BRNO UNIVERSITY OF TECHNOLOGY
Faculty of Mechanical Engineering
Institute of Machine and Industrial Design

Ing. David Rebenda

EFFECT OF VISCOSUPPLEMENTTATION ON FRICTION OF ARTICULAR CARTILAGE

VLIV VISKOSUPLEMENTACE NA TŘENÍ KLOUBNÍ CHRUPAVKY

Shortened version of PhD Thesis

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CONTENTS

1 INTRODUCTION

Osteoarthritis (OA) is one of the most common diseases of the musculoskeletal system among older people in developed countries. According to the OARSI, 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.

Viscosupplementation is one of the non-invasive methods for the treatment of OA for more than 30 years. It consists in hyaluronic acid (HA)-based injection into the joint capsule. The original idea of viscosupplementation was the resumption of healthy synovial fluid (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 viscosupplements (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

2.1 RHEOLOGY OF SF AND HA

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. [2]. Results of steady shear tests with model SF and HA were very similar. Therefore, the concentration of albumin and γ-globulin did not affect SF viscosity. The authors conclude that proteoglycan 4 (PRG4), which is also part of the synovial fluid, could affect the rheology. However, this SF constituent was not tested in the study.

Changes in SF composition and rheology due to the progression of OA were analyzed by Tyrnenopoulou et al. [3]. Sixteen samples of equine SFs were extracted from osteoarthritic fetlock joints. Results were compared with 6 samples obtained from normal joints. Total protein concentration (Figure 2-1) 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 viscoelastic properties analysis. 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

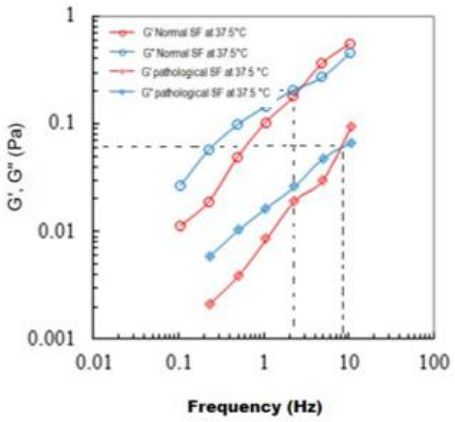

Figure 2-1 Left: Protein and HA concentration in normal and osteoarthritic SF. Right: Frequency sweeps of SFs [3]

Viscosity and viscoelastic properties of 13 SF samples aspirated from osteoarthritic joints were analyzed by Mathieu [4]. The rheological properties of pure SF, as well as SF mixed with native HA or commercial VS based on cross-linked HA, were discussed. HA had a molecular weight of 1.14 MDa. As a VS, Synvisc® based on a cross-linked Hylan G-F 20 was used. 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. 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, 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.

Viscosity of different SFs was also investigated by Bingöl et al. [5]. 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 highest measured zero shear viscosity of gathered SF was 445 Pa·s (Figure 2-2a). This value is even outside of the commonly reported range of normal SF [6]. 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. The results (Figure 2-2b) also 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.

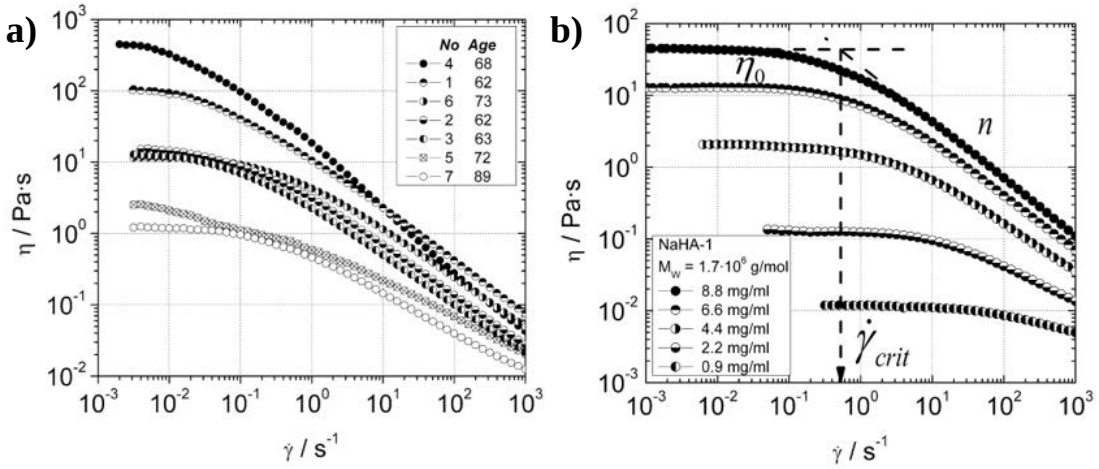

Figure 2-2 a) Shear viscosities of human SFs, b) Influence of concentration on the viscosity of HA [5]

Another rheological characterization of SFs from 22 patients undergoing total knee arthroplasty and three commercially available VSs was performed by Bhuanatanondh [7]. The commercial VSs were represented by Orthovisc® and Suplasyn®, which are based on linear HA, and Synvisc®. 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 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 2-3a), 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 2-3b). Low molecular weight HA reported viscous-like behavior.

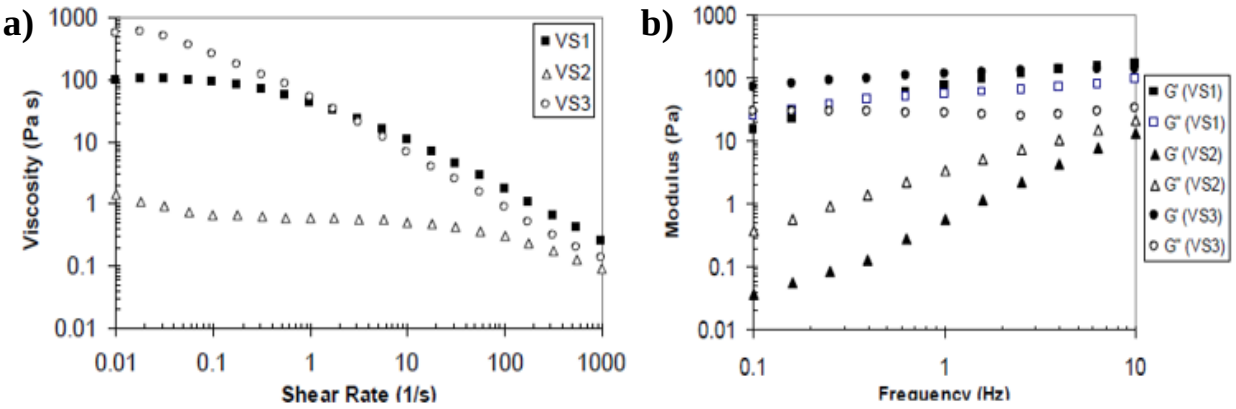

Figure 2-3 a) Viscosity of tested VSs, b) Viscoelastic properties of tested VSs (VS1 = Orthovisc®, VS2 = Suplasyn®, VS3 = Synvisc®) [7]

In a consequent study by Bhuanatanondh et al. [8], rheological changes after mixing of osteoarthritic SF with the same VSs were analyzed. Mixing of the VS with SF led to an increase in viscosity over the whole range of shear rate (Figure 2-4a). 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. Figure 2-4b 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.

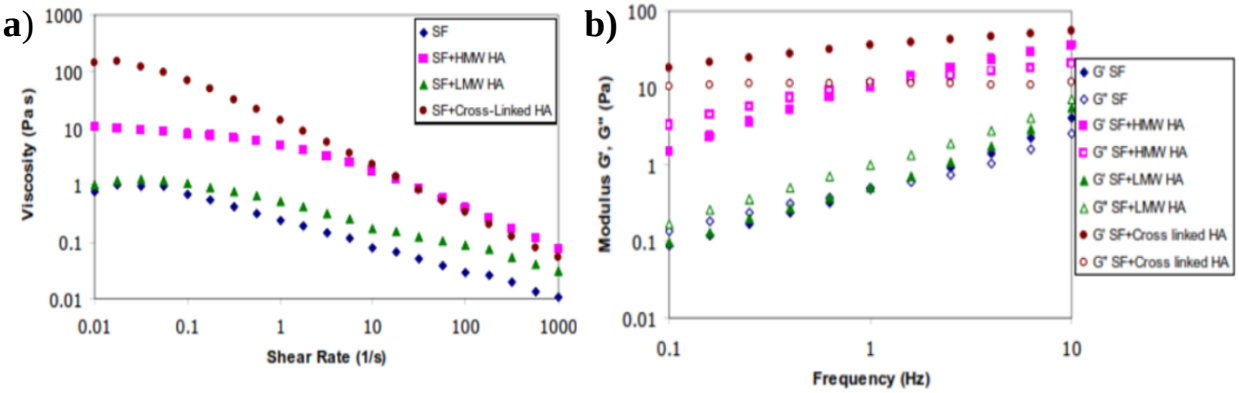

Figure 2-4 a) Viscosity of SF and mixtures with VSs, b) Viscoelastic properties of SF and mixtures [8]

Several commercial VSs in their pure form were analyzed by Finelli et al. [9]. Results in Figure 2-5b showed that Hymovis®, Durolane® and Synvisc® exhibited

gel-like behavior, whereas the crossover frequency lay below the range of knee motion frequency. 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 2-5a). 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®.

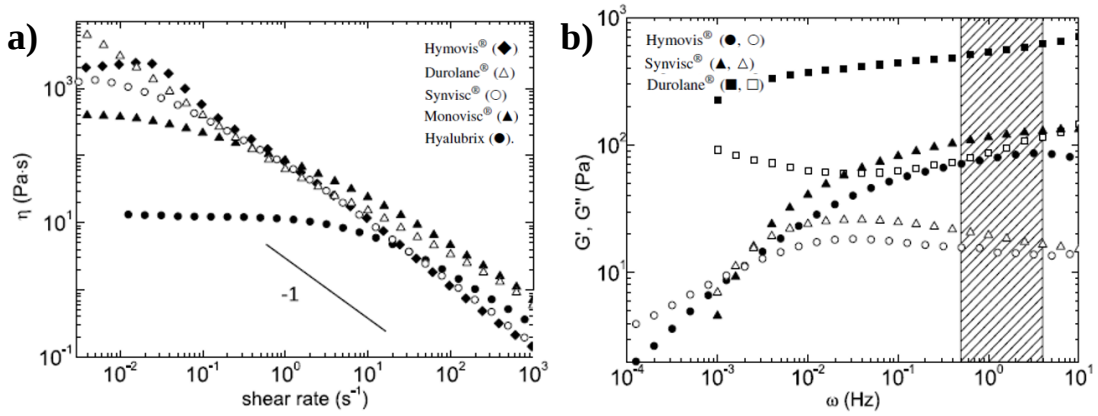

Figure 2-5 a) Viscosity curves of VSs, b) Viscoelastic properties of VSs [9]

Rheological analysis of pure commercial HA-based VSs was also conducted by Nicholls et al. [10]. 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® were the most similar to a healthy knee SF in terms of viscosity and viscoelasticity.

2.2 EFFECT OF SF COMPOSITION ON CARTILAGE FRICTION

In 2006, Forsey et al. [11] analyzed the frictional behavior of cartilage-on-cartilage contact lubricated by HA, dipalmitoylphosphatidylcholine (DPPC) or their combinations. From the results in Figure 2-6, it can be concluded that the concentration of HA has no significant effect on friction. On the other hand, the DPPC concentration significantly affected the values of CoF. The highest decrease of COF was observed for the solution containing 10 mg/ml HA and 200 mg/ml DPPC.

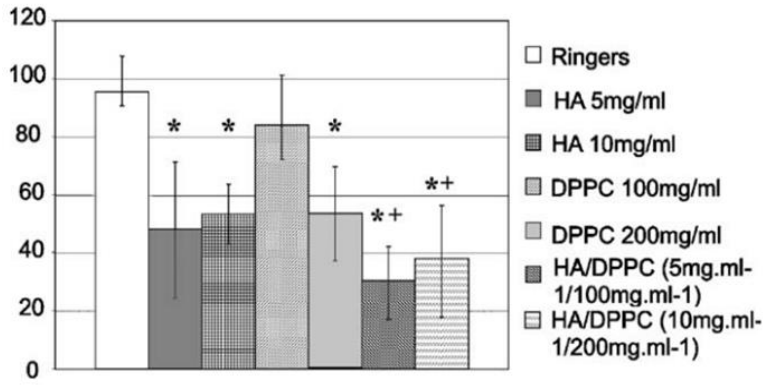

Figure 2-6 Percentage difference in CoF for various lubricants [11]

The effectiveness of interactions between HA, PRG4 and surface active phospholipids (SAPL) within the boundary lubrication of cartilage-on-cartilage contact was investigated by Schmidt et al. [12]. Results were also compared with bovine SF. The highest value of the CoF was measured for PBS and the lowest value was measured for SF. 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, proteins also contribute to the reduction of friction.

The role of HA within articular cartilage friction was further investigated by Kwiecinski et al. [13]. The main attention was paid to the HA molecular weight. Results showed that the reduction of friction caused by HA strongly depends on the HA molecular weight. Based on the results in Figure 2-7a, 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 2-7b). 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.

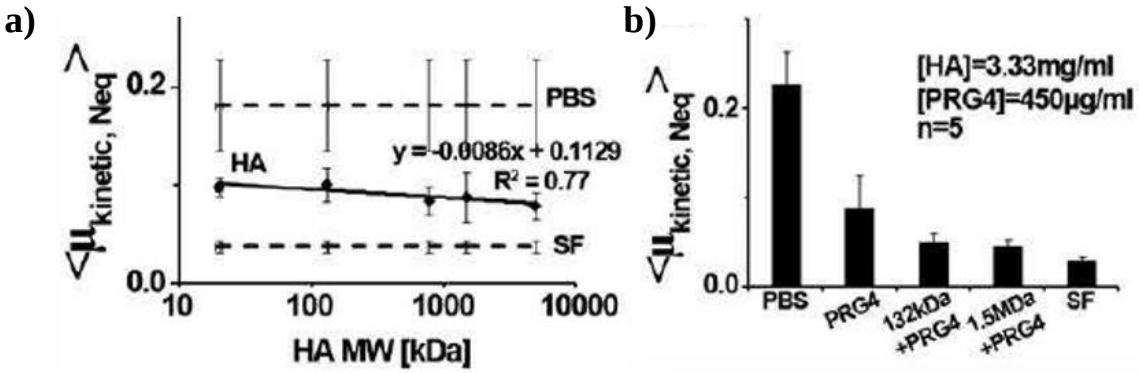

Figure 2-7 Dependence of the CoF on the HA molecular weight for: a) pure solutions, b) mixtures with PRG4 [13]

In another study by Murakami et al. [14], differences in friction between healthy and osteoarthritic cartilage lubricated by HA solutions were investigated. HA

solutions were also mixed with albumin and γ-globulin. Results (Figure 2-8) indicated 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. 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.

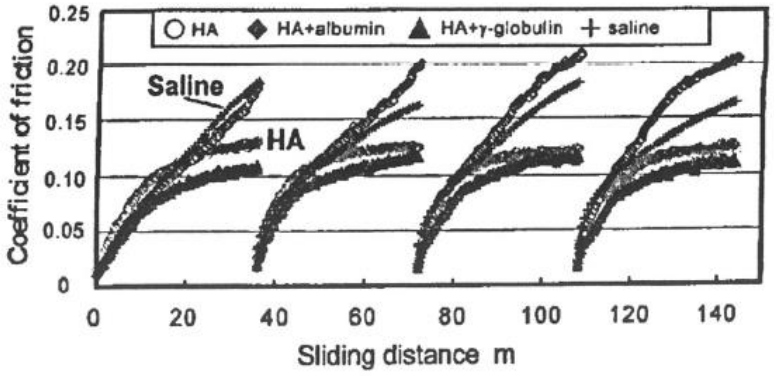

Figure 2-8 CoF for a HA solution mixed with proteins [14]

A complex study about the effect of individual SF constituents within the articular cartilage friction was published by Murakami et al. [15]. From the one-component solutions (Figure 2-9a), 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. 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 2-9b), which indicates the formation of complex structures formed by these two components. Each component of the SF has a role in cartilage lubrication. However, their interactions are also very important. The complex model SF exhibited the lowest values of the coefficient of friction.

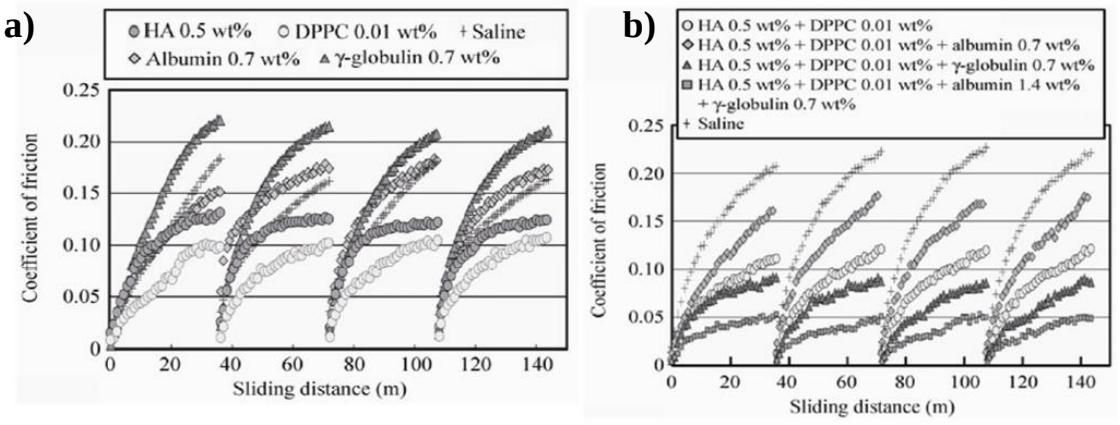

Figure 2-9 Frictional behavior of: a) one-component solutions, b) complex solution [15]

The concentration-dependent frictional behavior of γ-globulin and HA within the boundary lubrication regime was also investigated by Park et al. [16]. Results of all fictional measurements are summarized in Table 2-1. 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.

Table 2-1 CoF values for human cartilage [16]

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. [12] were further investigated by Ludwig et al. [17]. 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 were used as lubricants. Results (Figure 2-10) 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 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.

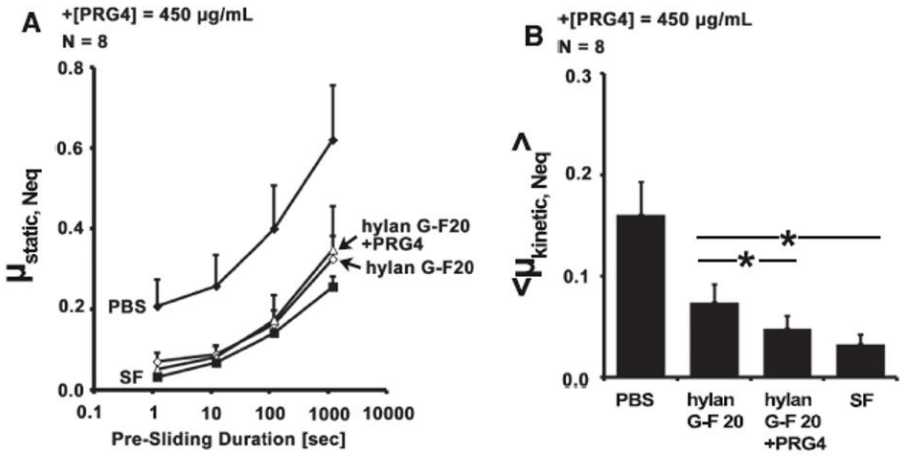

Figure 2-10 Effect of Hylan G-F 20 on: a) Static CoF, b) Kinetic CoF [17]

In another study, Bonnevie et al. [18] investigated the role of HA and PRG4 within articular cartilage lubrication. PBS, HA (500 – 730 kDa, 10mg/ml), hexadecyl derivate of HA (HYADD4, 8mg/ml) and rh-Lubricin (20 µg/ml) were used as lubricants. 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. 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.

Another mechanism of synergy, this time between HA and phospholipids, was introduced by Seror et al. [19]. 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. 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 2-11) composed of HA and DPPC is an essential thing.

Figure 2-11 Scheme of HA-DPPC synergy [19]

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. [20] 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, hydrogenated soy phosphatydylcholine (HSPC) and dopamine. Based on the result in Figure 2-12, 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.

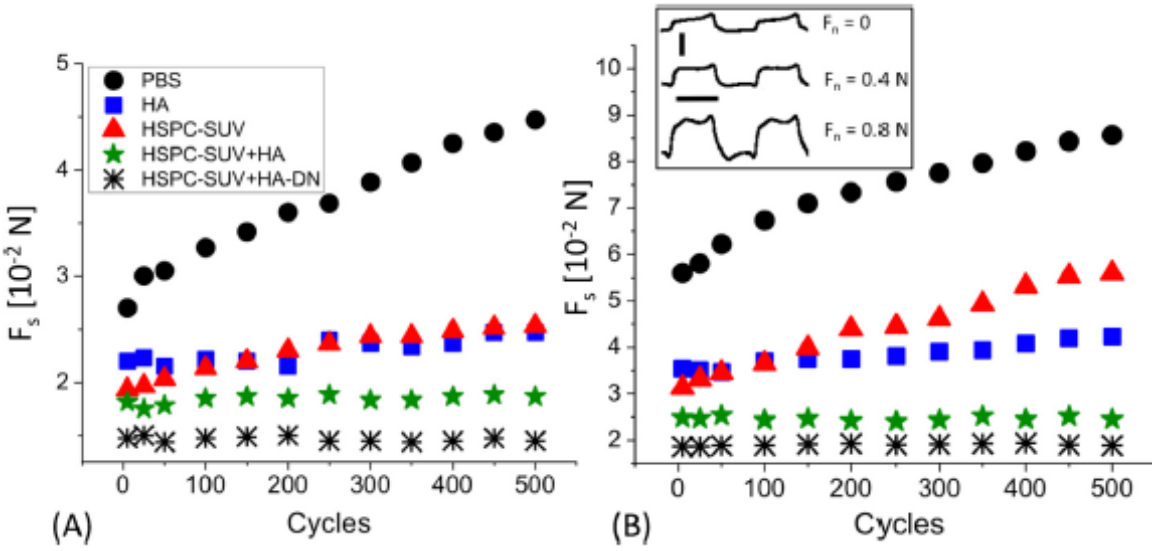

Figure 2-12 Friction force as a function of cycles number under a normal load of: a) 0.4 N; b) 0.8 N [20]

Liu et al. [21] 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 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.

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 dealt with the friction of VSs was published by Cherniakova et al. [22]. The results of the CoF measurements are shown in Figure 2-13. 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 rubbing surface during the experiement. 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.

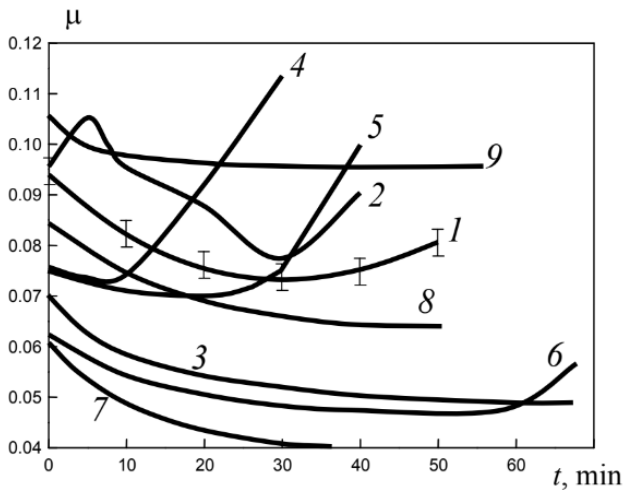

Figure 2-13 CoF of lubricating drugs: 1 – hydrocortisone, 2 – Kenalog®-40, 3 – Diprospan®, 4 – lincomycin, 5 – Synvisc®, 6 – Hyalgan®, 7 – SF + blood serum, 8 – hydrocortisone + blood serum, 9 - chondrosamine [22]

Results of a study combining rheological and tribological analysis of the commercial VSs were published by Bonnevie et al. [23]. In total, six commercial VSs were tested. The main conclusion was that the widely varying rheological properties of the tested VSs (Figure 2-14a) did not predict their frictional behavior within articular cartilage contact (Figure 2-14b). Adsorption of HA on the cartilage surface may cause a previously reported local increase in viscosity [18]. 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.

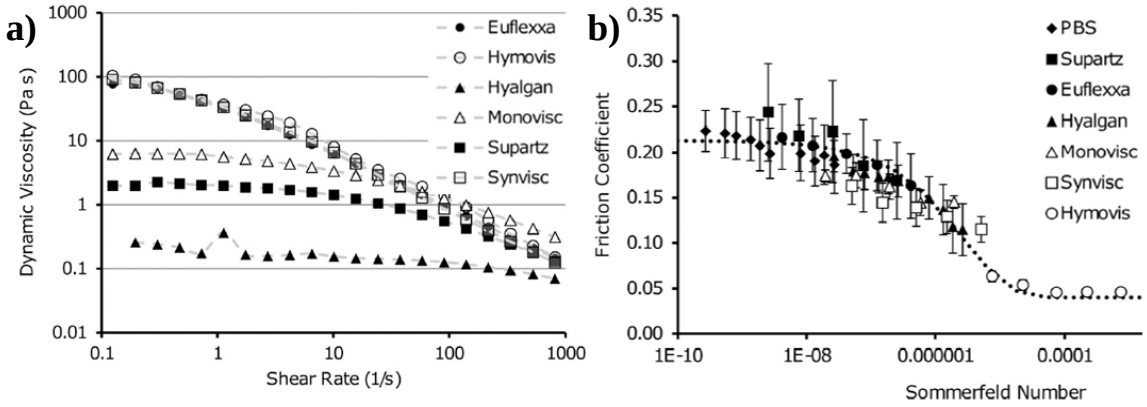

Figure 2-14 a) Viscosity curves of tested VSs, b) CoF as a function of the Sommerfeld number [23]

2.4 ARTIFICIAL ARTICULAR CARTILAGE

For articular cartilage friction and lubrication research, natural articular cartilage is occasionally replaced by artificial materials like PVA hydrogels. One of the first studies about PVA hydrogel as artificial cartilage was published by Nakashima et al. [24]. Based on the results, a protein adsorption model was introduced. 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.

Further in situ observation and frictional measurements of PVA hydrogel-on-glass contact were performed in a study by Murakami et al. [25]. The main attention was paid to the role of albumin:γ-globulin ratio within the formation of a boundary lubricating layer. Results showed relatively weak adsorption of albumin. 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 2-15) 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.

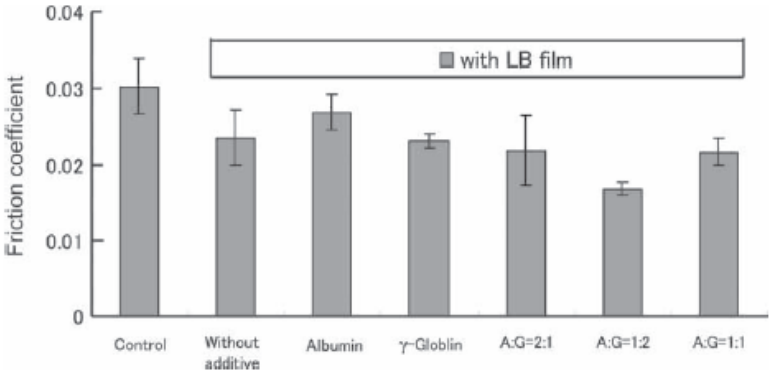

Figure 2-15 CoF values for protein solutions [25]

Previous studies were further expanded by Yarimitsu et al. [26]. 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.

The role of phospholipid DPPC within the lubrication of hydrogel-on-hydrogel contact was also investigated by Yarimitsu et al. [27]. CoF was reduced in a concentration-dependent manner for simple DPPC solutions. 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.

Previously mentioned results were expanded by another study by Murakami et al. [28]. The roles of individual SF constituents and their mixtures within PVA hydrogel friction were investigated. Two types of PVA hydrogels prepared by freeze-thawing (FT) and cast-drying (CD) methods were also compared with porcine articular cartilage. As shown in Figure 2-16a, the articular cartilage and PVA-FT hydrogel exhibited low initial friction with a subsequent gradual increase while PVA-FT hydrogel exhibited higher values of CoF. On the other hand, PVA-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. 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 2-16b) can maintain low friction with or without the addition of proteins albumin and γ-globulin.

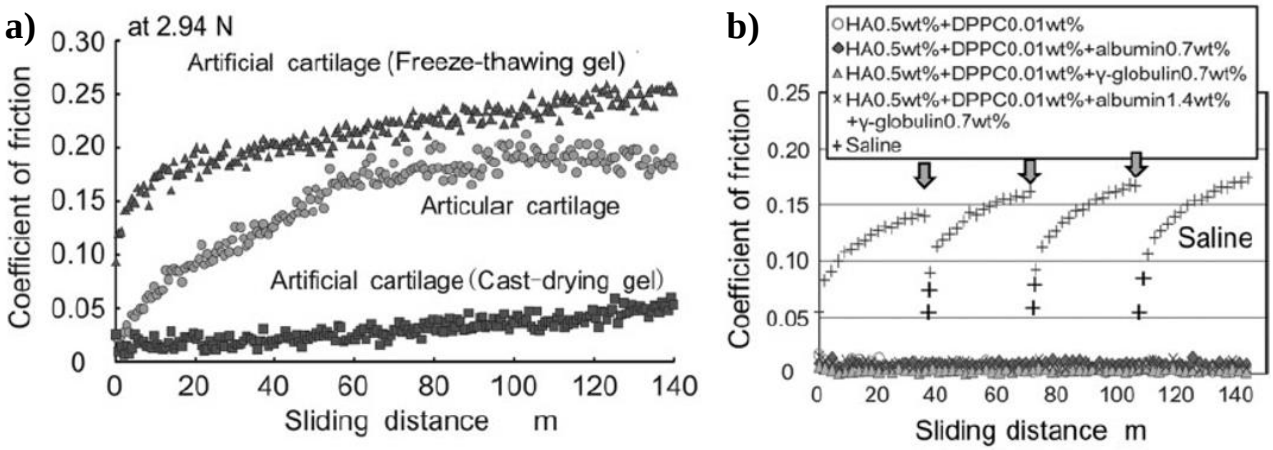

Figure 2-16 a) Friction of articular and artificial cartilage in saline, b) Influence of synovia constituents combinations on the friction of FT PVA hydrogel [28]

3 SUMMARY AND CONCLUSION OF STATE OF THE ART

During the progression of OA, the SF rheological properties are deteriorated due to a decrease in HA concentration [3] 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. [6], zero shear viscosities of a healthy SF in a range between 1 Pa·s and 175 Pa·s were measured. Bingöl et al. [5] reported an even higher zero shear viscosity of 445 Pa·s. 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. [4] varied by 3 orders of magnitude between 0.1 Pa·s and 10 Pa·s. As a result of lower HA concentration and molecular weight, SF viscoelastic properties are also aggravated. For example, Balazs [29] reported a crossover frequency of 0.4 Hz for a healthy synovial fluid. Mazzucco et al. reported a crossover frequency of 1.8 Hz for patients undergoing primary arthroplasty. The higher crossover frequency of equine osteoarthritic SF was also reported by Tyrnenopoulou et al. [3].

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 [7, 9, 10, 23]. Significant differences in viscosity between linear and cross-linked HA are also evident [7, 9, 10]. 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 [7, 9]. 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.

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 [25, 30]. On the other hand, γ-globulin exhibited a highly hydrophobic nature. It forms an even and stable lubricating film on the cartilage surface [25, 30]. During boundary lubrication, this protein layer protects the articular cartilage surfaces against direct contact of rubbing surfaces. When proteins are mixed, the presence of albumin in solution reduces the adsorption properties of γ-globulin [26]. 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. [12] reported a HA concentration-dependent decrease in articular cartilage friction. The reduction in cartilage friction lubricated by HA should also depend on HA molecular weight. In a study by Kwiecinski et al. [13], 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. In a study by Liu et al. [21], 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. This phenomenon was attributed to the higher adhesion energy between HA coated mica surface.

When HA and proteins were mixed, a significant difference between albumin and γ-globulin was reported [14]. 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 [15]. 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 [12, 13, 15, 17].

Bonnevie et al. [16] 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. [19]. 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 [20]. 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. [22] 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. [23] 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.

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. [28] 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. The lubrication of PVA hydrogels was intensively studied by Murakami et al. [25, 28] and Yarimitsu et al. [26, 27]. 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 AIM 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.

1.1 SCIENTIFIC QUESTIONS

Q1 What is the effect of changes in SF composition due to OA on the friction of articular cartilage?

Q2 How are the viscosity and viscoelastic properties of HA solutions connected with the friction of articular cartilage?

Q3 How is the friction of articular cartilage influenced by the molecular weight of HA contained in the VSs?

1.2 HYPOTHESES

H1: *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 [13, 18] or phospholipids

[15, 19]. Due to the decrease of HA concentration, formation of this layer worsens. This will lead to a higher CoF within contact.

H2: *The higher viscosity of HA and VSs will cause a more pronounced decrease of friction within a synovial joint model.*
The higher molecular weight of HA solutions or VSs leads to a higher value of zero shear viscosity [7, 9, 10]. Kwiecinski et al. [13] reported an proximately 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.

H3: *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.

1.3 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.

A. 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).

B. 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).

C. 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)**

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 - 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. 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 consists 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 an 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.

5.1.2 Pin-on-plate tribometer

For the frictional measurements, commercially available tribometer UMT TriboLab was used. All frictional measurements were conducted in a pin-on-plate configuration (Figure 5-1). 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 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. Cartilage samples were mounted in a loading mechanism of the tribometer. Two types of cartilage were used during the experiments - 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 which is mounted in the loading mechanism of the tribometer. From these data, the values of CoF were calculated.

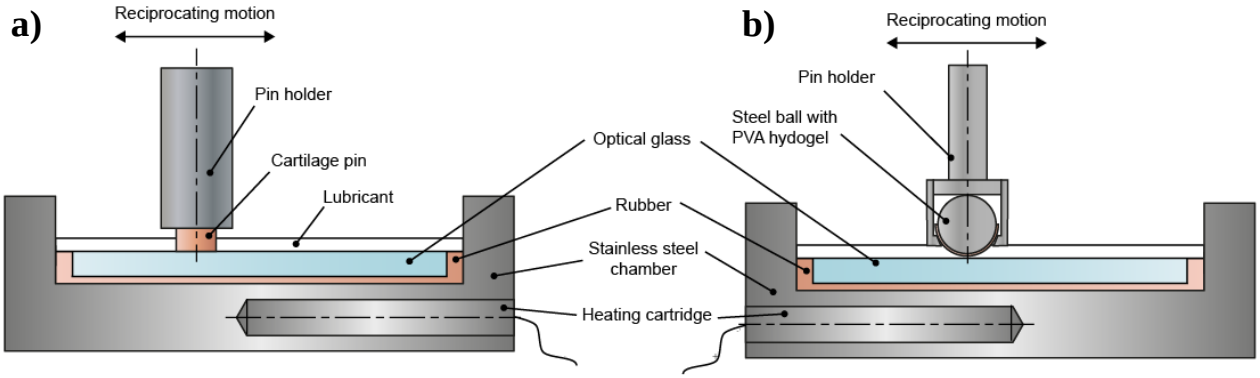

Figure 5-1 Schemes of frictional measurements: a) cartilage-on-glass, b) PVA hydrogel-on-glass

5.2 TEST SAMPLES

5.2.1 Lubricants

The first paper was focused on the effect of SF composition on friction within articular cartilage 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. Table 5-1 displays the concentration of individual constituents in model SFs. During the solutions preparation, individual constituents were dissolved in PBS overnight at 4 °C using a rocker-shaker. Consequently, the solutions were mixed together and deeply frozen at -22 °C. Before the experiments, test tubes with solutions were thawed at laboratory temperature.

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

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

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 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 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. From the range of products which were currently available in the Czech Republic, Erectus®, Hyalgan®, Monovisc®, Optivisc Single® and Synvisc One® 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. 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.

The methodology of PVA hydrogel preparation, as artificial articular cartilage, was based on the study by Yarimitsu et al. [31]. The first step was the preparation of PVA 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 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.

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. 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.

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. 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. During the 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.

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 small amplitude oscillatory shear (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, 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. [15] 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. [25], 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. [14] 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. [27]. 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.

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 ± 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 [32] whereas zero shear viscosity of the osteoarthritic SF ranges from 0.01 to 11 Pa·s [4, 7, 33]. 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} [6, 8]. Three out of four tested HA samples were consistent with results for commercial VSs. Nicholls et al. [10] 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 [29]. 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 [9]). 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 [34, 35]. 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. [13] 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. 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 [14, 18, 31]. On the other hand, the reactions between HA and phospholipids are crucial for the effectiveness of HA within articular cartilage friction [11, 12, 15]. According to the hydration lubrication theory by Klein et al. [19, 36, 37], 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®. The viscosity of VSs varied by the order of magnitudes.

Some of the tested VSs fell beneath the reported range for healthy SF viscosity [32]. Therefore, they should not be able to restore healthy SF rheology. Shear-thinning behavior 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. [32]. None of the tested VSs fell inside this range.

Another part of the rheological measurements was the analysis of viscoelastic properties. VSs exhibited all three types of viscoelastic behavior. Monovisc® and Erectus® exhibited a purely viscous behavior. 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 [9]. 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 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 [11] or molecular weight [13] 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. [11], low molecular weight HA can penetrate deeper layers of cartilage structure. Liu et al. [21] 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.

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:

H1: *"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.**

H2: *"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.**

H3: *"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.**

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CURRICULUM VITAE

Ing. David Rebenda
Date and place of birth: 13. 7. 1992, Hodonín

Education

- **2016 – 2021** Doctoral study at Institute of Machine and Industrial Design, Faculty of Mechanical Engineering, Brno University of Technology.
  Topic of the dissertation thesis: *Effect of Viscosupplementation on Friction of Articular cartilage.*
- **2014 – 2016** Master study at Institute of Machine and Industrial Design, Faculty of Mechanical Engineering, Brno University of Technology. Topic of the diploma thesis: *Lubrication of Hip Joint Replacements*.
- **2011 – 2014** Bachelor study at Faculty of Mechanical Engineering, Brno University of Technology. Topic of the bachelor thesis: *Design of Mechanism for Pronation and Supination of the Upper Limb Prostheses.*
- **2003 – 2011** Secondary Grammar School, Břeclav.

Teaching activities – seminars:

- Machine design – Machine Elements (5KS)
- Machine design – Mechanical Drives (6KT)
- Mechanical Design Project (ZIP)
- Team project (ZKP)
- Tribology (ZTR)

Participations in scientific projects

- 2017 – 2019: Study of the influence of lubricants rheology in elastohydrodynamic lubricated contacts (FSI-S-17-4415).
- 2017 – present: Influence of tribological processes on the service life of knee replacements (LTAUSA17150)
- 2020 – present: Study of tribological properties of small joint replacement (FSI-S-20-6443)
- 2020 – present: An Investigation of synovial Fluid Viscosupplementation and its Impact on Friction and Lubrication (20-00483S)

Language skills

Czech, English, German

Scientific activities

- Lubrication and friction of hip joint replacement
- Friction of articular cartilage
- Rheology of hyaluronic acid

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 propertiesof 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.

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.