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

David Rebenda [a,*], Martin Vrbka [a], David Nečas [a], Evgeniy Toropitsyn [b,c], Seido Yarimitsu [d], Pavel Čípek [a], Martin Pravda [b], Martin Hartl [a]

[a] Faculty of Mechanical Engineering, Brno University of Technology, Technická 2896/2, 616 69 Brno, Czech Republic
[b] Contipro a.s., Dolní Dobrouč 401, 561 02 Dolní Dobrouč, Czech Republic
[c] Biocev, First Faculty of Medicine, Charles University, Průmyslová 595, 252 50 Vestec, Czech Republic
[d] Faculty of System Design, Tokyo Metropolitan University, 1-1, Minami-osawa, Tokyo 192-0364, Hachioji, Japan

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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.

1. Introduction

A natural articular cartilage is a biphasic material composed of an extracellular matrix [1] with a high content of water. Thanks to its unique structure and synergy between the solid and fluid phase, 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. In general, the cartilage lubrication mechanism is called adaptive multimode lubrication [4,5]. It assumes that under certain circumstances, boundary lubrication [6,7], biphasic lubrication [8,9], hydration lubrication [10,11] and others may occur.

During biphasic lubrication, exudation of interstitial fluid from cartilage contributes to the fluid film formation [12]. Therefore, time-dependent frictional behavior is expected under compressive loading while the interstitial fluid pressurization is controlled by permeability and pressure. Low friction could be sustained for a long time if the fluid load support is preserved at sufficient levels. Therefore, migrating contact area [13], rehydration by cartilage unloading [14] or hydrodynamic effect [15] 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. It has already been reported that the main constituents of adsorbed film on cartilage surfaces are proteins [6,7,16], glycoproteins [17,18], hyaluronic acid (HA) [16,19,20] and phospholipids [6,20,21]. 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 [22,23]. Murakami et al. [24] reported that the adaptive multimode lubrication can be adapted for three types of PVA hydrogels – freeze-thawing (FT), cast-drying (CD) and hybrid. Li et al. [25] 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. [22], CD

* Corresponding author.
E-mail address: David.Rebenda@vut.cz (D. Rebenda).

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hydrogel-on-glass exhibited even lower friction than the cartilage-on-glass contact. Nakashima et al. [26] 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. However, 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 osteoarthritis [27]. The imbalance between the cartilage synthesis and wear leads to the progressive damage of cartilage tissue. This cartilage degeneration is surely connected with a distraction of cartilage lubricating mechanism [28,29] and changes in the composition of SF [30] 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 [31,32]. 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 [33,34]. Despite these various proven effects of intra-articular HA, clinical studies report inconsistent results of this treatment method in clinical practice [35]. For example, Maheu et al. [36] reported a reduction of pain after viscosupplementation. On the other hand, Bannuru et al. [37] or Jevsevar et al. [38] did not observe substantial differences between viscosupplements (VSs) and placebo or anti-inflammatory drugs. These conflicting conclusions also lead to inconsistent suggestions of international medical associations [39].

Many in vitro studies about viscosupplementation are focused on rheology. Higher concentration and molecular weight lead to higher viscosity and dynamic modulus [40,41]. Better results are also obtained for cross-linked hyaluronic based solutions [41,42]. However, the VSs in vivo efficacy cannot be fully described by flow properties measured by rotational rheometers. HA interacts with proteins and other constituents of SF [43] 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 [21]. Seror et al. [20] reported extremely low values of the coefficient of friction (CoF) 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 [14,44]. Despite all this evidence, not many tribological studies about VSs were published. Cherniakova et al. [45] 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. [46]. 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 a poly(vinyl alcohol) (PVA) hydrogel prepared by freeze-thawing method (PVA-FT hydrogel). We also hope that our conclusions will have an overlap in medicine and help orthopedists with the choice of viscosupplement (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, Fig. 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, Fig. 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 0.05–5 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.

2.2. Tested viscosupplements and model SF

In total, five different HA-based commercially available VSs (Fig. 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.

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. [30] 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

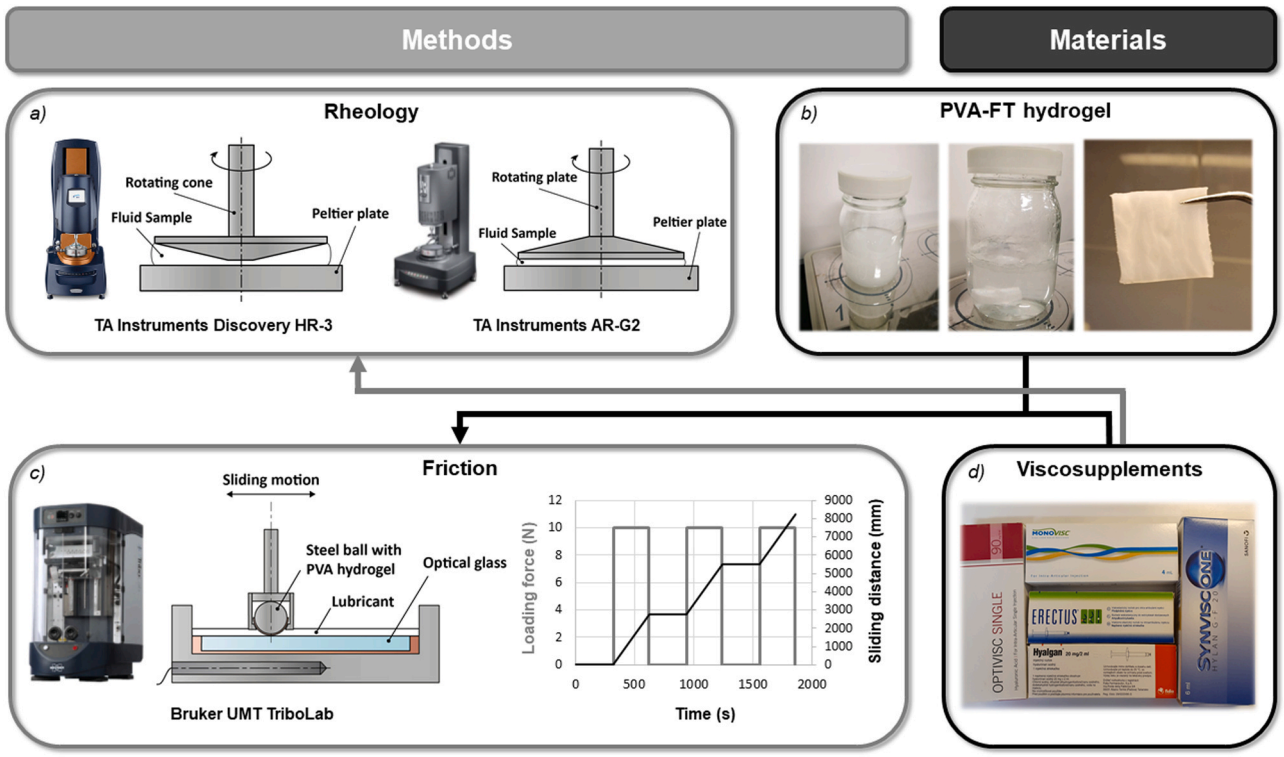

Fig. 1. Research plan: a) Rheological measurements, b) Preparation of PVA-FT hydrogel, c) Frictional measurements, d) Tested VSs.

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

(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 (Fig. 1c) while the 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 (Fig. 1c). Each phase lasted 300 s 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 s. 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 (Fig. 1b) was prepared according to the study by Yarimitsu et al. [47]. 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 h of freezing at -20 °C and 16 h 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 × 2 cm samples were carved from the hydrogel plate.

3. Results

3.1. Rheology

Firstly, steady shear experiments with all tested lubricants were performed. Fig. 2 contains data of shear rate-dependent viscosity for five tested VSs. They were tested as clear solutions (Fig. 2a) and also as mixtures in a 1:1 ratio with model osteoarthritic SF (Fig. 2b). As expected, a shear-thinning behavior was found in each of the VS. However,

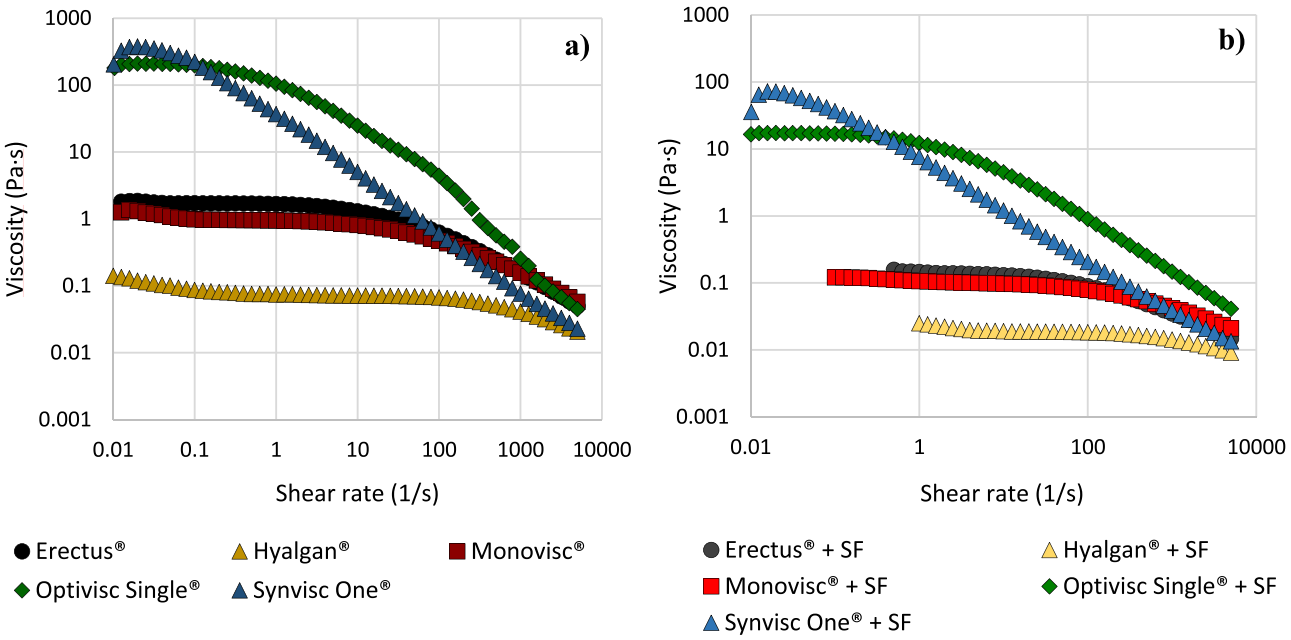

Fig. 2. Viscosity as a function of a shear rate: a) clear VSs, b) VSs mixed with model SF.

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.

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. Fig. 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.

Frequency sweep curves over a frequency range from 0.05 H to 5 Hz for clear VSs are shown in Fig. 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

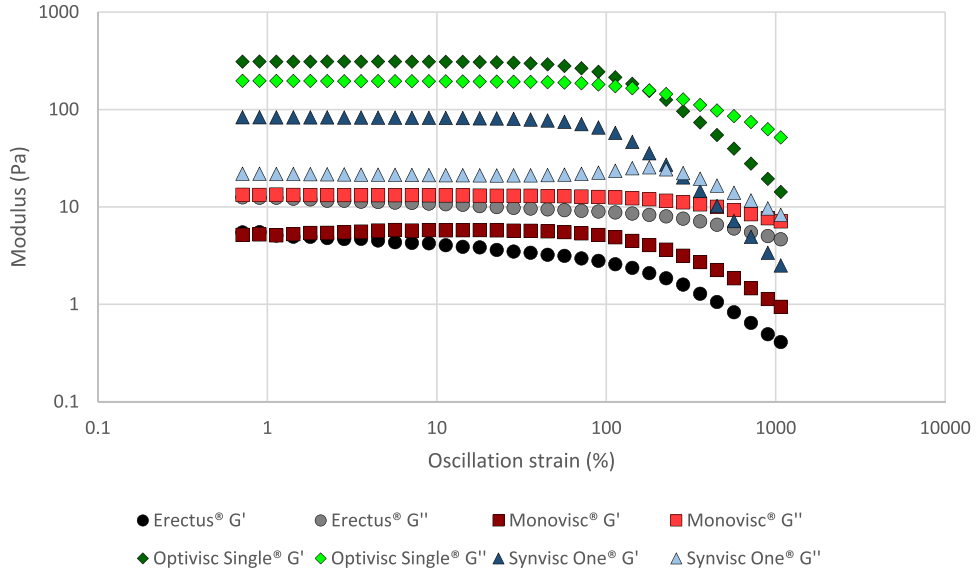

Fig. 3. Dynamic modulus as a function of strain at ω = 1 Hz.

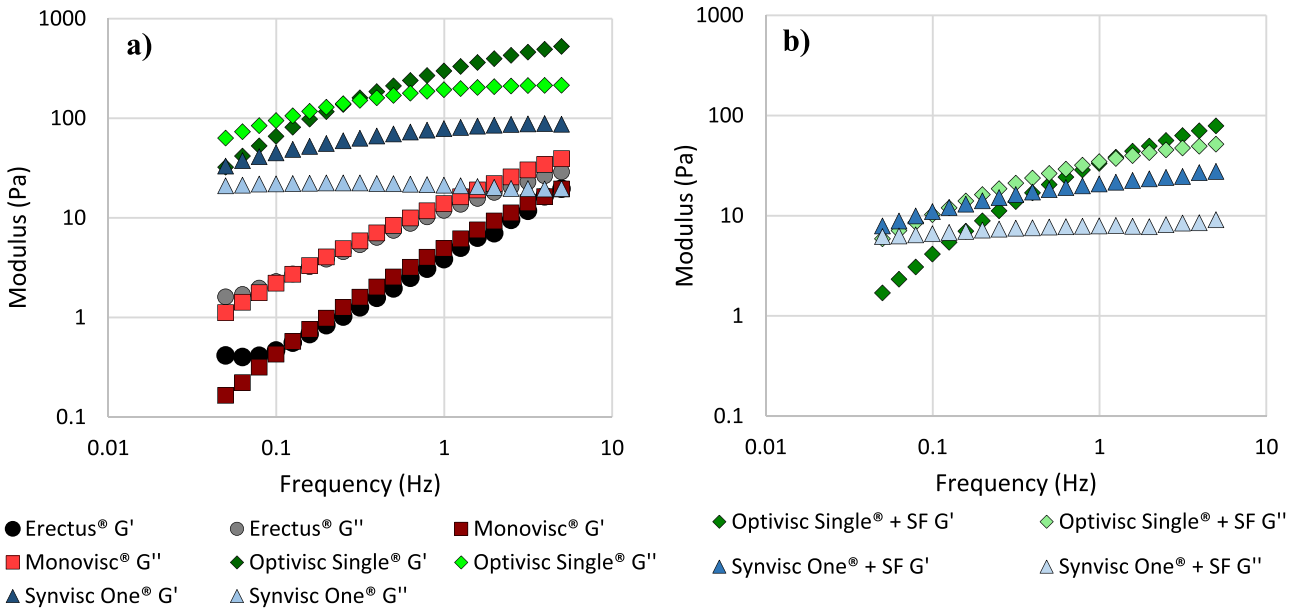

Fig. 4. Dynamic modulus as a function of the frequency: a) clear VSs, b) VSs mixed with model SF.

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.

Fig. 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.

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 Fig. 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® (Fig. 5a) exhibited a time-dependent frictional behavior similar to pure SF. On the other hand, values of CoF for Optivisc Single® (Fig. 5d) and Synvisc One® (Fig. 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.

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. [48], 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 [49]. 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) 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 [50]. 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. [51] reported zero shear rate viscosities of healthy SF in a range between 1 and

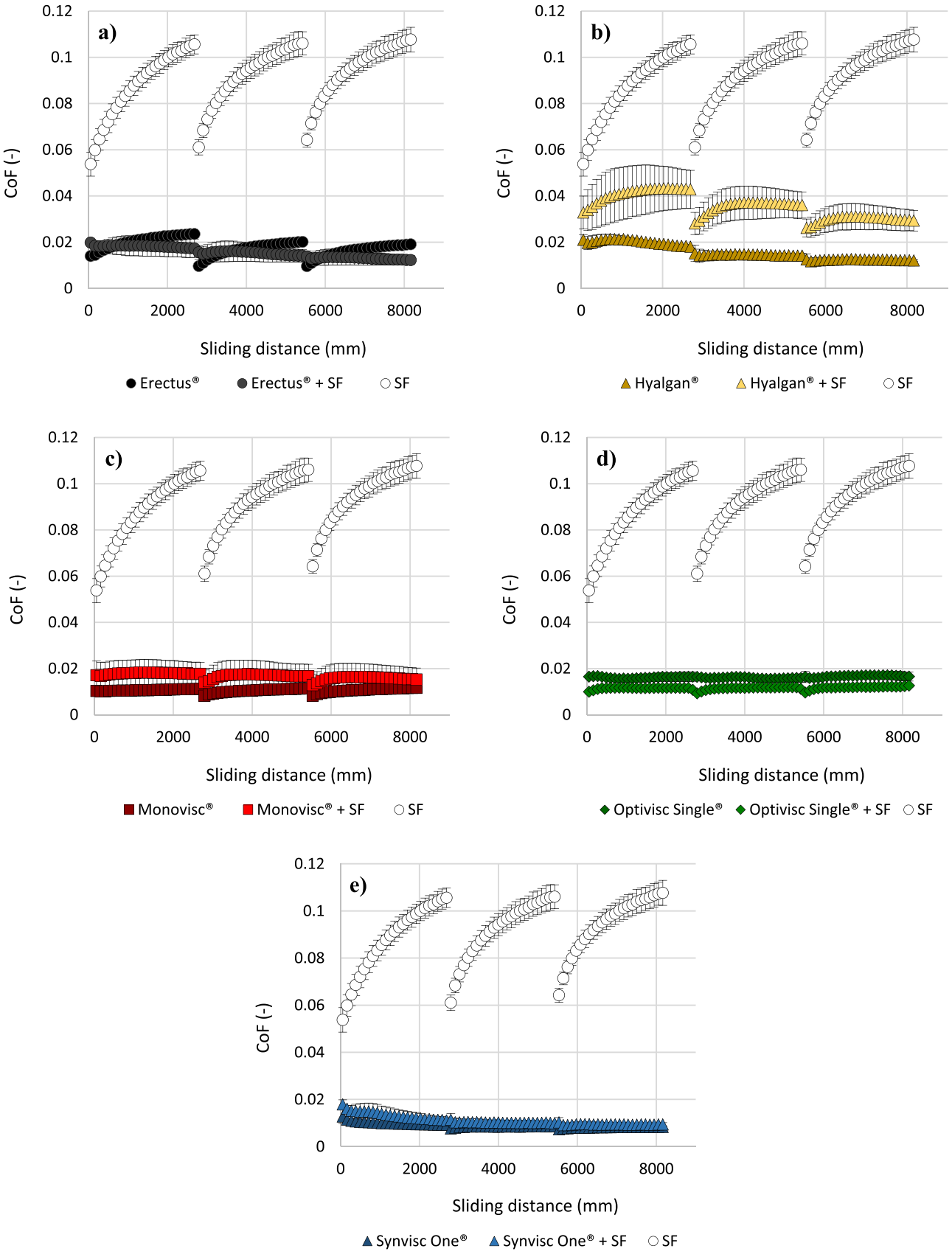

Fig. 5. CoF as a function of the sliding distance for clear VSs and mixtures with model SF: a) Erectus®, b) Hyalgan®, c) Monovisc®, d) Optivisc Single®, e) Synvisc One®.

175 Pa s. For comparison, zero shear rate viscosity of osteoarthritic SF ranges from 0.01 to 11 Pa s [41,52]. Some of the tested VSs fell beyond the range for healthy SF, even in their pure forms. From our results, only VSs based on a high molecular weight HA (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® with a molecular weight of 6 MDa and 37.76 ± 3.1 Pa s for Synvisc One® with a molecular weight of 3 MDa.

All tested solutions exhibited the non-Newtonian shear-thinning behavior. In general, VSs with higher molecular weight exhibited a stronger shear thinning behavior, i.e., the rate of viscosity decline with increasing shear rate was 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 [42,53]. 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® (molecular weight between 500 and 730 kDa) - 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. [51] 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.

The crossover frequency for healthy SF reported by Balazs et al. [54] was 0.41 Hz. Mazzucco et al. [52] 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 [55]. 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. In general, only VSs with very high HA molecular weight exhibited partial or complete elastic behavior during the measurements. Therefore, under certain conditions, they can absorb mechanical energy and protect the articular cartilage structure against direct contact of the rubbing surfaces.

Fig. 6 summarizes the values of CoF at the end of the frictional measurements. VSs significantly lowered friction compare to the pure SF. However, 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 [42,56] and concentration [57]. Dependency between HA molecular weight [58] or concentration [27] and friction within the cartilage contact was also reported. Still, no direct connection between viscosity and CoF was observed. These results are partially in agreement with our previous study [59] where no dependency between CoF within cartilage-on-glass contact and molecular weight of pure HA mixed with osteoarthritic SF was observed. HA tends to bind to lubricin on the articular cartilage surface and creates a highly viscous layer [18] whose viscosity can significantly vary from the viscosity measured by a rotational rheometer with stainless steel geometry. Human joints can also operate under shear rates up to 10^5 s^{-1} [35]. Under these conditions, the differences between individual VSs can change due to the different values of shear-thinning ratio or low viscosity plateau.

No clear dependency between the rheological and frictional properties of VSs was observed. From these two types of analyzes, frictional measurements seem to be more predictive of VSs clinical outcomes [46]. However, even for VS with the lowest exhibited values of CoF – Synvisc One®, the results of clinical studies are contradictory. Some of them reported long-term positive relieve of pain [60], whereas no clinical benefits over placebo [61] were also reported. Moreover, cross-linked VSs are connected with a higher incidence of post-injection effusion [62]. Same contradictory results of clinical outcomes were reported for VS with higher measured values of CoF - Hyalgan® [63,64].

Two types of frictional behavior were observed in Fig. 5. Solutions with low molecular weight (Hyalgan® of Erectus®) 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. In contrast, solutions with high molecular weight (Optivisc Single® or Synvisc One®) 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 [2,5], 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 [27]. Liu et al. [65] 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 (Fig. 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 Fig. 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 (Fig. 4a). Logarithmical shapes of their frictional curves in Fig. 5a and c rather correspond

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

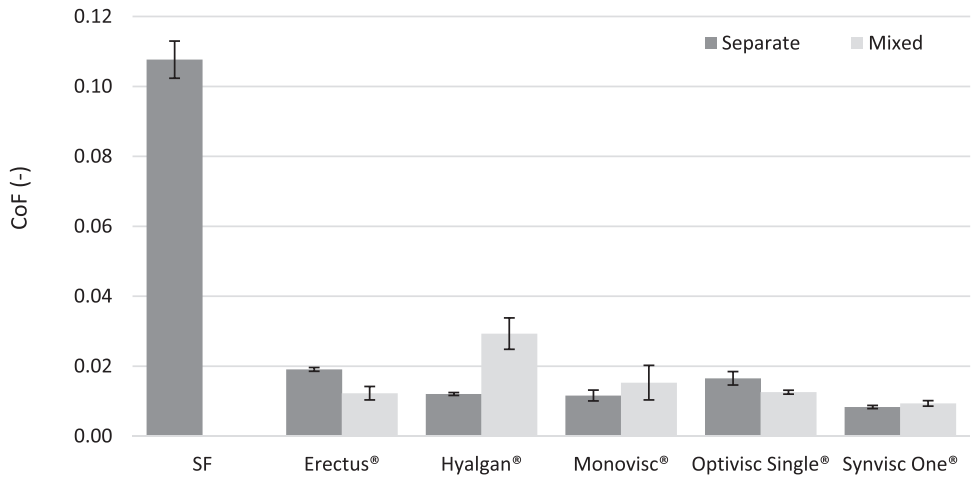

Fig. 6. CoF at the end of measurements for all tested lubricants.

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 (Fig. 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 [20]. Due to different electric charges, HA is also able to form complex structures with γ-globulin, which contributes to the lower friction [21].

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 Fig. 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 [40, 41, 52] 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 [30], and our previous study [66] 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 [25,67] 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 [68]. 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 [69]. 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.

CRediT authorship contribution statement

David Rebenda: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **Martin Vrbka:** Writing - review & editing, Supervision, Project administration. **David Nečas:** Conceptualization, Formal analysis, Writing - review & editing. **Evgeniy Toropitsyn:** Methodology, Validation, Formal analysis, Writing - review & editing. **Seido Yarimitsu:** Resources, Methodology, Writing - review & editing. **Pavel Čípek**: Methodology, Validation, **Martin Pravda:** Resources, Writing - review & editing. Supervision. **Martin Hartl:** Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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