THE EFFECT OF THE MOLECULAR WEIGHT OF HYALURONIC ACID ON THE RHEOLOGICAL AND TRIBOLOGICAL PROPERTIES OF THE BASE FOR ARTIFICIAL SYNOVIAL FLUID PREPARATIONS

Monika Izabela KARPOWICZ*, **Marcin KLEKOTKA***, **Jan Ryszard DĄBROWSKI***

*Faculty of Mechanical Engineering, Bialystok University of Technology, Wiejska 45C, 15-351 Białystok, Poland

monika.grykin@sd.pb.edu.pl, m.klekotka@pb.edu.pl, j.dabrowski@pb.edu.pl

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Abstract: The synovial fluid is responsible for adequately lubricating, moisturizing, and nutritional human joints. This liquid should have appropriate viscoelastic properties and ensure a low coefficient of friction in biotribological systems. Many artificial synovial fluid preparations used in viscosupplementation treatments are based on hyaluronic acid. This work aimed to evaluate the influence of molecular weight on the functional properties of solutions based on hyaluronic acid. 1% solutions based on hyaluronic acid with five different molecular weights from 50,000 Da to 2 MDa were made. Rheological (viscosity, viscoelasticity), tribological (coefficient of friction, wear assessment), and wettability tests were carried out. Significant differences were observed in the rheological tests, where the viscosity strictly depends on the molecular weight of the hyaluronic acid. It has been shown that the molecular weight of HA has little effect on the coefficient of friction. On the other hand, the differences in the tribological wear are much more significant. The molecular weight of biopolymers is one of the essential parameters in developing new artificial synovial fluids. Using a higher molecular weight of hyaluronic acid increases viscosity and wettability, resulting in less tribological wear.

Keywords: artificial synovial fluid, hyaluronic acid, viscosity, friction, wear

1. INTRODUCTION

A lubricant called synovial fluid is an essential component in the lubrication system of synovial joints. It is responsible for the proper functioning of joints and supports all movements performed by this biotribological system (1,2). Synovial fluid is a composition of blood plasma dialysate and particles secreted by cells in the synovial space. These particles include hyaluronic acid and proteoglycan 4 (lubricin and surface zone protein). (3,4). Albumin and globulin also play an important role in the composition of the synovial fluid. γ globulin particles combine with hyaluronic acid molecules. These ingredients are characterized by a high degree of interaction with each other. This combination exhibits strong adsorption with the substrate, creating a lubricating boundary layer. Albumin is mainly responsible for filling the lubricating layer by self-accumulation, while exhibiting low shear. Albumin has the ability to bind to the boundary layer, which is formed by globulin with HA molecules (5,6). The synovial fluid fills the entire joint cavity and mainly performs metabolic functions, i.e., it is responsible for the nutrition of the joint cartilage (3,4,7). It is a clear, viscous liquid with a slightly yellow color. Cellular and molecular components of synovial fluid describe unique properties in maintaining proper joint homeostasis (7,8). The volume of synovial fluid may depend on many aspects. This amount can be different for each person. This is mainly determined by the quality of a person's life, e.g. whether he or she practices sports or has a sedentary lifestyle. It also depends on genetic and physiological conditions. Lifestyle, diet and body posture also have a significant impact. Synovial fluid volume is reported in a wide range in the literature. Hui A.Y. (7) and Blewis M.E. (4) suggest that in the human knee joints, synovial fluid volume oscillates between 0.5 – 2 ml. Kraus V. (9) and Brannan S. (10) present that the volume of synovial fluid is around 4 ml. However, Gait A., et.al. (11) claim that in a healthy knee joint, the synovial fluid reaches a volume of up to 9.6 ml. Synovial joints are characterized by very complicated mechanics. Their very low motion resistance (μ = 0.001-0.01) makes it difficult to define the type of lubrication clearly. In joints, there is mixed lubrication, which consists of hydrostatic and boundary lubrication (12). Hydrostatic lubrication takes advantage of the unevenness on the joint surfaces and the viscosity of the lubricant provided by hyaluronic acid. It consists of squeezing the intercellular fluid out of the cartilage and creating a layer that separates the surfaces exposed to high friction (1,12). Boundary lubrication occurs mainly during periods of very high load at low speeds, i.e., when the lubricant film is the same as or less than the roughness of the joint surfaces (1,13). Boundary lubrication occurs at the boundaries of the articular surfaces, protecting the rubbing surfaces before cartilage decompression takes place, where fluid desaturation occurs, cartilage can increase in volume by up to 10% (1,12).

The synovial fluid is the main factor contributing to the synovial joints' longevity. The articular surfaces are characterized by very good lubrication and high wear resistance and usually show no signs of wear for most or even the whole life. Despite these advantages, in the case of any injury, the healing of the joint is complicated and time-consuming. The joints in the human body are subject to many debilitating diseases. Any health problems related to the incorrect chemical composition of the lubricant or its incorrect secretion can lead to biological damage to the synovial joints caused by excessive friction (1,2,13–19).

Due to its high biocompatibility, hyaluronic acid is widely used

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in medicine and bioengineering (20). Hyaluronic acid is a negatively charged biopolymer. It consists of alternating units of D-glucuronic acid and N-acetylglucosamine. In a healthy synovial joint, its concentration is about 1-4 mg/ml (3,12,21). Hyaluronic acid is mainly responsible for rheological and tribological properties. Molecular weight plays an important role in the organization and arrangement of hyaluronic acid molecules and ensures the ability of synovial fluid to dissipate energy (8,22–24). In addition, many nanotribological studies suggest that the chemical bonding of hyaluronic acid particles with the joint surface is necessary to reduce friction in the biotribological system. As a result, it also reduces the wear of the articular surfaces (1,3). Natural synovial fluid has a molecular weight of 6 000 – 7 000 kDa, while synovial fluid with rheumatoid disease has a lower mass of 3 000 – 5 000 kDa. When any chemical or physical disorder occurs in the synovial joints, the quality of hyaluronic acid deteriorates. This is related to reduced lubricating capacity and reduced rheological properties. (25,26). The properties of hyaluronic acid depend on the molecular weight. With a molecular weight of 0.4 - 4 kDa, hyaluronic acid has non-apoptotic properties and is an inducer of heat shock proteins. At higher molecular weights, such as 20 - 200 kDa, biological processes, such as wound healing or embryonic development and ovulation, are already involved. With a molecular weight above 500 kDa, hyaluronic acid can be used as a space filler (20,27–29). Hyaluronic acid also shows the ability to improve surface wettability, which is a crucial aspect during lubrication. Wettability is related to surface friction; higher wettability, lower wear (30).

In the event of a disease or injury to the synovial joint, there is immediate inflammation and a very large reduction in the viscosity of the synovial fluid. As a result, the lubrication system deteriorates, which can lead to the destruction of the articular surfaces (8). Viscosupplementation is the most commonly used method for treating joint pain caused by the disease. It is a procedure in which preparation is injected into the joint cavity to replace the natural synovial fluid (8,31–37). The literature confirms the appropriateness of administering hyaluronic acid during viscosupplementation. It is effective, especially during degenerative synovitis. Hyaluronic acid improves the fluidity of the synovial fluid, inhibits the degradation of HA in the remains of the natural synovial fluid, increases the viscosity of the synovial fluid, and additionally helps to fight inflammation and relieve pain in the joint (7,8,12,13,21,38).

Although there has been a lot of research into the development of artificial synovial fluid, there are still many aspects that would be good to expand upon. Many of the substitutes used in viscosupplementation today provide relief to patients, but the results are very short-lived or inadequate. For this reason, research has been conducted on the potential basis of artificial synovial fluid. This manuscript focuses on the molecular weight of hyaluronic acid and its influence on the rheological and tribological properties of tested solutions.

Hyaluronic acid with a mass of 750kDa - 1MDa is within the molecular weight range of one of the currently used commercial preparations, which is Hyalgan (39). To demonstrate the differences in lubricity, the molecular weights of the hyaluronic acids were examined over a fairly wide range from 30 kDa to 2 MDa. After dissolving HA in water, the solutions were mixed on a magnetic stirrer until a homogeneous mixture was obtained. All preparations were stored in closed glass containers in a laboratory incubator at 37oC for 24 hours before the test. Each solution was tested three times for statistical purposes.

2. MATERIALS AND METHODS

To evaluate the influence of the molecular weight of hyaluronic acid on the rheological and tribological properties, the solutions shown in Tab. 1 were prepared.

Tab. 1. Chemical composition of hyaluronic acid solutions

Concentration (wt %)	Molecular Weight Hyaluronic acid	Solvent
1 %	30 000 – 50 000 Da (Chemat, CM61910C)	deionized water
	50 000 – 100 000 Da (Chemat, CM00050C)	
	200 – 400 kDa (Chemat, CM01180C)	
	750 kDa – 1 MDa (Chemat, CM77330C)	
	1 – 1,5 MDa (Chemat, CM45820C)	
	2 MDa (Chemat CM39020C)	

Rheological tests were performed on a Rheostress 6000 (Thermo Fisher Scientific, Waltham, MA, USA). Measurements of viscosity and viscoelasticity were conducted in the titanium plate-plate system at 37oC (Fig. 1).

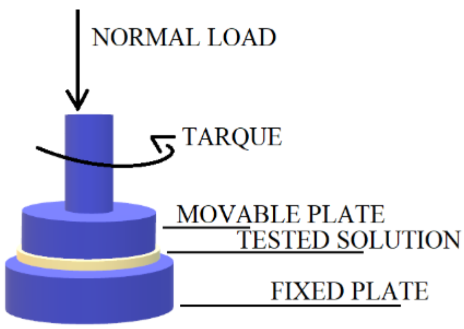

Fig. 1. Scheme of rheological measurement system

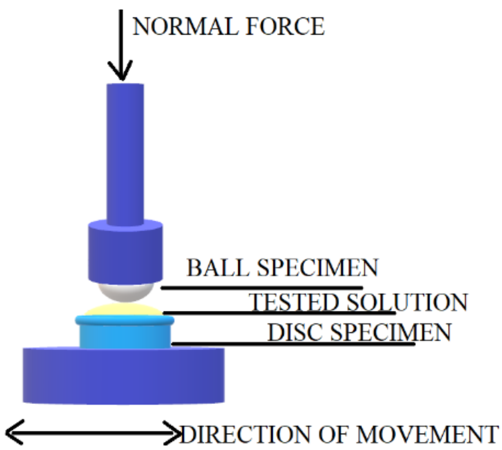

Fig. 2 Ball-on disc tribological system

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A moving plate with a diameter of 35 mm was used during the viscosity and viscoelastic tests. The gap between the movable and fixed plates was 1 mm. 1 ml of the solution was used for each test. Viscosity was tested in the range of 0.01 - 100 1/s. In the viscoelastic tests, the loss and storage modulus were determined at a constant strain value of γ = 0.01, in the frequency range of 0.1 – 10 Hz. All measurements were repeated three times to confirm the reliability of the study, and the results were presented as the average value of all samples.

Tribological tests were performed on a UMT TriboLab (Bruker, Billerica, MA, USA) with a ball-on-disc system (Fig. 2).

A metal-ceramic rubbing pair was used in the friction node. The sample was CoCrMo discs (Tab. 2) with a height of 5 mm and a diameter of 8 mm, while the counter-sample was an aluminum oxide (99,5% Al2O3) ball with a diameter of 6 mm.

Table 2. Chemical composition of the CoCrMo sample

Co	Cr	Mo	Mn	Si
rest	27,72	5,78	0,65	0,37
	C	**Al**	**Zr**	**Ti**
	0,036	<0,02	<0,01	<0,01

Before the tribological test, the metal samples were thoroughly ground and polished to a mirror effect. Sandpaper with gradations of 2 500, 3 000, and 5 000 was used for grinding, while Al2O3 suspension was used for polishing. Before the measurement, each sample was washed in an ultrasonic bath in ethanol and deionized water. In tribological tests, a special holder connected to a thermostat was used to maintain the temperature of 37oC. Measurements were made in reciprocating motion at a constant frequency of 2 Hz for 30 minutes. The normal force was 5N. All measurements were repeated three times to confirm the reliability of the study, and the results were presented as the average value of all samples.

Analysis of wear marks was performed using a LEXT OLS4000 confocal microscope (CLSM, Olympus, Tokyo, Japan). The laser in a microscope scans horizontally, but it does it layer by layer. The scanning step pitch was 0.05 µm. The size of the scanned surface, including the friction point, was 1400x480 µm. The images were taken without any filters. The measurements were carried out using special computer software cooperating with a microscope that allows it to work in 3D space and evaluate the volume of wear tracks.

Contact angles were determined using an Ossilla goniometer. A drop of 5 µl of the tested solution was applied to each CoCrMo metal sample using a micropipette (Fig. 3).

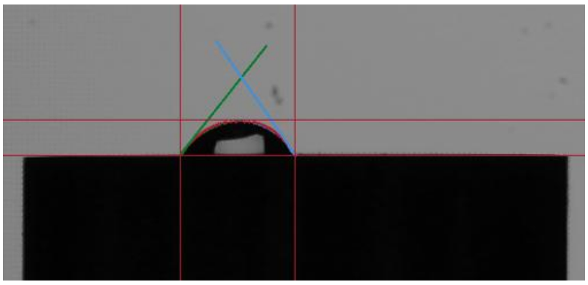


Fig. 3. The image of the sample droplet application and the designated angles

Then, the contact angles were determined using specialized goniometer software cooperating with a computer. The red lines at the bottom of the camera image (Fig. 3) indicate the area of the drop. These are lines corresponding to the border points of the preparation drop, which can be set with a slider. The green and blue lines indicate the tilt angle, which allows the left and right angles to be determined. The average of these angles gives the contact angle.

3. RESULTS AND DISCUSSION

The effect of molecular weight on the viscosity of tested solutions is shown in Fig. 4.

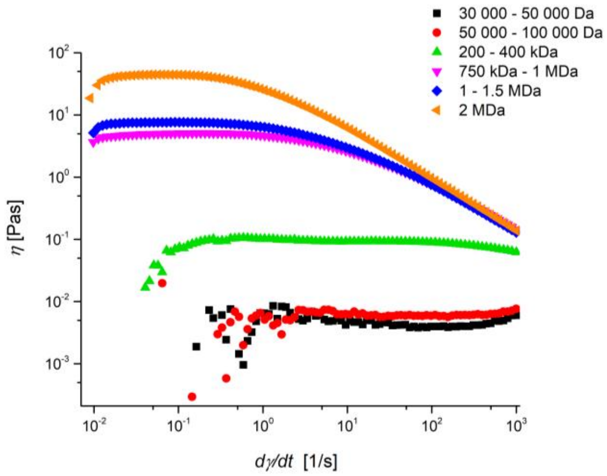
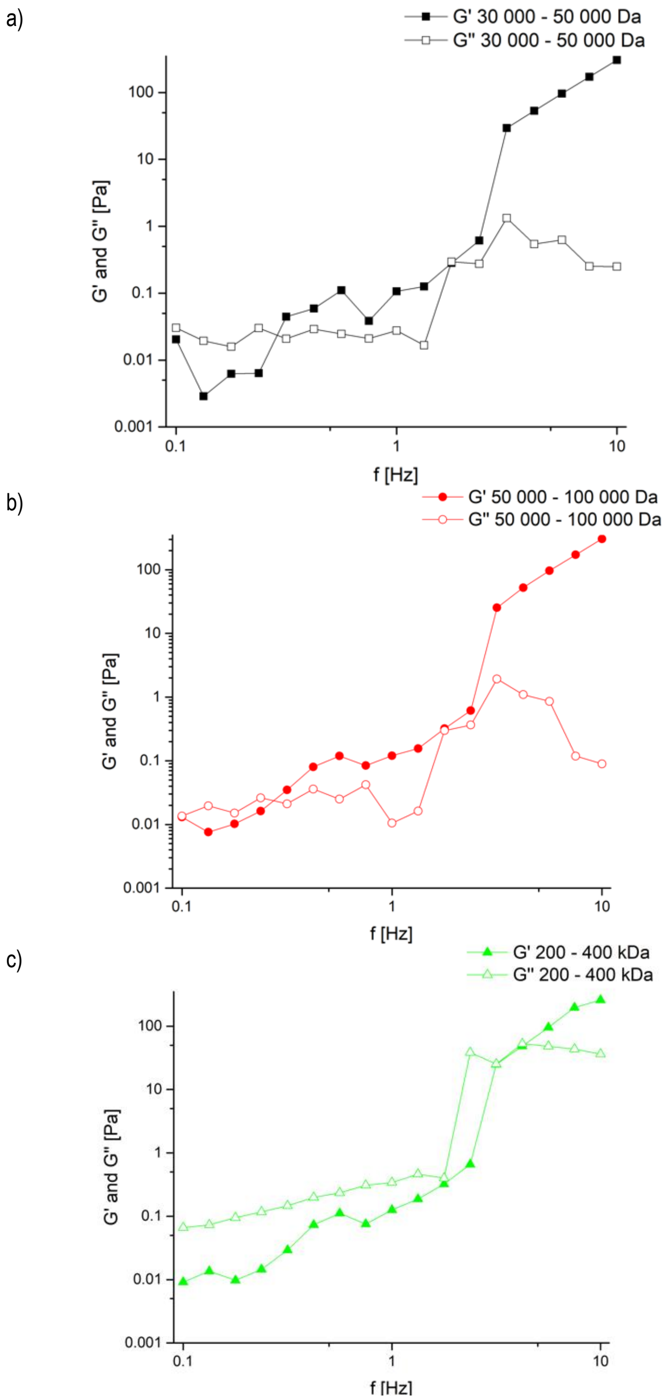


Fig. 4. Viscosity of hyaluronic acid based solutions

The viscosity of tested solutions increases with increasing molecular weight, which was confirmed by Snetkov et al. (20). High molecular weight hyaluronic acid exhibits interesting rheological properties by forming a tangled network of flexible polysaccharide molecules. Viscosity is a measure of the flow resistance of a given solution. This means that the molecular weight affects the flow. The lower the molecular weight, the lower the effect on flow, and therefore the lower the viscosity (40). It can be seen that preparations with a higher molecular weight in the range of 750 kDa to 2 MDa are in the viscosity range of natural synovial fluid (1 - 175 Pas) (31). According to the literature, solutions with a viscosity below 1 Pas are considered almost watery, while above this value, solutions are considered viscous (40). Three solutions with the highest molecular weights show a downward trend in viscosity with increasing shear rate. This indicates that these solutions are non-Newtonian fluids, consistent with the behavior of naturally occurring hyaluronic acid in the synovial fluid. The literature shows that the synovial fluid is non-Newtonian because the dynamic interactions between individual hyaluronic acid polymers depend mainly on the shear rate (21,40). Dynamic viscosity is constant at lower shear rates. As the shear rate is increased, it is observed that the solutions are characterized by fluid dilution adequate to the increase in shear rate. Shear-thinning effect occurs. This is characteristic of pseudoplastic fluid (21,41,42). At low shear rates, strong intermolecular interactions occur, creating strong network entanglement, which results in high flow resistance, i.e. high viscosity. However, at high shear rates, intermolecular interactions weaken, which is associated with the disentangling of molecular networks. This causes the resistance to decrease and the particles to align in the direction of flow, assuming a much lower viscosity (43). Shear rate is a measure of flow. Fluid shear occurs whenever it is forced to move. The highest shear rate tested corresponds to the shear rate of the fluid passing through the syringe. Therefore, the viscosity drops significantly and is independent of the molecular weight. This is due to the arrangement of the particles in the flow current lines. This is a desirable effect in viscosupplementation (20,40,44,45). In contrast, the viscosity at low shear rates is considered zero shear viscosity. It is strongly related to the molecular weight value (20,40,46).

In Fig. 5 is shown the behavior of the storage modulus G' and the loss modulus G" as a function of frequency, with a constant strain of 0.01.



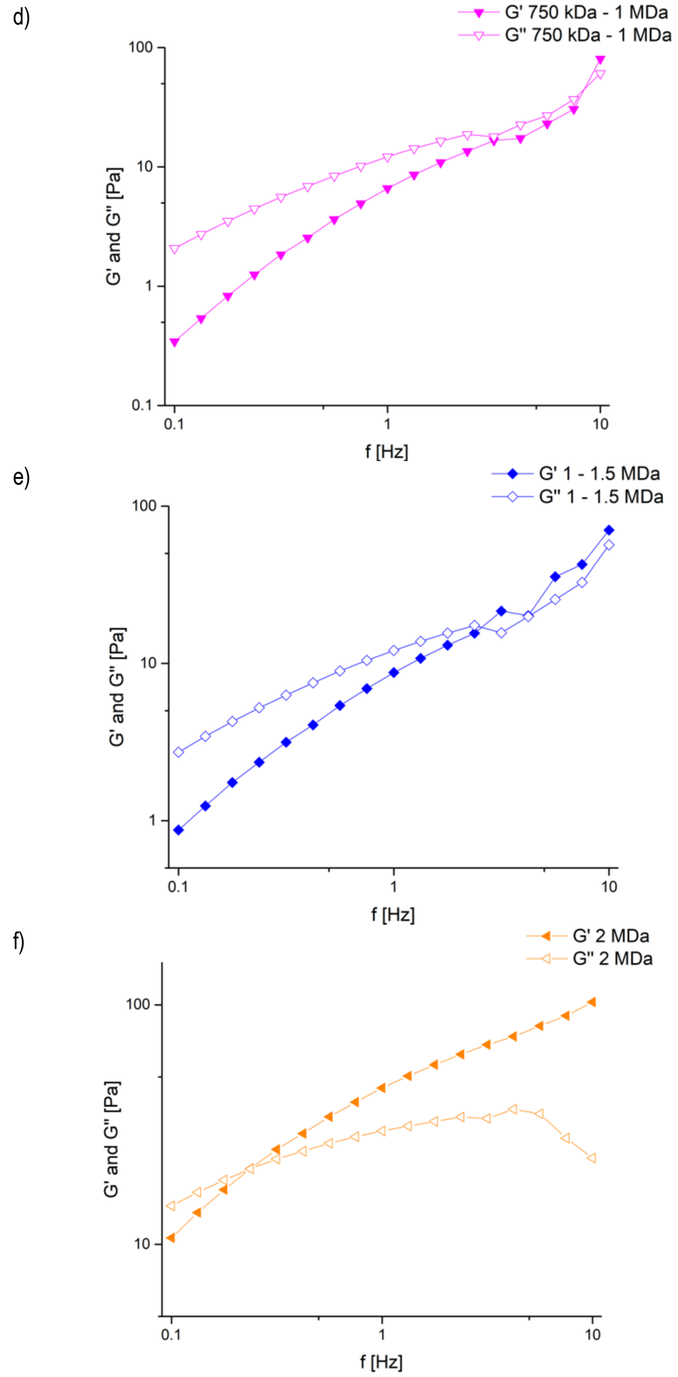


Fig. 5. Storage modules G' and loss modulus G'' as a function of the oscillating frequency f for solutions based on hyaluronic acid of different molecular weight: a) 30 000 – 50 000 Da, b) 750 kDa – 1MDa, c) 200 – 400 kDa, d) 750 kDa – 1 MDa, e) 1 – 1,5 MDa, f) 2 MDa

Obtained data can be divided into two groups, according to the previously described viscous and aqueous preparations dependencies. In the upper row, there are preparations based on low molecular weight hyaluronic acid, while in the lower row, there are preparations with high molecular weight hyaluronic acid. For more aqueous formulations, these characteristics are less clear, and the results are more unstable, while in the case of more viscous formulations, the characteristics match literature assumptions. The loss modulus G" (viscosity) is a measure of the energy dissipated into the material in which the deformation has been imposed. The storage modulus G' (elasticity) is a measure of the energy that has been stored in the material in which the deformation has been imposed and is adequate to elastic deformations. The solutions retain a viscous liquid nature at low frequencies (G''>G') and become more flexible at higher frequencies (G'>G'') (7). This parameter is significant in artificial synovial fluids due to the preservation of viscous and elastic properties. Such a fluid can absorb mechanical energy and protect the cartilage from damage or increased wear (20). It should be noted that the higher the molecular weight, the higher the loss modulus G' and the storage modulus G''. It can also be seen that the higher the molecular weight, the intersection of G' and G'' occurs at lower frequencies, this is visible for viscous solutions (7,8,20,47).

Tribological tests were carried out for a broader analysis of the influence of the molecular weight of hyaluronic acid on the functional properties of artificial synovial fluid solutions. The behavior of lubricating solutions during tribological tests is shown in Fig. 6.

All of the tested preparations have similar coefficients of friction, ranging from 0.18 to 0.25. In the results presented, the differences can be considered minor. Also, almost all measurements are within the margin of error. Based on the literature and the results obtained, it can be concluded that different molecular weights of hyaluronic acid do not clearly influence the resistance to movement (48,49).

Although the molecular weight has very little effect on the coefficient of friction, more significant wear trends can be observed. The average volume of the wear tracks is shown in Fig. 7.

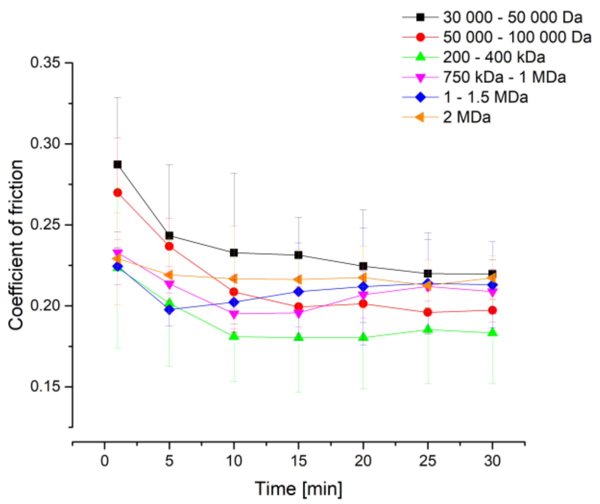


Fig. 6. Coefficient of friction as a function of time for solutions based on hyaluronic acid

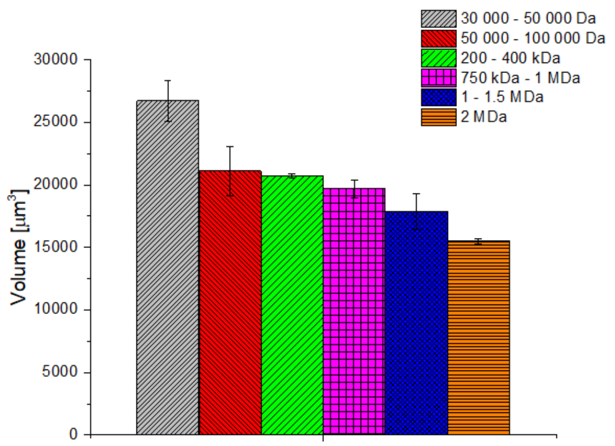


Fig. 7. Volume of wear tracks

Solutions based on hyaluronic acid with a molecular weight of 2 MDa showed the lowest wear (15 483 ± 209 µm3). However, the highest decrement of metal disc (26 702 ± 1607 µm3) has been achieved for the lowest molecular weight solution. The results obtained confirm that wear does not always correlate with friction coefficient. An example is a preparation based on hyaluronic acid with a high molecular weight of 2MDa, which provides the best protection against wear among the tested solutions, while exhibiting one of the highest coefficients of friction. Moreover, there are significant wear protection benefits due to the higher molecular weight. It is likely that the high molecular weight provides better wear protection due to its better ability to capture and immobilize other molecules that initiate loss in the sample material. This is also related to the viscoelasticity, which is higher depending on the higher molecular weight of hyaluronic acid. Higher viscoelastic values provide better adhesion to surfaces while increasing elasticity between surfaces. Protecting surfaces from excessive wear may be more related to the adhesive and elastic properties of the fluid than to the coefficient of friction itself. (49–52).

In this study, contact angle measurements were also performed to evaluate how changes in the molecular weight of hyaluronic acid affect wettability. The results of the contact angle measurements are shown in Fig. 8.

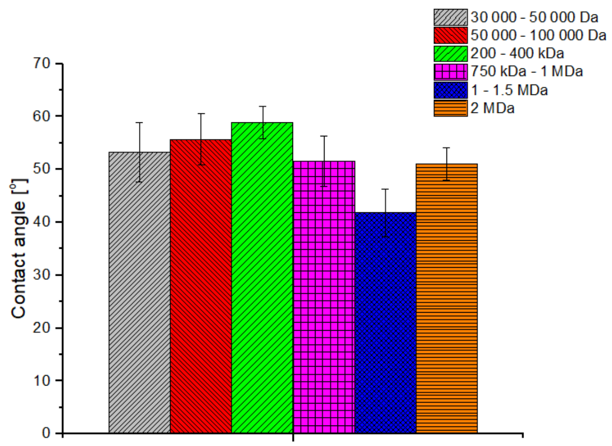


Fig. 8. Contact angle for solutions based on hyaluronic acid

Contact angle measurements indicate that molecular weight has no significant effect on wettability. All solutions have contact angles below 90o, which means that they all have a hydrophilic nature and good wettability. There is a slight tendency for higher molecular weight solutions, where the solution is viscous, to have slightly higher contact angles than low molecular weight hyaluronic acid based solutions. Literature reports confirm that the contact angle is not strictly related to the molecular weight of solutions (20,46,53).

Low molecular weight acid is often used for skin regeneration. This is due to its ease of penetration through the skin, one of the benefits of which is to accelerate the wound healing process. In addition, low molecular weight acid has unstable rheological properties, which was confirmed in tests (Fig. 4), and is subject to rapid degradation (54–59). High molecular weight hyaluronic acid is suitable for injection and can act as a space filler, which provides better cushioning. Such an effect is highly desirable because viscosupplementation is used for damaged joints that need support. Furthermore, the literature has confirmed better analge-

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sic and anti-inflammatory effects for high-molecular-weight hyaluronic acid. It is definitely longer lasting than using low molecular weight acid (20,27,29,60–62). However, not all commercial artificial synovial fluid preparations are based on a high molecular weight acid, and this work confirms the legitimacy of its use.

4. CONCLUSIONS

The correlation between wear and viscosity is responsible for the proper functional properties of lubricants. The viscosity of the solution depends on the molecular weight. It has a significant effect on whether the solution will be aqueous or viscous. The viscosity of the tested compositions increases with increasing molecular weight. The coefficient of friction is not directly correlated to the wear rating. More relevant information can be deduced from the wear volume - the higher the molecular weight, the lower wear. Good protection of the surface against wear is ensured by high viscoelasticity, which provides a protective layer and ensures better adhesion, eliminating excessive abrasion. The molecular weight of hyaluronic acid solutions does not significantly affect wettability. Higher molecular weight hyaluronic acid solutions show more favorable viscoelasticity, viscosity, and wear results. It should be noted that the results of this study may be helpful in selecting an appropriate base for artificial synovial fluid preparation and are only a starting point for further investigation.

REFERENCES

1. Jay GD, Waller KA. The biology of Lubricin: Near frictionless joint motion. Matrix Biol [Internet]. 2014;39:17–24. Available from: http://dx.doi.org/10.1016/j.matbio.2014.08.008
2. Schmidt TA, Sah RL. Effect of synovial fluid on boundary lubrication of articular cartilage. Osteoarthr Cartil. 2007;15(1):35–47.
3. Chang DP, Abu-Lail NI, Coles JM, Guilak F, Jay GD, Zauscher S. Friction Force Microscopy of Lubricin and Hyaluronic Acid between Hydrophobic and Hydrophilic Surfaces. Soft Matter. 2009;5(18): 3438–45.
4. Blewis ME, Nugent-Derfus GE, Schmidt TA, Schumacher BL, Sah RL. A model of synovial fluid lubricant composition in normal and injured joints. Eur Cells Mater. 2007;13(858):26–38.
5. Nečas D, Vrbka M, Marian M, Rothammer B, Tremmel S, Wartzack S, et al. Towards the understanding of lubrication mechanisms in total knee replacements – Part I: Experimental investigations. Tribol Int. 2021;156(January).
6. Yarimitsu S, Nakashima K, Sawae Y, Murakami T. Influences of lubricant composition on forming boundary film composed of synovia constituents. Tribol Int [Internet]. 2009;42(11–12):1615–23. Available from: http://dx.doi.org/10.1016/j.triboint.2008.11.005
7. Hui AY, McCarty WJ, Masuda K, Firestein GS, Sah RL. A systems biology approach to synovial joint lubrication in health, injury, and disease. Wiley Interdiscip Rev Syst Biol Med. 2012;4(1):15–37.
8. Prekasan D, Saju KK. Tribological effectiveness of viscosupplements for osteoarthritis in knee joint. SN Appl Sci [Internet]. 2019;1(9):1–6. Available from: https://doi.org/10.1007/s42452-019-1030-2
9. Kraus VB, Stabler T V., Kong SY, Varju G, McDaniel G. Measurement of synovial fluid volume using urea. Osteoarthr Cartil. 2007;15(10):1217–20.
10. Brannan SR, Jerrard DA. Synovial fluid analysis. J Emerg Med. 2006;30(3):331–9.
11. Gait AD, Hodgson R, Parkes MJ, Hutchinson CE, O'Neill TW, Maricar N, et al. Synovial volume vs synovial measurements from dynamic contrast enhanced MRI as measures of response in osteoarthritis. Osteoarthr Cartil [Internet]. 2016;24(8):1392–8. Available from: http://dx.doi.org/10.1016/j.joca.2016.03.015
12. Moskalewski S, Jankowska-Steifer E. Hydrostatic and Boundary Lubrication of Joints – Nature of Boundary Lubricant. Ortop Traumatol Rehabil. 2012;14(1):13–21.
13. Waller KA, Zhang LX, Elsaid KA, Fleming BC, Warman ML, Jay GD. Role of lubricin and boundary lubrication in the prevention of chondrocyte apoptosis. Proc Natl Acad Sci U S A. 2013;110(15):5852–7.
14. Zappone B, Ruths M, Greene GW, Jay GD, Israelachvili JN. Adsorption, lubrication, and wear of lubricin on model surfaces: Polymer brush-like behavior of a glycoprotein. Biophys J. 2007;92(5): 1693–708.
15. Morrell KC, Hodge WA, Krebs DE, Mann RW. Corroboration of in vivo cartilage pressures with implications for synovial joint tribology and osteoarthritis causation. Proc Natl Acad Sci U S A. 2005;102(41):14819–24.
16. Skou ST, Koes BW, Grønne DT, Young J, Roos EM. Comparison of three sets of clinical classification criteria for knee osteoarthritis: a cross-sectional study of 13,459 patients treated in primary care. Osteoarthr Cartil [Internet]. 2020;28(2):167–72. Available from: https://doi.org/10.1016/j.joca.2019.09.003
17. Lotz MK. New developments in osteoarthritis. Posttraumatic osteoarthritis: Pathogenesis and pharmacological treatment options. Arthritis Res Ther. 2010;12(3).
18. Anderson DD, Chubinskaya S, Guilak F, Martin JA, Oegema TR, Olson SA, et al. Post-traumatic osteoarthritis: Improved understanding and opportunities for early intervention. J Orthop Res. 2011;29(6):802–9.
19. Elsaid KA, Fleming BC, Oksendahl HL, Machan JT, Fadale PD, Hulstyn MJ, et al. Decreased lubricin concentrations and markers of joint inflammation in the synovial fluid of patients with anterior cruciate ligament injury. Arthritis Rheum. 2008;58(6):1707–15.
20. Snetkov P, Zakharova K, Morozkina S, Olekhnovich R, Uspenskaya M. Hyaluronic acid: The influence of molecular weight on structural, physical, physico-chemical, and degradable properties of biopolymer. Polymers (Basel). 2020;12(8).
21. Jay GD, Torres JR, Warman ML, Laderer MC, Breuer KS. The role of lubricin in the mechanical behavior of synovial fluid. Proc Natl Acad Sci U S A. 2007;104(15):6194–9.
22. Freemont AJ. Role of cytological analysis of synovial fluid in diagnosis and research. Ann Rheum Dis. 1991;50(2):120–3.
23. Prekasan D, Saju KK. Review of the Tribological Characteristics of Synovial Fluid. Procedia Technol [Internet]. 2016;25(Raerest):1170–4. Available from: http://dx.doi.org/10.1016/j.protcy.2016.08.235
24. Rwei SP, Chen SW, Mao CF, Fang HW. Viscoelasticity and wearability of hyaluronate solutions. Biochem Eng J. 2008;40(2):211–7.
25. More S, Kotiya A, Kotia A, Ghosh SK, Spyrou LA, Sarris IE. Rheological properties of synovial fluid due to viscosupplements: A review for osteoarthritis remedy. Comput Methods Programs Biomed [Internet]. 2020;196:105644. Available from: https://doi.org/10.1016/j.cmpb.2020.105644
26. Rothammer B, Marian M, Rummel F, Schroeder S, Uhler M, Kretzer JP, et al. Rheological behavior of an artificial synovial fluid – influence of temperature, shear rate and pressure. J Mech Behav Biomed Mater [Internet]. 2021;115(March 2020):104278. Available from: https://doi.org/10.1016/j.jmbbm.2020.104278
27. Boeriu CG, Springer J, Kooy FK, van den Broek LAM, Eggink G. Production Methods for Hyaluronan. Int J Carbohydr Chem. 2013;2013:1–14.
28. Fraser JRE, Laurent TC, Laurent UBG. Hyaluronan: Its nature, distribution, functions and turnover. J Intern Med. 1997;242(1):27–33.
29. Gupta RC, Lall R, Srivastava A, Sinha A. Hyaluronic acid: Molecular mechanisms and therapeutic trajectory. Front Vet Sci. 2019;6(JUN):1–24.
30. Yamasaki K, Drolle E, Nakagawa H, Hisamura R, Ngo W, Jones L. Impact of a low molecular weight hyaluronic acid derivative on contact lens wettability. Contact Lens Anterior Eye [Internet]. 2021;44(3):101334. Available from: https://doi.org/10.1016/j.clae.2020.05.003

31. Mathieu P, Conrozier T, Vignon E, Rozand Y, Rinaudo M. Rheologic behavior of osteoarthritic synovial fluid after addition of hyaluronic acid: A pilot study. Clin Orthop Relat Res. 2009;467(11):3002–9.
32. Vincent HK, Percival SS, Conrad BP, Seay AN, Montero C, Vincent KR. Hyaluronic Acid (HA) Viscosupplementation on Synovial Fluid Inflammation in Knee Osteoarthritis: A Pilot Study. Open Orthop J. 2013;7(1):378–84.
33. Quilliot J, Couderc M, Giraud C, Soubrier M, Mathieu S. VISCO-SUPPLEMENTATION. Semin Arthritis Rheum [Internet]. 2019;49(1):e10–1. Available from: https://doi.org/10.1016/j.semarthrit.2019.02.001
34. van der Weegen W, Wullems JA, Bos E, Noten H, van Drumpt RAM. No Difference Between Intra-Articular Injection of Hyaluronic Acid and Placebo for Mild to Moderate Knee Osteoarthritis: A Randomized, Controlled, Double-Blind Trial. J Arthroplasty [Internet]. 2015; 30(5):754–7. Available from: http://dx.doi.org/10.1016/j.arth.2014.12.012
35. Bell CJ, Ingham E, Fisher J. Influence of hyaluronic acid on the time-dependent friction response of articular cartilage under different conditions. Proc Inst Mech Eng Part H J Eng Med. 2006;220(1):23–31.
36. Zhang J-H. Encyclopedia of Tribology. Encyclopedia of Tribology. 2013. 110–113 p.
37. Goldberg VM, Buckwalter JA. Hyaluronans in the treatment of osteoarthritis of the knee: Evidence for disease-modifying activity. Osteoarthr Cartil. 2005;13(3):216–24.
38. Ogston AG, Stanier JE. The physiological function of hyaluronic acid in synovial fluid; viscous, elastic and lubricant properties. J Physiol. 1953;119(2–3):244–52.
39. Nicholls M, Manjoo A, Shaw P, Niazi F, Rosen J. A Comparison Between Rheological Properties of Intra-articular Hyaluronic Acid Preparations and Reported Human Synovial Fluid. Adv Ther [Internet]. 2018;35(4):523–30. Available from: https://doi.org/10.1007/s12325-018-0688-y
40. Bothner H, Wik O. Rheology of hyaluronate. Acta Otolaryngol. 1987;104(S442):25–30.
41. Smith AM, Fleming L, Wudebwe U, Bowen J, Grover LM. Development of a synovial fluid analogue with bio-relevant rheology for wear testing of orthopaedic implants. J Mech Behav Biomed Mater [Internet]. 2014;32:177–84. Available from: http://dx.doi.org/10.1016/j.jmbbm.2013.12.009
42. Fallacara A, Baldini E, Manfredini S, Vertuani S. Hyaluronic acid in the third millennium. Polymers (Basel). 2018;10(7).
43. Simou K, Jones SW, Davis ET, Preece J, Zhang ZJ. Rheological and interface adhesive properties of osteoarthritic synovial fluids. Biotribology [Internet]. 2022;32:100227. Available from: https://doi.org/10.1016/j.biotri.2022.100227
44. Necas J, Bartosikova L, Brauner P, Kolar J. Hyaluronic acid (hyaluronan): A review. Vet Med (Praha) [Internet]. 2008;53(8):397–411. Available from: https://doi.org/10.17221/1930-VETMED
45. Fakhari A, Berkland C. Applications and emerging trends of hyaluronic acid in tissue engineering, as a dermal filler and in osteoarthritis treatment. Acta Biomater [Internet]. 2013;9(7):7081–92. Available from: http://dx.doi.org/10.1016/j.actbio.2013.03.005
46. Falcone SJ, Palmeri DM, Berg RA. Rheological and cohesive properties of hyaluronic acid. J Biomed Mater Res - Part A. 2006; 76(4):721–8.
47. Bhuanantanondh P, Grecov D, Kwok E. Rheological study of viscosupplements and synovial fluid in patients with osteoarthritis. J Med Biol Eng. 2012;32(1):12–6.
48. Rebenda D, Vrbka M, Čípek P, Toropitsyn E, Nećas D, Pravda M, et al. On the dependence of rheology of hyaluronic acid solutions and frictional behavior of articular cartilage. Materials (Basel). 2020;13(11).
49. Lee DW, Banquy X, Das S, Cadirov N, Jay G, Israelachvili J. Effects of molecular weight of grafted hyaluronic acid on wear initiation. Acta Biomater [Internet]. 2014;10(5):1817–23. Available from: http://dx.doi.org/10.1016/j.actbio.2014.01.013
50. Das S, Banquy X, Zappone B, Greene GW, Jay GD, Israelachvili JN. Synergistic interactions between grafted hyaluronic acid and lubricin provide enhanced wear protection and lubrication. Biomacromolecules. 2013;14(5):1669–77.
51. Yu J, Banquy X, Greene GW, Lowrey DD, Israelachvili JN. The boundary lubrication of chemically grafted and cross-linked hyaluronic acid in phosphate buffered saline and lipid solutions measured by the surface forces apparatus. Langmuir. 2012;28(4):2244–50.
52. Raviv U, Giasson S, Kampf N, Gohy JF, Jérôme R, Klein J. Lubrication by charged polymers. Nature. 2003;425(6954):163–5.
53. Yin C, Qi X, Wu J, Guo C, Wu X. Therapeutic contact lenses fabricated by hyaluronic acid and silver incorporated bovine serum albumin porous films for the treatment of alkali-burned corneal wound. Int J Biol Macromol [Internet]. 2021;184(June):713–20. Available from: https://doi.org/10.1016/j.ijbiomac.2021.06.155
54. Rayahin JE, Buhrman JS, Zhang Y, Koh TJ, Gemeinhart RA. High and Low Molecular Weight Hyaluronic Acid Differentially Influence Macrophage Activation. ACS Biomater Sci Eng. 2015;1(7):481–93.
55. Drozdova M, Vodyakova M, Tolstova T, Chernogortseva M, Sazhnev N, Demina T, et al. Composite Hydrogels Based on Cross-Linked Chitosan and Low Molecular Weight Hyaluronic Acid for Tissue Engineering. Polymers (Basel). 2023;15(10).
56. Yasin A, Ren Y, Li J, Sheng Y, Cao C, Zhang K. Advances in Hyaluronic Acid for Biomedical Applications. Front Bioeng Biotechnol. 2022;10(July):1–12.
57. Cui L, Li J, Guan S, Zhang K, Zhang K, Li J. Injectable multifunctional CMC/HA-DA hydrogel for repairing skin injury. Mater Today Bio [Internet]. 2022;14(April):100257. Available from: https://doi.org/10.1016/j.mtbio.2022.100257
58. Park HY, Kweon DK, Kim JK. Molecular weight-dependent hyaluronic acid permeability and tight junction modulation in human buccal TR146 cell monolayers. Int J Biol Macromol [Internet]. 2023;227:182–92. Available from: https://doi.org/10.1016/j.ijbiomac.2022.12.106
59. Vu TT, Gulfam M, Jo SH, Rizwan A, Joo S Bin, Lee B, et al. The effect of molecular weight and chemical structure of cross-linkers on the properties of redox-responsive hyaluronic acid hydrogels. Int J Biol Macromol [Internet]. 2023;238(March):124285. Available from: https://doi.org/10.1016/j.ijbiomac.2023.124285
60. Chavda S, Rabbani SA, Wadhwa T. Role and Effectiveness of Intra-articular Injection of Hyaluronic Acid in the Treatment of Knee Osteoarthritis: A Systematic Review. Cureus. 2022;14(4).
61. Sunte J, Waghmare V. An Electrohydrodynamic Lubrication of Synovial Lubricant on Human Body. 2022;25–35.
62. Farah H, Wijesinghe SN, Nicholson T, Alnajjar F, Certo M, Alghamdi A et al. Differential Metabotypes in Synovial Fibroblasts and Synovial Fluid in Hip Osteoarthritis Patients Support Inflammatory Responses. Int J Mol Sci. 2022;23(6):1–13.

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Monika Izabela Karpowicz: https://orcid.org/0000-0002-2808-1274

Marcin Klekotka: https://orcid.org/0000-0002-9751-2939

Jan Ryszard Dąbrowski: https://orcid.org/0000-0002-0175-0669