rheology is the molecular weight. Therefore, the second article was focused on the effect of HA molecular weight on the viscosity and viscoelastic properties of tested solutions and on the friction of articular cartilage. To do that, four HA solutions with a concentration of 20 mg/ml and a molecular weight of 77 kDa, 640 kDa, 1 060 kDa and 2010 kDa were prepared for the experiments. To prepare all these solutions, the required amount of HA powder (Contipro, Dolní Dobrouč, Czech Republic) with defined molecular weight was dissolved in PBS. To ensure the proper dissolution of HA in PBS, the solutions were stirred by a magnetic stirrer (SMHS-3, Witeg Labortechnik, Wertheim, Germany) and heated to 60 °C for at least three hours.

The last article was focused on the rheological and frictional analysis of commercially available VSs. Based on the concentration, molecular weight and cross-linking of the contained HA, five different HA-based VSs were identified for the measurements. From the range of products which were currently available in the Czech Republic, Erectus® (Angelini Pharma Österreich, Vienna, Austria), Hyalgan® (Fidia Farmaceutici, Padua, Italy), Monovisc® (Anika Therapeutics, Bedford, MA, USA), Optivisc Single® (Moss Vision, Wembley, United Kingdom) and Synvisc One® (Sanofi Genzyme, Ridgefield, NJ, USA) were chosen for the experiments. Samples were used as provided by the local drugstore. Table 5-3 summarizes their basic properties based on the package leaflets and information from manufacturers' sites. To better analyze the changes in rheology and friction after viscosupplementation, VSs were tested as clear solutions and as mixtures in a 1:1 ratio with model osteoarthritic SF.

Table 5-3 Summary of tested HA-based VSs

Product	HA Concentration (mg/ml)	HA Molecular Weight (mg/ml)	Cross-linking	Package Volume (ml)
Erectus®	12	1 100	No	2
Hyalgan®	10	500 – 730	No	2
Monovisc®	22	1 000 – 2 900	Yes	3
Optivisc Single®	30	3 000	Yes	3
Synvisc One®	8	6 000	Yes	6

5.2.2 Cartilage samples

During the frictional measurements, two types of cartilage were used – natural porcine articular cartilage and PVA hydrogel-based artificial cartilage. Specimens from intact porcine cartilage were extracted even with underlying subchondral bone from porcine femoral heads (Figure 5-4). The bones from 6 to 8 months old mature pigs having a mass around 100 – 120 kg were collected from a local butchery within a few hours of slaughter. Cylindrical cartilage specimens with a diameter of 5.6 mm were extracted by a hollow drill. Just one cartilage specimen from approximately the same area of the femoral head was extracted from each femur in order to get samples with approximately the same curvature of the cartilage surface and similar mechanical properties. Specimens were stored for no more than 2 weeks in a freezer at - 20 °C. Half an hour before the experiments, cartilage samples were removed from the freezer and thawed at laboratory temperature.

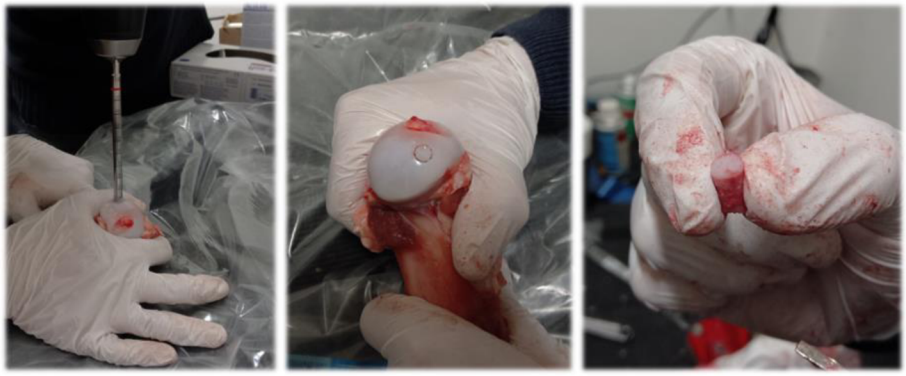

Figure 5-4 Extraction of articular cartilage samples

The methodology (Figure 5-5) of PVA hydrogel preparation, as artificial articular cartilage, was based on the study by Yarimitsu et al. [45]. The first step was the preparation of PVA (polymerization degree: 1700, saponification degree: 98.0 – 99.0 mol%, Kuraray, Tokyo, Japan) 15 wt% solution. The aqueous solution was poured into a previously manufactured acrylic mold and sealed. Filled and sealed mold was consequently closed into a temperature and humidity-controlled chamber (SH-242, ESPEC, Osaka, Japan) and treated by a repeated freeze-thawing method. In total, four cycles of freezing and thawing were repeated. Each cycle consisted of 8 hours of freezing at - 20 °C and 16 hours of thawing at 4 °C. The resulting PVA hydrogel had a shape of a plate and was 2 mm thick. PVA-FT hydrogel was stored in deionized water at laboratory temperature to prevent drying due to its porous structure.

Figure 5-5 Preparation of PVA hydrogel samples

5.3 Experimental design and conditions

5.3.1 CoF measurements

To analyze the frictional behavior of articular cartilage, Bruker UMT Tribolab in pin-on-plate configuration was used. Before the experiments, the glass plate was mounted in a stainless steel chamber and was preheated to 37 °C by heating cartridges. The glass plate

was also cleaned with sodium dodecyl sulphate and isopropyl alcohol to prevent the contamination of the tested lubricant. Subsequently, the steel chamber was filled with preheated lubricant. The cartilage sample was mounted in a sample holder and connected to the loading mechanism of the tribometer. During the experiment, the cartilage was loaded by a constant force of 5 N or 10 N and was doing a reciprocating sliding motion against the glass plate. According to the mechanical properties of glass and natural/artificial cartilage, the contact pressure was approximately between 0.3 and 0.5 MPa. The sliding speeds of 5 mm/s or 10 mm/s were selected to represent slow and normal walking conditions. The stroke length was set to 20 mm. Each experiment consisted of three loaded phases which were separated by two unloaded phases. During the unloaded phases, the cartilage sample was unloaded but still immersed in the tested solution to enable the rehydration of cartilage. Loaded phases lasted 250 s or 300 s. This corresponds to a sliding distance of 2 280 mm and 2 740 mm. Cartilage rehydration phases lasted 300 s. During loaded phases, the normal and frictional forces were continuously monitored. From these data, CoF dependency on time or sliding distance was analyzed. In the later stages of PhD thesis, measurements were repeated with fresh samples of cartilage and lubricant to obtain more relevant data. Raw data of articular cartilage friction were presented to point out on the differences between individual cartilage samples. However, in the case of PVA-FT hydrogel, satisfactory repeatability of measurements was observed. Therefore, the mean values and standard deviations of CoF were calculated.

5.3.2 Viscosity and viscoelastic properties

An important part of the second and third papers was the analysis of the rheological properties of HA solutions and VSs. Viscosity measurements were conducted on a TA Instrument Discovery HR-3 rheometer in a cone-plate configuration. The truncation gap was set to 52 μm. Based on the selected geometry and site of the truncation gap, approximately 1 ml of tested solution was necessary for every measurement. The required amount of tested solution was applied on the Peltier plate which heated the tested solution to 37 °C. Redundant amount of tested fluid should affect the result of measurement. Therefore, it was trimmed away when the cone geometry was in the measurement position. Before the experiments, tested samples were conditioned by pre-shear. Rotational motion with an angular velocity of 50 rad/s was applied to the tested solutions for 1 s. Consequently, viscosity measurements began. During these steady shear tests, the shear rates ranging from 0.01 to 5 000 s^{-1} were applied to the tested fluids. As a result, shear rate-dependent viscosity data were obtained. These data were fitted to the Carreau-Yasuda model to designate the pseudoplastic behavior of HA solutions and VSs. To avoid inaccuracies due to the contamination, geometry surfaces were cleaned with isopropyl alcohol after every measurement. To obtain more relevant data, each experiment was repeated three times with a fresh sample of tested solution. Insufficient repeatability was observed in some low viscosity samples (77 kDa HA, Hyalgan® mixed

with model SF, etc.) at low shear rates due to the wall slip. Concentric cylinders geometry was not available at the time of measurement. Therefore, these data were excluded from the results.

The second part of rheological measurements was an analysis of the viscoelastic properties. TA Instruments AR-G2 rheometer in a parallel plate configuration was used to perform the SAOS tests. SAOS test analyzes the values of storage and loss modulus when a tested sample is subjected to the sinusoidal strain. Based on the selected geometry and gap size between parallel plates (1 400 μm), 0.44 ml of the tested solution was needed for each measurement. Same as the viscosity measurements, the tested solution was heated to 37 °C via Peltier plate. In the first step of the measurement, the linear response region of the tested sample was determined. Strain sweep with an oscillatory strain with an increasing amplitude ranging between 0.001 and 1.5 rad at a constant frequency of 1 Hz was applied to the solution. Consequently, based on the result of a strain sweep, frequency sweep was conducted at 5 % oscillatory shear strain over a frequency range of 0.05 to 5 Hz. All experiments were repeated three times. As a result, data of dynamic modulus dependency on oscillation strain and frequency were obtained.

6 RESULTS AND DISCUSSION

In the first experimental study, the effect of individual SF constituents or kinematic and loading conditions within articular cartilage friction was investigated. Frictional differences between physiologic and osteoarthritic SF were investigated as well as the role of individual SF constituents. The results showed that the CoF strongly depends on the lubricant composition and concentration. The highest values of CoF were measured for PBS. Addition of individual constituents always led to a decrease in CoF but the complex SF did not always report the lowest friction. For complex SFs, a concentration typical for patients suffering from OA, higher values of CoF were measured.

Only a limited effect of protein solutions concentration was observed. CoF at the end of measurement was approximately 0.2 for all protein solutions containing albumin, γ-globulin or both of them. Murakami et al. [25] reported a significant effect of proteins on the friction of articular cartilage. However, the concentration of their protein solutions was significantly lower. From our results, the protein frictional behavior seems to be not dependent on the concentration of the used proteins. These results were measured for simple protein solutions as well as their mixtures. According to another study by Murakami et al. [37], γ-globulin forms a more stable lubricating film than albumin. Therefore, the frictional behavior should be different. Our results did not confirm these findings probably due to the previously mentioned differences in protein concentrations.

Measurements with mixtures of HA and proteins pointed out on a synergistic effect between γ-globulin and HA whereas mixture of γ-globulin and HA with physiologic concertation reported markedly lower friction against mixture of the same constituents with osteoarthritic concentration. However, higher friction was observed for a higher relative concentration of HA mixed with albumin or both proteins. Based on these results, HA seems to be unhelpful for albumin adsorption on the cartilage surface. Murakami et al. [24] attributed different reactions between HA and albumin or γ-globulin to the same or opposite electric charges of the molecules. The admixing of PHs with protein solutions did not also have a synergic effect in most cases. Only insignificant frictional changes were observed when γ-globulin or both proteins were mixed with phospholipids. The values of CoF were similar to the simple protein solutions. In the case of albumin and phospholipids, a sufficient boundary lubricating layer was not even formed. The CoF was only slightly lower than during measurement with PBS. This ineffectiveness of simple phospholipids concentration was also reported by Yarimitsu et al. [39]. When HA and phospholipids were mixed with proteins, lubricant containing albumin achieved similar friction as the model SF whereas a decrease of CoF was observed after mixing of γ-globulin with HA and phospholipids. These mixtures reported the lowest values of CoF within all lubricants.

For complex SFs, a negligible impact of speed was observed as well as for simple γ-globulin solutions. Contrary to these results, there was a visible effect of speed for albumin-based

lubricants. According to the Kienle et al. [46], the value of CoF depends on the concentration of salt ions. Healthy concentrations of albumin and its mixture with HA reported an increase of friction with increased speed. On the contrary, a slight decrease was observed with same solution at osteoarthritic concentrations. It is likely that the adsorbed protein layer and HA have no effect on speed, and thus the whole effect is caused by the electric charge of albumin molecules. To sum it up, the effect of speed within the articular cartilage was complex and complicated to describe. The effect strongly depends on the composition of the lubricant and also on the concentration of individual constituents. This conclusion is also in accordance with the study by Yarimitsu et al. [39].

Results also showed that the higher load led to lower values of CoF. These results are caused by the biphasic structure of articular cartilage, respectively by the fluid load support of articular cartilage. Under load, cartilage interstitial fluid is squeezed out to the unloaded regions of the cartilage structure. This fluid flow is accompanied by high resistance due to the cartilage permeability. Higher load causes higher pressurization of the fluid and, therefore, higher portion of the load is transferred by the fluid phase. However, this mechanism does not work if articular cartilage is loaded for a long period of time. Under these conditions, interstitial fluid is completely squeezed out of cartilage structure and boundary lubrication regime occur. During this lubrication regime, the effect of load within the articular cartilage friction can be different.

The second article analyzed the effect of HA molecular weight on rheology and friction within articular cartilage contact. The results showed a strong dependency between HA molecular weight and viscosity. The lowest measured zero shear viscosity was $0.013 \pm 3 \times 10^{-3}$ Pa·s for 77 kDa HA, whereas the highest zero shear viscosity was 107.1 ± 1.7 Pa·s for 2 010 kDa HA. Based on the literature, the zero shear viscosity of a healthy SF ranges from 1 to 175 Pa·s [47] whereas zero shear viscosity of the osteoarthritic SF ranges from 0.01 to 11 Pa·s [11; 12; 15]. From these results, it can be assumed that low viscosity VSs will not perform well in a recovery of healthy SF rheology. Non-Newtonian shear-thinning behavior was observed in all HA samples. The rate of shear-thinning behavior was characterized by the value of η_0/η_{300} [14; 16]. Three out of four tested HA samples were consistent with results for commercial VSs. Nicholls et al. [18] reported values of shear-thinning ratio between 2.3 and 740.7 for commercial HA-based VSs.

The HA viscoelastic properties were analyzed as well. 640 kDa HA and 1 060 kDa HA exhibited a viscous-like behavior in the whole range of tested frequencies. Only the results of 2 010 kDa HA reported a viscoelastic behavior with a crossover point at 0.4 Hz. This almost matches a crossover point frequency for a healthy SF reported by Balazs [43]. The crossover point of 0.4 Hz means that the 2 010 kDa HA solution behaves like the elastic body during walking or running (correspond to a frequency of 0.5 and 2.5 Hz [17]). Therefore, under these conditions, the articular cartilage surface should be protected against direct contact of rubbing surfaces and thus against wear or mechanical damage.

Frictional measurements with PBS reported very low initial values of CoF, just between 0.01 and 0.015. However, at the end of the measurement substeps, CoF has increased up to 0.18. This behavior was attributed to the biphasic lubrication theory [48; 49]. Experiments with HA solutions showed a significant decrease in friction compared to the PBS. The lowest value of CoF measured at the end of loading phase was 0.009. Overall, HA solutions reported a large scatter of data. Therefore, no clear dependence between the HA molecular weight and the CoF within the cartilage-on-glass contact was observed. Contrary to these results, Kwiecinski et al. [23] reported a linear dependency between the HA molecular weight and cartilage friction. The results showed different interactions between HA and invidual cartilage samples. These differences were attributed to the differences in the geometry, structure and mechanical properties of cartilage samples. Cartilage Young's modulus or shear modulus may be affected by a different content of collagen fibers and proteoglycans. Different content of collagen fibers and proteoglycans across the tibia plateau were reported by Appleayrd et al. [50] or by Kiviranta et al. [51]. Moreover, differences in Young's modulus and Poisson ratio of various healthy cartilage samples from different people were reported by Richard et al. [52]. All these individualities also affect the friction within the articular cartilage contact [53; 54]. Results may also be affected by interactions between HA and SF residues which remained on the cartilage surface after extraction. Reactions with proteins can be either synergistic or unbeneficial for cartilage friction [24; 28; 45]. On the other hand, the reactions between HA and phospholipids are crucial for the effectiveness of HA within articular cartilage friction [19; 21; 25]. According to the hydration lubrication theory by Klein et al. [5; 29; 55], HA bounds to the collagen fibers or PRG4 presented in the articular cartilage structure to provide a robust boundary layer (composed of phospholipids) with extremely low friction.

Finally, the last article was aimed at the rheological and frictional analysis of five commercially available HA-based VSs – Hyalgan®, Erectus®, Monovisc®, Synvisc One® and Optivisc Single®. VSs were analyzed in their pure form as well as mixtures in a 1:1 ratio with osteoarthritic SF. As with the previous article, shear-thinning behavior was found in all tested solutions. Nevertheless, the viscosity of VSs varied by the order of magnitudes. Some of the tested VSs fell beneath the reported range for healthy SF viscosity [47]. Therefore, they should not be able to restore healthy SF rheology. Shear.thinning in a wider ranges of shear rate and higher values of shear-thinning ratio were measured for VSs with high viscosity. For example, the highest value of the shear-thinning ratio was calculated for Synvisc One® - 983.86. Viscosity of VSs decreased approximately by one order of magnitude after mixing with model SF. Based on the results, Optivisc Single® and Synvisc One® were the only VSs which should be able to restore the reported rheology of a healthy SF. The zero shear viscosity measured for a mixture of Synvisc One® and model SF was 37.76 ± 3.1 Pa·s and 18.56 ± 1.73 Pa·s for a mixture of Optivisc Single® and model SF. Mixtures of VSs and model SF also reported a decrease of shear-thinning ratio. The shear-thinning ratio of Synvisc One® decreased from 983.86 to 419.19. Shear-thinning

ratio of a healthy SF was reported by Fam et al. [47]. None of the tested VSs fell inside this range.

Another part of the rheological measurements was the analysis of viscoelastic properties. Firstly, region of linear viscoelastic response was identified by a strain sweep. The constant values of storage and loss modulus were measured in a region from 0.7 % to 45 % strain. Therefore, subsequent frequency sweeps were conducted at a 5 % strain. VSs exhibited all three types of viscoelastic behavior. Monovisc® and Erectus® exhibited a purely viscous behavior which means that the values of loss modulus were higher than the storage modulus. Optivisc Single® exhibited a viscoelastic behavior with a crossover frequency at 0.3 Hz. Only Synvisc One® reported the purely elastic behavior over the whole range of tested frequencies. However, from the measured data, an apparent crossover point beneath a frequency of 0.05 Hz may be considered. After mixing, VSs reported the lower values of dynamic modulus and the higher frequencies of crossover point but their type of viscoelastic behavior remained. For example, the results of Optivisc Single® mixture still exhibited the viscoelastic behavior with a crossover point at 1.2 Hz. This VS mixed with model SF was also the most similar solution to the healthy SF. However, the mixture of SF and Optivisc Single® exhibits the viscous response under the frequency of 0.5 Hz which corresponds to the walking frequency [17]. This may be a big shortcoming of this VS. Only mixed Synvisc One® preserved his gel-like behavior even at very low frequencies.

The last part of the VSs analysis was focused on the friction within the artificial cartilage (PVA hydrogel)-on-glass contact. Articular cartilage was substituted by PVA hydrogel due to unsatisfactory repeatability of measurements and ambiguity of the previous article's conclusions. Based on the current state of the art, PVA hydrogel is an acceptable substitute in terms of friction and lubrication. Clear SF reported even lower initial values of CoF than during measurements with natural porcine articular cartilage. The initial values of CoF ranged between 0.05 and 0.065. However, at the end of measurements, the values of CoF raised to 0.107. HA-based VSs exhibited considerably lower friction compared to the osteoarthritic SF. Differences in friction were not as significant as in the case of rheology even though the literature reports dependency between HA concentration [19] or molecular weight [23] and friction within articular cartilage contact. Moreover, different frictional behavior between individual VSs was observed. Some of them (Erectus® or Hyalgan®) exhibited time-dependent frictional behavior with friction drops caused by the rehydration of hydrogel structure. This type of behavior points to the biphasic lubrication regime within the contact. Other VS (Optivisc Single® or Synvisc One®) reported constant values of CoF during the measurements. Even the effect of rehydration was negligible. This type of frictional behavior corresponds more to the boundary lubrication regime within the contact. During this lubrication regime, friction is strongly influenced by a boundary film adsorbed on the surface of the hydrogel. This type of behavior may also be attributed to the large molecules of high molecular weight HA. According to Forsey et al. [19], low molecular weight HA can penetrate deeper layers of cartilage structure. Liu et al. [32] also

reported lower adhesion energy between low molecular weight HA and mica. Therefore, low molecular weight HA is not very effective during the boundary lubrication of articular cartilage. Another important role plays the HA viscoelastic properties. Based on the viscoelasticity measurements, Synvisc One® exhibited a gel-like behavior over the whole range of tested frequencies. Therefore, it behaves like an elastic body even during the oscillating motion with very low frequency. To sum it up, Synvisc One® seemed to be the most appropriate HA-based solution for the viscosupplementation of SF in terms of friction Its mixture with SF reported the lowest value of CoF at the end of measurement - 0.009 ± 0.0008.

materials

MDPI

Article

The Effect of Synovial Fluid Composition, Speed and Load on Frictional Behaviour of Articular Cartilage

Denis Furmann *, David Nečas *, David Rebenda, Pavel Čípek, Martin Vrbka, Ivan Křupka and Martin Hartl

Faculty of Mechanical Engineering, Brno University of Technology, Brno 616 69, Czech Republic; David.Rebenda@vut.cz (D.R.); Pavel.Cipek@vut.cz (P.Č.); Martin.Vrbka@vut.cz (M.V.); krupka@fme.vutbr.cz (I.K.); Martin.Hartl@vut.cz (M.H.)

* Correspondence: Denis.Furmann@vut.cz (D.F.); David.Necas@vut.cz (D.N.); Tel.: +420-541143-238 (D.F.); +420-541143-239 (D.N.)

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Abstract: Articular cartilage ensures smooth motion of natural synovial joints operating at very low friction. However, the number of patients suffering from joint diseases, usually associated with cartilage degradation, continuously increases. Therefore, an understanding of cartilage tribological behaviour is of great interest in order to minimize its degradation, preserving the reliable function of the joints. The aim of the present study is to provide a comprehensive comparison of frictional behaviour of articular cartilage, focusing on the effect of synovial fluid composition (i), speed (ii), and load (iii). The experiments were realized using a pin-on-plate tribometer with reciprocating motion. The articular cartilage pin was loaded against smooth glass plate while the tests consisted of loading and unloading phases in order to enable cartilage rehydration. Various model fluids containing albumin, γ-globulin, hyaluronic acid, and phospholipids were prepared in two different concentrations simulating physiologic and osteoarthritic synovial fluid. Two different speeds, 5 mm/s and 10 mm/s were applied, and the tests were carried out under 5 N and 10 N. It was found that protein-based solutions exhibit almost no difference in friction coefficient, independently of the concentration of the constituents. However, the behaviour is considerably changed when adding hyaluronic acid and phospholipids. Especially when interacting with γ-globulin, friction coefficient decreased substantially. In general, an important role of the interaction of fluid constituents was observed. On the other hand, a limited effect of speed was detected for most of the model fluids. Finally, it was shown that elevated load leads to lower friction, which corresponds well with previous observations. Further study should concentrate on specific explored phenomena focusing on the detailed statistical evaluation.

Keywords: biotribology; articular cartilage; coefficient of friction; tribological properties; synovial fluid

1. Introduction

Joint osteoarthritis (OA) represents one of the most common diseases all over the world. It is estimated that more 50% of the adult population in the US will be diagnosed with joint OA by 2040 [1]. Arthritis is a degenerative disease of articular cartilage, usually occurring in large synovial joints such as hips and knees [2]. Nevertheless, it is reported that the occurrence of arthritis increases also in the case of small joints such as elbows, wrists, or fingers [3,4]. OA mainly affects older people [5] and its presence gradually grows with age. It is characterized by a strong joint pain which often leads to movement disabilities. Because of the avascular and aneural nature of the articular cartilage, there are limited possibilities of curing OA [6]. One of the options is to apply hyaluronic acid (HA)-based

viscosupplements. However, the efficiency is not guaranteed and the treatment effect often lasts for a limited time [7]. Therefore, the only chance for the patients suffering from OA is often represented by the joint replacement, the number of which gradually increases with rapid growth reported within the last decade [8]. To prevent further substantial increases in the number of joint replacements, preventing the degradation of articular cartilage seems to be a challenging task for upcoming decades. In that case, knowledge of the tribological properties of synovial fluid constituents and their interactions could help to develop new therapeutic procedures for the treatment of OA.

The main function of the articular cartilage is to ensure the transfer of load during movement while keeping a very low coefficient of friction [9]. The joints operate in several lubrication regimes, dependent on the loading conditions. The tribological performance of articular cartilage is mostly associated with its bi-phasic character [10]. Solid-phase consists mostly of the collagen fibres and proteoglycans, while the liquid phase is represented by the water and dissolved salt ions [6]. The biphasic character of lubrication was described in [11]. The authors showed that the liquid bound to the cartilage tissue is able to transfer part of the load. In the case of permanent load, the liquid is pushed out of the contact zone to the unloaded regions. A minor portion of the load is being supported by the liquid, and the gradual increase in friction is measured. The lubrication mode steadily changes from the so-called biphasic to boundary.

The dependence of friction on time was demonstrated by Forster and Fisher [12,13]. It was shown that the value of the friction factor grows until the whole load is transmitted only by a solid phase of the cartilage. Thus, the ability to rehydrate pushed liquid back to the previously loaded tissue significantly affects lubrication performance. However, rehydration itself is influenced by sliding speed [14] or whether the contact point is moved to another location [15]. Since the lubrication regime depends not only on the composition of the lubricant but also on the structure of the cartilage, several studies analysed the effect of the degradation of the cartilage tissue. Bell et al. [16] analysed the effect of the collagen network disruption. It was shown that the damaged cartilage samples reached higher values of the friction coefficient.

The region between contact pairs is filled with the synovial fluid (SF), which also acts as a boundary lubricant [15]. Recent studies analysed the adsorption properties of synovia constituents. It was shown that proteins significantly affect the adsorption of the HA [17–19]. Synergic effect of γ-Globulin and HA was further observed. γ-globulin adsorbs on the cartilage surface while HA adsorbs on the protein layer, forming stable lubricating film exhibiting lower friction. However, such an effect was not measured when using albumin and HA. An amount of studies was aimed at determining the constituents responsible for lubricating properties. Hills and Buttler [20] studied the effect of enzymatic degradation of various constituents. No significant change in friction was observed with the dissolving HA. The strongest impact was observed with the degradation of phospholipids (PHs). However, a slight improvement in lubrication properties was observed in terms of the degradation of the proteins. On the contrary, Bell et al. [21] examined the effect of the concentration of the ions in the salt solution and HA, with significant improvement in tribological properties being observed when adding HA to the solution. Forsey et al. [22] examined the impact of HA and PHs. It was shown that the efficiency of HA was not dependent on the concentration. However, the concentration had a major impact on the tribological properties of PHs. In the following study [23], the influence of PHs and proteins was observed, showing a slight increase in the friction coefficient for lipid and protein-impaired cartilage compared to the reference cartilage sample. The differences were apparent especially at shorter loading times. The effect of lubricin was analysed in the upcoming study [24], in which the synovial fluid was collected from the individuals with decreased lubricin presence. Synovial fluid with less lubricin had much worse tribological properties compared to the synovial fluid of a healthy individuals. Moreover, Ludwig et al. [25] and Schmidt et al. [26] showed that the combination of lubricin, HA and PHs results in a synergic improvement of the tribological properties.

It was shown above that extensive research has been carried out regarding articular cartilage behaviour. However, with respect to limited abilities of cartilage treatment, various authors aimed at

the possibility of the cartilage replacement in recent years. In that case, polyvinyl alcohol hydrogel (PVA) seems to be a suitable anticipating material [27]. Recently, several studies have focused on the analysis of PVA hydrogel tribological properties. Hydrogel has similar properties to the natural cartilage [17]. The impact of the individual components of synovial fluid on friction was investigated. It was found that the constituents in contact with the glass plate had the same effect as in natural cartilage. The effect of the adsorbed protein layer was studied in [18] concluding that the adsorbed proteins have a significant effect on friction. In particular, γ-globulin led to reduction of friction value. However, in the following study [28], where the separate impact of the constituents was evaluated, the effect of γ-globulin was shown to be the opposite. In the next paper [29], the effect of a PHs in combination with proteins was investigated. It was shown, that the concentration of PHs has a major impact on the tribological properties. In the studies led by Klein [30–32], it was shown that the presence of proteoglycans has a positive effect on the frictional properties, and that friction depends on the type of the PH used when combined with HA.

Based on the above references, it can be concluded that articular cartilage has been extensively investigated over the last few decades. Various studies focusing on cartilage behaviour may be found; however, there is a lack of complex frictional assessment so far. Therefore, the aim of the present study is to provide a comprehensive analysis of the frictional response of articular cartilage considering the effect of synovial fluid composition, speed, and load. The loading and kinematic conditions are designed based on literature in order to mimic common daily activities (slow, normal walking, stair climbing—elevated load). In addition, since it is assumed that synovial fluid composition plays a major role in joint lubrication, the main attention is paid to the concentration and mutual interactions of individual synovial fluid constituents. The present study should point to the fundamentals of cartilage friction. Future study should concentrate on specific observed phenomena, focusing on the improved statistical evaluation.

2. Material and Methods

The experiments were carried out in pin-on-plate configuration using commercial tribometer Bruker UMT TriboLab (Bruker, Billerica, MA, USA). The contact of porcine articular cartilage pin and smooth glass plate was investigated. The cartilage specimens were prepared from a porcine femoral head. The bones were collected from a local butchery shortly after slaughtering of six to eight months old mature pigs having a mass around 100–120 kg. Pins of a diameter of 5.6 mm were cored out using a hollow drill from the femoral heads. The subchondral bone was retained along with the cartilage tissue for proper fixation during the friction tests. After preparation, cartilage pins were stored in the phosphate buffered saline (PBS) solution for one hour in order to ensure sufficient tissue hydration, and were then frozen at −22 °C until further use. Optical glass plates were used, since the nature of glass mimics the superficial layer of articular cartilage under wet conditions, as discussed below. The tests were conducted at two different sliding speeds of 5 mm/s and 10 mm/s, representing slow and normal walking conditions. To explore the role of load, two different levels of normal force were considered; 5 N and 10 N, in particular. Following the elastic properties of the articular cartilage [33], the glass (technical datasheet), and the curvature of the cartilage pin, the contact pressure should correspond to approximately 0.3 to 0.5 MPa. Similar load was applied in previous references dealing with cartilage investigation [34,35]. Due to the soft nature of the articular cartilage, the size of the contact area corresponds to the whole pin contact surface. In all the tests, the stroke length of 20 mm was used. The research plan is schematically shown in Figure 1. Initially, the tests under the given conditions with six randomly chosen model fluids (no. 2, 4, 6, 10, 12, 13, see Table 1) were performed two times in order to check the repeatability of the experiments. As satisfactory compliance of the data was observed together with confirmation of some expectations based on the literature, the rest tests were performed only once following the below described procedure. However, in the case of any doubt, the experiments were repeated again in order to confirm the validity of the conclusions.

Before the test, the glass plate was cleaned using a sodium dodecyl sulphate solution and isopropyl alcohol and then dried by pressed air. The lubricants and the cartilage specimens were thawed at room temperature for 30 min and were stored in a fluid bath to avoid dehydration. Each experiment consisted of five steps—three friction (loading) steps and two unloading steps in order to enable cartilage rehydration [18,28]. Focusing on daily activities such as walking, the articular cartilage is repeatedly loaded and unloaded within very short time intervals. This is difficult to simulate using a laboratory tester. Therefore, each loading phase lasting 250 s was followed by 300 s unloading. Therefore, the overall duration of the experiment was 22.5 min (12.5 min of friction steps and 10 min of rehydration). The schematic illustration of the test procedure is shown in Figure 2. Focusing on Figure 3, the displayed data correspond to the third (last) friction step. The values of friction presented in Figures 4–6 correspond to the friction at the end of the third test.

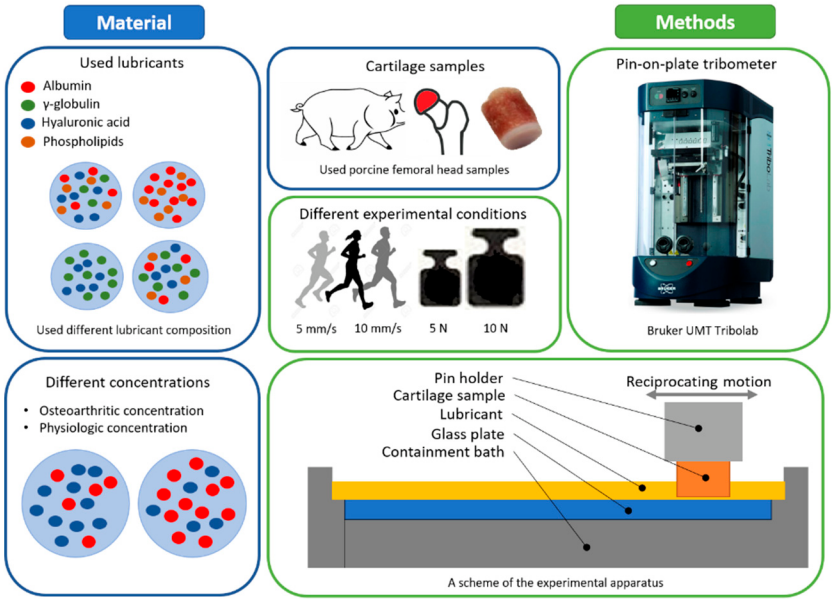

Figure 1. A research plan of the study.

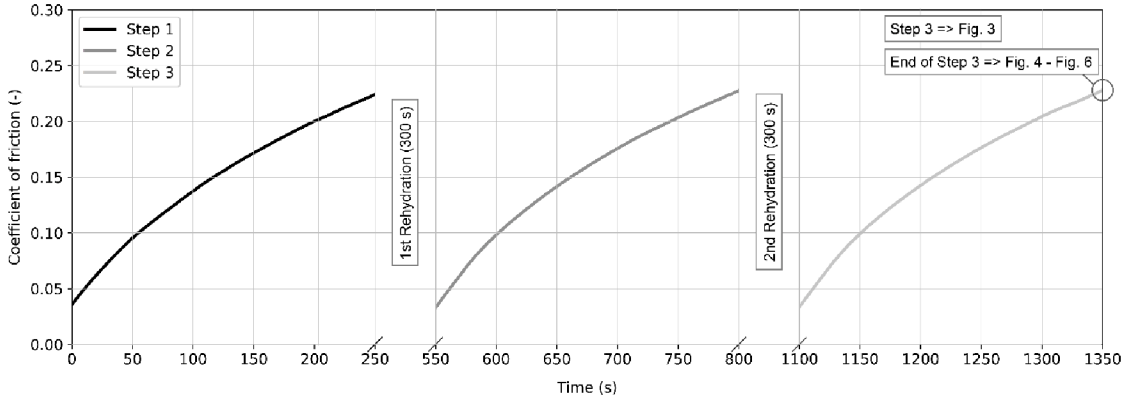

Figure 2. A schematic illustration of the test procedure.

In the present study, various model solutions were tested. PBS was used as a base fluid, while albumin, γ-globulin, HA, and PHs were added. The constituents were initially separately solved with PBS overnight at 4 °C using laboratory rocker-shaker (MR-12, Biosan, Riga, Latvia). Subsequently, the individual solutions were mixed together in order albumin, γ-globulin, HA, PHs. Focusing on the specific constituents, the following products were used. Bovine serum albumin (powder, ≥96%; A2153, Sigma-Aldrich, St. Louis, MO, USA), γ-globulin from bovine blood (powder, ≥99%; G5009, Sigma-Aldrich, St. Louis, MO, USA), HA = Sodium Hyaluronate HySilk (powder, quality

class—cosmetic; molecular weight = 820–1020 kDa, Contipro, Dolní Dobrouč, Czech Republic), and PHs = L-α-Phosphatidylcholine (powder, Type XVI-E, lyophilized powder; ≥99%; vesicles form; P3556, Sigma-Aldrich, St. Louis, MO, USA). After preparation, the lubricants were frozen at −22 °C until further use. The fluids of two different concentrations were prepared, representing both healthy (physiologic) and osteoarthritic synovial fluid concentrations [36]. The combinations of the chosen lubricant constituents can be seen in Table 1, and the used concentrations in Table 2.

Table 1. The combinations of the constituents in the lubricants.

	Model Fluid Constituents				
1	PBS	—	—	—	—
2	PBS	Albumin	—	—	—
3	PBS	—	γ-globulin	—	—
4	PBS	Albumin	γ-globulin	—	—
5	PBS	Albumin	—	Hyaluronic acid	—
6	PBS	—	γ-globulin	Hyaluronic acid	—
7	PBS	Albumin	γ-globulin	Hyaluronic acid	—
8	PBS	Albumin	—	—	Phospholipids
9	PBS	—	γ-globulin	—	Phospholipids
10	PBS	Albumin	γ-globulin	—	Phospholipids
11	PBS	Albumin	—	Hyaluronic acid	Phospholipids
12	PBS	—	γ-globulin	Hyaluronic acid	Phospholipids
13	PBS	Albumin	γ-globulin	Hyaluronic acid	Phospholipids

Table 2. Used concentrations of the constituents [36].

Constituents	Physiologic Concentration (mg/mL)	Osteoarthritic Concentration (mg/mL)
Albumin	20.00	24.90
γ-globulin	3.60	6.10
Hyaluronic Acid	2.50	1.49
Phospholipids	0.15	0.34

3. Results

3.1. The Effect of Fluid Composition

The results of friction coefficient during the third step of the test for various lubricants are summarized in Figure 3. The results for lubricants of physiological concentration (PC) of the constituents are shown in the left, while osteoarthritic concentration (OC) fluids are shown in the right figures. As can be seen in Figure 3, the values of the friction coefficient for pure PBS reach the highest values for the whole duration of the experiment. Thus, the constituents always led to lower friction. It is also evident from the figure that the model synovial fluid does not always reach the lowest friction.

For the lubricants at PC, it is seen that the values of the friction coefficient are very similar to those of a model synovial fluid during the test for protein-based lubricants (Figure 3a). The differences are small, and it is not possible to clearly assess which lubricant leads to better performance. In the case of a concentration typical for patients suffering from osteoarthritis, the model fluid has inferior tribological properties than for the PC. However, for the lubricants containing only the proteins, only a slight change of the friction coefficient is observed. All the observed lubricants achieved lower values of friction than pure PBS.

Addition of HA does not considerably change the results compared to the protein solution lubricants (Figure 3c,d). In the case of a PC (Figure 3c), the lowest values of friction were achieved with the γ-globulin and HA. However, in the case of albumin and both proteins mixtures with HA, no visible change occurred. Similar to the protein solution lubricants without the addition of HA, these lubricants have achieved comparable values as the model fluid. At OC (Figure 3d) a similar result

is observed. Likewise, as in the case of the protein solution lubricants, these achieved lower values of friction than a model fluid. When comparing the values of the friction coefficient with lubricants containing only proteins, a slight decrease in friction is seen when the HA is added indicating positive lubricating effect of HA.

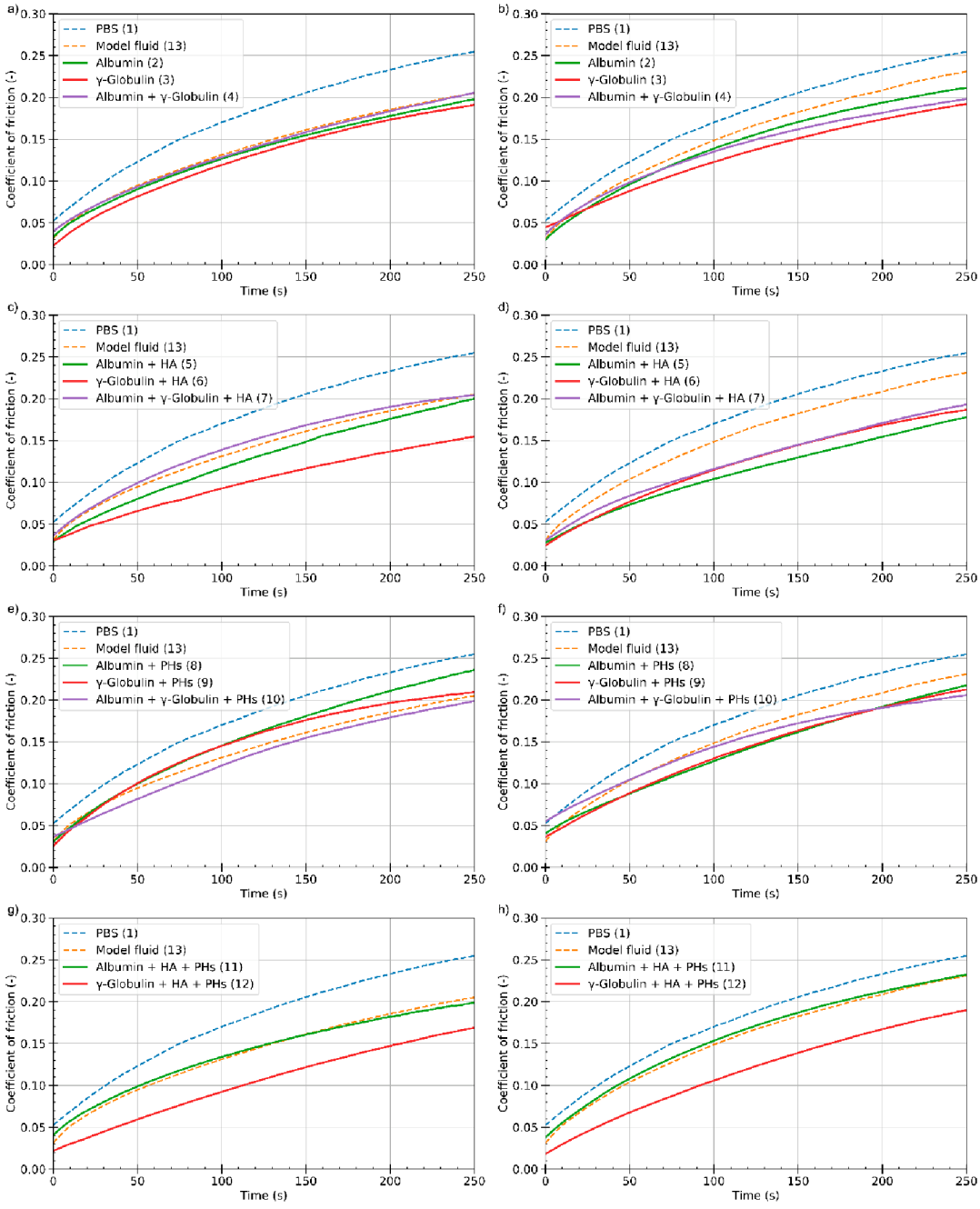

Figure 3. Dependence of the friction coefficient on time for various protein- (**a**,**b**), HA- (**c**,**d**), (PHs)- (**e**,**f**), and HA + PHs- (**g**,**h**) based lubricants; PC (**left**) and OC (**right**). HA: hyaluronic acid; PH: phospholipids; PC: physiologic concentration; OC: osteoarthritic concentration.

A further set of the experiments was conducted with the lubricants based on PHs (Figure 3e,f). At the PC (Figure 3e), it can be seen that the addition of PHs does not have a synergic effect in most cases. In the case of γ-globulin and albumin containing lubricants, the gradual growth of the friction is almost the same for the first 120 s. However, in the case of albumin, the growth continues during the test, and the end value is a bit higher. In the case of a combination of proteins, the value is similar to

the model fluid. Results of OC fluids are shown in Figure 3f. Focusing on the combination of albumin and γ-globulin, although the initial value is higher than for the model fluid, friction growth is less steep than that of the model fluid. For sole protein lubricants, the value of friction is lower than that of the model fluid during the test. Moreover, the growth curves are steeper than that of the lubricant containing both the proteins. There is a cross point at around 200 s when the friction of simple protein solutions exceeds the friction of mixed solution. Nevertheless, the observed friction is always lower than that of the model fluid.

When HA and PHs are present in the lubricant, similar results are seen at both concentrations (Figure 3g,h). The growth curves are similar for model fluid and the albumin lubricants. The values start at the same value at each concentration. However, at OC, the growth is steeper and the friction reaches higher values. The lowest friction is observed for the γ-globulin lubricant during the test. Likewise, the growth is steeper at the OC.

3.2. The Effect of Concentration

It is evident from Figure 4b that HA with γ-globulin has a synergic effect. At PC, it achieves the lowest friction for all the investigated lubricants. However, when the concentration of HA was lower (OC), there was an obvious increase of friction similar to that of the model fluid. Nevertheless, in the case of a combination of proteins or simple albumin with HA, only a limited change of friction is observed.

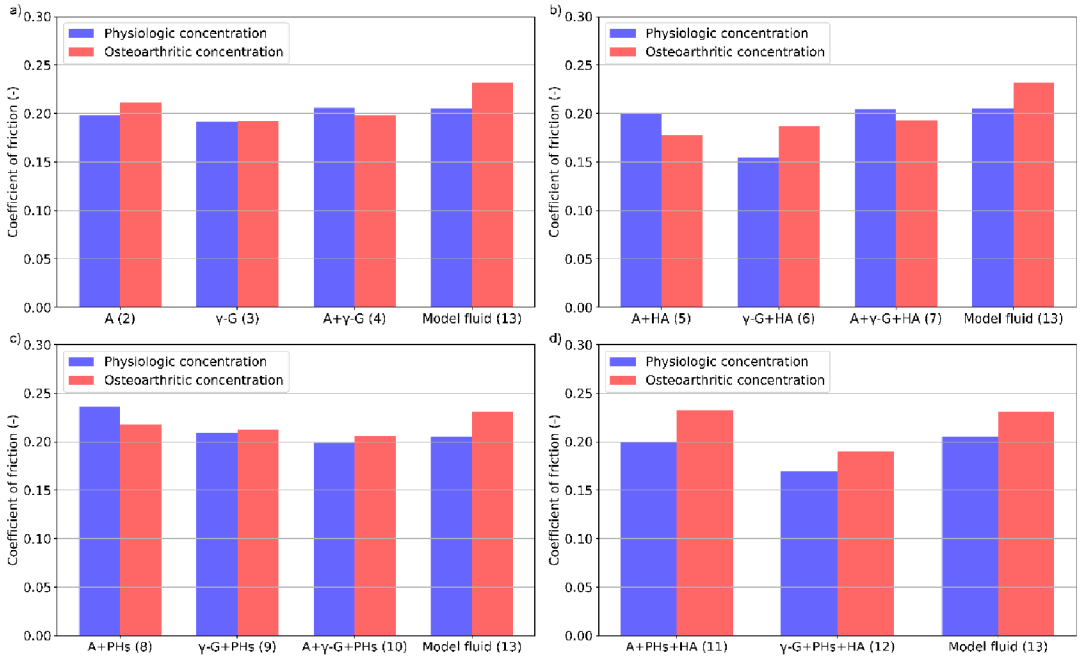

Figure 4. The effect of concentration on the friction coefficient for various protein- (**a**), protein + HA- (**b**), PHs- (**c**), and HA + PHs- (**d**) based lubricants. (A—Albumin, γ-G—γ-Globulin).

In the case of addition of PHs to the solutions (Figure 4c), a very slight decrease in friction can be seen for the lubricants containing albumin. During the test, this lubricant exhibited the highest values of the friction coefficient. When the concentration was altered, the concentration of both the components (albumin, PHs) decreased; therefore, it is evident that their combination affects the frictional behaviour. On the contrary, no change of friction is observed when γ-globulin or combination of albumin and γ-globulin was used. For HA and PHs-based lubricants (Figure 4d), a major increase of friction is observed for all the analysed lubricants. Values of friction are similar at each concentration for model fluid and albumin lubricant. However, when only γ-globulin was used, a lower value of friction was observed. Furthermore, the friction was the lowest from all the observed lubricants during the test.

3.3. The Effect of Speed

For a complex model fluid, a negligible impact of speed is observed independently of concentration, as is shown in Figure 5. On the other hand, there is a visible effect of speed for albumin-based lubricant. By lowering the speed to 5 mm/s, a decrease in friction is observed at PC, but there is a slight increase at OC for single protein lubricants. For pure γ-globulin, the effect of speed seems to be not as important. At PC, a little improvement of the friction is observed, but at OC a small increase of the friction is evident. However, compared to albumin, these changes are much lower. For combination of the proteins, the change in the values at both the studied concentrations is similar. In both cases, a slight reduction in the friction coefficient occurs when the speed decreases. The results for protein-based lubricants under two different speeds are shown in Figure 5a,b.

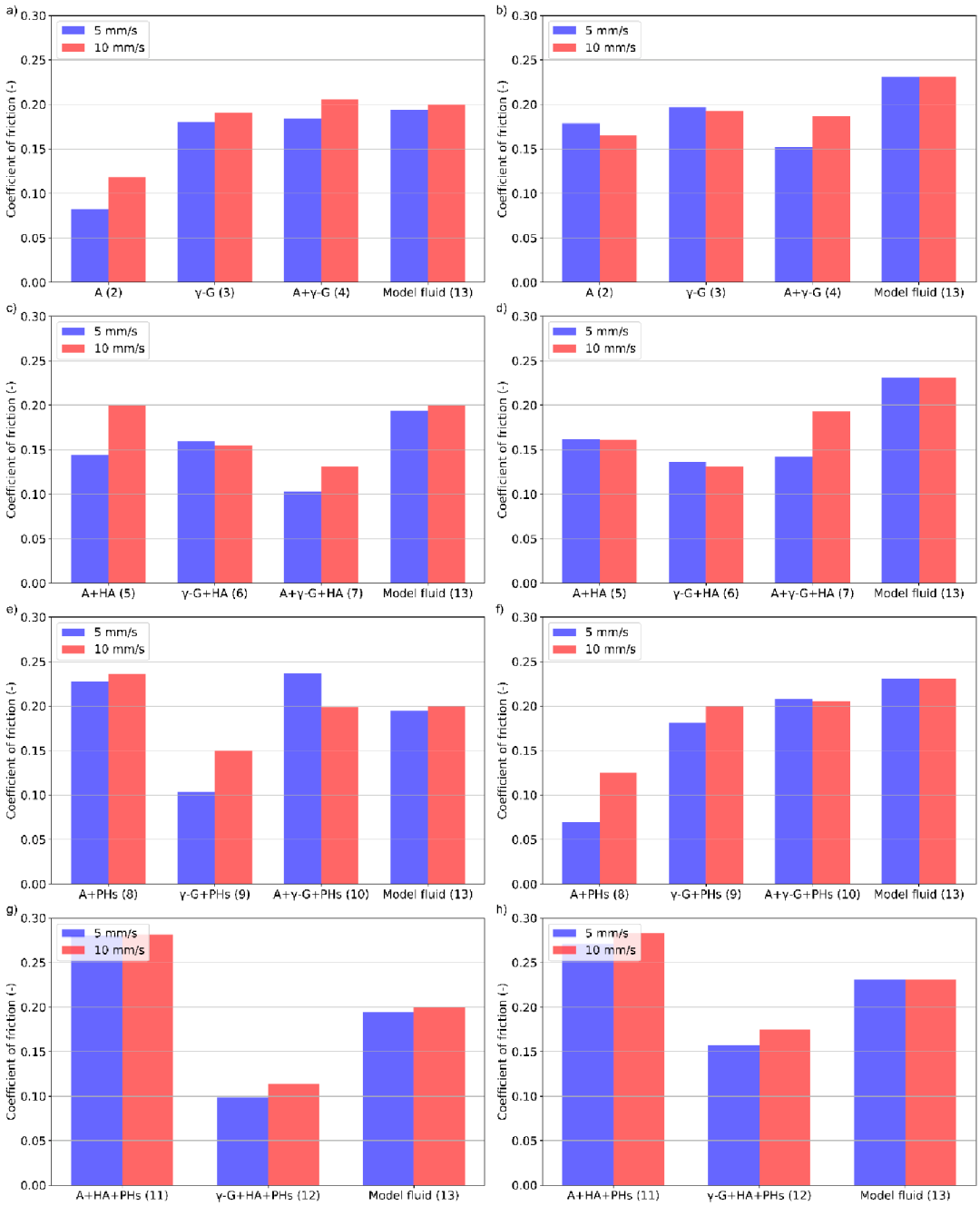

Figure 5. The effect of speed on the friction coefficient for various protein- (**a**,**b**), HA- (**c**,**d**), PHs- (**e**,**f**), and HA + PHs- (**g**,**h**) based lubricants; PC (**left**) and OC (**right**).

In the case of addition of HA to albumin, the impact of speed is very similar to the pure albumin (Figure 5c,d). At the PC, an apparent decrease of friction is observed when the speed is lowered, but no effect is observed at the OC. For γ-globulin, a very small increase in the coefficient of friction is observed at both concentrations. However, compared to the albumin, this increase is negligible. Also, albumin with γ-globulin has a similar effect at both observed concentrations. With a reduction in speed, an evident reduction of the friction coefficient occurs.

Addition of PHs led to opposite speed effect for albumin solution at OC (Figure 5f). As is displayed in the figure, with the decrease of speed, a substantial reduction of the coefficient of friction occurs. However, at PC, only a minimum effect is observed (Figure 5e). The effect of PHs on pure γ-globulin is the opposite. For the combination of albumin and γ-globulin, a slight increase of friction is observed at PC; however, no effect is observed at the OC. For the combination of HA and PHs-based lubricants (Figure 5g,h), a decrease in friction is observed with the reduction of the speed for the γ-globulin containing lubricant. However, there is no considerable change in the friction coefficient for the model fluid and the albumin-containing solution.

3.4. The Effect of Load

Based on Figure 6, it can be concluded that the impact of load on friction is the same for all the lubricants and concentrations. Only the difference in terms of percentage change can be identified. Independently of the test lubricant, lower friction coefficients were observed for higher levels of load. Figure 6a,b shows the load dependence for lubricants containing only the proteins. The most apparent friction reduction occurs at both the concentrations when simple γ-globulin solution is applied. In particular, at OC, the decrease is more pronounced. Likewise, the decrease is more pronounced at OC when the lubricant contains only albumin. A slight change is observed for lubricants containing the mixture of albumin and γ-globulin. The decrease in friction for the complex PC is considerable; nevertheless, very little effect is observed at OC where the difference in friction values is the lowest.

After adding HA to the protein solutions (Figure 6c,d), the greatest decrease of friction is observed for albumin containing lubricant at PC. On the contrary, for the lubricant with γ-globulin, only a negligible effect is achieved. For a combination of proteins, the decrease is similar to that of a model fluid. At OC, the most apparent decrease is observed for a combination of proteins. For the lubricants containing only one of the proteins, the observed effect is similar but much lower than that of the combination of the proteins.

Figure 6e,f show the effect of load considering the lubricants with the addition of PHs. At PC, a major reduction of friction is observed for single protein lubricants. For a combination of proteins, the decrease is slightly lower than that of the model fluid. Contrary, at OC the most considerable decrease of friction is observed for the lubricant containing albumin. The following are a γ-globulin and the combination of proteins. The decrease of these is similar; however, it is more pronounced than for the OC. The impact of the load with the addition of both HA and PHs is shown in Figure 6g,h. The most considerable decrease considering PC lubricants is observed for the complex model fluid. However, at the OC, the decrease of friction is the lowest for the model fluid. The decrease of friction for the single protein lubricants is similar at PC.

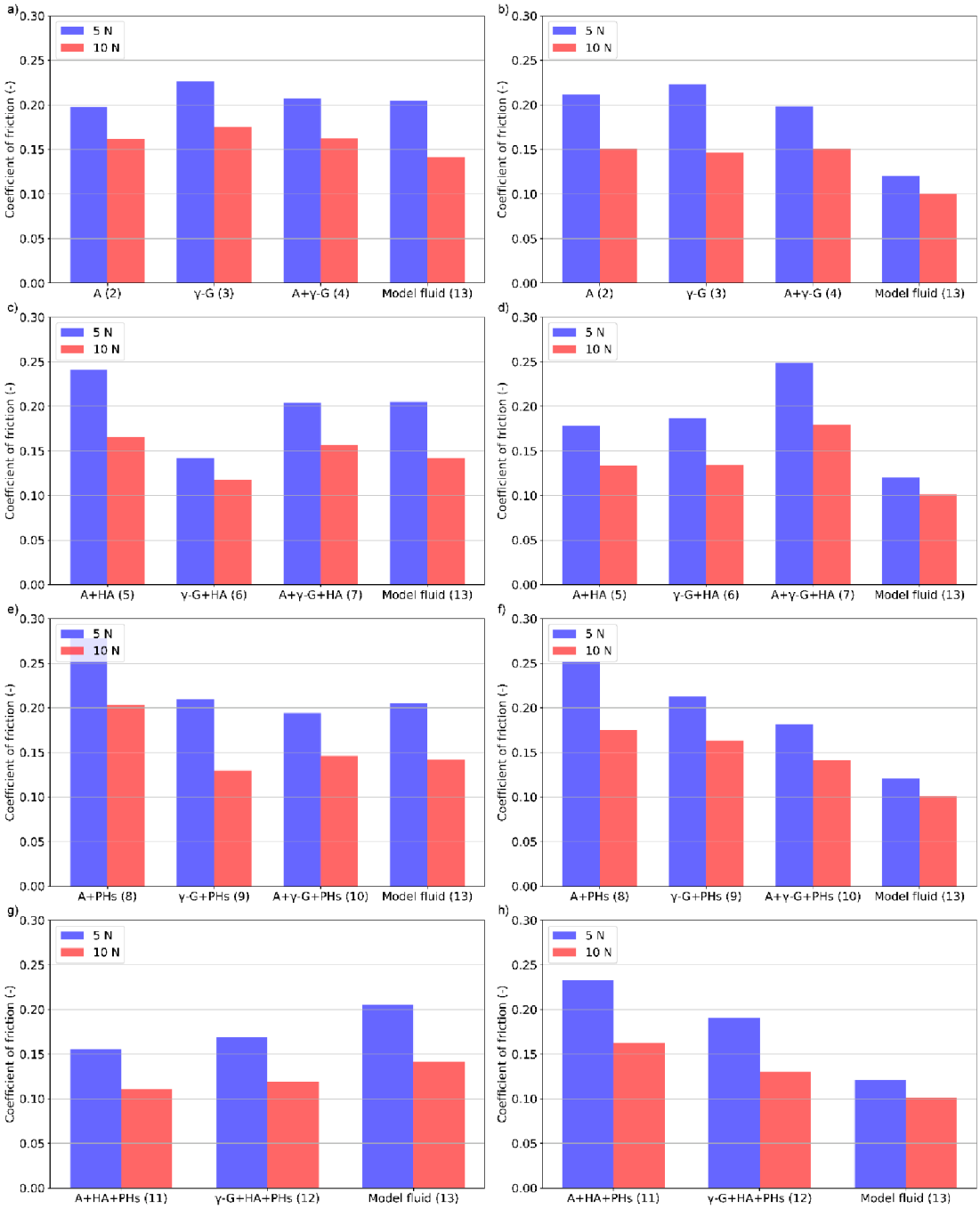

Figure 6. The effect of load on the friction coefficient for various protein- (**a**,**b**), HA- (**c**,**d**), PHs- (**e**,**f**), and HA + PHs- (**g**,**h**) based lubricants; PC (**left**) and OC (**right**).

4. Discussion

The results show that the friction coefficient strongly depends on both the used composition and the selected lubricant concentration. In the case of lubricants containing only proteins, the limited effect of the concentration or the composition is observed. Each lubricant reached approximately the value of the friction coefficient at around 0.2. The effect of HA or PHs has been analysed in several studies. Seror and co-workers, as shown in the works [30,31], used avidin, which is a protein extracted from egg whites. Murakami et al. [17,18,28,29] predominantly examined the characteristics of the hydrogel applying different concentrations of the constituents. However, compared to the present

results, their chosen concentrations were one order of magnitude lower than the concentrations used at the present study.

The quantity of each constituent was chosen to represent the actual concentrations occurring in the human body. Study [28] used the same set of lubricants; however, the concentrations employed were lower by approximately one order of magnitude. With the use of such low concentrations, a substantial effect of the proteins on lubrication properties was observed. However, when using a higher amount of protein, as was used in the present study, no difference in the lubrication properties was demonstrated. Proteins are adsorbed onto the surface of the articular cartilage and create a boundary lubricating film. According to Figure 3, the lubricating capacity seems to be not dependent on the concentration or the used protein. It rather looks like each protein is sufficient in order to create a lubricating film. Moreover, the same effect was observed when both the proteins were used. Proteins that do not contribute to the formation of the lubrication film probably do not have any substantial effect on friction. In reference [37], the authors analysed the adsorption properties of the proteins on the cartilage surface. It was shown that γ-globulin forms a more stable lubricating film than albumin, and the values of friction should therefore be different from each other. However, as stated before, no such effect was observed. It is assumed that this influence is caused primarily by the different concentrations of the proteins.

In Table 2, it can be seen that the percentage change of concentration (considering PC and OC) is not the same for all the constituents. Therefore, in order to study the effect of HA or PHs, concentration changes were mostly taken to be related to the concentration of the protein at each chosen concentration set. For lubricants with γ-globulin, Figure 4 shows a rapid increase of friction that is dependent on the concentration of HA. The synergic effect of these components is thus evident. However, when focusing on albumin and a combination of proteins, higher friction is observed at a higher relative concentration of HA. In addition, HA seems to have a negative effect on the adsorption of albumin on the articular cartilage surface. This finding is in compliance with previous observations [38].

The concentration of PHs and γ-globulin is higher in the case of OC. However, the relative concentration of these constituents is very similar. In Figure 4, no change of the friction coefficient for lubricants containing γ-globulin as well as a combination of proteins was observed. The friction value is similar to that when only protein containing lubricants are used. On the contrary, it is apparent that the combination of albumin and PHs at PC and OC does not form a sufficient boundary lubricant. The friction level at PC is only slightly lower than that of PBS. Although the friction at OC is lower, the value is still higher than lubricant containing only albumin. In the study [29], it was shown that addition of PHs is not always necessarily associated with the reduction of friction. Furthermore, in [22] it was demonstrated that friction is significantly affected by the concentration of the PHs and proteins.

In the case of combination of HA and PHs, it is evident from Figure 4 that each lubricant exhibited slightly elevated values of the friction coefficient at OC. In general, the lowest friction was achieved with the lubricant containing γ-globulin. However, the lubricants where albumin was presented achieved a similar friction as the model fluid at both concentrations. In the previous reference [29], the lowest friction was detected for the combination of all the components of the model fluid. It is therefore likely that, as is the case with the addition of PHs, friction is strongly dependent on the concentration of the proteins in the lubricant.

Focusing on the effect of speed, a very interesting similarity between protein-based and HA-based lubricants is observed. The percentage change is not as considerable for the lubricant with γ-globulin, as is also the case with the model fluid. However, it is evident from Figure 5 that for lubricants containing albumin, the magnitude of the effect of speed depends on the concentration. The impact of speed has been studied in the literature previously [14]. The authors found that the coefficient of friction depends on the concentration of salt ions. A similar dependence was observed with the albumin containing lubricant, even if HA is added. For lower concentrations, which is a PC for albumin, the increase of friction is observed with increased speed. On the contrary, when albumin concentration is increased to OC, which represents growth by around 20%, a slight lowering of friction

can be seen in Figure 5. It is likely that the adsorbed protein layer and HA have no effect on speed, and thus the whole effect is caused by the present charge of albumin molecules. However, when HA and PHs are added, the limited effect of speed is observed. PHs are also charged molecules; however, the lubrication mechanism is different. When only PHs are added to the albumin solution, a strong effect of speed is observed only at OC. At the PC, a small increase of friction is observed; however, it is much less pronounced as in the case of OC. The effect thus apparently depends on which proteins and which concentration is considered, similar as it is with the effects of composition and concentration. The findings are in accordance with the previous observations [29], where strong influence of concentration was observed as well.

It is shown that with increasing load, the friction coefficient decreases for all the applied lubricants. The impact of load was previously explained by Walker et al. [39]. Under a constant load, the fluid is slowly pushed into a region where the cartilage is not loaded. However, the porous structure of the articular cartilage causes high resistance which is accompanied by the increase of pressure inside of the cartilage tissue. A higher load causes higher pressure, and thus the liquid phase can transfer a larger portion of the load. That explains the decrease of the friction coefficient. The decrease is thus caused by the structure of the cartilage and not by the properties of the lubricant. It can be assumed that the level of friction reduction may be related to the thickness and permeability of the lubricating film. Therefore, the creation of boundary lubricating film affects only the rate of the friction drop.

The authors recognize several drawbacks of the performed study. The most important point is related to measurement repeatability. Since the articular cartilage is a biological tissue, the repeatability is usually not as good as in the case of technical materials. The main motivation for the performed study was to provide a very complex comparison of cartilage frictional behaviour focusing on the effect of speed, load, and synovial fluid composition. It is generally recommended to provide seven repetitions at minimum for biological samples. When summarizing all the studied conditions and fluids, this would lead to nearly 600 separate experiments, each lasting nearly 23 min. Clearly, this could not be done due to (i) amount of cartilage samples, (ii) costs of model fluids, and (iii) required time for testing. However, it should be pointed out that the authors followed very strict experimental procedure. The cartilage specimens were extracted from the same parts of the porcine femoral bones collected shortly after slaughtering from a local butcher. In the case of any suspicion about the condition of the bone, or extraction of the cartilage pin, these samples were immediately excluded. At the beginning of the study, six random lubricants were chosen, and the experiments were repeated two times in order to check the repeatability. As very satisfactory compliance was observed, further measurements were realized only once. Nevertheless, the results of the tests were analysed independently by various members of the research team in order to prevent unclear conclusions. If necessary, the experiments under some conditions were repeated more times. Therefore, even if the authors could not provide extensive statistical analysis in the present study, it is assumed that the findings are relevant showing clear findings and dependencies. This statement is supported by a comprehensive data set with clear effects across a wide range of lubricants and experimental conditions. In addition, excellent repeatability could be obtained for three consequence friction tests performed with each specimen, as illustrated in Figure 2. According to the author's best knowledge, such a complex study has not been introduced before. Our further motivation is to combine the information about frictional behaviour with direct in situ observation of the contact using fluorescent microscopy method [40]. This technique makes it possible to qualitatively assess the film thickness together with the determination of the role of individual synovial fluid constituents [41]. Moreover, the main motivation for the present study was to provide a comprehensive assessment of the cartilage frictional behaviour. Specific observed phenomena are going to be investigated in the upcoming study. This should focus on specific conditions and fluids, concentrating on fully relevant statistical description.

Focusing on the previous research, it was found that human synovial fluid exhibits significant differences in viscosity [36]. However, it should be emphasized that all the fluids employed in the present study were prepared artificially from the same constituents of the same series provided by the

same producers. Although there is an apparent difference in content of individual parts regarding physiologic and osteoarthritic fluid, the viscosity difference was found to be negligible varying from 2.6 to 2.8 mPa·s for PC and OC, respectively. Therefore, it is assumed that the difference in friction corresponds to interaction of the constituents rather than to the effect of viscosity.

A further limitation of the study is related to the contact mechanics. The present paper uses cartilage-on-glass contact. It is particularly complicated to investigate real cartilage-on-cartilage contact in the laboratory. The point is that it is nearly impossible to get a flat cartilage sample of sufficient size in order to enable mutual motion in the range of a couple of millimetres at least. Therefore, one of the rubbing surfaces is often substituted by technical material [12,13,23,28]. Glass as a counter face was used by Murakami et al. [28], among others. It has been discussed in the literature that selection of glass may benefit from its properties. Glass is generally very smooth, hard, nonporous, and impermeable, compared to articular cartilage; however, it exhibits a hydrophilic negatively charged nature, corresponding to proteoglycan on superficial layer of articular cartilage under wet conditions [42]. Thus, glass is considered to be a suitable counterpart material. Further motivation for using glass is related to the possibility of visualization of lubricant film formation [43] using optical fluorescent microscopy [40] in future. The results will be confronted in the upcoming study. Moreover, it should be emphasized that glass apparently has a very limited effect on frictional behaviour. This could be clearly seen during the experiments. After rehydration, friction dropped to the same level as was at the beginning of the test; indicating that there were no molecules adsorbed on the glass surface which was flooded all the time.

When summarizing the drawbacks of the paper, simplified loading and kinematic conditions should be highlighted as well. It is apparent that human large synovial joints (hips, knees) which usually suffer from osteoarthritis, operate under transient load and kinematics. However, it should be mentioned that laboratory pin-on-plate reciprocating testing became a routine established technique when examining friction, lubrication, and wear mechanisms in biotribology area. However, variable change of load throughout the stroke, different length of individual strokes, or more complex eight-like or spiral-like motion might be a motivation for future research. This idea is supported by findings provided by Myant et al. [44], who described a clear effect of transient motion on lubrication behaviour of artificial joints.

5. Conclusion

The present study dealt with complex assessment of frictional behaviour of articular cartilage. The experiments were realized using pin-on-plate reciprocating tribometer while the contact of porcine femoral cartilage and smooth glass plate was studied. Thirteen model lubricants were compared, focusing on the role of concentration and mutual interaction of individual synovial fluid constituents. The experiments were carried out under two speeds corresponding to slow and normal walking. Furthermore, two levels of load mimicking walking and stair climbing were considered. The main findings are summarized in the following points.

- Focusing on the differences in concentration of fluid constituents corresponding to physiologic and osteoarthritic synovial fluid, only a limited effect on friction was observed when protein-based lubricants were applied.
- The lowest friction was generally observed for the mixture of γ-globulin, HA, and PHs, indicating the apparent role of interaction of the constituents. In addition, the importance of HA and PHs despite its lower concentration was demonstrated.
- The effect of HA and PHs can be further discussed in relation to sliding speed. The results showed that these constituents lead to stabilized friction with a negligible speed effect.
- An increased load led to lower friction, in general. This finding is in agreement with previous scientific observations.

- Regarding the implication for biotribological testing of articular cartilage, the importance of the interaction of proteins with HA and PHs should be highlighted. Therefore, it is strongly recommended to employ complex model fluids when examining cartilage behaviour.
- Further research should explore the role of transient kinematic and loading conditions, among other things [44]. In addition, the role of other constituents (e.g., lubricin) should be clarified, as it has been indicated in the literature that it may influence cartilage lubrication properties [25,26,45].

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References

1. Hootman, J.M.; Helmick, C.G.; Barbour, K.; Theis, K.A.; Boring, M.A. Updated Projected Prevalence of Self-Reported Doctor-Diagnosed Arthritis and Arthritis-Attributable Activity Limitation Among US Adults, 2015–2040. *Arthritis Rheumatol.* **2016**, *68*, 1582–1587. [CrossRef] [PubMed]
2. Kopec, J.; Sayre, E.C.; Schwartz, T.A.; Renner, J.B.; Helmick, C.G.; Badley, E.M.; Cibere, J.; Callahan, L.F.; Jordan, J.M. Occurrence of radiographic osteoarthritis of the knee and hip among African Americans and whites: A population-based prospective cohort study. *Arthritis Care Res.* **2013**, *65*, 928–935. [CrossRef] [PubMed]
3. Yamamoto, M.; Chung, K.C. Joint Fusion and Arthroplasty in the Hand. *Clin. Plast. Surg.* **2019**, *46*, 479–488. [CrossRef] [PubMed]
4. Haara, M.; Manninen, P.; Kröger, H.; Arokoski, J.; Kärkkäinen, A.; Knekt, P.; Aromaa, A.; Heliövaara, M. Osteoarthritis of finger joints in Finns aged 30 or over: Prevalence, determinants, and association with mortality. *Ann. Rheum. Dis.* **2003**, *62*, 151–158. [CrossRef]
5. Australian Institute of Health and Welfare. Available online: https://www.aihw.gov.au/ (accessed on 10 January 2020).
6. Lees, D.; Partington, P. Articular cartilage. *Orthop. Trauma* **2016**, *30*, 265–272. [CrossRef]
7. Cherniakova, Y.M.; Pinchuk, L.S. Tribological aspects of joint intraaricular therapy. *Acta Bioeng. Biomech.* **2011**, *13*, 57.
8. Colombo, F. *Help Wanted? OECD Health Policy Studies*; OECD: Paris, France, 2011; pp. 1–8. ISBN 9789264097582.
9. Katta, J.; Pawaskar, S.S.; Jin, Z.; Ingham, E.; Fisher, J. Effect of load variation on the friction properties of articular cartilage. *Proc. Inst. Mech. Eng. Part J J. Eng. Tribol.* **2007**, *221*, 175–181. [CrossRef]
10. Sakai, N.; Hagihara, Y.; Furusawa, T.; Hosoda, N.; Sawae, Y.; Murakami, T. Analysis of biphasic lubrication of articular cartilage loaded by cylindrical indenter. *Tribol. Int.* **2012**, *46*, 225–236. [CrossRef]
11. Mow, V.C.; Kuei, S.C.; Lai, W.M.; Armstrong, C.G. Biphasic Creep and Stress Relaxation of Articular Cartilage in Compression: Theory and Experiments. *J. Biomech. Eng.* **1980**, *102*, 73–84. [CrossRef]
12. Forster, H.; Fisher, J. The Influence of Loading Time and Lubricant on the Friction of Articular Cartilage. *Proc. Inst. Mech. Eng. Part H J. Eng. Med.* **1996**, *210*, 109–119. [CrossRef]
13. Forster, H.; Fisher, J. The influence of continuous sliding and subsequent surface wear on the friction of articular cartilage. *Proc. Inst. Mech. Eng. Part H J. Eng. Med.* **1999**, *213*, 329–345. [CrossRef] [PubMed]
14. Kienle, S.; Boettcher, K.; Wiegleb, L.; Urban, J.; Burgkart, R.H.; Lieleg, O.; Hugel, T. Comparison of friction and wear of articular cartilage on different length scales. *J. Biomech.* **2015**, *48*, 3052–3058. [CrossRef] [PubMed]
15. Caligaris, M.; Ateshian, G.A. Effects of sustained interstitial fluid pressurization under migrating contact area, and boundary lubrication by synovial fluid, on cartilage friction. *Osteoarthr. Cartil.* **2008**, *16*, 1220–1227. [CrossRef] [PubMed]

16. Bell, C.J.; Carrick, L.M.; Katta, J.; Jin, Z.; Ingham, E.; Aggeli, A.; Boden, N.; Waigh, T.A.; Fisher, J. Self-assembling peptides as injectable lubricants for osteoarthritis. *J. Biomed. Mater. Res. Part A* **2006**, *78*, 236–246. [CrossRef] [PubMed]
17. Murakami, T.; Yarimitsu, S.; Nakashima, K.; Yamaguchi, T.; Sawae, Y.; Sakai, N.; Suzuki, A. Superior lubricity in articular cartilage and artificial hydrogel cartilage. *Proc. Inst. Mech. Eng. Part J J. Eng. Tribol.* **2014**, *228*, 1099–1111. [CrossRef]
18. Nakashima, K.; Sawae, Y.; Murakami, T. Influence of protein conformation on frictional properties of poly (vinyl alcohol) hydrogel for artificial cartilage. *Tribol. Lett.* **2007**, *26*, 145–151. [CrossRef]
19. Yarimitsu, S.; Nakashima, K.; Sawae, Y.; Murakami, T. Influences of lubricant composition on forming boundary film composed of synovia constituents. *Tribol. Int.* **2009**, *42*, 1615–1623. [CrossRef]
20. A Hills, B.; Butler, B.D. Surfactants identified in synovial fluid and their ability to act as boundary lubricants. *Ann. Rheum. Dis.* **1984**, *43*, 641–648. [CrossRef]
21. Bell, C.J.; Ingham, E.; Fisher, J. Influence of hyaluronic acid on the time-dependent friction response of articular cartilage under different conditions. *Proc. Inst. Mech. Eng. Part H J. Eng. Med.* **2006**, *220*, 23–31. [CrossRef]
22. Forsey, R.W.; Fisher, J.; Thompson, J.; Stone, M.H.; Bell, C.; Ingham, E. The effect of hyaluronic acid and phospholipid based lubricants on friction within a human cartilage damage model. *Biomaterials* **2006**, *27*, 4581–4590. [CrossRef]
23. Pickard, J.; Fisher, J.; Ingham, E.; Egan, J. Investigation into the effects of proteins and lipids on the frictional properties of articular cartilage. *Biomaterials* **1998**, *19*, 1807–1812. [CrossRef]
24. Jay, G.D.; Torres, J.R.; Rhee, D.K.; Helminen, H.J.; Hytinnen, M.M.; Cha, C.-J.; Elsaid, K.A.; Kim, K.-S.; Cui, Y.; Warman, M.L. Association between friction and wear in diarthrodial joints lacking lubricin. *Arthritis Rheum.* **2007**, *56*, 3662–3669. [CrossRef] [PubMed]
25. Ludwig, T.E.; Hunter, M.; Schmidt, T.A. Cartilage boundary lubrication synergism is mediated by hyaluronan concentration and PRG4 concentration and structure. *BMC Musculoskelet. Disord.* **2015**, *16*, 386. [CrossRef] [PubMed]
26. Schmidt, T.A.; Gastelum, N.S.; Nguyen, Q.T.; Schumacher, B.L.; Sah, R.L. Boundary lubrication of articular cartilage: Role of synovial fluid constituents. *Arthritis Rheum.* **2007**, *56*, 882–891. [CrossRef]
27. Nakashima, K.; Sawae, Y.; Murakami, T. Study on Wear Reduction Mechanisms of Artificial Cartilage by Synergistic Protein Boundary Film Formation. *JSME Int. J. Ser. C* **2005**, *48*, 555–561. [CrossRef]
28. Murakami, T.; Yarimitsu, S.; Nakashima, K.; Sawae, Y.; Sakai, N. Influence of synovia constituents on tribological behaviors of articular cartilage. *Friction* **2013**, *1*, 150–162. [CrossRef]
29. Yarimitsu, S.; Nakashima, K.; Sawae, Y.; Murakami, T. Influence of Phospholipid and Protein Constituents on Tribological Properties of Artificial Hydrogel Cartilage Material. *J. Biomech. Sci. Eng.* **2013**, *8*, 257–267. [CrossRef]
30. Zhu, L.; Seror, J.; Day, A.; Kampf, N.; Klein, J. Ultra-low friction between boundary layers of hyaluronan-phosphatidylcholine complexes. *Acta Biomater.* **2017**, *59*, 283–292. [CrossRef]
31. Seror, J.; Merkher, Y.; Kampf, N.; Collinson, L.; Day, A.; Maroudas, A.; Klein, J. Articular Cartilage Proteoglycans As Boundary Lubricants: Structure and Frictional Interaction of Surface-Attached Hyaluronan and Hyaluronan–Aggrecan Complexes. *Biomacromolecules* **2011**, *12*, 3432–3443. [CrossRef]
32. Seror, J.; Zhu, L.; Goldberg, R.; Day, A.; Klein, J. Supramolecular synergy in the boundary lubrication of synovial joints. *Nat. Commun.* **2015**, *6*, 6497. [CrossRef]
33. Stolz, M.; Raiteri, R.; Daniels, A.U.; VanLandingham, M.R.; Baschong, W.; Aebi, U. Dynamic Elastic Modulus of Porcine Articular Cartilage Determined at Two Different Levels of Tissue Organization by Indentation-Type Atomic Force Microscopy. *Biophys. J.* **2004**, *86*, 3269–3283. [CrossRef]
34. Li, F.; Wang, A.; Wang, C. Analysis of friction between articular cartilage and polyvinyl alcohol hydrogel artificial cartilage. *J. Mater. Sci. Mater. Electron.* **2016**, *27*, 27. [CrossRef]
35. Burris, D.L.; Moore, A. Cartilage and Joint Lubrication: New Insights Into the Role of Hydrodynamics. *Biotribology* **2017**, *12*, 8–14. [CrossRef]
36. Galandakova, A.; Ulrichova, J.; Langová, K.; Hanáková, A.; Vrbka, M.; Hartl, M.; Gallo, J. Characteristics of synovial fluid required for optimization of lubrication fluid for biotribological experiments. *J. Biomed. Mater. Res. Part B: Appl. Biomater.* **2016**, *105*, 1422–1431. [CrossRef] [PubMed]

37. Murakami, T.; Sawae, Y.; Nakashima, K.; Yarimitsu, S.; Sato, T. Micro- and nanoscopic biotribological behaviours in natural synovial joints and artificial joints. *Proc. Inst. Mech. Eng. Part J J. Eng. Tribol.* **2007**, *221*, 237–245. [CrossRef]
38. Murakami, T.; Nakashima, K.; Yarimitsu, S.; Sawae, Y.; Sakai, N. Effectiveness of adsorbed film and gel layer in hydration lubrication as adaptive multimode lubrication mechanism for articular cartilage. *Proc. Inst. Mech. Eng. Part J J. Eng. Tribol.* **2011**, *225*, 1174–1185. [CrossRef]
39. Walker, P.S.; Dowson, D.; Longfield, M.D.; Wright, V. Lubrication of human joints. *Ann. Rheum. Dis.* **1969**, *28*, 194. [CrossRef]
40. Nečas, D.; Vrbka, M.; Urban, F.; Krupka, I.; Hartl, M. The effect of lubricant constituents on lubrication mechanisms in hip joint replacements. *J. Mech. Behav. Biomed. Mater.* **2016**, *55*, 295–307. [CrossRef]
41. Nečas, D.; Vrbka, M.; Galandáková, A.; Křupka, I.; Hartl, M. On the observation of lubrication mechanisms within hip joint replacements. Part I: Hard-on-soft bearing pairs. *J. Mech. Behav. Biomed. Mater.* **2019**, *89*, 237–248. [CrossRef]
42. Higaki, H.; Murakami, T.; Nakanishi, Y.; Miura, H.; Mawatari, T.; Iwamoto, Y. The lubricating ability of biomembrane models with dipalmitoyl phosphatidylcholine and γ-globulin. *Proc. Inst. Mech. Eng. Part H J. Eng. Med.* **1998**, *212*, 337–346. [CrossRef]
43. Čípek, P.; Rebenda, D.; Nečas, D.; Vrbka, M.; Křupka, I.; Hartl, M. Visualization of Lubrication Film in Model of Synovial Joint. *Tribol. Ind.* **2019**, *41*, 387–393. [CrossRef]
44. Myant, C.; Cann, P. The effect of transient conditions on synovial fluid protein aggregation lubrication. *J. Mech. Behav. Biomed. Mater.* **2014**, *34*, 349–357. [CrossRef] [PubMed]
45. Mazzucco, D.; Spector, M. THE JOHN CHARNLEY AWARD PAPER: The Role of Joint Fluid in the Tribology of Total Joint Arthroplasty. *Clin. Orthop. Relat. Res.* **2004**, *429*, 17–32. [CrossRef] [PubMed]

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On the Dependence of Rheology of Hyaluronic Acid Solutions and Frictional Behavior of Articular Cartilage

David Rebenda [1,*], Martin Vrbka [1], Pavel Čípek [1], Evgeniy Toropitsyn [2], David Nečas [1], Martin Pravda [2] and Martin Hartl [1]

[1] Faculty of Mechanical Engineering, Brno University of Technology, 616 69 Brno, Czech Republic; Martin.Vrbka@vut.cz (M.V.); Pavel.Cipek@vut.cz (P.Č.); David.Necas@vut.cz (D.N.); Martin.Hartl@vut.cz (M.H.)

[2] Contipro a.s., Dolní Dobrouč 401, 561 02 Dolní Dobrouč, Czech Republic; Evgeniy.Toropitsyn@contipro.com (E.T.); Martin.Pravda@contipro.com (M.P.)

* Correspondence: David.Rebenda@vut.cz

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Abstract: Hyaluronic acid (HA) injections represent one of the most common methods for the treatment of osteoarthritis. However, the clinical results of this method are unambiguous mainly because the mechanism of action has not been clearly clarified yet. Viscosupplementation consists, inter alia, of the improvement of synovial fluid rheological properties by injected solution. The present paper deals with the effect of HA molecular weight on the rheological properties of its solutions and also on friction in the articular cartilage model. Viscosity and viscoelastic properties of HA solutions were analyzed with a rotational rheometer in a cone–plate and plate–plate configuration. In total, four HA solutions with molecular weights between 77 kDa and 2010 kDa were tested. The frictional measurements were realized on a commercial tribometer Bruker UMT TriboLab, while the coefficient of friction (CoF) dependency on time was measured. The contact couple consisted of the articular cartilage pin and the plate made from optical glass. The contact was fully flooded with tested HA solutions. Results showed a strong dependency between HA molecular weight and its rheological properties. However, no clear dependence between HA molecular weight and CoF was revealed from the frictional measurements. This study presents new insight into the dependence between rheological and frictional behavior of the articular cartilage, while such an extensive investigation has not been presented before.

Keywords: articular cartilage; hyaluronic acid; rheology; friction

1. Introduction

An articular cartilage is a kind of hyaline cartilage which covers sliding surfaces in large synovial joints (e.g., the hip or knee). Under physiological conditions, the articular cartilage creates sliding surfaces with extremely low friction and minimal wear. It can also absorb impact loads quite well. The cartilage structure consists of a fluid and solid phase, which determine its mechanical properties [1]. A solid phase is composed of an extracellular matrix from collagen fibrils and proteoglycans. According to the orientation of collagen fibrils, the cartilage structure can be divided into several layers: superficial, middle, deep and calcified zones [2]. Collagen fibrils are oriented tangentially to the cartilage surface in the superficial zone whereas they are mostly perpendicular to the surface in the deep zone. The collagen content is the highest in the superficial zone and decreases towards the deep zone. On the contrary, the proteoglycan content is the lowest in the superficial zone. The superficial zone also has the highest

porosity, which means the highest content of fluid phase. This interstitial fluid is mainly composed of water and electrolytes [3].

A cartilage-on-cartilage motion exhibits very low friction under physiological conditions. However, unhealthy lifestyle, obesity, or traumatic injuries can lead to the damage of the articular cartilage and can cause diseases such as osteoarthritis, chondropathy, etc. Osteoarthritis is one of the most common diseases of the musculoskeletal system. These days, it afflicts about 70% of people older than 70 years [4]. Osteoarthritis is characterized by an imbalance between the synthesis and wear of the articular cartilage. The surface of the cartilage shows areas of softening, fibrillations, or erosions. In the later stage, there may even be areas of cartilage loss. This cartilage damage leads to a distraction of the cartilage lubrication mechanism and a higher friction. Progression of osteoarthritis is also connected with changes in the composition of the synovial fluid [5]. Osteoarthritic synovial fluid is diluted by inflammatory effusion and the concentration of the individual components is changing.

Viscosupplementation is one of the noninvasive methods for the curing of osteoarthritis. This method consists of intra-articular injections with hyaluronic acid (HA) into the joint capsule. The original theory about viscosupplementation [6] assumed the improvement of the rheological properties of synovial fluid by exogenous HA. Higher viscosity and improved viscoelastic properties should lead to better lubrication and lower friction of the osteoarthritic articular cartilage. Exogenous HA can be detected in the synovial fluid for only a few days after the injection but medical studies [7] reported positive effects of this treatment method even after 6 months. This pointed out another physiological effect of this treatment method such as a synthesis of endogenous HA or an anti-inflammatory effect [8]. However, the mechanism of action of viscosupplementation has not been sufficiently clarified yet.

Hyaluronic acid (HA) is a polymer of disaccharides composed of D-glucuronic acid and N-acetyl D-glucosamine. It is one of the primary constituents of synovial fluid. In a healthy synovial joint, the concentration varies between 1 and 4 mg/mL [9,10], and the molecular weight ranges from 4 kDa up to 8 MDa [11]. HA is the main constituent which affects the rheology of synovial fluid [12]. Concentration and molecular weight are the key parameters which affect the viscosity and viscoelastic properties of HA solutions. Solutions with a higher concentration exhibit a higher viscosity [13,14], the same as solutions with higher molecular weights [13,15]. Solutions with higher molecular weights also report higher values of the storage (G') and loss (G") modulus [13,15], and the value of crossover frequency is decreasing [14]. Longer polymer chains need more time to disentangle so the molecular transition from a predominantly viscous response to an elastic response occurs at lower frequencies. In osteoarthritic synovial fluid, the concentration and molecular weight of HA is decreased [16]. Exogenous HA which is injected into the joint capsule during viscosupplementation, should restore the rheological properties of healthy synovial fluid. Due to the low concentration and molecular weight of endogenous HA, the rheological properties of mixed synovial fluid with viscosupplement are primarily dependent on exogenous HA. The best results are obtained for synovial fluids mixed with cross-linked HA [17,18].

The superior tribological properties (low friction and minimal wear) of the articular cartilage under physiological conditions seem to be established by an interaction between the solid and fluid phase. However, a detailed cooperation between the cartilage structure and the synovial fluid has not been clarified yet. The superior tribological performance was attributed to many lubrication modes such as boundary lubrication [19], weeping lubrication [20], or micro-elastohydrodynamic lubrication [21]. Human synovial joints operate under variable loading and motions including rolling and sliding during various daily activities. Therefore, the lubrication of natural synovial joints is likely to be actualized not by a single lubrication mode but by a synergistic combination of them. In recent years, theories about adaptive multimode lubrication [22,23], biphasic lubrication [24], or hydration lubrication [25] have been published.

The importance of HA within these various lubrication regimes of the articular cartilage was already proved. HA solutions showed lower values of coefficient of friction compared to the simple solutions such as phosphate buffer saline (PBS) [26] or Ringer's solution [4]. Unlike the rheology,

the interaction between HA and other synovial fluid constituents plays an important role. A mixture of HA and phospholipids [27] leads to a lower friction compared to a simple phospholipid solution. Surface-anchored HA molecules complex synergistically with lipids present in the synovial fluid to form a boundary lubricating layer with very low friction ($\mu \approx 0.001$) [28]. Molecules of protein γ-globulin and HA have a different electric charge so their molecules attract each other and form complex structures, which contributes to the lower friction [29,30]. On the other hand, albumin and HA have the same electric charge and they repel each other. This interaction is not useful for the reduction of friction [29,30].

The HA molecular weight has a significant impact on the rheology of synovial fluid. Rheological studies have shown that HA with a higher molecular weight exhibits higher viscosity and better viscoelastic properties. HA also plays an important role in the reduction in friction in the osteoarthritic joint. Tribological studies showed a significant decrease in friction after the addition of HA to the tested lubricant and also the importance of HA in the formation of boundary lubricating layers on the cartilage surface. However, so far, no one has focused on how the HA molecular weight affects the friction of the articular cartilage. Therefore, this study is focused on the changes in cartilage friction caused by differences in HA molecular weight. The investigation is based on the combination of the detailed rheology description of HA-based solutions along with its impact on the frictional behavior of the articular cartilage. According to the author's best knowledge, such methodology, possibly bringing an important implication for clinical practice, has not been applied before. Viscosupplementation is a procedure which is commonly used to cure osteoarthritis in many human joints but results reported by patients show substantial differences. Therefore, it is important to examine how the main constituent of viscosupplement (i.e., HA) affects the friction of the articular cartilage.

2. Materials and Methods

The rheological properties of tested hyaluronan solutions were determined using a TA Instruments Discovery HR-3 rheometer (TA Instruments, New Castle, DE, USA, Figure 1a). Experiments were conducted using a stainless-steel cone and plate geometry (60 mm diameter cone with a 1° cone angle). The temperature was set to 37 °C during all experiments. In the steady shear test, the shear rates ranging from 0.01 to 5000 s^{-1} were applied to the tested fluids. Dependency of viscosity on shear rate (viscosity curves) was evaluated. The viscoelastic properties of the tested solutions with a higher viscosity were analyzed by performing a small-amplitude oscillatory shear test (SAOS) using a TA Instruments AR-G2 rheometer (TA Instruments, New Castle, DE, USA) Figure 1a) in a plate–plate configuration (20 mm diameter plate). The SAOS test measures the elastic and viscous modulus, when the tested material is subjected to sinusoidal strain. Frequency sweep measurements were conducted at 5% strain over a frequency range of 0.05–5 Hz. Frequency sweeps were performed at strain amplitude which was determined to be in the linear viscoelastic range. Temperature was set to 37 °C. Dynamic moduli were determined as a function of the angular frequency. All experiments were repeated three times with a fresh sample of HA. From these data, average values and standard deviations were counted.

In an effort to understand the effect of HA molecular weight on the rheology and friction of cartilage, all experiments were performed using simple HA solutions with different molecular weights. In total, four HA solutions with a concentration of 20 mg/mL and a molecular weight of 77 kDa, 640 kDa, 1060 kDa and 2010 kDa were tested. Solutions were prepared from HA powder (Contipro, Dolní Dobrouč, Czech Republic) by dissolution of the required amount of powder in PBS. The solution was stirred by a magnetic stirrer and heated to 60 °C for at least three hours to ensure the proper dissolution of HA.

The reciprocating sliding tests were conducted on a commercial tribometer Bruker UMT TriboLab (Bruker, Billerica, MA, USA) in a pin-on-plate configuration (Figure 1c). The coefficient of friction was investigated as a function of time for the sliding pair of the stationary glass plate made from the optical glass B270 and the moving porcine cartilage specimen. This specimen was loaded with

a constant load of 5 N. The sliding speed of 10 mm/s was selected and the reciprocating stroke was 20 mm. The contact was fully flooded with HA solution. To mimic the temperature of the human body, the lubricant was heated to 37 °C via heating cartridges in a steel chamber. Before each experiment, an unloaded cartilage sample was immersed in lubricant for 320 s to let the cartilage soak with lubricant. At the end of this preliminary phase, the cartilage was loaded and the friction test started immediately. After 300 s (75 cycles, sliding distance of 2740 mm), the sliding test was interrupted and the cartilage was unloaded for another 320 s. This unloaded phase is important for the rehydration of the cartilage specimen. Subsequently, the reciprocating test was restarted immediately after reloading and continued for another 300 s. The unloading phase was repeated twice so three tests under the same conditions were performed (Figure 1c). The friction and the normal force were continuously monitored through a biaxial force sensor connected to the pin holder. From these data, the coefficient of friction was calculated. Sliding tests were repeated four times under the same conditions with four different cartilage samples and fresh samples of tested lubricant. Between tests with different lubricants, cartilage samples were immersed in PBS.

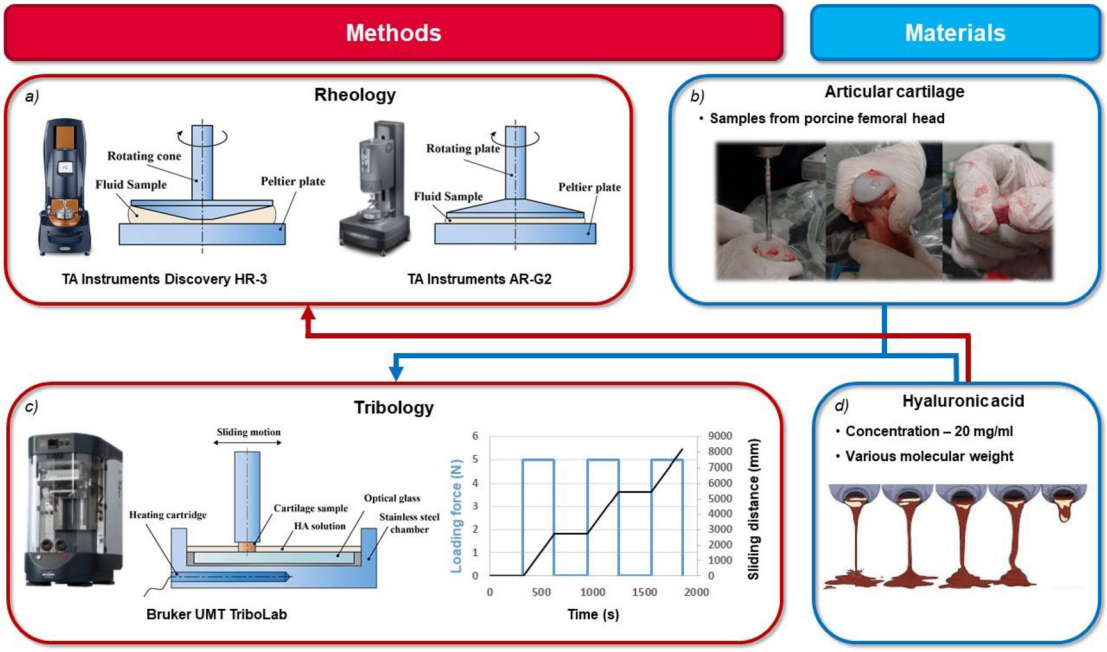

Figure 1. Scheme of research plan: (**a**) rheological measurements; (**b**) cartilage sample preparation; (**c**) frictional measurements; (**d**) tested solutions.

Intact cartilage specimens with underlying subchondral bone were prepared from porcine femoral heads (Figure 1b). Porcine femurs were obtained from the local slaughterhouse within a few hours of slaughter. Cylindrical cartilage specimens with diameters of 5.6 mm were extracted from the femoral heads using a hollow drill. Just one cartilage specimen from approximately the same area of the femoral head was extracted from each femur. After preparation, the specimen was stored in the freezer at −20 °C in a PBS solution for no more than 2 weeks. This procedure should slow down the biological degradation of the cartilage tissue. It has also been reported [31] that storing the articular cartilage under these condition does not change its mechanical properties. Half an hour before the experiment, the cartilage was removed from the freezer to thaw at room temperature.

3. Results and Discussion

3.1. Rheology of HA

Firstly, the viscosity of all the tested HA solutions was measured. The viscosity curves (a viscosity dependence on shear rate) of all four HA samples with different molecular weights are shown in Figure 2a. The results showed a strong dependency between the viscosity and molecular weight of HA. The highest viscosity was measured for HA with a molecular weight of 2010 kDa and the lowest for 77 kDa HA over the whole range of shear rate. The measured zero shear viscosity (viscosity for the lowest tested shear rate – 0.01 1/s) for all tested samples was (mean value ± standard deviation): 107.1 ± 1.7 Pa·s; 11.6 ± 0.4 Pa·s; 1.67 ± 0.05 Pa·s. The viscosity of 77 kDa HA was not measured at very low shear rates due to the limitations of the experimental methodology. Viscosity was measured on the rheometer in a cone–plate configuration; the measurement of such low viscosity fluid at very low shear rates in this configuration was not possible. The zero shear rate viscosity of this sample was measured at 0.1 1/s and the measured value is $0.013 \pm 3 \times 10^{-3}$ Pa·s. The zero shear rate viscosity of the synovial fluid from a healthy joint ranges from 1 to 175 Pa·s [32], while the zero shear rate viscosity of the synovial fluid obtained from osteoarthritic joint ranges from 0.01 to 11 Pa·s [15,17,32,33]. Based on the zero shear viscosities, tested HA solutions correspond to the osteoarthritic synovial fluid or to the low viscosity synovial fluid from a healthy joint even though the concentration of HA is much higher. Interestingly, studies with commercial viscosupplements also exhibit a large dispersion in results. The zero shear rate viscosity of commercial viscosupplements can vary between 0.5 and 190 Pa·s [34,35]. It can be assumed that these low viscosity HA solutions will not perform well in the recovery of the rheological properties of osteoarthritic synovial fluid after mixing with it. Resumption of the rheological properties of healthy synovial fluid after mixing osteoarthritic synovial fluid with viscosupplement is one of the main objectives of viscosupplementation.

HA exhibited the non-Newtonian shear thinning behavior (viscosity decrease with increasing shear rate). Molecules of HA are entangled and most resistant to flow at low shear rates. At high shear rates, molecules disentangle and align in the shear field. The strongest shear thinning behavior can be observed with 2010 kDa HA. HA with longer polymer chains allow for a greater number of entanglements and consequently for a higher value of zero shear viscosity and a stronger shear thinning behavior [18]. On the other hand, the shear thinning behavior for 77 kDa HA is relatively weak and can be observed in a very small range of shear rates. This HA sample exhibits Newtonian behavior most of the time. For example, the rate of shear thinning behavior can be analyzed by the value of $\frac{\eta_0}{\eta_x}$, which is the ratio of the zero shear rate viscosity and the viscosity at some defined values of shear rate [18,36]. Calculated values of shear thinning ratio $\frac{\eta_0}{\eta_{300}}$ for all four HA samples are stated in Table 1. Shear thinning ratios for synovial fluid from normal joints vary between 70 and 250 or between 5 and 40 for the synovial fluid aspirated from joints with osteoarthritis. For commercial viscosupplements, this ratio ranges between 2.3 and 651.2 [37]. Three out of four tested HA samples are consistent with results of osteoarthritic synovial joint or viscosupplements with low molecular weight HA again.

The second part of the rheological measurements was an analysis of the viscoelastic properties of the tested HA solutions. Figure 2b contains results of the frequency sweep measurements. The graph contains the storage and loss modulus dependency on the frequency of oscillating motion for three HA solutions. Viscoelastic properties of 77 kDa HA were not measurable in the plate–plate configuration. HA with molecular weights of 640 kDa and 1060 kDa exhibited a viscous-like behavior in the whole range of tested frequencies (i.e., the values of G" were always higher than the values of G'). Only the results of HA with the highest molecular weight exhibited a viscoelastic behavior, presenting a crossover point at 0.4 Hz. This point indicates a transition from the viscous to elastic behavior. The solution shows the viscous behavior at low frequencies, because the molecular chains can release stress by disentanglement and molecular rearrangement during the period of oscillation. However, at high frequencies, chains cannot disentangle during the short period of oscillating motion; therefore, the solution exhibits elastic behavior [38]. The crossover frequency is also important because it

determines to what extent the fluid absorbs or dissipates energy [18]. Balazs [39] reported nearly the same value of crossover point frequency for healthy synovial fluid obtained from the knees of individuals over the age of 52. The crossover point of 0.4 Hz means that, during normal daily activities, such as walking or running (frequency of 0.5 and 2.5–3 Hz [40]), the 2 010 kDa HA solution behaves like the elastic body. It can adsorb mechanical energy and thereby it could protect the articular cartilage against mechanical damage or wear. Values of G′ and G″ of all tested HA solutions at the frequencies of 0.5 Hz and 2.5 Hz are stated in Table 1. The results showed that the magnitudes of G′ and G″ increase with the molecular weight of HA and the potential crossover point moves to lower frequencies.

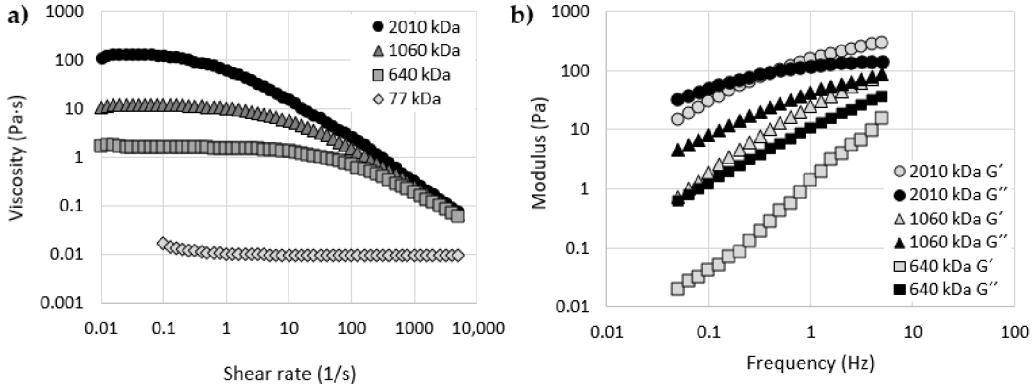

Figure 2. (**a**) Viscosity as a function of shear rate for HA solutions with different molecular weights; (**b**) Elastic (G′) and viscous (G″) moduli as a function of frequency for HA solutions with different molecular weights.

Table 1. Rheological properties of tested HA solutions.

MW (kDa)	Zero Shear Viscosity (Pa·s)	$\frac{\eta_0}{\eta_{300}}$	0.5 Hz		2.5 Hz		Crossover Frequency (Hz)
			G′ (Pa)	G″ (Pa)	G′ (Pa)	G″ (Pa)	
2010	107 ± 1.7	113.9	101 ± 3.5	92.3 ± 4	220 ± 9.5	125 ± 6.3	0.4
1060	11.6 ± 0.4	17.8	13.5 ± 1.5	29 ± 2.5	55.8 ± 5.6	67.5 ± 5.3	-
640	1.67 ± 0.05	4.1	0.4 ± 0.04	5.8 ± 0.03	5.4 ± 0.3	22.2 ± 0.2	-
77	$0.013 \pm 3 \times 10^{-3}$	1.3	-	-	-		-

3.2. Cartilage Friction Analysis

A distance-dependent frictional behavior for four cartilage samples lubricated by PBS is shown in Figure 3. The initial friction is very low, as is typical for the intact cartilage. The values of the coefficient of friction (CoF) are between 0.01 and 0.015. However, the CoF is gradually increasing with sliding distance. At the end of the first measurement substep (sliding distance of 2740 mm), the values of CoF have increased to 0.15–0.18. This behavior corresponds to the theory of biphasic lubrication by Ateshian et al. [3,24,41]. The friction is strongly influenced by load support from pressurized interstitial fluid (i.e., by exudation and rehydration of the cartilage porous structure during the loaded and unloaded phases of gait cycle). After the sliding distance of 2740 mm, the cartilage specimen was unloaded for 320 s. When the cartilage sample was reloaded and the reciprocating test restarted, friction was significantly decreased from the previous high level. This recovery of low friction was caused by the previously mentioned rehydration of cartilage, but the initial values of CoF are slightly higher than in the first measurement substep. This phenomenon could be caused by insufficient rehydration of the cartilage or by partial removal of the boundary lubricating layer from the cartilage surface. In the second and third measurement substeps, a very similar frictional behavior to the first substep was observed. However, the initial and final values of CoF were slightly higher compared to the previous substep.

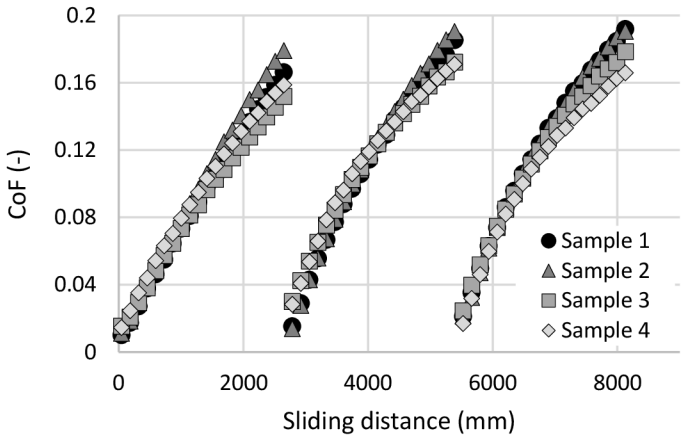

Figure 3. Coefficient of friction as a function of a sliding distance for four cartilage samples lubricated by phosphate buffer saline (PBS).

After this initial set of experiments, all four cartilage samples were tested with all four HA solutions as lubricants. The results of these experiments are stated in Figure 4. Each graph contains data measured with one cartilage specimen and four HA solutions with different molecular weights. The results showed a significant decrease in friction compared to the pure base solution (i.e., PBS). Similar trends were published in studies [4,30,42]. The most significant decrease can be observed with sample 4 (Figure 4d). At the end of the frictional tests, the values of CoF measured with HA with different molecular weights varied between 0.009 and 0.03 compared to the value of 0.16 measured with pure PBS. On the other hand, sample 2 (Figure 4b) exhibited the highest values of CoF. At the end of the frictional tests, values of CoF varied between 0.05 and 0.9 depending on the molecular weight of HA. The highest friction for sample 2 was measured with 77 kDa HA and the lowest friction with 1060 kDa HA. Nevertheless, the friction measured with sample 2 and 1060 kDa HA was still higher than any results of sample 4.

Overall, HA solutions showed a larger scatter of data compared with the rheological measurements, and no clear dependence between the molecular weight (viscosity) of HA and the friction in the cartilage model can be observed. The study by Kwiecinski et al. [43] reported an approximately linear dependency between the HA molecular weight and the coefficient of friction in cartilage-on-cartilage contact. Higher molecular weight of HA leads to lower values of CoF. This trend can be partially observed with sample 1 (Figure 4a) and 3 (Figure 4c).

Figure 5 contains the same data as Figure 4 but each graph contains data of measurements with all four cartilage samples and one HA solution. The data showed that the HA solution interacts differently with each cartilage sample. Overall, the results showed relatively significant friction differences between cartilage samples. These differences could be affected by differences in the geometry, structure and mechanical properties of cartilage samples. Each cartilage sample was extracted from one porcine femoral head from approximately the same area. However, studies by Appleayrd et al. [44] or by Kiviranta et al. [45] showed a different content of collagen fibrils and proteoglycans across the tibia plateau, patellae, etc. These differences affect the mechanical properties of the cartilage, such as Young's modulus or Shear modulus. Moreover, Richard et al. [46] reported differences in Young's modulus and Poisson ratio between the healthy cartilages from six patients with femoral neck fracture only. Samples from four different cartilage areas were tested on each femoral head. These differences in the mechanical properties of the cartilage sample, resp. the location of sample extraction, also affect the friction of the cartilage [47,48]. Some differences in the geometry of samples can also be caused by the methodology of their extraction.

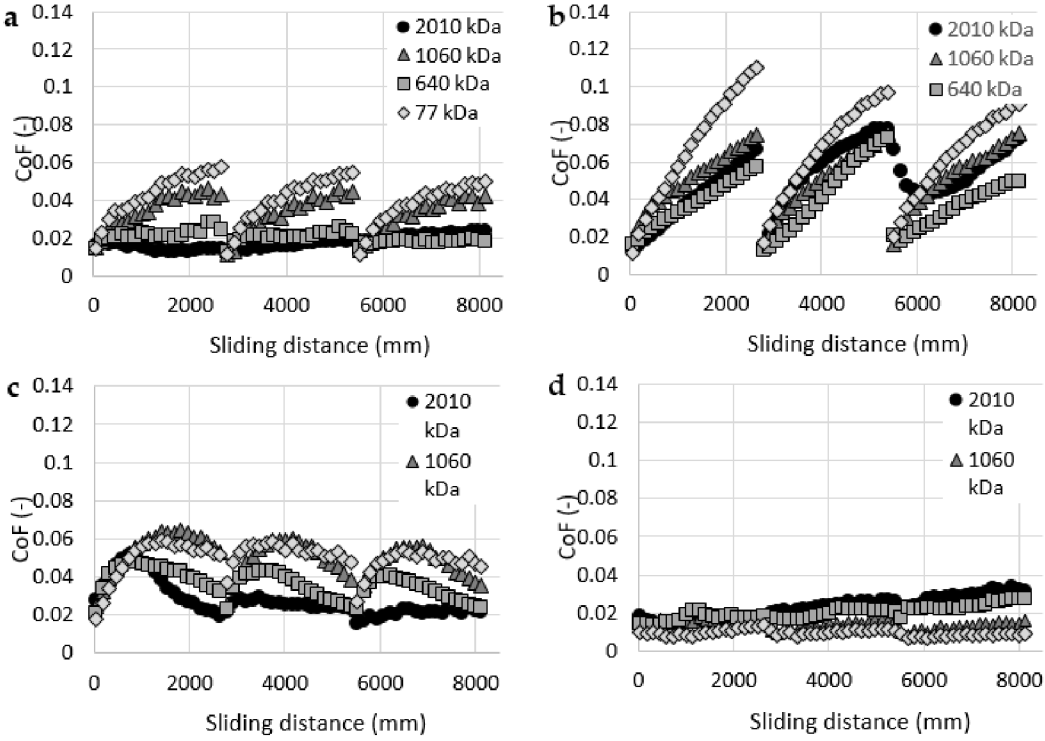

Figure 4. Coefficient of friction as a function of a sliding distance for hyaluronic acid (HA) solutions with a molecular weight of: (**a**) cartilage sample 1, (**b**) cartilage sample 2, (**c**) cartilage sample 3, (**d**) cartilage sample 4.

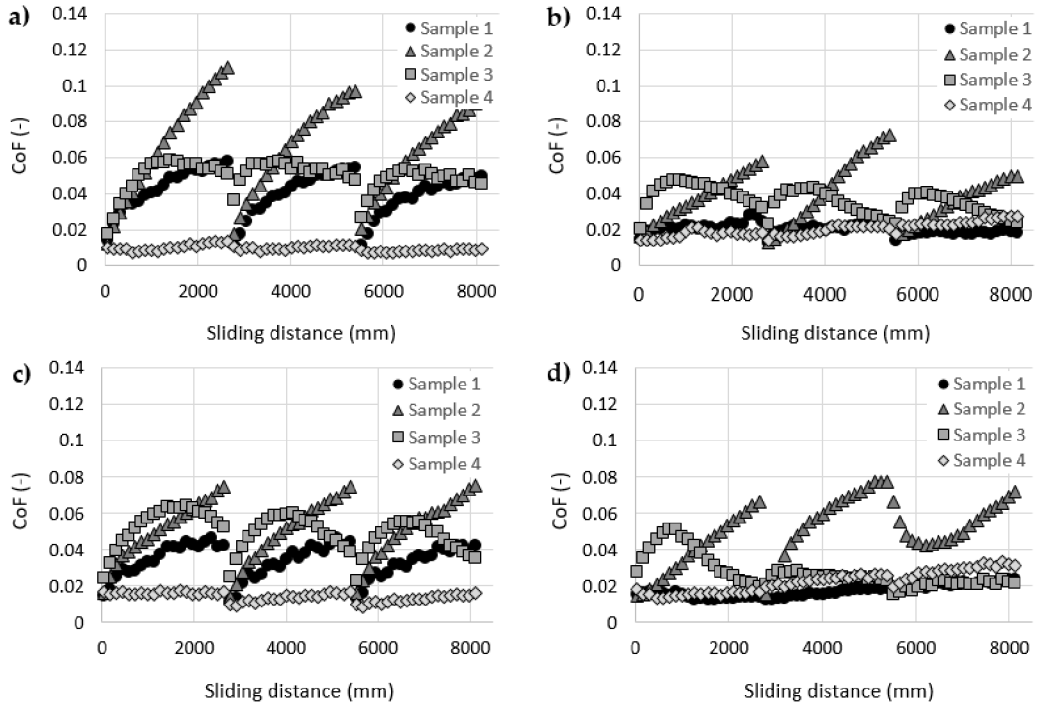

Figure 5. Coefficient of friction as a function of a sliding distance for four cartilage samples and HA solutions with a molecular weight of: (**a**) 77 kDa, (**b**) 640 kDa, (**c**) 1060 kDa, (**d**) 2010 kDa.

An important role may also be played by possible interactions between HA and the residues of synovial fluid on the surface of the cartilage. All samples were bathed in PBS prior to the frictional

measurements. Solutions, such as sodium dodecyl sulphate or alcohol, are commonly used prior to the biotribological experiments to clean the surfaces of the proteins, phospholipids, etc. [49–51]. However, these solutions could possibly damage the structure of the cartilage. Reactions between HA and proteins can be either synergistic or unbeneficial for cartilage friction [29,30,42]. However, the reactions between HA and phospholipids seem to be crucial for the effectiveness of HA in lowering friction [4,27,52]. The theory by Klein et al. [28,53–55] assumes that HA may complex with lipids such as Dipalmitoylphosphatidylcholine (DPPC), that are present in the articular cartilage and in the surrounding synovial fluid, to provide a robust boundary layer with extremely low friction.

Different results of every cartilage specimen should also be related to the inconsistent results of viscosupplementation in clinical practice. Some studies report positive effects of viscosupplementation. Maheu et al. [56] report an improvement in the pain or function of osteoarthritic joints up to 40 months after viscosupplementation. Tikiz et al. [57] reported a significant reduction in VAS (Visual Analogue Scale) and WOMAC (Western Ontario and McMaster Universities Osteoarthritis Index) indexes for a period of 6 months in patients with hip osteoarthritis. Nevertheless, authors did not find any significant differences between viscosupplements with high and low molecular weights. On the other hand, many studies did not find differences between HA and anti-inflammatory drugs [58] or placebo [59]. Some of them even report an increased risk of serious adverse event after therapy [60,61]. Ambiguity of results leads to the non-uniform recommendations of international medical associations. For example, the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) recommends viscosupplementation for the advanced pharmacological management of knee osteoarthritis in patients who remain symptomatic despite the use of non-steroidal anti-inflammatory drugs [62]. On the other hand, Osteoarthritis Research Society International (OARSI) considers viscosupplementation as uncertain but possible for the treatment of knee osteoarthritis [63].

Experiments with the articular cartilage showed a large dispersion in results. The main reasons are likely to be the differences in structure and shape between the individual cartilage specimens. Therefore, for our future studies on viscosupplementation, a cartilage substitution is one of the possibilities to improve the repeatability of the measurements. The substitutional material should be more homogenous in its mechanical properties but it should still have similar mechanical properties, be porous and exhibit low values of CoF. One option may be the use of hydrogels based on polyvinyl alcohol (PVA). These materials are, among others, developed and tested in the long term as a suitable material for the replacement of damaged osteoarthritic cartilage [64–68]. Figure 6 contains the results of the initial experiments with PVA hydrogel as a cartilage replacement. The test rig, experimental conditions and tested lubricants were the same as before. Samples from freeze-thawing PVA hydrogel were made according to the study by Yarimitsu et al. [29]. The hydrogel sample was replaced after each experiment. Results showed a decrease in CoF during the initial run-in phase. After this, most of the HA solutions exhibited constant friction. Values of CoF were even lower than during the experiments with cartilage samples. Continuously, even in this case, no direct dependence between the HA molecular weight and CoF can be seen.

For a detailed study on the effect of viscosupplementation on the friction of the articular cartilage, more complex lubricants should be tested. Interaction between HA and other constituents plays an important role in the lubrication of the articular cartilage [27–30]. Therefore, lubricants containing other synovial fluid constituents (proteins, phospholipids, etc.) should be tested. More reliable results may also be obtained from measurements with commercial viscosupplements rather than from experiments with pure HA solutions. The methodology of frictional experiments also has some shortcomings. Constant speed and load during sliding motion do not correspond to the conditions in real human joints. The cartilage-on-cartilage configuration is likely to exhibit lower values of CoF which will be closer to the real joints. However, important findings will allow the in-situ observation of the cartilage-on-glass contact by optical methods, which assumes the transparent material of one of the surfaces. Fluorescent microscopy should be suitable for this application. It allows the study of the behavior of the individual components of complex lubricants and is already used for lubrication analysis of joint

replacements in the author's laboratory [49,69]. The pin-on-plate tribometer for the in-situ observation of cartilage-on-glass contact by fluorescent microscopy is currently under development [70].

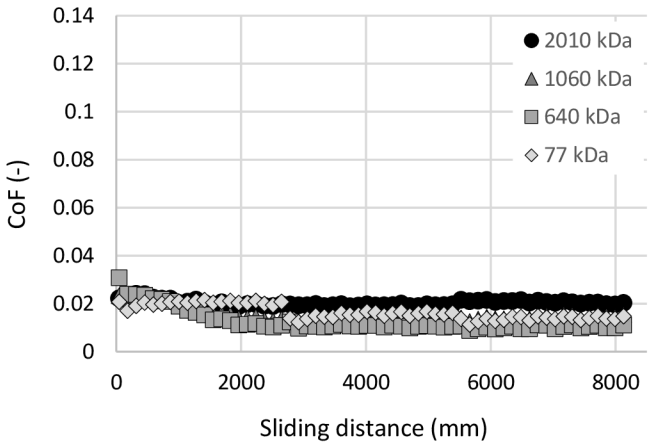

Figure 6. Coefficient of friction as a function of sliding distance for polyvinyl alcohol (PVA) hydrogel-on-glass configuration and HA solutions with different molecular weights.

4. Conclusions

The present paper analyzed the rheological properties of HA solutions with different molecular weights and also the frictional behavior of these solutions in the cartilage-on-glass contact during reciprocating sliding tests. Rotational rheometers were employed in order to analyze the viscosity and viscoelastic properties of HA solutions with molecular weights varying between 77 kDa and 2010 kDa. The pin-on-plate tribometer was later employed to analyze the CoF dependency on time in the cartilage-on-glass contact lubricated by these HA solutions. The main conclusions which emerged from the measured data are summarized in the following points:

- Rheological measurements showed a strong dependency between the molecular weight and the viscosity or viscoelastic properties of HA solutions. HA solutions with higher molecular weights exhibited higher viscosity, dynamic moduli and shear thinning ratio.
- The crossover point was measured only for one of the tested HA samples. Based on the obtained data, it can be assumed that a higher molecular weight of HA leads to lower values of crossover frequency.
- CoF measurements showed a substantial dispersion in the results, showing no clear dependency between the HA molecular weight and the friction in the cartilage-on-glass contact.
- Mechanical properties and overall conditions of individual cartilage samples can significantly affect the effectiveness of HA solutions during the reciprocating sliding motion. In most cases, each cartilage sample exhibited the highest and the lowest values of CoF during measurements with different HA solution.
- Unclear results may support the contradictory conclusions of medical studies whose results are strongly dependent on the individual patient's conditions. The cartilage condition and composition of synovial fluid can significantly affect the effectiveness of viscosupplementation.
- Different results of rheological and frictional measurements might also show the insufficiency of rheological measurements in the assessment of viscosupplements effectiveness.

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References

1. Lees, D.; Partington, P. Articular cartilage. *Orthop. Trauma* **2016**, *30*, 265–272. [CrossRef]
2. Goldring, M.B. Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis. *Ther. Adv. Musculoskelet. Dis.* **2012**, *4*, 269–285. [CrossRef] [PubMed]
3. Ateshian, G.A. The role of interstitial fluid pressurization in articular cartilage lubrication. *J. Biomech.* **2009**, *42*, 1163–1176. [CrossRef] [PubMed]
4. Forsey, R.; Fisher, J.; Thompson, J.; Stone, M.; Bell, C.; Ingham, E. The effect of hyaluronic acid and phospholipid-based lubricants on friction within a human cartilage damage model. *Biomaterials* **2006**, *27*, 4581–4590. [CrossRef] [PubMed]
5. Galandáková, A.; Ulrichová, J.; Langová, K.; Hanáková, A.; Vrbka, M.; Hartl, M.; Gallo, J. Characteristics of synovial fluid required for optimization of lubrication fluid for biotribological experiments. *J. Biomed. Mater. Res. B Appl. Biomater.* **2017**, *105*, 1422–1431. [CrossRef]
6. Balazs, E.A.; Denlinger, J.L. Viscosupplementation: A new concept in the treatment of osteoarthritis. *J. Rheumatol. Suppl.* **1993**, *39*, 3–9.
7. Ghosh, P.; Guidolin, D. Potential mechanism of action of intra-articular hyaluronan therapy in osteoarthritis: Are the effects molecular weight dependent? *Semin. Arthritis Rheum.* **2002**, *32*, 10–37. [CrossRef]
8. Altman, R.D.; Manjoo, A.; Fierlinger, A.; Niazi, F.; Nicholls, M. The mechanism of action for hyaluronic acid treatment in the osteoarthritic knee: A systematic review. *BMC Musculoskelet. Disord.* **2015**, *16*, 321. [CrossRef]
9. Watterson, J.R.; Esdaile, J.M. Viscosupplementation: Therapeutic Mechanisms and clinical potential in osteoarthritis of the knee. *J. Am. Acad. Orthop. Surg.* **2000**, *8*, 277–284. [CrossRef]
10. Mazzucco, D.; Scott, R.; Spector, M. Composition of joint fluid in patients undergoing total knee replacement and revision arthroplasty: Correlation with flow properties. *Biomaterials* **2004**, *25*, 4433–4445. [CrossRef]
11. Ghosh, P. The role of hyaluronic acid (hyaluronan) in health and disease: Interactions with cells, cartilage and components of synovial fluid. *Clin. Exp. Rheumatol.* **1994**, *12*, 75–82. [PubMed]
12. Zhang, Z.; Barman, S.; Christopher, G.F. The role of protein content on the steady and oscillatory shear rheology of model synovial fluids. *Soft Matter* **2014**, *10*, 5965–5973. [CrossRef] [PubMed]
13. Balazs, E.A. Viscoelastic properties of hyaluronan and its therapeutic use. In *Chemistry and Biology of Hyaluronan*, 1st ed.; Garg, G.G., Hales, C.A., Eds.; Elsevier Ltd.: Amsterdam, The Netherlands, 2004; Volume 1, pp. 415–455.
14. Falcone, S.J.; Palmeri, D.M.; Berg, R.A. Rheological and cohesive properties of hyaluronic acid. *J. Biomed. Mater. Res. A* **2006**, *76A*, 721–728. [CrossRef]
15. Bhuanantanondh, P.; Grecov, D.; Kwok, E. Rheological Study of viscosupplements and synovial fluid in patients with osteoarthritis. *J. Med. Biol. Eng.* **2010**, *32*, 12–16. [CrossRef]
16. Altman, R.D. Status of hyaluronan supplementation therapy in osteoarthritis. *Curr. Rheumatol. Rep.* **2003**, *5*, 7–14. [CrossRef]
17. Mathieu, P.; Conrozier, T.; Vignon, E.; Rozand, Y.; Rinaudo, M. Rheologic behavior of osteoarthritic synovial fluid after addition of hyaluronic acid: A pilot study. *Clin. Orthop. Relat. Res.* **2009**, *467*, 3002–3009. [CrossRef]
18. Bhuanantanondh, P.; Grecov, D.; Kwok, E.; Guy, P. Rheology of osteoarthritic synovial fluid mixed with viscosupplements: A pilot study. *Biomed. Eng. Lett.* **2011**, *1*, 213–219. [CrossRef]
19. Dowson, D. Modes of lubrication in human joints. *Proc. Inst. Mech. Eng.* **1996**, *181*, 45–54.
20. McCutchen, C.W. The frictional properties of animal joints. *Wear* **1962**, *5*, 1–17. [CrossRef]
21. Dowson, D.; Jin, Z.-M. Micro-elastohydrodynamic lubrication of synovial joints. *Eng. Med.* **1986**, *15*, 63–65. [CrossRef]
22. Murakami, T. The lubrication in natural synovial joints and joint prostheses. *JSME Int. J. Ser. III Vib. Control Eng. Eng. Ind.* **1990**, *33*, 465–474. [CrossRef]
23. Murakami, T.; Higaki, H.; Sawae, Y.; Ohtsuki, N.; Moriyama, S.; Nakanishi, Y. Adaptive multimode lubrication in natural synovial joints and artificial joints. *Proc. Inst. Mech. Eng. H* **2006**, *212*, 23–35. [CrossRef]

24. Ateshian, G.A. A theoretical formulation for boundary friction in articular cartilage. *J. Biomech. Eng.* **1997**, *119*, 81–86. [CrossRef]
25. Ikeuchi, K. Origin and future of hydration lubrication. *Proc. Inst. Mech. Eng. J.* **2007**, *221*, 301–305. [CrossRef]
26. Bell, C.J.; Ingham, E.; Fisher, J. Influence of hyaluronic acid on the time-dependent friction response of articular cartilage under different conditions. *Proc. Inst. Mech. Eng. H* **2006**, *220*, 23–31. [CrossRef]
27. Murakami, T.; Yarimitsu, S.; Nakashima, K.; Sawae, Y.; Sakai, N. Influence of synovia constituents on tribological behaviors of articular cartilage. *Friction* **2013**, *1*, 150–162. [CrossRef]
28. Seror, J.; Zhu, L.; Goldberg, R.; Day, A.J.; Klein, J. Supramolecular synergy in the boundary lubrication of synovial joints. *Nat. Commun.* **2015**, *6*, 6497. [CrossRef]
29. Yarimitsu, S.; Sasaki, S.; Murakami, T.; Suzuki, A. Evaluation of lubrication properties of hydrogel artificial cartilage materials for joint prosthesis. *Biosurf. Biotribol.* **2016**, *2*, 40–47. [CrossRef]
30. Murakami, T.; Nakashima, K.; Yarimitsu, S.; Sawae, Y.; Sakai, N. Effectiveness of adsorbed film and gel layer in hydration lubrication as adaptive multimode lubrication mechanism for articular cartilage. *Proc. Inst. Mech. Eng. J.* **2011**, *225*, 1174–1185. [CrossRef]
31. Szarko, M.; Muldrew, K.; Bertram, J.E.A. Freeze-thaw treatment effects on the dynamic mechanical properties of articular cartilage. *BMC Musculoskelet. Disord.* **2010**, *11*, 281. [CrossRef]
32. Fam, H.; Bryant, J.T.; Kontopoulou, M. Rheological properties of synovial fluids. *Biorheology* **2007**, *44*, 59–74.
33. Mazzucco, D.; McKinley, G.; Scott, R.D.; Spector, M. Rheology of joint fluid in total knee arthroplasty patients. *J. Orthop. Res.* **2002**, *20*, 1157–1163. [CrossRef]
34. Lapasin, R. Rheological Studies Dedicated to the Development of a Novel Injectable Polymeric Blend for Viscosupplementation Treatment. *Chem. Biochem. Eng. Q.* **2016**, *29*, 511–518. [CrossRef]
35. Bonnevie, E.D.; Galesso, D.; Secchieri, C.; Bonassar, L.J.; Awad, H.A. Frictional characterization of injectable hyaluronic acids is more predictive of clinical outcomes than traditional rheological or viscoelastic characterization. *PLoS ONE* **2019**, *14*, e0216702. [CrossRef]
36. Rainer, F.; Ribitsch, V. Viscoelastic properties of normal human synovia and their relation to biomechanics. *Zeitschrift fur Rheumatologie* **1985**, *44*, 114–119.
37. Nicholls, M.; Manjoo, A.; Shaw, P.; Niazi, F.; Rosen, J. A Comparison Between Rheological Properties of Intra-articular Hyaluronic Acid Preparations and Reported Human Synovial Fluid. *Adv. Ther.* **2018**, *35*, 523–530. [CrossRef]
38. Borzacchiello, A.; Mayol, L.; Schiavinato, A.; Ambrosio, L. Effect of hyaluronic acid amide derivative on equine synovial fluid viscoelasticity. *J. Biomed. Mater. Res. A* **2009**, *92A*, 1162–1170. [CrossRef]
39. Balazs, E.A. The physical properties of synovial fluid and the special role of hyaluronic acid. In *Disorders of the Knee*, 1st ed.; Helfet, A.J., Ed.; JB Lippincott & Co.: Philadelphia, PA, USA, 1974; Volume 2, pp. 63–75.
40. Finelli, I.; Chiessi, E.; Galesso, D.; Renier, D.; Paradossi, G. A new viscosupplement based on partially hydrophobic hyaluronic acid: A comparative study. *Biorheology* **2011**, *48*, 263–275. [CrossRef]
41. Krishnan, R.; Kopacz, M.; Ateshian, G.A. Experimental verification of the role of interstitial fluid pressurization in cartilage lubrication. *J. Orthop. Res.* **2004**, *22*, 565–570. [CrossRef]
42. Bonnevie, E.D.; Galesso, D.; Secchieri, C.; Cohen, I.; Bonassar, L.J.; Awad, H.A. Elastoviscous transitions of articular cartilage reveal a mechanism of synergy between lubricin and hyaluronic acid. *PLoS ONE* **2015**, *10*, e0143415. [CrossRef]
43. Kwiecinski, J.J.; Dorosz, S.G.; Ludwig, T.E.; Abubacker, S.; Cowman, M.K.; Schmidt, T.A. The effect of molecular weight on hyaluronan's cartilage boundary lubricating ability—Alone and in combination with proteoglycan 4. *Osteoarthr. Cartil.* **2011**, *19*, 1356–1362. [CrossRef]
44. Appleyard, R.C.; Burkhardt, D.; Ghosh, P.; Read, R.; Cake, M.; Swain, M.V.; Murrell, G.A.C. Topographical analysis of the structural, biochemical and dynamic biomechanical properties of cartilage in an ovine model of osteoarthritis. *Osteoarthr. Cartil.* **2003**, *11*, 65–77. [CrossRef]
45. Kiviranta, P.; Lammentausta, E.; Töyräs, J.; Kiviranta, I.; Jurvelin, J.S. Indentation diagnostics of cartilage degeneration. *Osteoarthr. Cartil.* **2008**, *16*, 796–804. [CrossRef]
46. Richard, F.; Villars, M.; Thibaud, S. Viscoelastic modeling and quantitative experimental characterization of normal and osteoarthritic human articular cartilage using indentation. *J. Mech. Behav. Biomed. Mater.* **2013**, *24*, 41–52. [CrossRef]

47. DuRaine, G.; Neu, C.P.; Chan, S.M.T.; Komvopoulos, K.; June, R.K.; Reddi, A.H. Regulation of the friction coefficient of articular cartilage by TGF-β1 and IL-1β. *J. Orthop. Res.* **2009**, *27*, 249–256. [CrossRef]
48. Chan, S.M.T.; Neu, C.P.; Komvopoulos, K.; Reddi, A.H. The role of lubricant entrapment at biological interfaces: Reduction of friction and adhesion in articular cartilage. *J. Biomech.* **2011**, *44*, 2015–2020. [CrossRef] [PubMed]
49. Nečas, D.; Vrbka, M.; Urban, F.; Křupka, I.; Hartl, M. The effect of lubricant constituents on lubrication mechanisms in hip joint replacements. *J. Mech. Behav. Biomed. Mater.* **2016**, *55*, 295–307. [CrossRef]
50. Myant, C.W.; Cann, P. The effect of transient conditions on synovial fluid protein aggregation lubrication. *J. Mech. Behav. Biomed. Mater.* **2014**, *34*, 349–357. [CrossRef] [PubMed]
51. Ma, L.; Rainforth, W.M. The effect of lubrication on the friction and wear of Biolox®delta. *Acta Biomater.* **2012**, *8*, 2348–2359. [CrossRef] [PubMed]
52. Schmidt, T.A.; Gastelum, N.S.; Nguyen, Q.T.; Schumacher, B.L.; Sah, R.L. Boundary lubrication of articular cartilage: Role of synovial fluid constituents. *Arthritis Rheum.* **2007**, *56*, 882–891. [CrossRef] [PubMed]
53. Seror, J.; Sorkin, R.; Klein, J. Boundary lubrication by macromolecular layers and its relevance to synovial joints. *Polym. Adv. Technol.* **2014**, *25*, 468–477. [CrossRef]
54. Klein, J. Hydration lubrication. *Friction* **2013**, *1*, 1–23. [CrossRef]
55. Jahn, S.; Klein, J. Lubrication of articular cartilage. *Physics Today* **2018**, *71*, 48–54. [CrossRef]
56. Maheu, E.; Rannou, F.; Reginster, J.-Y. Efficacy and safety of hyaluronic acid in the management of osteoarthritis: Evidence from real-life setting trials and surveys. *Semin. Arthritis Rheum.* **2016**, *45*, S28–S33. [CrossRef]
57. Tıkız, C.; Ünlü, Z.; Şener, A.; Efe, M.; Tüzün, Ç. Comparison of the efficacy of lower and higher molecular weight viscosupplementation in the treatment of hip osteoarthritis. *Clin. Rheum.* **2005**, *24*, 244–250. [CrossRef]
58. Bannuru, R.R.; Vaysbrot, E.E.; Sullivan, M.C.; McAlindon, T.E. Relative efficacy of hyaluronic acid in comparison with NSAIDs for knee osteoarthritis: A systematic review and meta-analysis. *Semin. Arthritis Rheum.* **2014**, *43*, 593–599. [CrossRef]
59. Jevsevar, D.; Donnelly, P.; Brown, G.A.; Cummins, D.S. Viscosupplementation for osteoarthritis of the knee. *J. Bone Jt. Surg. Am.* **2015**, *97*, 2047–2060. [CrossRef]
60. Rutjes, A.W.S.; Jüni, P.; da Costa, B.R.; Trelle, S.; Nüesch, E.; Reichenbach, S. Viscosupplementation for osteoarthritis of the knee. *Ann. Intern. Med.* **2012**, *157*, 180–191. [CrossRef]
61. Arrich, J. Intra-articular hyaluronic acid for the treatment of osteoarthritis of the knee: Systematic review and meta-analysis. *Can. Med. Assoc. J.* **2005**, *172*, 1039–1043. [CrossRef]
62. Bruyère, O.; Cooper, C.; Pelletier, J.-P.; Branco, J.; Luisa Brandi, M.; Guillemin, F.; Hochberg, M.C.; Kanis, J.A.; Kvien, T.K.; Martel-Pelletier, J.; et al. An algorithm recommendation for the management of knee osteoarthritis in Europe and internationally: A report from a task force of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). *Semin. Arthritis Rheum.* **2014**, *44*, 253–263. [CrossRef]
63. McAlindon, T.E.; Bannuru, R.R.; Sullivan, M.C.; Arden, N.K.; Berenbaum, F.; Bierma-Zeinstra, S.M.; Hawker, G.A.; Henrotin, Y.; Hunter, D.J.; Kawaguchi, H.; et al. OARSI guidelines for the non-surgical management of knee osteoarthritis. *Osteoarthr. Cartil.* **2014**, *22*, 363–388. [CrossRef] [PubMed]
64. Pan, Y.-S.; Xiong, D.-S.; Ma, R.-Y. A study on the friction properties of poly (vinyl alcohol) hydrogel as articular cartilage against titanium alloy. *Wear* **2007**, *262*, 1021–1025. [CrossRef]
65. Katta, J.K.; Marcolongo, M.; Lowman, A.; Mansmann, K.A. Friction and wear behavior of poly (vinyl alcohol)/poly (vinyl pyrrolidone) hydrogels for articular cartilage replacement. *J. Biomed. Mater. Res. A* **2007**, *83A*, 471–479. [CrossRef]
66. Murakami, T.; Sakai, N.; Yamaguchi, T.; Yarimitsu, S.; Nakashima, K.; Sawae, Y.; Suzuki, A. Evaluation of a superior lubrication mechanism with biphasic hydrogels for artificial cartilage. *Tribol. Int.* **2015**, *89*, 19–26. [CrossRef]
67. Murakami, T.; Yarimitsu, S.; Nakashima, K.; Yamaguchi, T.; Sawae, Y.; Sakai, N.; Suzuki, A. Superior lubricity in articular cartilage and artificial hydrogel cartilage. *Proc. Inst. Mech. Eng. J.* **2014**, *228*, 1099–1111. [CrossRef]
68. Murakami, T.; Yarimitsu, S.; Sakai, N.; Nakashima, K.; Yamaguchi, T.; Sawae, Y.; Suzuki, A. Superior lubrication mechanism in poly (vinyl alcohol) hybrid gel as artificial cartilage. *Proc. Inst. Mech. Eng. J.* **2017**, *231*, 1160–1170. [CrossRef]

69. Nečas, D.; Vrbka, M.; Galandáková, A.; Křupka, I.; Hartl, M. On the observation of lubrication mechanisms within hip joint replacements. Part I: Hard-on-soft bearing pairs. *J. Mech. Behav. Biomed. Mater.* **2019**, *89*, 237–248.
70. Čípek, P.; Rebenda, D.; Nečas, D.; Vrbka, M.; Křupka, I.; Hartl, M. Visualization of lubrication film in model of synovial joint. *Tribol. Ind.* **2019**, *41*, 387–393. [CrossRef]

Rheological and frictional analysis of viscosupplements towards improved lubrication of human joints

David REBENDA [1,], Martin VRBKA [1], David NEČAS [1], Evgeniy TOROPITSYN [2,3], Seido YARIMITSU [4], Pavel ČÍPEK [1], Martin PRAVDA [2], Martin HARTL [1]*

[1] Faculty of Mechanical Engineering, Brno University of Technology, Technická 2896/2, 616 69 Brno, Czech Republic

[2] Contipro a.s., Dolní Dobrouč 401, 561 02 Dolní Dobrouč, Czech Republic

[3] Biocev, First Faculty of Medicine, Charles University, Průmyslová 595, 252 50 Vestec, Czech Republic

[4] Faculty of System Design, Tokyo Metropolitan University, 1-1, Minami-osawa, Hachioji, Tokyo 192-0364, Japan

[*] Corresponding author

E-mail address: David.Rebenda@vut.cz (David Rebenda)

Telephone number: +420 541 143 221

Address: Faculty of Mechanical Engineering

Brno University of Technology

Technická 2896/2

616 69 Brno

Czech Republic

Abstract

The present paper explores the effect of viscosupplementation on the friction of articular cartilage depending on the rheology of viscosupplements. The experiments were realized on rotational rheometers and a tribometer in pin-on-plate configuration. Five commercially available viscosupplements and their mixtures with synovial fluid were tested. The results showed differences by the order of magnitudes between viscosupplements viscosity and no viscoelastic properties in some of them. The friction was substantially affected by the addition of viscosupplement into the model synovial fluid. In most cases, mixtures of synovial fluid and viscosupplement even showed similar friction as clear viscosupplements. This study is the basis for a better understanding of the short-term changes in articular cartilage frictional behavior after the viscosupplementation of synovial joint.

Keywords

articular cartilage, rheology, friction, viscosupplementation

1. Introduction

A natural articular cartilage is a biphasic material composed of an extracellular matrix of collagen fibers, proteoglycans and chondrocytes [1]. The interstitial fluid is composed of water which makes up to 70 – 80 % of the cartilage structure. Thanks to its unique structure and synergy between the solid and fluid phase, the cartilage plays an important role in the lubrication mechanisms of heavily loaded synovial joints, such as hip or knee [2]. Under physiological conditions, the cartilage-on-cartilage contact is characterized by extremely low friction and excellent wear resistance[3]. However, there are still many ambiguities about the lubrication mechanism of the articular cartilage. It seems that various mechanisms of fluid film or boundary lubrication may occur in the synovial joint according to the kinematic and loading conditions [4, 5, 6]. In general, the cartilage lubrication mechanism is called adaptive multimode lubrication [5, 7, 8]. Under certain circumstances, boundary lubrication [9, 10], weeping lubrication [3], biphasic lubrication [11, 12], hydration lubrication [13, 14] and others may occur.

During biphasic lubrication, exudation of interstitial fluid from cartilage is expected to contribute to the fluid film formation [15]. Therefore, time-dependent frictional behavior is expected under compressive loading while the interstitial fluid pressurization is controlled by permeability and pressure. The articular cartilage has low permeability; thus it can considerably contribute to load support by fluid pressurization [16]. Ateshian et al. [12] assumed that the time-dependent frictional behavior depends on the rate of interstitial fluid exudation from the cartilage extracellular matrix and rehydration of cartilage. Low friction could be sustained for a long time if the fluid load support is preserved at sufficient levels. Therefore, migrating contact area [17], rehydration by cartilage unloading [18] or hydrodynamic effect [19] are crucial for the proper function of the cartilage.

Under severe conditions, a direct contact between cartilage surfaces in a synovial joint may occur. In these conditions, adsorbed film formation on the cartilage surface is an essential prerequisite for cartilage low friction and minimal wear maintenance. Interaction between the cartilage complex structure and the interaction with individual constituents of an adsorbed film should also be considered. It has already been reported that the main constituents of adsorbed

film on cartilage surfaces are proteins [9, 10, 20], glycoproteins [21, 22], hyaluronic acid (HA) [20, 23, 24] and phospholipids [9, 24, 25]. However, a detailed interaction and synergy between individual constituents of synovial fluid (SF) and the cartilage structure is still a subject of many scientific studies.

The articular cartilage is considered as a natural high-water content hydrogel. Reproduction of its structure and tribological performance using artificial materials could lead to the improvement of artificial joints lifetime. Polyvinyl alcohol (PVA) hydrogel seems to be a suitable candidate for artificial articular cartilage due to great biocompatibility and mechanical strength [26, 27, 28]. The main drawback of hydrogels that prevents their implantation into a human joint is the excessive wear under loading conditions of heavily loaded joints [29, 30]. Murakami et al. [31] reported that the adaptive multimode lubrication can be adapted for three types of PVA hydrogels – freeze-thawing (FT), cast-drying (CD) and hybrid, whereas CD on FT hybrid gel had the best frictional results. Li et al. [32] also reported a biphasic lubrication behavior in cartilage-on-FT hydrogel contact with a reduction of friction after the addition of HA into a basic solution. In another study by Murakami et al. [26], CD hydrogel-on-glass exhibited even lower friction than the cartilage-on-glass contact. Nakashima et al. [30] analyzed the PVA hydrogel-on-glass contact by fluorescent microscopy. The appropriate concentration ratio of albumin and γ-globulin mixed with HA remarkably reduced wear which indicates that the adsorbed boundary lubricating film with optimum structure plays an important role in the long-time durability of hydrogels as with natural articular cartilage.

Due to the adaptive multimode lubrication, the cartilage can maintain low friction and wear under physiological conditions for a long period of time. These conditions can significantly change during joint diseases, such as osteoarthritis or rheumatoid arthritis. Osteoarthritis is one of the most common diseases of locomotor system and a major pathology among the elderly in western societies. In these days, about 70 % of people older than 70 years suffer from mobility disorders which are caused by primary osteoarthritis [33]. Secondary osteoarthritis, caused by joint injuries such as trauma, surgery or infection [34], also becomes a problem among younger people with an active lifestyle. The imbalance between the cartilage synthesis and wear leads to the progressive damage of cartilage tissue and considerable pain. The cartilage surface shows areas of softening, fibrillation or erosion [35] during various stages of osteoarthritis. In the later stage, the areas of cartilage loss with revealing of the underlying subchondral bone can be detected. This cartilage degeneration is surely connected with a distraction of cartilage lubricating mechanism [36, 37, 38] and changes in the composition of SF [39] which is diluted by an inflammatory effusion.

Intra-articular injections with HA have been a method for improvement of lubrication conditions preventing pain and motion disabilities due to osteoarthritis for more than 30 years [40, 41]. This therapy is called viscosupplementation based on the restoration of pathological SF viscosity. Many biological mechanisms of HA in the osteoarthritic joint have also been reported [42, 43, 44]. Despite these various proven effects of intra-articular HA, clinical studies report inconsistent results of this treatment method in clinical practice. For example, Maheu et al. [45] or Tikiz et al. [46] reported a reduction of pain after viscosupplementation. On the other hand, Bannuru et al. [47] or Jevsevar et al. [48] did not observe substantial differences between viscosupplements (VSs) and placebo or anti-inflammatory drugs. These conflicting conclusions lead to contradictory clinical recommendations [49] and also to inconsistent suggestions of international medical associations [50, 51].

Many in vitro studies about viscosupplementation are focused on rheology. Higher concentration and molecular weight lead to higher viscosity and dynamic modulus [52, 53]. Furthermore, the viscosity dependency on molecular weight can be affected by partial cross-linking. Better results are usually obtained for cross-linked hyaluronic based solutions [53, 54]. It is well understood, that VSs exhibit non-Newtonian shear thinning behavior but their in vivo efficacy cannot be fully described by flow properties measured by rotational rheometers. HA interacts with proteins and other constituents of SF [55] and creates complex structures which can significantly improve the boundary lubrication of cartilage. For example, a mixture of HA and phospholipids exhibits lower friction in comparison with pure phospholipids solution [25]. Seror et al. [24] reported extremely low values of CoF ($\mu \approx 0.001$) in cartilage-on-mica contact due to a boundary lubricating layer in which the surface-anchored HA complex synergistically with lipids. Interaction between HA and proteins, especially with γ-globulin, will also play an important role in boundary lubrication of articular cartilage. According to Murakami et al. [18] and Yarimitsu et al. [56], γ-globulin and HA have opposite electric charges which contribute to the creation of complex structures with a positive impact on the cartilage friction. Despite all this evidence, not many tribological studies about VSs were published. Cherniakova et al. [57] analyzed the frictional behavior of various drugs (antibacterial, anti-inflammatory or VSs) which are injected into the joint cavity during synovitis. The frictional analysis of six VSs in cartilage-on-glass contact was performed by Bonnevie et al. [58]. However, changes in VSs rheology and friction after mixing with osteoarthritic SF were not measured or analyzed.

Although a relatively large attention has already been dedicated to the issues of intra-articular HA injections, many ambiguities still need to be clarified. Therefore, this study aimed to evaluate the rheological and frictional behavior of five commercially available VSs that are currently used in the Czech Republic. We tried to designate the extent to which their rheological and viscoelastic properties measured by conventional rotational rheometers are correlated with their lubricating properties in a model of synovial joint in which the natural articular cartilage is replaced by PVA hydrogel. We also hope that our conclusions will have an overlap in medicine and help orthopedists with the choice of VS for a specific patient. From our best knowledge, the selection of VS is usually based on the orthopedic clinical experience.

2. Materials and methods

2.1. Rheological measurements

To designate the impact of VSs and SF rheological properties on friction, the shear rate-dependent viscosity and frequency-dependent dynamic modulus were measured by commercial rotational rheometers. Viscosity measurements were performed using a TA Instruments Discovery HR-3 rheometer (TA Instruments, New Castle, DE, USA, Figure 1a). A 60 mm diameter cone-plate set up with a 1° cone angle was used. In steady shear tests, the shear rates ranging from 0.01 to 5 000 s^{-1} were applied to the tested fluids while these fluids were heated up to 37 °C by a built-in Peltier plate. The data of shear rate-dependent viscosity were fitted to the Carreau-Yasuda model to designate the pseudoplastic behavior of the commercial VSs and their mixtures with model SF. Additionally, TA Instruments AR-G2 (TA Instruments, New Castle, DE, USA, Figure 1a) rheometer was used to perform small-amplitude oscillatory (SAOS) tests to analyze the viscoelastic properties of tested solutions. A 20 mm plate-plate configuration was used and lubricant samples were heated to 37 °C during the tests. The SAOS test analyzes the dynamic modulus when a fluid sample is subjected to sinusoidal strain. In all

measurements, an initial strain sweep with an oscillatory strain of a 1 Hz constant frequency and an amplitude between 0.001 and 1.5 rad was applied to the VSs to determine the linear response region of the tested samples. Based on these results, subsequent frequency sweeps were conducted at 5 % oscillatory shear strain over a frequency range of 0.05 to 5 Hz. All rheological experiments were conducted three times with a fresh sample of tested fluid. From these data, average values were calculated and presented in graphs in the following chapter.

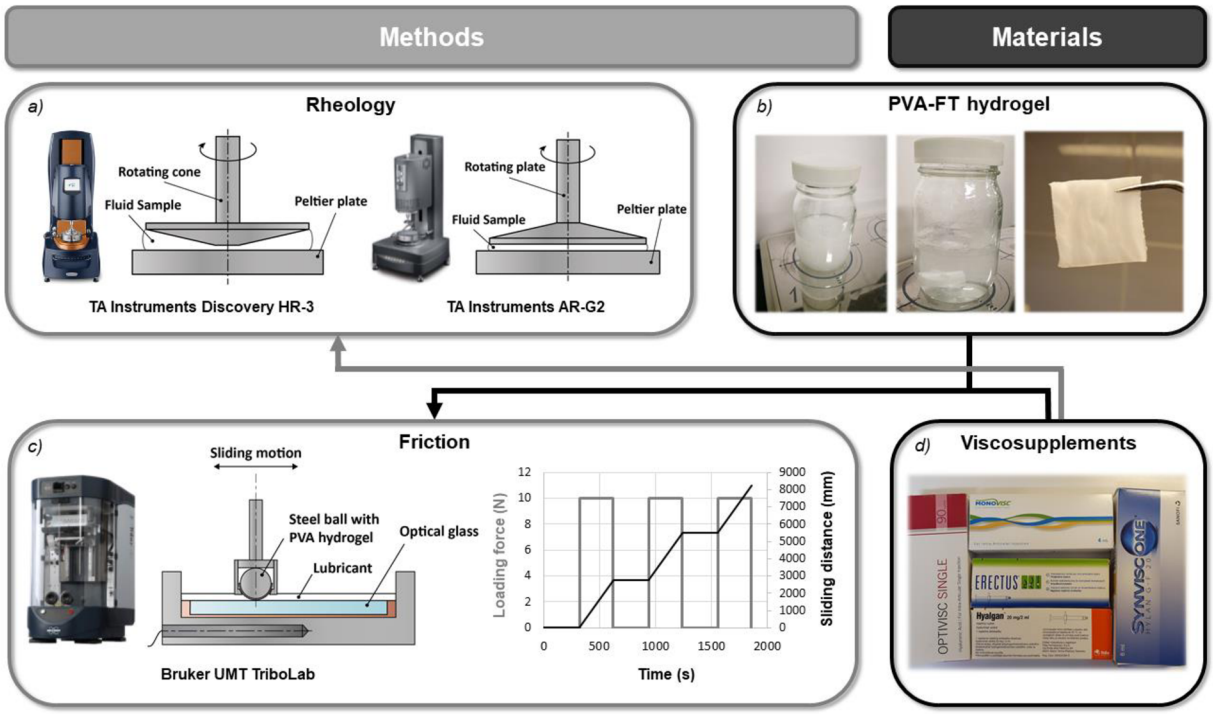

Figure 1 *Research plan: a) Rheological measurements, b) Preparation of PVA-FT hydrogel, c) Frictional measurements, d) Tested VSs*

2.2. Tested viscosupplements and model SF

In total, five different HA-based commercially available VSs (Figure 1d) were identified for rheological and frictional measurements - Erectus® (Angelini Pharma Österreich, Vienna, Austria), Hyalgan® (Fidia Farmaceutici, Padua, Italy), Monovisc® (Anika Therapeutics, Bedford, MA, USA), Optivisc Single® (Moss Vision, Wembley, United Kingdom) and Synvisc One® (Sanofi Genzyme, Ridgefield, NJ, USA). VSs were selected based on the concentration, molecular weight and cross-linking of contained HA. Samples were used as provided by the local suppliers. Table 1 summarizes basic information on VSs based on the package leaflets.

Table 1 *Summary of tested HA-based VSs*

Product	HA Concentration (mg/ml)	HA Molecular Weight (kDa)	Crosslinking	Package Volume (ml)
Erectus®	12	1 100	No	2
Hyalgan®	10	500 – 730	No	2
Monovisc®	22	1 000 – 2 900	Yes	4
Optivisc Single®	30	3 000	Yes	3
Synvisc One®	8	6 000	Yes	6

All VSs were tested as clear solutions and also as mixtures in a 1:1 ratio with model SF to better examine the effect of VSs on the rheology of SF and also on friction in the osteoarthritic joint. The model SF composition was based on the research of Galandáková et al. [39] and should correspond to the composition of SF of orthopedic patients who suffer from osteoarthritis. Phosphate buffer saline (PBS) was used as a basic solution to which albumin (24.9 mg/ml), γ-globulin (6.1 mg/ml), HA (1.49 mg/ml) and phospholipids (0.34 mg/ml) were added. The following products were used for preparation – Bovine serum albumin (powder, ≥ 96 %; A2153, Sigma-Aldrich, St. Louis, MO, USA), γ-globulin from bovine blood (powder, ≥ 99 %; G5009, Sigma-Aldrich, St. Louis, MO, USA), Sodium Hyaluronate HySilk (powder, quality class – cosmetic; molecular weight = 820 – 1 020 kDa, Contipro, Dolní Dobrouč, Czech Republic) and L-α-Phosphatidylcholine (powder, Type XVI-E, lyophilized powder; ≥ 99 %; vesicles form; P3556, Sigma-Aldrich, St- Louis, MO, USA). The model synovial fluid constituents were solved with PBS overnight at 4 °C using a rocker-shaker (MR-12, Biosan, Riga, Latvia). After this, solutions were mixed together and deeply frozen at -22 °C until further experiments. Prior to the experiments, test tubes with model SF were taken out of the freezer to thaw at laboratory temperature. Model SF was mixed with VSs by a magnetic stirrer (SMHS-3, Witeg Labortechnik, Wertheim, Germany).

2.3. Frictional measurements

In order to determine the frictional properties of tested HA-based VSs, two series of reciprocating sliding tests with clear and mixed VSs were performed on the commercial tribometer Bruker UMT TriboLab (Bruker, Billerica, MA, USA). Sliding tests were conducted in pin-on-plate configuration (Figure 1c) while the coefficient of friction (CoF) as a function of sliding distance was investigated. The contact pair consisted of the stationary glass plate made from optical glass B270 and the moving specimen made from PVA-FT hydrogel. The plate from PVA-FT hydrogel was deployed on the AISI 52100 steel ball with a diameter of 19 mm and mounted in the loading mechanism of the tribometer. PVA-FT hydrogel was loaded with a constant load of 10 N and was performing a reciprocating sliding motion with a sliding speed of 10 mm/s. The stroke length was set to 20 mm. The contact pair was fully flooded with clear VS or mixture with SF during tests and the lubricant was heated to 37 °C via heating cartridges mounted in a stainless-steel chamber. Each test consisted of three of these loaded phases (Figure 1c). Each phase lasted 300 seconds and the PVA-FT hydrogel sample traveled a sliding distance of 2740 mm. Loaded phases were separated by two unloaded phases. During unloaded phases, the PVA-FT hydrogel sample was unloaded but still immersed in tested lubricant for another 300 seconds. These unloaded phases are important for the rehydration of the PVA-FT hydrogel sample. During the experiments, frictional and loading forces were constantly monitored by a biaxial load cell which was connected to the loading mechanism of the tribometer. From these data, the values of CoF were calculated. All frictional measurements were conducted three times under the same conditions with fresh samples of PVA-FT hydrogel and lubricant. Average values and standard deviations from these three experiments were calculated and presented in the following chapter.

2.4. PVA-FT hydrogel

PVA-FT hydrogel (Figure 1b) was prepared according to the study by Yarimitsu et al. [59]. Firstly, 15 wt% aqueous solution of PVA (polymerization degree: 1700, saponification degree: 98.0 – 99.0 mol%, Kuraray, Tokyo, Japan) was prepared. The liquid hydrogel was poured into an acrylic mold and sealed. The PVA solution-containing mold was treated by a

repeated freeze-thawing method in a temperature and humidity-controlled chamber (SH-242, ESPEC, Osaka, Japan). Four FT cycles were repeated while each cycle consisted of 8 hours of freezing at - 20 °C and 16 hours of thawing at 4 °C. The PVA-FT hydrogel was 2 mm thick in its swollen state. PVA-FT hydrogel was stored in deionized water at laboratory temperature to prevent the hydrogel from drying out. Before experiments, approximately 2 x 2 cm samples were carved from the hydrogel plate.

3. Results

3.1. Rheology

Firstly, steady shear experiments with all tested lubricants were performed. Figure 2 contains data of shear rate-dependent viscosity for five tested VSs. They were tested as clear solutions (Figure 2a) and also as mixtures in a 1:1 ratio with model osteoarthritic SF (Figure 2b). As expected, a shear-thinning behavior was found in each of the VS. However, viscosities vary by the order of magnitudes between various VSs. Based on the zero shear rate viscosity (viscosity at 0.01 1/s), VSs can be divided into three groups – with high (Synvisc One®, Optivisc Single®), medium (Monovisc®, Erectus®) and low (Hyalgan®) viscosity. The highest zero shear rate viscosity was measured at Synvisc One® - 325.8 ± 3.4 Pa·s and the lowest viscosity was measured at Hyalgan® - 0.139 ± 0.016 Pa·s. VSs with higher viscosity also exhibit shear-thinning behavior in wider ranges of shear rate.

Mixing of VSs with model SF led to a significant decrease of viscosity compared to the clear VSs. The decrease of viscosity for all samples was approximately by one order of magnitude but differences between individual mixtures and a division into three groups by the viscosity of solutions remained. The highest zero shear rate viscosity was also measured for Synvisc One® mixed with model SF - 37.76 ± 3.1 Pa·s and the lowest viscosity was measured for a mixture of Hyalgan® and SF - 0.0244 ± 0.0005 Pa·s.

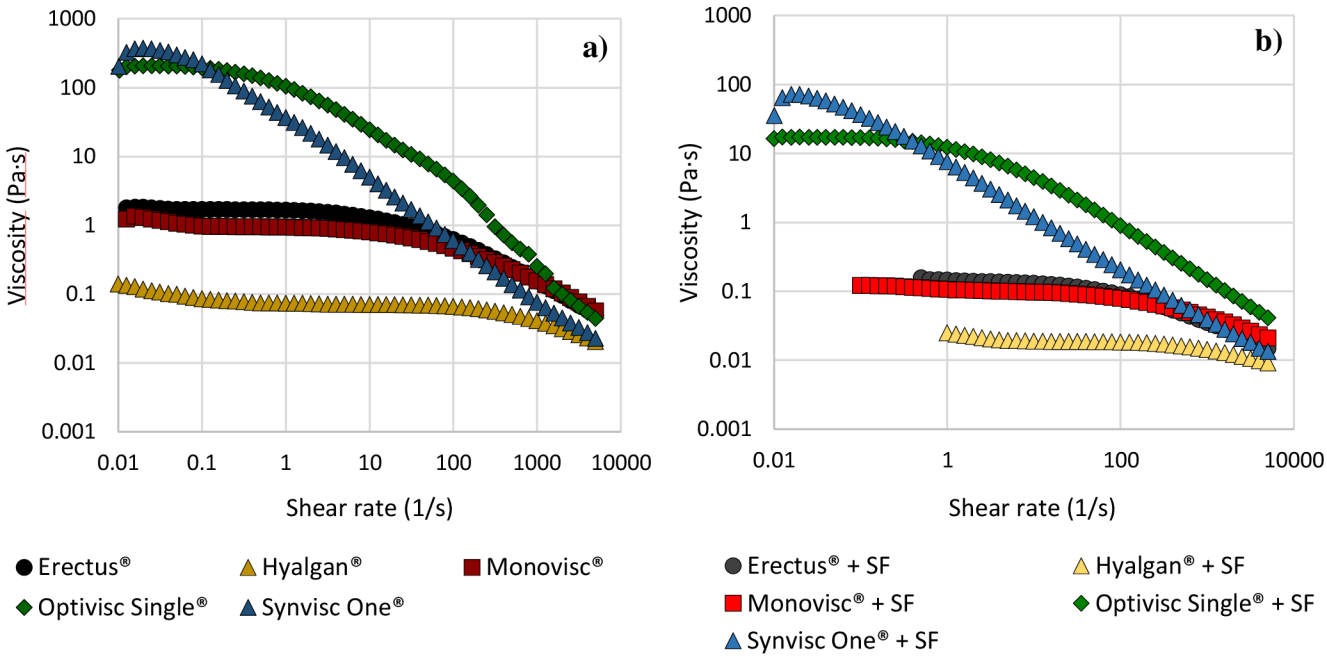

Figure 2 *Viscosity as a function of a shear rate:* ***a)*** *clear VSs,* ***b)*** *VSs mixed with model SF*

The subsequent part of the rheological measurements was the analysis of VSs viscoelastic properties. Before a frequency sweep, an initial strain sweep with a constant frequency of 1 Hz and an increasing amplitude was applied to all tested solutions to identify the region of VSs linear viscoelastic response. Figure 3 shows the dynamic moduli-strain dependence of four of the tested VSs. Hyalgan® was excluded from the viscoelastic analysis due to its low viscosity. Nearly all VSs showed no dependency between storage (G´) or loss (G´´) modulus and a strain in a wide region from 0.7 to 45 %. Therefore, subsequent frequency sweeps were measured at 5 % strain.

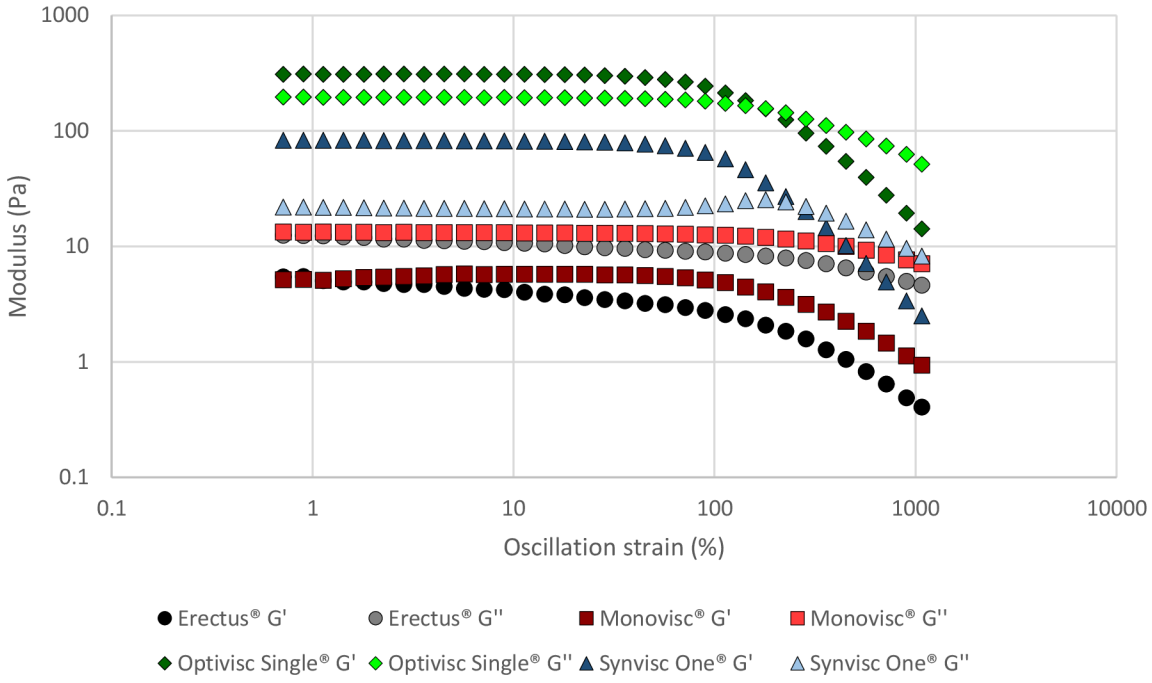

Figure 3 *Dynamic modulus as a function of strain at ω = 1 Hz*

Frequency sweep curves over a frequency range from 0.05 to 5 Hz for clear VSs are shown in Figure 4a. All three types of viscoelastic behavior were observed between tested solutions. Very similar results were obtained for Monovisc® and Erectus®. These two solutions exhibited a purely viscous behavior, i.e., values of loss modulus were higher than the storage modulus over the whole tested range of oscillation frequency. The highest values of dynamic moduli were measured for Optivisc Single®. In addition, this VS exhibited a viscoelastic behavior with a crossover frequency at 0.3 Hz. The value of dynamic modulus at this point was 134.2 Pa. The crossover point represents a transition between the viscous and elastic behavior of tested solution. During low frequency oscillating motion, linear molecular chains untangle to release stress. However, linear chains cannot untangle during high frequency oscillating motion due to the short period of movement. Thus, elastic or gel-like behavior of the solution can be observed. Synvisc One® exhibited the gel-like behavior over the whole range of oscillation frequency. From data, we can still assume that the crossover frequency lies beneath 0.05 Hz.

Figure 4b shows frequency sweep curves for VSs mixed with model SF. Only data for Optivisc Single® and Synvisc One® are presented. Accurate data for the lower viscosity samples were not measurable due to the limitations of plate-plate geometry. In general, the mixing of VS with model SF led to the lower values of dynamic modulus and the crossover point was moved to a higher frequency. Optivisc Single® still exhibits the viscoelastic behavior with a

crossover point at 1.2 Hz and Synvisc One® preserved its gel-like behavior over the whole range of oscillation frequency.

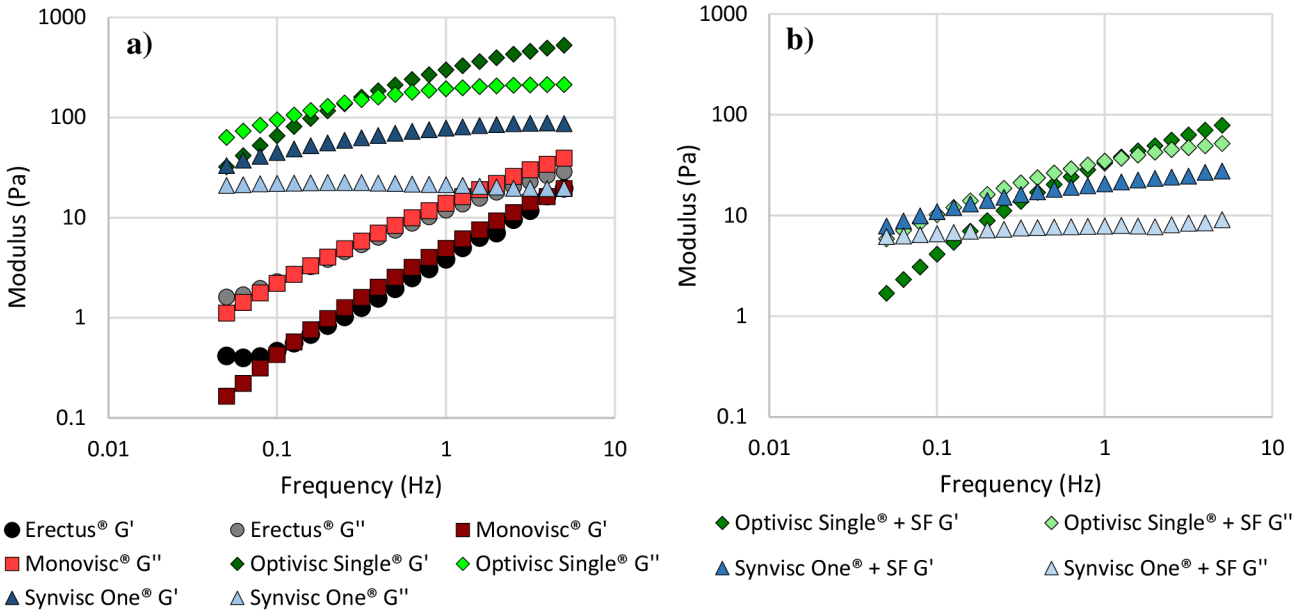

Figure 4 *Dynamic modulus as a function of the frequency:* ***a)*** *clear VSs,* ***b)*** *VSs mixed with model SF*

3.2. Friction

The following part of the VS analysis was focused on friction. CoF dependency on a sliding distance in the contact between the PVA-FT hydrogel and the optical glass B270 was measured. The contact was fully flooded with VS, model SF or a mixture of these two solutions in a 1:1 ratio. All results can be seen in Figure 5. It is noted that, even for clear SF, the initial values of CoF are very low, between 0.05 and 0.065. Nevertheless, CoF rapidly increases with sliding distance until the loaded phase finishes. At the end of the loaded phases, the values of CoF range between 0.105 and 0.107. All commercial VSs exhibited a considerably lower friction compared to the pure SF but there are differences in the shape of frictional curves between the individual VSs. For example, Erectus® (Figure 5a) exhibited a time-dependent frictional behavior similar to pure SF. On the other hand, values of CoF for Optivisc Single® (Figure 5d) and Synvisc One® (Figure 5e) are nearly constant. Synvisc One® just reports the decrease of friction during the running-in phase of the measurement. Even the effect of rehydration is, in the case of Optivisc Single® and Synvisc One®, negligible, which points to the different lubrication regime. Overall, the lowest friction was measured for Synvisc One®. At the end of measurement, the value of CoF was 0.008 ± 0.0004.

The addition of VS into the model SF caused a significant decrease in friction. In most cases, the frictional behavior of mixed solutions is very similar to that of clear VSs. The only exception is Hyalgan® which also exhibited a greater dispersion of data compared to other mixtures. In the case of Erectus® and Optivisc Single®, the friction of mixtures with model SF exhibited even lower friction than the clear VS. Nevertheless, in terms of friction in hydrogel-on-glass contact, the Synvisc One® seems to be the most suitable VS whereas its mixture exhibited the lowest value of CoF at the end of frictional measurement - 0.009 ± 0.0008.

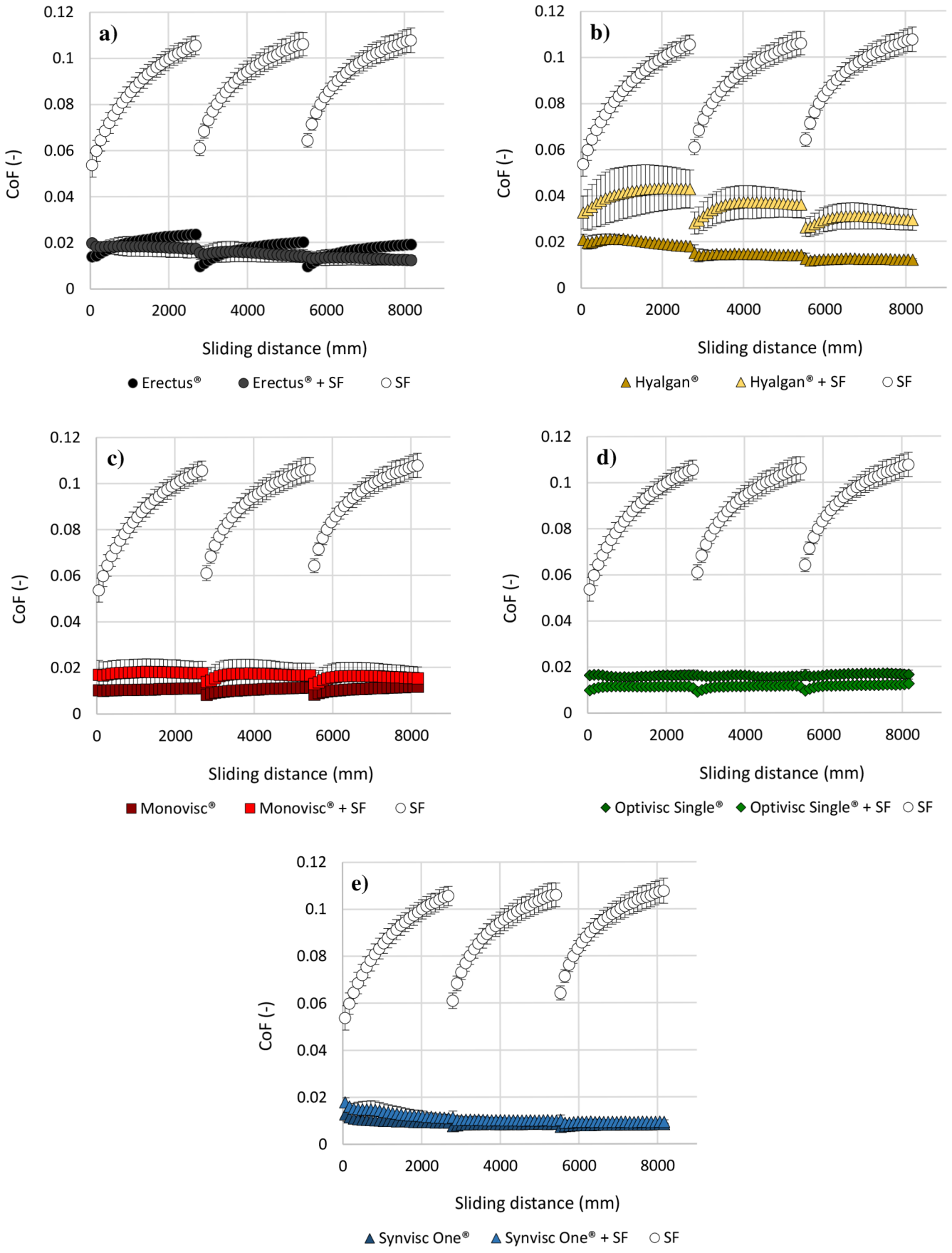

Figure 5 *CoF as a function of the sliding distance for clear VSs and mixtures with model SF:* ***a)*** *Erectus®,* ***b)*** *Hyalgan®,* ***c)*** *Monovisc®,* ***d)*** *Optivisc Single®,* ***e)*** *Synvisc One®*

4. Discussion

4.1. General discussion

The present study was aimed at the evaluation of the rheological and frictional properties of five commercially available HA-based solutions which are used for viscosupplementation of SF in the osteoarthritic joint. The research was carried out using a conventional rotational rheometer enabling to analyze viscosity and viscoelastic properties of tested solutions. Besides, a pin-on-plate tribometer was utilized for CoF measurements in a model of a synovial joint. In order to get more relevant data, all experiments were repeated three times. Sufficient repeatability of results was observed under most conditions.

HA is a main constituent of SF which contributes to its viscoelastic properties. According to Zhang et al. [60], protein aggregation or any other interactions do not affect the rheological properties of SF in comparison with pure HA solution. In a healthy synovial joint, SF contains a linear chain structure HA with a molecular weight of approximately 5 MDa [61]. From the tested VSs, only Synvisc One® contains HA with higher molecular weight (Table 1). However, Synvisc One® is composed of a mixture of two cross-linked HA derivatives (Hylan A and B) [62] which have a branched structure of HA chains. From these two derivatives, only Hylan A can penetrate the cartilage structure and interact with CD44 receptors [63]. This makes a cross-linked products different from the linear chain structure of HA within the healthy synovial joint.

The original idea of viscosupplementation was the resumption of rheological properties of a healthy SF. However, the literature reports a marked difference between healthy SF viscosities. Fam et al. [64] reported zero shear rate viscosities of healthy SF in a range between 1 and 175 Pa·s. For comparison, zero shear rate viscosity of osteoarthritic SF ranges from 0.01 to 11 Pa·s [52, 53, 65]. Some of the tested VSs fell beyond the range for healthy SF, even in their pure forms. From our results, only Optivisc Single® and Synvisc One® should be able to restore the rheological properties of a healthy SF. Measured zero shear rate viscosities after mixing of these VSs with osteoarthritic SF were 18.56 ± 1.73 Pa·s for Optivisc Single® and 37.76 ± 3.1 Pa·s for Synvisc One®.

All tested solutions exhibited the non-Newtonian shear-thinning behavior. In general, VSs with higher viscosity also exhibit a much stronger shear thinning behavior, i.e., the rate of viscosity decline with increasing shear rate is more pronounced. The rate of shear-thinning behavior can be, for example, characterized by the value of η_0/η_{300}, which is the ratio of the zero shear rate viscosity and the viscosity at the shear rate of 300 1/s [54, 66]. Calculated values of the shear-thinning ratio are stated in Table 2. The highest value of the shear-thinning ratio was calculated for Synvisc One® - 983.86 and the lowest value for Hyalgan® - 2.48. Mixing of VSs with model SF led to a reduction of shear thinning behavior of solutions. For example, the shear-thinning ratio of clear Synvisc One® drops from 983.86 to 419.19 for a mixed solution with model SF. Fam et al. [64] reported a shear-thinning ratio in the range between 70 and 250 for healthy SF and in the range between 5 and 40 for SF aspirated from the osteoarthritic joint. None of the VSs mixtures fell inside the range of healthy SF. These differences may result in a SF that does not operate similarly to a healthy SF within the joint under severe conditions.

Table 2 *Summary of VSs rheological and frictional properties*

Product	Zero Shear Viscosity (Pa·s)	$\frac{\eta_0}{\eta_{300}}$	0.5 Hz		Crossover Frequency (Hz)	CoF (-)
			G‘ (Pa)	G“ (Pa)		
ERECTUS®	1.53 ± 0.171	5.38	1.9 ± 0.7	6.7 ± 1	> 5	0.019 ± 0.0005
ERECTUS® + SF	0.145 ± 0.010	2.49	-	-	-	0.012 ± 0.0019
HYALGAN®	0.139 ± 0.016	2.48	-	-	-	0.012 ± 0.0004
HYALGAN® + SF	0.0244 ± 0.0005	1.44	-	-	-	0.029 ± 0.0045
MONOVISC®	1.02 ± 0.032	3.47	2.4 ± 0.2	7.9 ± 0.4	> 5	0.012 ± 0.0016
MONOVISC® + SF	0.112 ± 0.012	1.89	-	-	-	0.015 ± 0.0050
OPTIVISC SINGLE®	176.2 ± 6.1	193.15	197.2 ± 14	159 ± 9.1	0.3 ± 0.01	0.017 ± 0.0019
OPTIVISC SINGLE® + SF	18.56 ± 1.73	47.85	23.7 ± 0.5	28.5 ± 0.4	1.2 ± 0.03	0.013 ± 0.0006
SYNVISC ONE®	325.8 ± 3.4	983.86	71.5 ± 2.9	22.3 ± 0.6	< 0.05	0.008 ± 0.0004
SYNVISC ONE® + SF	37.76 ± 3.1	419.19	17.6 ± 1.6	8.1 ± 0.9	< 0.05	0.009 ± 0.0008

The crossover frequency for healthy SF reported by Balazs et al. [67] was 0.41 Hz. Mazzucco et al. [65] reported a crossover point of 1.8 Hz for osteoarthritic SF. From the tested VSs, Optivisc Single® mixed with osteoarthritic HA was the most similar to the healthy SF with a crossover frequency of 1.2 Hz. However, the physiological frequencies of the knee joint were defined as 0.5 Hz for walking and 2.5 Hz for running [68]. This means that a mixture of SF and Optivisc Single® exhibits the viscous response during walking and the elastic response during running. Only the mixture of Synvisc One® and osteoarthritic SF behaves like the elastic body, even at low frequencies of the joint movement. Therefore, it can absorb mechanical energy and protect the articular cartilage structure against direct contact of the rubbing surfaces.

Figure 6 summarizes the values of CoF at the end of the frictional measurements. Differences in frictional properties of VSs are not so significant as in the case of rheological properties. The viscosity of HA is primarily influenced by molecular weight [54, 69] and concentration [70]. Dependency between HA molecular weight [71, 72] or concentration [33] and friction within the cartilage contact was also reported. Still, no direct connection between viscosity and CoF was observed.

Two types of frictional behavior were observed in Figure 5. Some solutions exhibited approximately logarithmical dependency between CoF and the sliding distance and substantial declines in friction caused by the rehydration of PVA-FT hydrogel during the unloaded phases of experiments. This type of behavior points to the biphasic lubrication within the contact. Other solutions exhibited approximately a constant CoF with no declines after the rehydration. This type of behavior corresponds more to the boundary lubrication. In this lubrication regime, the cartilage low friction is controlled by the adsorbed film which is, among others, composed of HA. According to the Stribeck curve for articular and artificial cartilage [2, 8], the lubrication regime is strongly influenced by the viscosity of the lubricant. However, there are several other explanations for these results. A low molecular weight HA is able to penetrate the cartilage structure [33]; therefore, it is more biologically active [73]. Liu et al. [74] also reported a lower adhesion energy between the low molecular weight HA chains and the gelatin layer on the mica surface in comparison with high molecular weight HA. Therefore, the high molecular weight

HA is more effective within the formation of a boundary lubricating layer on the cartilage surface. Viscoelastic properties of HA may also affect the lubrication regime within the contact. Pure and mixed Synvisc One® exhibited a gel-like behavior over the whole range of frequencies (Figure 4). This means that Synvisc One® behaves like an elastic body during the oscillating motion which corresponds with constant friction, i.e., the boundary lubrication regime in Figure 5e. An apparent decline of CoF can be seen during the first phase of measurements. This is probably caused by the formation of HA boundary layer on the surface of PVA hydrogel. On the other hand, VSs, such as Erectus® and Monovisc®, exhibited the viscous-like behavior during the measurement of viscoelastic properties (Figure 4a). Logarithmical shapes of their frictional curves in Figure 5a and Figure 5c rather correspond with biphasic lubrication, i.e., fluid lubrication within the contact.

Surprisingly, mixed Erectus® and Optivisc Single® reported even lower values of CoF than the clear VS (Figure 6). This surely points out on some synergistic reactions between individual components of SF and HA within VSs. Lipids presented in synovial fluid can interact with surface-anchored HA to form a boundary lubricating layer with extremely low friction [24]. Due to different electric charges, HA is also able to form complex structures with γ-globulin, which contributes to the lower friction [25].

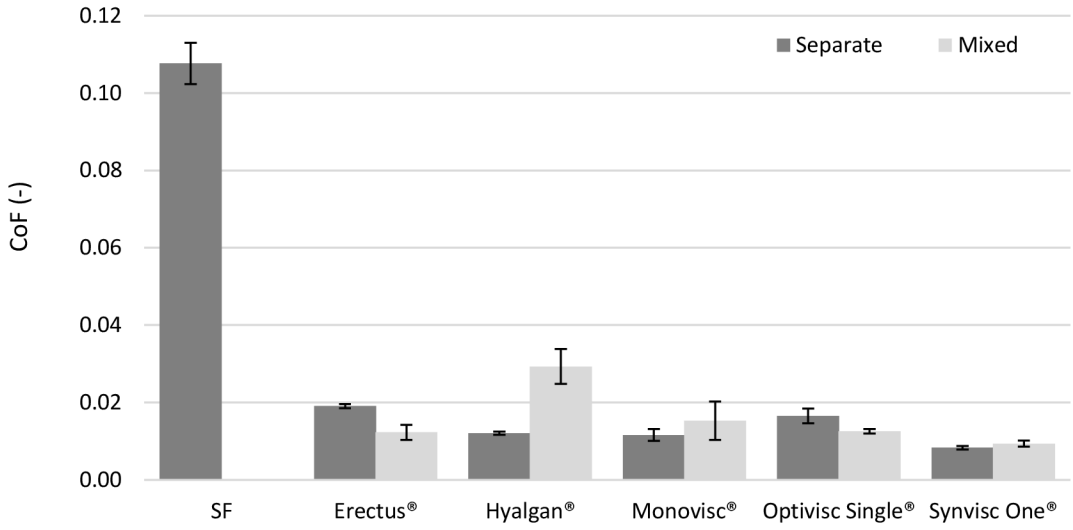

Figure 6 *CoF at the end of measurements for all tested lubricants*

4.2. Limitations

Shortcomings of the performed analysis and the motivation for further research should be pointed out. Viscosities of Erectus®, Hyalgan® and Monovisc® mixtures were generally very low. Viscosity measurements of these low viscosity fluids at low shear rates were not possible due to the limitations of cone-plate geometry. Therefore, these data are missing in Figure 2b. Viscoelastic properties of some VSs and their mixtures were not measurable. Coaxial cylinder geometry or double-gap cylinder geometry of the rotational rheometer should be more appropriate for the rheological analysis of these solutions. More samples of osteoarthritic SF should be involved in the study to emphasize the individual patient's condition. Zero shear rate viscosity of osteoarthritic SF ranges between 0.01 and 11 Pa·s [52, 53, 65] whereas we used only one sample with zero shear rate viscosity of 0.03 Pa·s. The concentration of individual synovial fluid components also changes during the progression of osteoarthritis [39], and our

previous study [75] showed that these changes may affect friction within the cartilage-on-glass contact. Frictional measurements under constant speed and load do not correspond with complex kinematic and loading conditions within synovial joints. Many studies about articular cartilage and PVA hydrogels [32, 76, 77] pointed out the effect of experimental conditions on the values of CoF within the contact. Although we highlighted many similarities between the articular cartilage and PVA hydrogels in the introduction section, the PVA hydrogel cannot fully mitigate the structure of articular cartilage. In recent years, publications denying the fluid load support theory in hydrogels were also published [78, 79]. As a counterpart to the PVA hydrogel, we used the optical glass which is not suitable to mitigate the cartilage due to its artificial structure and different mechanical properties or wettability. Therefore, future studies should focus on frictional measurements in cartilage-on-cartilage contact. For a deeper understanding of tribological changes in synovial joints after viscosupplementation, in situ observation of the contact area should also be a very powerful tool. In our laboratory, we have already developed a simulator which enables contact visualization by fluorescent microscopy with simultaneous measurement of CoF within the contact [80]. Fluorescence microscopy will allow for the study of the behavior of fluorescently labeled synovial fluid components within the contact. Fluorescence microscopy as an optical method requires transparent material at one of the rubbing surfaces. Therefore, a cartilage-on-cartilage configuration is not possible for these types of experiments. We suggest replacing one of the cartilage surfaces by transparent PVA hydrogel or polymethyl methacrylate (PMMA). From our point of view, this will be the best model for the study of articular cartilage lubrication and its changes after the injection of HA into the synovial joint capsule.

5. Conclusions

The present paper aimed at the rheological and frictional analysis of five commercially available solutions for viscosupplementation of osteoarthritic SF. Rotational rheometers in cone-plate and plate-plate configuration were used to analyze the rheological properties of clear VSs and their mixtures with osteoarthritic SF. A pin-on-plate tribometer was used to evaluate the frictional behavior of these solutions in the contact between the PVA hydrogel as a model of articular cartilage and the glass. The main conclusions are summarized in the following points:

- Substantial differences in the rheological properties of individual VSs were observed.
- Mixtures of osteoarthritic SF with Optivisc Single® or Synvisc One® exhibited the most similar results when compared to the SF within the healthy synovial joint.
- Widely varying rheological properties of tested VSs did not predict their frictional properties. Differences in the frictional behavior of individual VSs were not as substantial as differences in their rheological properties.
- Mixing of osteoarthritic SF with a specific VS led to a significant decrease of viscosity and deterioration of viscoelastic properties compared to the clear VS.
- On the contrary, the worsening of frictional properties was not so noticeable. Values of CoF measured for clear VSs and their mixtures were similar for most of the tested VS. In some cases, the mixing of VS and osteoarthritic SF leads to even lower values of CoF compared with the clear VS. This refers to some synergistic reactions between HA and synovial fluid components.

- The molecular weight of HA and its viscoelastic properties can possibly affect the lubrication regime within hydrogel-on-glass contact.

Further investigation should focus on (a) measurements with more samples of osteoarthritic SF, (b) application of transient loading and kinematic conditions, (c) frictional measurements of cartilage-on-cartilage contact (d) in situ observation of cartilage-on-hydrogel (potentially glass or PMMA) contact by fluorescent microscopy.

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References

[1] Mow VC, Kuei SC, Lai WM, Armstrong CG. Biphasic Creep and Stress Relaxation of Articular Cartilage in Compression: Theory and Experiments. Journal Of Biomechanical Engineering 1980;102:73-84. https://doi.org/10.1115/1.3138202.

[2] Murakami T, Yarimitsu S, Nakashima K, Sakai N, Yamaguchi T, Sawae Y, et al. Biphasic and boundary lubrication mechanisms in artificial hydrogel cartilage: A review. Proceedings Of The Institution Of Mechanical Engineers, Part H: Journal Of Engineering In Medicine 2015;229:864-878. https://doi.org/10.1177/0954411915611160.

[3] McCutchen CW. The frictional properties of animal joints. Wear 1962;5:1-17. https://doi.org/10.1016/0043-1648(62)90176-X.

[4] Unsworth A, Dowson D, Wright V. Some new evidence on human joint lubrication. Annals Of The Rheumatic Diseases 1975;34:277-285. https://doi.org/10.1136/ard.34.4.277.

[5] Murakami T. The lubrication in natural synovial joints and joint prostheses. Jsme International Journal. Ser. 3, Vibration, Control Engineering, Engineering For Industry 1990;33:465-474. https://doi.org/10.1299/jsmec1988.33.465.

[6] Ikeuchi K. The Role of Synovial Fluid in Joint Lubrication. Lubricants And Lubrication - Proceedings Of The 21Th Leeds-Lyon Symposium On Tribology 1995:65-69. https://doi.org/10.1016/S0167-8922(08)70617-5.

[7] Murakami T, Higaki H, Sawae Y, Ohtsuki N, Moriyama S, Nakanishi Y. Adaptive multimode lubrication in natural synovial joints and artificial joints. Proceedings Of The Institution Of Mechanical Engineers, Part H: Journal Of Engineering In Medicine 1998;212:23-35. https://doi.org/10.1243/0954411981533791.

[8] Murakami T. Importance of adaptive multimode lubrication mechanism in natural and artificial joints. Proceedings Of The Institution Of Mechanical Engineers, Part J: Journal Of Engineering Tribology 2012;226:827-837. https://doi.org/10.1177/1350650112451377.

[9] Higaki H. Role of Constituents in Synovial fluid and Surface Layer on Articular Cartilage in Joint Lubrication (Part 2) – The Boundary Lubrication Ability of Proteins. Japanese Journal of Tribology 1995;40:691–700.

[10] Higaki H, Murakami T, Nakanishi Y, Miura H, Mawatari T, Iwamoto Y. The lubricating ability of biomembrane models with dipalmitoyl phosphatidylcholine and γ-globulin. Proceedings Of The Institution Of Mechanical Engineers, Part H: Journal Of Engineering In Medicine 1998;212:337-346. https://doi.org/10.1243/0954411981534114.

[11] Forster H, Fisher J. The Influence of Loading Time and Lubricant on the Friction of Articular Cartilage. Proceedings Of The Institution Of Mechanical Engineers, Part H: Journal Of Engineering In Medicine 1996;210:109-119. https://doi.org/10.1243/PIME_PROC_1996_210_399_02.

[12] Ateshian GA. A Theoretical Formulation for Boundary Friction in Articular Cartilage. Journal Of Biomechanical Engineering 1997;119:81-86. https://doi.org/10.1115/1.2796069.

[13] Ikeuchi K. Origin and future of hydration lubrication. Proceedings Of The Institution Of Mechanical Engineers, Part J: Journal Of Engineering Tribology 2007;221:301-305. https://doi.org/10.1243/13506501JET214.

[14] Klein J. Hydration lubrication. Friction 2013;1:1-23. https://doi.org/10.1007/s40544-013-0001-7.

[15] Mansour JM, Mow VC. On the Natural Lubrication of Synovial Joints: Normal and Degenerate. Journal Of Lubrication Technology 1977;99:163-172. https://doi.org/10.1115/1.3453003.

[16] Krishnan R, Kopacz M, Ateshian GA. Experimental verification of the role of interstitial fluid pressurization in cartilage lubrication. Journal Of Orthopaedic Research 2004;22:565-570. https://doi.org/10.1016/j.orthres.2003.07.002.

[17] Caligaris M, Ateshian GA. Effects of sustained interstitial fluid pressurization under migrating contact area, and boundary lubrication by synovial fluid, on cartilage friction. Osteoarthritis And Cartilage 2008;16:1220-1227. https://doi.org/10.1016/j.joca.2008.02.020.

[18] Murakami T, Nakashima K, Yarimitsu S, Sawae Y, Sakai N. Effectiveness of adsorbed film and gel layer in hydration lubrication as adaptive multimode lubrication mechanism for articular cartilage. Proceedings Of The Institution Of Mechanical Engineers, Part J: Journal Of Engineering Tribology 2011;225:1174-1185. https://doi.org/10.1177/1350650111415756.

[19] Burris DL, Moore AC. Cartilage and Joint Lubrication: New Insights Into the Role of Hydrodynamics. Biotribology 2017;12:8-14. https://doi.org/10.1016/j.biotri.2017.09.001.

[20] Murakami T, Nakashima K, Sawae Y, Sakai N, Hosoda N. Roles of adsorbed film and gel layer in hydration lubrication for articular cartilage. Proceedings Of The Institution Of Mechanical Engineers, Part J: Journal Of Engineering Tribology 2009;223:287-295. https://doi.org/10.1243/13506501JET536.

[21] Zappone B, Ruths M, Greene GW, Jay GD, Israelachvili JN. Adsorption, Lubrication, and Wear of Lubricin on Model Surfaces: Polymer Brush-Like Behavior of a Glycoprotein. Biophysical Journal 2007;92:1693-1708. https://doi.org/10.1529/biophysj.106.088799.

[22] Bonnevie ED, Galesso D, Secchieri C, Cohen I, Bonassar LJ, Awad HA. Elastoviscous Transitions of Articular Cartilage Reveal a Mechanism of Synergy between Lubricin and Hyaluronic Acid. Plos One 2015;10. https://doi.org/10.1371/journal.pone.0143415.

[23] Park J-Y, Duong C-T, Sharma AR, Son K-M, Thompson MS, Park S, et al. Effects of Hyaluronic Acid and γ–Globulin Concentrations on the Frictional Response of Human Osteoarthritic Articular Cartilage. Plos One 2014;9. https://doi.org/10.1371/journal.pone.0112684.

[24] Seror J, Zhu L, Goldberg R, Day AJ, Klein J. Supramolecular synergy in the boundary lubrication of synovial joints. Nature Communications 2015;6. https://doi.org/10.1038/ncomms7497.

[25] Murakami T, Yarimitsu S, Nakashima K, Sawae Y, Sakai N. Influence of synovia constituents on tribological behaviors of articular cartilage. Friction 2013;1:150-162. https://doi.org/10.1007/s40544-013-0010-6.

[26] Murakami T, Yarimitsu S, Nakashima K, Yamaguchi T, Sawae Y, Sakai N, et al. Superior lubricity in articular cartilage and artificial hydrogel cartilage. Proceedings Of The Institution Of Mechanical Engineers, Part J: Journal Of Engineering Tribology 2014;228:1099-1111. https://doi.org/10.1177/1350650114530273.

[27] Oka M, Noguchi T, Kumar P, Ikeuchi K, Yamamuro T, Hyon SH, et al. Development of an artificial articular cartilage. Clinical Materials 1990;6:361-381. https://doi.org/10.1016/0267-6605(90)90053-X.

[28] Sardinha VM, Lima LL, Belangero WD, Zavaglia CA, Bavaresco VP, Gomes JR. Tribological characterization of polyvinyl alcohol hydrogel as substitute of articular cartilage. Wear 2013;301:218-225. https://doi.org/10.1016/j.wear.2012.11.054.

[29] Katta JK, Marcolongo M, Lowman A, Mansmann KA. Friction and wear behavior of poly(vinyl alcohol)/poly(vinyl pyrrolidone) hydrogels for articular cartilage replacement. Journal Of Biomedical Materials Research Part A 2007;83A:471-479. https://doi.org/10.1002/jbm.a.31238.

[30] Nakashima K, Sawae Y, Murakami T. Study on Wear Reduction Mechanisms of Artificial Cartilage by Synergistic Protein Boundary Film Formation. Jsme International Journal Series C 2005;48:555-561. https://doi.org/10.1299/jsmec.48.555.

[31] Murakami T, Sakai N, Yamaguchi T, Yarimitsu S, Nakashima K, Sawae Y, et al. Evaluation of a superior lubrication mechanism with biphasic hydrogels for artificial cartilage. Tribology International 2015;89:19-26. https://doi.org/10.1016/j.triboint.2014.12.013.

[32] Li F, Wang A, Wang C. Analysis of friction between articular cartilage and polyvinyl alcohol hydrogel artificial cartilage. Journal Of Materials Science: Materials In Medicine 2016;27. https://doi.org/10.1007/s10856-016-5700-y.

[33] Forsey R, Fisher J, Thompson J, Stone M, Bell C, Ingham E. The effect of hyaluronic acid and phospholipid based lubricants on friction within a human cartilage damage model. Biomaterials 2006;27:4581-4590. https://doi.org/10.1016/j.biomaterials.2006.04.018.

[34] Axe MJ, Shields CL. Potential Applications of Hyaluronans in Orthopaedics. Sports Medicine 2005;35:853-864. https://doi.org/10.2165/00007256-200535100-00003.

[35] Cicuttini FM, Wluka AE, Stuckley SL. Tibial and femoral cartilage changes in knee osteoarthritis. Annals Of The Rheumatic Diseases 2001;60:977-980. https://doi.org/10.1136/ard.60.10.977.

[36] Tanaka E, Iwabe T, Dalla-Bona DA, Kawai N, van Eijden T, Tanaka M, Kitagawa S, Takata T, Tanne K. The effect of experimental cartilage damage and impairment and restoration of synovial lubrication on friction in the temporomandibular joint. Journal of Orofacial Pain 2005;19:331–336.

[37] Caligaris M, Canal CE, Ahmad CS, Gardner TR, Ateshian GA. Investigation of the frictional response of osteoarthritic human tibiofemoral joints and the potential beneficial tribological effect of healthy synovial fluid. Osteoarthritis And Cartilage 2009;17:1327-1332. https://doi.org/10.1016/j.joca.2009.03.020.

[38] Shi L, Brunski DB, Sikavitsas VI, Johnson MB, Striolo A. Friction coefficients for mechanically damaged bovine articular cartilage. Biotechnology And Bioengineering 2012;109:1769-1778. https://doi.org/10.1002/bit.24435.

[39] Galandáková A, Ulrichová J, Langová K, Hanáková A, Vrbka M, Hartl M, et al. Characteristics of synovial fluid required for optimization of lubrication fluid for biotribological experiments. Journal Of Biomedical Materials Research Part B: Applied Biomaterials 2017;105:1422-1431. https://doi.org/10.1002/jbm.b.33663.

[40] Dixon AS, Jacoby RK, Berry H, Hamilton EB. Clinical trial of intra-articular injection of sodium hyaluronate in patients with osteoarthritis of the knee. Current Medical Research and Opinion 1988;11:205–213.

[41] Balazs EA, Denlinger JL. Viscosupplementation: A new concept in the treatment of osteoarthritis. The Journal of Rheumatology Supplement 1993;39:3–9.

[42] Altman RD, Manjoo A, Fierlinger A, Niazi F, Nicholls M. The mechanism of action for hyaluronic acid treatment in the osteoarthritic knee: a systematic review. Bmc Musculoskeletal Disorders 2015;16. https://doi.org/10.1186/s12891-015-0775-z.

[43] Li J, Gorski DJ, Anemaet W, Velasco J, Takeuchi J, Sandy JD, et al. Hyaluronan injection in murine osteoarthritis prevents TGFbeta 1-induced synovial neovascularization and fibrosis and maintains articular cartilage integrity by a CD44-dependent mechanism 2012;14. https://doi.org/10.1186/ar3887.

[44] Creamer P, Sharif M, George E, Meadows K, Cushnaghan J, Shinmei M, et al. Intra-articular hyaluronic acid in osteoarthritis of the knee: an investigation into mechanisms of action. Osteoarthritis And Cartilage 1994;2:133-140. https://doi.org/10.1016/S1063-4584(05)80063-9.

[45] Maheu E, Rannou F, Reginster J-Y. Efficacy and safety of hyaluronic acid in the management of osteoarthritis: Evidence from real-life setting trials and surveys. Seminars In Arthritis And Rheumatism 2016;45:S28-S33. https://doi.org/10.1016/j.semarthrit.2015.11.008.

[46] Tıkız C, Ünlü Z, Şener A, Efe M, Tüzün Ç. Comparison of the efficacy of lower and higher molecular weight viscosupplementation in the treatment of hip osteoarthritis. Clinical Rheumatology 2005;24:244-250. https://doi.org/10.1007/s10067-004-1013-5.

[47] Bannuru RR, Vaysbrot EE, Sullivan MC, McAlindon TE. Relative efficacy of hyaluronic acid in comparison with NSAIDs for knee osteoarthritis: A systematic review and meta-analysis. Seminars In Arthritis And Rheumatism 2014;43:593-599. https://doi.org/10.1016/j.semarthrit.2013.10.002.

[48] Jevsevar D, Donnelly P, Brown GA, Cummins DS. Viscosupplementation for Osteoarthritis of the Knee. The Journal Of Bone And Joint Surgery-American Volume 2015;97:2047-2060. https://doi.org/10.2106/JBJS.N.00743.

[49] Altman RD, Schemitsch E, Bedi A. Assessment of clinical practice guideline methodology for the treatment of knee osteoarthritis with intra-articular hyaluronic acid. Seminars In Arthritis And Rheumatism 2015;45:132-139. https://doi.org/10.1016/j.semarthrit.2015.04.013.

[50] Bruyère O, Cooper C, Pelletier J-P, Branco J, Luisa Brandi M, Guillemin F, et al. An algorithm recommendation for the management of knee osteoarthritis in Europe and internationally: A report from a task force of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). Seminars In Arthritis And Rheumatism 2014;44:253-263. https://doi.org/10.1016/j.semarthrit.2014.05.014.

[51] McAlindon TE, Bannuru RR, Sullivan MC, Arden NK, Berenbaum F, Bierma-Zeinstra SM, et al. OARSI guidelines for the non-surgical management of knee osteoarthritis. Osteoarthritis And Cartilage 2014;22:363-388. https://doi.org/10.1016/j.joca.2014.01.003.

[52] Bhuanantanondh P, Grecov D, Kwok E. Rheological Study of Viscosupplements and Synovial Fluid in Patients with Osteoarthritis. Journal of Medical and Biological Engineering 2012;31:12–16.

[53] Mathieu P, Conrozier T, Vignon E, Rozand Y, Rinaudo M. Rheologic Behavior of Osteoarthritic Synovial Fluid after Addition of Hyaluronic Acid: A Pilot Study. Clinical Orthopaedics And Related Research® 2009;467:3002-3009. https://doi.org/10.1007/s11999-009-0867-x.

[54] Bhuanantanondh P, Grecov D, Kwok E, Guy P. Rheology of osteoarthritic synovial fluid mixed with viscosupplements: A pilot study. Biomedical Engineering Letters 2011;1:213-219. https://doi.org/10.1007/s13534-011-0034-7.

[55] Das S, Banquy X, Zappone B, Greene GW, Jay GD, Israelachvili JN. Synergistic Interactions between Grafted Hyaluronic Acid and Lubricin Provide Enhanced Wear Protection and Lubrication. Biomacromolecules 2013;14:1669-1677. https://doi.org/10.1021/bm400327a.

[56] Yarimitsu S, Nakashima K, Sawae Y, Murakami T. Influences of lubricant composition on forming boundary film composed of synovia constituents. Tribology International 2009;42:1615-1623. https://doi.org/10.1016/j.triboint.2008.11.005.

[57] Cherniakova YM, Pinchuk LS, Tribological aspects of joint intraarticular therapy. Acta of Bioengineering and Biomechanics 2011;13(1):57–63.

[58] Bonnevie ED, Galesso D, Secchieri C, Bonassar LJ, Awad HA. Frictional characterization of injectable hyaluronic acids is more predictive of clinical outcomes than traditional rheological or viscoelastic characterization. Plos One 2019;14. https://doi.org/10.1371/journal.pone.0216702.

[59] Yarimitsu S, Sasaki S, Murakami T, Suzuki A. Evaluation of lubrication properties of hydrogel artificial cartilage materials for joint prosthesis. Biosurface And Biotribology 2016;2:40-47. https://doi.org/10.1016/j.bsbt.2016.02.005.

[60] Zhang Z, Barman S, Christopher GF. The role of protein content on the steady and oscillatory shear rheology of model synovial fluids. Soft Matter 2014;10:5965-5973. https://doi.org/10.1039/C4SM00716F.

[61] Watterson JR, Esdaile JM. Viscosupplementation: Therapeutic Mechanisms and Clinical Potential in Osteoarthritis of the Knee. Journal Of The American Academy Of Orthopaedic Surgeons 2000;8:277-284. https://doi.org/10.5435/00124635-200009000-00001.

[62] Ishikawa M, Yoshioka K, Urano K, Tanaka Y, Hatanaka T, Nii A. Biocompatibility of cross-linked hyaluronate (Gel-200) for the treatment of knee osteoarthritis. Osteoarthritis And Cartilage 2014;22:1902-1909. https://doi.org/10.1016/j.joca.2014.08.002.

[63] Jackson DW, Simon TM. Intra-articular distribution and residence time of Hylan A and B: a study in the goat knee. Osteoarthritis And Cartilage 2006;14:1248-1257. https://doi.org/10.1016/j.joca.2006.05.015.

[64] Fam H, Bryant J T, Kontopoulou M. Rheological properties of synovial fluids. Biorheology 2007;44(2):59–74.

[65] Mazzucco D, McKinley G, Scott RD, Spector M. Rheology of joint fluid in total knee arthroplasty patients. Journal Of Orthopaedic Research 2002;20:1157-1163. https://doi.org/10.1016/S0736-0266(02)00050-5.

[66] Rainer F, Ribitsch V. Viscoelastic properties of normal human synovia and their relation to biomechanics. Zeitschrift für Rheumatologie 1985:44(3):114–119.

[67] Balazs EA. The psysical properties of synovial fluid and the special role of hyaluronic acid. In: Helfet AJ, editor. Disorders ot the Knee, Philadelphia: JB Lippincott Company; 1974, p. 63-75.

[68] Finelli I, Chiessi E, Galesso D, Renier D, Paradossi G. A new viscosupplement based on partially hydrophobic hyaluronic acid: A comparative study. Biorheology 2011;48:263-275. https://doi.org/10.3233/BIR-2011-0596.

[69] Cowman MK, Schmidt TA, Raghavan P, Stecco A. Viscoelastic Properties of Hyaluronan in Physiological Conditions. F1000Research 2015;4. https://doi.org/10.12688/f1000research.6885.1.

[70] Bingöl AÖ, Lohmann D, Püschel K, Kulicke W-M. Characterization and comparison of shear and extensional flow of sodium hyaluronate and human synovial fluid. Biorheology 2010;47:205-224. https://doi.org/10.3233/BIR-2010-0572.

[71] Kwiecinski JJ, Dorosz SG, Ludwig TE, Abubacker S, Cowman MK, Schmidt TA. The effect of molecular weight on hyaluronan's cartilage boundary lubricating ability – alone and in combination with proteoglycan 4. Osteoarthritis And Cartilage 2011;19:1356-1362. https://doi.org/10.1016/j.joca.2011.07.019.

[72] Rebenda D, Vrbka M, Čípek P, Toropitsyn E, Nečas D, Pravda M, et al. On the Dependence of Rheology of Hyaluronic Acid Solutions and Frictional Behavior of Articular Cartilage. Materials 2020;13. https://doi.org/10.3390/ma13112659.

[73] Ghosh P, Guidolin D. Potential mechanism of action of intra-articular hyaluronan therapy in osteoarthritis: Are the effects molecular weight dependent? Seminars In Arthritis And Rheumatism 2002;32:10-37. https://doi.org/10.1053/sarh.2002.33720.

[74] Liu Z, Lin W, Fan Y, Kampf N, Wang Y, Klein J. Effects of Hyaluronan Molecular Weight on the Lubrication of Cartilage-Emulating Boundary Layers. Biomacromolecules 2020;21:4345-4354. https://doi.org/10.1021/acs.biomac.0c01151.

[75] Furmann D, Nečas D, Rebenda D, Čípek P, Vrbka M, Křupka I, et al. The Effect of Synovial Fluid Composition, Speed and Load on Frictional Behaviour of Articular Cartilage. Materials 2020;13. https://doi.org/10.3390/ma13061334.

[76] Li F, Zhang G, Wang A, Guo F. The Effects of Surface Mechanical Deformation and Bovine Serum Albumin on the Tribological Properties of Polyvinyl Alcohol Hydrogel as an Artificial Cartilage. Advances In Materials Science And Engineering 2017;2017:1-9. https://doi.org/10.1155/2017/4502904.

[77] Oliveira AS, Seidi O, Ribeiro N, Colaço R, Serro AP. Tribomechanical Comparison between PVA Hydrogels Obtained Using Different Processing Conditions and Human Cartilage. Materials 2019;12. https://doi.org/10.3390/ma12203413.

[78] Porte E, Cann P, Masen M. A lubrication replenishment theory for hydrogels. Soft Matter 2020;16:10290-10300. https://doi.org/10.1039/D0SM01236J.

[79] Porte E, Cann P, Masen M. Fluid load support does not explain tribological performance of PVA hydrogels. Journal Of The Mechanical Behavior Of Biomedical Materials 2019;90:284-294. https://doi.org/10.1016/j.jmbbm.2018.09.048.

[80] Čípek P, Vrbka M, Rebenda D, Nečas D, Křupka I. Biotribology of Synovial Cartilage: A New Method for Visualization of Lubricating Film and Simultaneous Measurement of the Friction Coefficient. Materials 2020;13. https://doi.org/10.3390/ma13092075.

7 CONCLUSIONS

The present PhD thesis deals with the effect of viscosupplementation on friction within the osteoarthritic articular cartilage. Viscosupplementation is an OA treatment method for more than 30 years but the effectiveness of this treatment method is still debatable. Mainly due to the unexplained mechanisms which occur in a synovial joint after viscosupplementation. The main attention of the research was paid to the rheological analysis of VSs and osteoarthritic SF so far. However, little is known about the effect of viscosupplementation on the friction of articular cartilage. Moreover, dependency between the rheological properties of VSs and friction within articular cartilage has not been proven yet. Such knowledge could lead to the development of new, more effective VSs and also help in clinical practice with the picking of appropriate VSs for individual patients.

The first part of this PhD thesis describes the current state of the art in the field of viscosupplementation and related areas. Studies focused on the rheology of healthy and osteoarthritic SF are presented. The second part of the current state of the art is focused on the effect of SF composition and viscosupplementation on friction within the articular cartilage. Studies about artificial articular cartilage are presented too. Based on the critical analysis of the current state of the art, the main goal of this PhD thesis was to clarify the changes of articular cartilage frictional behavior after viscosupplementation. Attention was also paid to the rheology of VSs and the changes of articular cartilage friction due to OA.

The following sections of PhD thesis deal with the employed experimental devices and experimental conditions. Subsequently, the original results of this thesis are presented in the form of three articles which were published in journals with impact factor. The first article was focused on the effect of SF composition, speed and load on the frictional behavior of articular cartilage. For this purpose, several protein solutions and two model SFs were used in order to analyze the role of individual SF constituents during articular cartilage friction. Apart from this, differences in frictional behavior between a healthy and osteoarthritic SF were examined. The most important conclusion was that the interaction between HA and phospholipids plays an important role in the friction of articular cartilage. Focusing on the differences between physiologic and osteoarthritic SF, only a limited effect on friction was observed. The second paper's goal was to clarify the effect of HA molecular weight on the rheology of SF and friction within articular cartilage. Molecular weight significantly affected HA viscosity and viscoelastic properties but no clear dependency between HA molecular weight and friction within cartilage-on-glass contact was observed. In the last article, the effectiveness of five commercially available VSs was examined. Substantial differences in VSs rheology were observed. However, widely varying rheological properties did not predict VSs frictional properties. Differences in the frictional behavior of individual VSs were not as significant as differences in their rheology.

Nevertheless, changes in the lubrication regime due to the HA molecular weight were observed.

The current PhD thesis presents original results which extend the knowledge in the field of articular cartilage friction. Description of the frictional changes in a synovial joint after the addition of exogenous HA is one of the key things to clarify the issues of viscosupplementation. Presented data could lead to the development of new more effective VSs. Results could also be useful in clinical practice, whereas the literature reported a correlation between friction and pain reduction among patients with OA. Further investigation should focus on the measurements with even more complex model SFs. Glycoprotein PRG4 and molecular weight of HA should be taken into account. Application of transient loading and kinematic conditions during the cartilage-on-cartilage frictional measurements would bring the results closer to the real situation. Some of the results should be examined in more detail. In situ observation of articular cartilage by florescent microscopy should bring more information about the changes in articular cartilage tribology after viscosupplementation. The main contribution of the thesis can be summarized into the following points:

- Frictional differences between physiologic and osteoarthritic SFs with an emphasis on individual constituents were analyzed.
- Analysis of the effect of HA molecular weight on rheology and friction within articular cartilage was conducted.
- The effectiveness of five commercially available VSs was investigated. Their rheology and ability of a healthy SF rheology resumption was analyzed. The changes in friction within the articular cartilage model after the addition of VS in the osteoarthritic SF were also described.

Regarding the scientific questions, the obtained knowledge can be summarized in the following bullet points:

- *"A lower concentration of HA in osteoarthritic SF fluid will increase the friction within articular cartilage model."*
  Despite the expectation, changes in SF composition due to the progression of OA did not have a significant effect on the cartilage friction. However, the progression of OA is also connected with a decrease in HA molecular weight. This information was not taken into account during the preparation of the model SFs. **The first hypothesis was falsified.**
- *"Higher viscosity of HA and VSs will cause a more pronounced decrease of friction within a synovial joint model."*
  The HA solutions and cross-linked HA-based VSs with high molecular weight exhibited higher viscosity and better viscoelastic properties. The lowest value of CoF within artificial cartilage contact was measured for VS with the highest molecular weight - Synvisc One®. Still, no direct dependence between HA rheology and friction within natural or artificial cartilage was observed. **The second hypothesis was falsified.**

- *"Large molecules of high molecular weight/cross-linked HA will perform better in reduction of friction within articular cartilage model."*
  No direct dependence between molecular weight and CoF within a model of synovial joint was observed, even though the lowest CoF was measured for VS with the highest molecular weight - Synvisc One®. However, frictional measurements with high molecular weight or cross-linked HA VSs (Optivisc Single®, Synvisc One®) mostly exhibited frictional behavior which corresponds to the boundary lubrication regime. The results of some other VSs, like Hyalgan® or Erectus®, corresponded more to the biphasic lubrication regime. Therefore, changes in a lubrication regime due to the HA molecular weight were observed. These findigs could be further tested by in situ measurements of articular cartilage contact by fluorescent microscopy. The frictional measurements with fluorescently stained HA solutions with different molecular weight and consequent cartilage histology would show if HA is able to penetrate the cartilage structure or just adheres on the cartilage surface to create a boundary lubricating layer. Nevertheless, to the extent of work that was carried out within this dissertation thesis, **the third hypothesis was falsified.**

8 LIST OF PUBLICATIONS

8.1 Papers published in journals with impact factor

ČÍPEK, P., M. VRBKA, D. REBENDA, D. NEČAS and I. KŘUPKA. 2020. Biotribology of Synovial Cartilage: A New Method for Visualization of Lubricating Film and Simultaneous Measurement of the Friction Coefficient. *Materials*. **13**(9).

FURMANN, D., D. NEČAS, D. REBENDA, P. ČÍPEK, M. VRBKA, I. KŘUPKA and Martin HARTL. 2020. The Effect of Synovial Fluid Composition, Speed and Load on Frictional Behaviour of Articular Cartilage. *Materials*. **13**(6).

LU, X., D. NEČAS, Q. MENG, D. REBENDA, M. VRBKA, M. HARTL and Z. JIN. 2020. Towards the direct validation of computational lubrication modelling of hip replacements. *Tribology International*. **146**.

REBENDA, D., M. VRBKA, P. ČÍPEK, E. TOROPITSYN, D. NEČAS, M. PRAVDA and M. HARTL. 2020. On the Dependence of Rheology of Hyaluronic Acid Solutions and Frictional Behavior of Articular Cartilage. *Materials*. **13**(11).

NEČAS, D., M. VRBKA, D. REBENDA, J. GALLO, A. GALANDÁKOVÁ, L. WOLFOVÁ, I. KŘUPKA and M. HARTL. 2018. In situ observation of lubricant film formation in THR considering real conformity: The effect of model synovial fluid composition. *Tribology International*. **117**, 206-216.

CHOUDHURY, D., D. REBENDA, S. SASAKI, P. HEKRLE, M. VRBKA, M. ZOU, I. KŘUPKA and M. HARTL. 2018. Enhanced lubricant film formation through micro-dimpled hard-on-hard artificial hip joint: An in-situ observation of dimple shape effects. *Journal of the Mechanical Behavior of Biomedical Materials*. **81**, 120-129.

REBENDA, D., M. VRBKA, D. NEČAS, E. TOROPITSYN, S. YARIMITSU, P. ČÍPEK, M. PRAVDA and M. HARTL. 2021. Rheological and frictional Analysis of Viscosupplements Towards Improved Lubrication of Human Joints. *Tribology International.* **(UNDER REVIEW)**

ČÍPEK, P., M. VRBKA, D. REBENDA, D. NEČAS and I. KŘUPKA. 2021. Biotribology of Synovial Cartilage: The Role of Albumin in Lubricant Film Formation. *Friction.* **(UNDER REVIEW)**

8.2 Papers published in peer-reviewed journals

ČÍPEK, P., D. REBENDA, D. NEČAS, M. VRBKA, I. KŘUPKA and M. HARTL. 2019. Visualization of Lubrication Film in Model of Synovial Joint. *Tribology in Industry*. **41**(3), 387-393.

RUFAQUA, R., M. VRBKA, D. HEMZAL, D. CHOUDHURY, D. REBENDA, I. KŘUPKA and M. HARTL. 2021. Raman analysis of chemisorbed tribo-film for metal-on-polyethylene hip joint prostheses. *Biosurface and Biotribology.* **(UNDER REVIEW)**

RUFAQUA, R., M. VRBKA, D. HEMZAL, D. CHOUDHURY, D. REBENDA, I. KŘUPKA and M. HARTL. 2021. Analysis of chemisorbed tribo-film for ceramic-on-ceramic hip joint prostheses by Raman spectroscopy. *Journal of Functional Biomaterials.* **(UNDER REVIEW)**

8.3 Papers in conference proceedings

REBENDA, David, Pavel ČÍPEK, Martin VRBKA and Ivan KŘUPKA. 2019. Effect of Hyaluronic Acid Molecular Weight on Friction of Articular Cartilage. In: *Proceedings on Engineering Sciences*. **1**(1), p. 693-697.

ČÍPEK, Pavel, David REBENDA, Martin VRBKA and Martin HARTL. 2019. Observation of Lubrication Film in Synovial Joint. In: *Proceedings on Engineering Sciences*. **1**(1), p. 687-692.

REBENDA, David, Pavel ČÍPEK, David NEČAS, Martin VRBKA and Martin HARTL. 2018. Effect of Hyaluronic Acid on Friction of Articular Cartilage. In: *Engineering Mechanics 2018,* p. 709-712.

ČÍPEK, Pavel, David REBENDA, David NEČAS, Martin VRBKA and Ivan KŘUPKA. 2018. Development of Reciprocating Tribometer for Testing Synovial Joint. In: *Engineering Mechanics 2018,* p. 169-172.

9 LITERATURE

[1] OARSI, *Osteoarthritis: A Serious Disease* [online]. Osteoarthritis Research Society International, ©2016. [Cit. 27.2.2021]. Available from: https://oarsi.org/sites/default/files/library/2018/pdf/oarsi_white_paper_oa_serious_disease121416_1.pdf

[2] MURAKAMI, T. 1990. The lubrication in natural synovial joints and joint prostheses. *JSME international journal. Ser. 3, Vibration, control engineering, engineering for industry*. **33**(4), 465-474. ISSN 0914-8825.

[3] LEES, D. and P. PARTINGTON. 2016. Articular cartilage. *Orthopaedics and Trauma*. **30**(3), 265-272.

[4] SOPHIA FOX, A. J., A. BEDI and S. A. RODEO. 2009. The Basic Science of Articular Cartilage: Structure, Composition, and Function. *Sports Health: A Multidisciplinary Approach*. **1**(6), 461-468.

[5] JAHN, S. and J. KLEIN. 2018. Lubrication of articular cartilage. *Physics Today*. **71**(4), 48-54.

[6] WONG, B. L., W. C. BAE, J. CHUN, K. R. GRATZ, M. LOTZ and R. L. SAH. 2008. *Biomechanics of cartilage articulation: Effects of lubrication and degeneration on shear deformation*. **58**(7), 2065-2074.

[7] COVA, M. and R. TOFFANIN. 2002. MR microscopy of hyaline cartilage: current status. *European Radiology*. **12**(4), 814-823.

[8] ZHANG, Z., S. BARMAN and G. F. CHRISTOPHER. 2014. The role of protein content on the steady and oscillatory shear rheology of model synovial fluids. *Soft Matter*. **10**(32), 5965-5973.

[9] TYRNENOPOULOU, P., E. RIZOS, S. CHAINTOUTIS, M. PATSIKAS, P. PAPADOPOULOU, Z. POLIZOPOULOU, A. AGGELI, L. PAPAZOGLOU and N. DIAKAKIS. 2020. Alterations in the Viscoelastic Properties of Equine Synovial Fluid from Fetlock Joints with Naturally Occurring Osteoarthritis. *Archives of Veterinary Science and Medicine*. **01**(01), 4-12.

[10] BORZACCHIELLO, A., L. MAYOL, A. SCHIAVINATO and L. AMBROSIO. 2009. Effect of hyaluronic acid amide derivative on equine synovial fluid viscoelasticity. *Journal of Biomedical Materials Research Part A*. **92**(3), 1162-1170.

[11] MAZZUCCO, D., G. MCKINLEY, R. D. SCOTT and M. SPECTOR. 2002. Rheology of joint fluid in total knee arthroplasty patients. *Journal of Orthopaedic Research*. **20**(6), 1157-1163.

[12] MATHIEU, P., T. CONROZIER, E. VIGNON, Y. ROZAND and M. RINAUDO. 2009. Rheologic Behavior of Osteoarthritic Synovial Fluid after Addition of Hyaluronic Acid: A Pilot Study. *Clinical Orthopaedics and Related Research®*. **467**(11), 3002-3009.

[13] BINGÖL, A. Ö., D. LOHMANN, K. PÜSCHEL and W.-M. KULICKE. 2010. Characterization and comparison of shear and extensional flow of sodium hyaluronate and human synovial fluid. *Biorheology*. **47**(3-4), 205-224.

[14] RAINER, F. and V. RIBITSCH. 1985. Viscoelastic properties of normal human synovia and their relation ti biomechanics. *Zeitschrift fur Rheumatologie.* **44**(3), 114-119.

[15] BHUANANTANONDH, P., D. GRECOV and E. KWOK. 2010. Rheological Study of Viscosupplements and Synovial Fluid in Patients with Osteoarthritis. *Journal of Medical and Biological Engineering*. **32**(1), 12-16.

[16] BHUANANTANONDH, P., D. GRECOV, E. KWOK and P. GUY. 2011. Rheology of osteoarthritic synovial fluid mixed with viscosupplements: A pilot study. *Biomedical Engineering Letters*. **1**(4), 213-219.

[17] FINELLI, I., E. CHIESSI, D. GALESSO, D. RENIER and G. PARADOSSI. 2011. A new viscosupplement based on partially hydrophobic hyaluronic acid: A comparative study. *Biorheology*. **48**(5-6), 263-275.

[18] NICHOLLS, M., A. MANJOO, P. SHAW, F. NIAZI and J. ROSEN. 2018. A Comparison Between Rheological Properties of Intra-articular Hyaluronic Acid Preparations and Reported Human Synovial Fluid. *Advances in Therapy*. **35**(4), 523-530.

[19] FORSEY, R., J. FISHER, J. THOMPSON, M. STONE, C. BELL and E. INGHAM. 2006. The effect of hyaluronic acid and phospholipid based lubricants on friction within a human cartilage damage model. *Biomaterials*. **27**(26), 4581-4590.

[20] BELL, C. J., E. INGHAM and J. FISHER. 2006. Influence of hyaluronic acid on the time-dependent friction response of articular cartilage under different conditions. *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine*. **220**(1), 23-31.

[21] SCHMIDT, T. A., N. S. GASTELUM, Q. T. NGUYEN, B. L. SCHUMACHER and R. L. SAH. 2007. *Boundary lubrication of articular cartilage: Role of synovial fluid constituents*. **56**(3), 882-891.

[22] MURAKAMI, T., K. NAKASHIMA, Y. SAWAE, N. SAKAI and N. HOSODA. 2009. Roles of adsorbed film and gel layer in hydration lubrication for articular cartilage. *Proceedings of the Institution of Mechanical Engineers, Part J: Journal of Engineering Tribology*. **223**(3), 287-295.

[23] KWIECINSKI, J. J., S. G. DOROSZ, T. E. LUDWIG, S. ABUBACKER, M. K. COWMAN and T. A. SCHMIDT. 2011. The effect of molecular weight on hyaluronan's cartilage boundary lubricating ability – alone and in combination with proteoglycan 4. *Osteoarthritis and Cartilage*. **19**(11), 1356-1362.

[24] MURAKAMI, T., K. NAKASHIMA, S. YARIMITSU, Y. SAWAE and N. SAKAI. 2011. Effectiveness of adsorbed film and gel layer in hydration lubrication as adaptive multimode lubrication mechanism for articular cartilage. *Proceedings of the Institution of Mechanical Engineers, Part J: Journal of Engineering Tribology*. **225**(12), 1174-1185.

[25] MURAKAMI, T., S. YARIMITSU, K. NAKASHIMA, Y. SAWAE and N. SAKAI. 2013. Influence of synovia constituents on tribological behaviors of articular cartilage. *Friction*. **1**(2), 150-162.

[26] PARK, J.-Y., C.-T. DUONG, A. R. SHARMA, et al. 2014. Effects of Hyaluronic Acid and γ–Globulin Concentrations on the Frictional Response of Human Osteoarthritic Articular Cartilage. *PLoS ONE*. **9**(11).

[27] LUDWIG, T. E., M. M. HUNTER and T. A. SCHMIDT. 2015. Cartilage boundary lubrication synergism is mediated by hyaluronan concentration and PRG4 concentration and structure. *BMC Musculoskeletal Disorders*. **16**(1).

[28] BONNEVIE, E. D., D. GALESSO, C. SECCHIERI, I. COHEN, L. J. BONASSAR and H. A. AWAD. 2015. Elastoviscous Transitions of Articular Cartilage Reveal a Mechanism of Synergy between Lubricin and Hyaluronic Acid. *PLOS ONE*. **10**(11).

[29] SEROR, J., L. ZHU, R. GOLDBERG, A. J. DAY and J. KLEIN. 2015. Supramolecular synergy in the boundary lubrication of synovial joints. *Nature Communications*. **6**(1).

[30] ZHU, L., J. SEROR, A. J. DAY, N. KAMPF and J. KLEIN. 2017. Ultra-low friction between boundary layers of hyaluronan-phosphatidylcholine complexes. *Acta Biomaterialia*. **59**, 283-292.

[31] LIN, W., R. MASHIAH, J. SEROR, A. KADAR, O. DOLKART, T. PRITSCH, R. GOLDBERG and J. KLEIN. 2019. Lipid-hyaluronan synergy strongly reduces intrasynovial tissue boundary friction. *Acta Biomaterialia*. **83**, 314-321.

[32] LIU, Z., W. LIN, Y. FAN, N. KAMPF, Y. WANG and J. KLEIN. 2020. Effects of Hyaluronan Molecular Weight on the Lubrication of Cartilage-Emulating Boundary Layers. *Biomacromolecules*. **21**(10), 4345-4354.

[33] CHERNIAKOVA, Y. and L. S. PINCHUK. 2011. Tribological aspects of joint intraarticular therapy. *Acta of bioengineering and biomechanice*. **13**(1), 57-63.

[34] BONNEVIE, E. D., D. GALESSO, C. SECCHIERI, L. J. BONASSAR and H. A. AWAD. 2019. Frictional characterization of injectable hyaluronic acids is more predictive of clinical outcomes than traditional rheological or viscoelastic characterization. *PLOS ONE*. **14**(5).

[35] PREKASAN, D. and K. K. SAJU. 2019. Tribological effectiveness of viscosupplements for osteoarthritis in knee joint. *SN Applied Sciences*. **1**(9).

[36] NAKASHIMA, K., Y. SAWAE and T. MURAKAMI. 2005. Study on Wear Reduction Mechanisms of Artificial Cartilage by Synergistic Protein Boundary Film Formation. *JSME International Journal Series C*. **48**(4), 555-561.

[37] MURAKAMI, T., Y. SAWAE, K. NAKASHIMA, S. YARIMITSU and T. SATO. 2007. Micro- and nanoscopic biotribological behaviours in natural synovial joints and artificial joints. *Proceedings of the Institution of Mechanical Engineers, Part J: Journal of Engineering Tribology*. **221**(3), 237-245.

[38] YARIMITSU, S., K. NAKASHIMA, Y. SAWAE and T. MURAKAMI. 2009. Influences of lubricant composition on forming boundary film composed of synovia constituents. *Tribology International*. **42**(11-12), 1615-1623.

[39] YARIMITSU, S., K. NAKASHIMA, Y. SAWAE and T. MURAKAMI. 2013. Influence of Phospholipid and Protein Constituents on Tribological Properties of Artificial Hydrogel Cartilage Material. *Journal of Biomechanical Science and Engineering*. **8**(3), 257-267.

[40] MURAKAMI, T., S. YARIMITSU, K. NAKASHIMA, T. YAMAGUCHI, Y. SAWAE, N. SAKAI and A. SUZUKI. 2014. Superior lubricity in articular cartilage and artificial hydrogel cartilage. *Proceedings of the Institution of Mechanical Engineers, Part J: Journal of Engineering Tribology*. **228**(10), 1099-1111.

[41] LI, F., G. ZHANG, A. WANG and F. GUO. 2017. The Effects of Surface Mechanical Deformation and Bovine Serum Albumin on the Tribological Properties of Polyvinyl Alcohol Hydrogel as an Artificial Cartilage. *Advances in Materials Science and Engineering*. **2017**, 1-9.

[42] OLIVEIRA, A. S., O. SEIDI, N. RIBEIRO, R. COLAÇO and A. P. SERRO. 2019. Tribomechanical Comparison between PVA Hydrogels Obtained Using Different Processing Conditions and Human Cartilage. *Materials*. **12**(20).

[43] BALAZS, E. A. The physical properties of synovial fluid and the special role of hyaluronic acid. In: *Disorders of the knee*. Philapelphia: JB Lippincott Company, 1974, s. 63-75.

[44] CALIGARIS, M., C.E. CANAL, C.S. AHMAD, T.R. GARDNER and G.A. ATESHIAN. 2009. Investigation of the frictional response of osteoarthritic human

tibiofemoral joints and the potential beneficial tribological effect of healthy synovial fluid. *Osteoarthritis and Cartilage*. **17**(10), 1327-1332.

[45] YARIMITSU, S., S. SASAKI, T. MURAKAMI and A. SUZUKI. 2016. Evaluation of lubrication properties of hydrogel artificial cartilage materials for joint prosthesis. *Biosurface and Biotribology*. **2**(1), 40-47.

[46] KIENLE, S., K. BOETTCHER, L. WIEGLEB, J. URBAN, R. BURGKART, O. LIELEG and T. HUGEL. 2015. Comparison of friction and wear of articular cartilage on different length scales. *Journal of Biomechanics*. **48**(12), 3052-3058.

[47] FAM, H., J. T. BRYANT and M. KONTOPOULOU. 2007. Rheological properties of synovial fluids. *Biorheology*. **44**(2), 59-74.

[48] ATESHIAN, G. A. 2009. The role of interstitial fluid pressurization in articular cartilage lubrication. *Journal of Biomechanics*. **42**(9), 1163-1176.

[49] KRISHNAN, R., M. KOPACZ and G. A. ATESHIAN. 2004. Experimental verification of the role of interstitial fluid pressurization in cartilage lubrication. *Journal of Orthopaedic Research*. **22**(3), 565-570.

[50] APPLEYARD, R.C., D. BURKHARDT, P. GHOSH, R. READ, M. CAKE, M.V. SWAIN and G.A.C. MURRELL. 2003. Topographical analysis of the structural, biochemical and dynamic biomechanical properties of cartilage in an ovine model of osteoarthritis. *Osteoarthritis and Cartilage*. **11**(1), 65-77.

[51] KIVIRANTA, P., E. LAMMENTAUSTA, J. TÖYRÄS, I. KIVIRANTA and J.S. JURVELIN. 2008. Indentation diagnostics of cartilage degeneration. *Osteoarthritis and Cartilage*. **16**(7), 796-804.

[52] RICHARD, F., M. VILLARS and S. THIBAUD. 2013. Viscoelastic modeling and quantitative experimental characterization of normal and osteoarthritic human articular cartilage using indentation. *Journal of the Mechanical Behavior of Biomedical Materials*. **24**, 41-52.

[53] DURAINE, G., C. P. NEU, S. M.T. CHAN, K. KOMVOPOULOS, R. K. JUNE and A. H. REDDI. 2009. Regulation of the friction coefficient of articular cartilage by TGF-β1 and IL-1β. *Journal of Orthopaedic Research*. **27**(2), 249-256.

[54] CHAN, S.M.T., C.P. NEU, K. KOMVOPOULOS and A.H. REDDI. 2011. The role of lubricant entrapment at biological interfaces: Reduction of friction and adhesion in articular cartilage. *Journal of Biomechanics*. **44**(11), 2015-2020.

[55] KLEIN, J. 2013. Hydration lubrication. *Friction*. **1**(1), 1-23.

LIST OF FIGURES AND TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

CD	cast-drying
CoF	coefficient of friction
DMPC	1,2-dimyristoyl-sn-glycero-3-phoshphocholine
DPPC	dipalmitoylphosphatidylcholine
FT	freeze-thawing
HA	hyaluronic acid
HSPC	hydrogenated soy phosphatydylcholine
OA	osteoarthritis
OARSI	Osteaorthritis Research Society International
PBS	phosphate buffered saline
POPC	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholin
PRG4	proteoglycan 4, lubricin
PVA	poly(vinyl alcohol)
SAOS	small-amplitude oscilátory shear
SAPL	surface active phospholipids
SF	synovial fluid
VS	viscosupplement
WOMAC	The Western Ontario and MacMaster Universities Osteoarthritis Index
η_0	zero shear viscosity
η_{300}	viscosity at a strain of 300 s^{-1}