Serbian Tribology Society

SERBIATRIB '19

16th International Conference on Tribology

Faculty of Engineering University of Kragujevac

Kragujevac, Serbia, 15 – 17 May 2019

EFFECT OF HYALURONIC ACID MOLECULAR WEIGHT ON FRICTION OF ARTICULAR CARTILAGE

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Abstract: *Hyaluronic acid (HA) is among others one of the component of synovial fluid (SF) and is the main component which affects the rheology of SF. However, during various degenerative joint diseases like osteoarthritis, the concentration and molecular weight of HA in SF is decreasing. In recent times, injections with exogenous HA are gaining popularity as a one of the methods for non-invasive treatment of osteoarthritis. Exogenous HA should improve rheological properties of osteoarthritic SF and has also many physiological effects. One of the key parameters which affects the rheology of HA and the efficiency of viscosupplementation is the molecular weight of HA. In this study, the changes in coefficient of friction between intact porcine cartilage and glass plate during reciprocating sliding tests were analyzed to understand how the changes in molecular weight of HA affects the friction of articular cartilage model. The changes in friction between osteoarthritic SF and mixtures of SF and HA with different molecular weight were also analyzed to assess the significance of exogenous HA molecular weight on cartilage friction after viscosupplementation.*

Keywords: *hyaluronic acid, friction, biotribology, synovial fluid, viscosupplementation*

1. INTRODUCTION

Articular cartilage is a biological tissue which covers sliding surfaces in big synovial joints like hip or knee. Its porous structure is mainly composed of collagen fibers and proteoglycans [1]. Gaps between matrix are filled with SF which is mainly composed of water, proteins, etc. Interaction between these two phases stands behind its excellent tribological characteristics. Cartilage maintains great lubricating properties with extremely low friction and minimal wear under physiological conditions. It can also absorb impact loads quite well.

Osteoarthritis is one of the most common diseases of musculoskeletal system. These days, it afflicts about 70 % of 70 year olds [2]. It is characterized by excessive wear of cartilage structure which leads to higher friction and distraction of cartilage lubrication mechanism. Progression of osteoarthritis is also connected to changes in composition of SF [3]. Osteoarthritic SF is diluted by inflammatory effusion and the concentration and molecular weight of HA is lower [4].

The original theory about viscosupplementation, presented by Balazs et al. [5], assumed improvement of SF rheology by exogenous HA. Higher viscosity and better viscoelastic properties of synovial fluid should lead to lower friction and better shock absorption abilities. However, the positive effect of viscosupplementation on patients can

be observed even after six months after viscosupplemenatiton [6], so exogenous HA has also some physiological effects such as reduction of inflammation or synthesis of endogenous HA in joint capsule.

One of the key parameters which affects the rheological properties of HA is its molecular weight. Other SF constituents do not have effect on SF rheology [7], so HA is also the main constituent which affects rheology of whole SF. HA with higher molecular weight has higher viscosity and exhibits better viscoelastic properties [8]. Even better results can be measured for cross-linked HA [9]. Addition of HA to osteoarthritic SF leads to better rheology of SF whilst results are strongly dependent on HA properties [10,11].

Reduction of friction by HA was already proved. HA solutions showed lower values of coefficient of friction compared to the simple solutions like phosphate buffer saline (PBS) [12] or Ringers solution [2]. Unlike the rheology, interaction between HA and other SF constituents plays an important role. Mixture of HA and phospholipids [13] leads to lower friction compared to simple phospholipids solution. Molecules of protein γ-globulin and HA have different electric charge so their molecules attract each other and form complex structures which contributes to the lower friction [14,15]. On the other hand, albumin and HA have the same electric charge and they repel each other. This interaction is unworthy for the reduction of friction [14,15]. Molecular weight of HA should also be one of the parameters which affects the friction of articular cartilage. However, no one focused on this problematic so far.

2. MATERIALS AND METHODS

2.1 Experimental methods

Frictional measurements were performed on a commercial tribometer Bruker UMT TriboLab in a pin-on-plate configuration (Fig. 1). Coefficient of friction was investigated as a function of time for the sliding pair of stationary glass plate from optical glass (B270) and moving porcine cartilage specimen. Cartilage specimen was loaded with constant load of 5 N which corresponds to contact pressure of 0.8 MPa. The sliding speed of 10 mm/s was selected and the reciprocating stroke was set to 20 mm. Contact was fully flooded with the tested lubricant. Both the lubricant and the contact pair were heated to 37 °C via heating cartridges mounted in the stainless steel chamber.

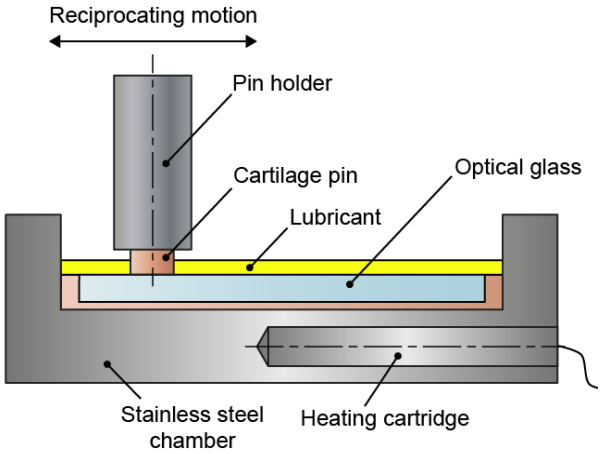

Figure 1. Scheme of experimental apparatus

Before each experiment, unloaded cartilage sample was immersed in the lubricant for 320 seconds. In the end of this preliminary phase, cartilage was loaded and the friction test started immediately. After 300 seconds (75 cycles, sliding distance of 2 780 mm), the sliding test was interrupted and the cartilage was unloaded for another 320 seconds. This unloaded phase is important for rehydration of cartilage specimen. Subsequently, the reciprocating test was restarted immediately after reloading and continued for another 300 seconds. The unloading phase was repeated two times so three tests under the same conditions were performed. Coefficient of friction was evaluated based on the values of normal and friction forces measured by biaxial force sensor mounted to the pin holder.

2.2 Materials

Cartilage specimen with underlying subchondral bone was taken from porcine femur. Cylindrical specimen of 5,6 mm diameter with intact cartilage layer was harvested from femoral head with a hollow drill. Specimen was prepared within few hours after slaughter.

Extracted cartilage was stored in PBS solution in freezer at -20 °C. This procedure should slow down the biological degradation of the cartilage tissue. Szarko et al. [16] also showed that this approach does not change the mechanical properties of the cartilage. Cartilage was stored in a freezer for no longer than one week. Half an hour before the experiments, cartilage was removed from the freezer to thaw naturally at room temperature.

In an effort to understand the effect of HA molecular weight on the friction of cartilage, the first series of experiments was performed using simple HA solutions with different molecular weight. In total, five HA solutions with HA concentration of 20 mg/ml and molecular weight of 77 kDa, 350 kDa, 640 kDa, 1 060 kDa and 2 010 kDa were tested. Solutions were prepared from HA powder by dissolution of required amount of powder in PBS. Solution was stirred by a magnetic stirrer and heated to 50 °C for at least 3 hours to ensure the proper dissolution of HA.

Table 1. Composition of model SF

	Albumin	γ-globulin	HA	Phospholipids
Concentration (mg/ml)	24.9	6.1	1.49	0.34

In the second series of the tests, HA solutions were mixed with model synovial fluid to analyze the effect of HA molecular weight on reaction between HA and SF components and to show how the changes in HA molecular weight affect the friction in cartilage-on-glass contact lubricated by HA and SF solution. Composition of synovial fluid was based on research performed by Galandáková el al. [3] where authors performed an extensive study focused on analysis of composition of SFs of different orthopedic patients. Composition of tested model SF should correspond to the SF of patients with osteoarthritis. Exact composition of SF and concentrations of individual components are stated in Table 1. SF was mixed with HA solution by magnetic stirrer in 1:1 ratio. This ratio is commonly used in medical practice during viscosupplementation.

3. RESULTS AND DISCUSSION

Higher molecular weight of pure HA solution leads to higher viscosity [8] but results of frictional measurements showed that higher viscosity of solution does not affect the friction in cartilage-on-glass contact. Figure 2 shows the results of coefficient of friction measurements for three HA solutions with different molecular weight – 350 kDa, 640 kDa and 2 010 kDa. Values of coefficient of friction varies for all the tested samples between 0,02 and 0,03 at the end of each measurement substep. Therefore, the effect of molecular weight of HA on friction in cartilage-on-glass contact seems to be negligible.

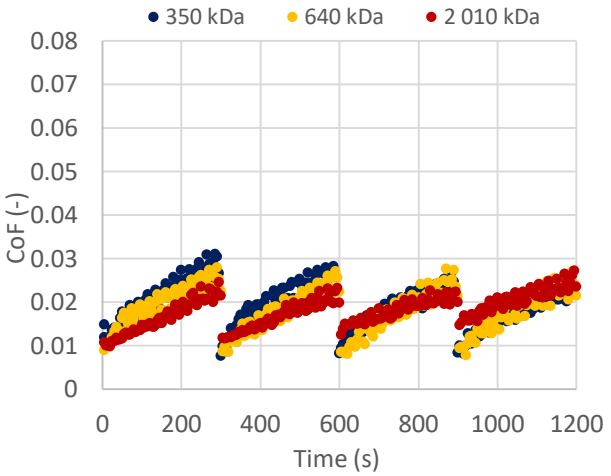

Figure 2. CoF as a function of a time for pure HA solutions with different molecular weight

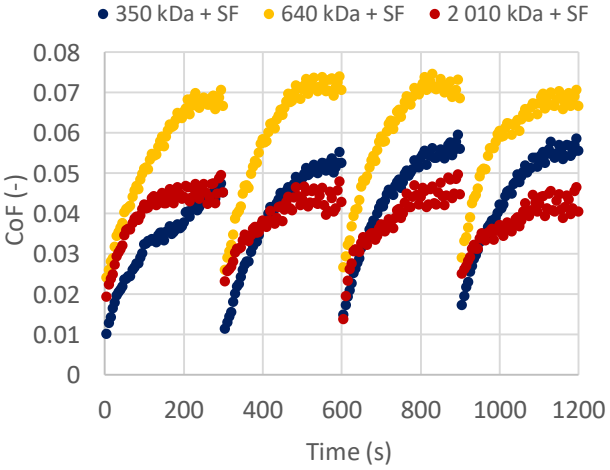

Figure 3. CoF as a function of a time for SF mixed with HA solutions with various molecular weight

Contrary to these results, Kwieciski et al. [17] observed linear dependence between HA

molecular weight and friction. Higher molecular weight led to lower values of coefficient of friction in cartilage-on-cartilage contact.

Figure 3 shows results of experiments with mixtures of model SF and HA solutions. Results show higher values of coefficent of friction compared to the tests with simple HA solutions. Higher friction is probably caused by dilution of HA with water from SF and by reactions between HA and other SF components [13-15]. The effect of molecular weight is still minimal. The difference in coefficient of friction between solutions in Fig. 3 is only about 0,03 at the end of experimental substeps.

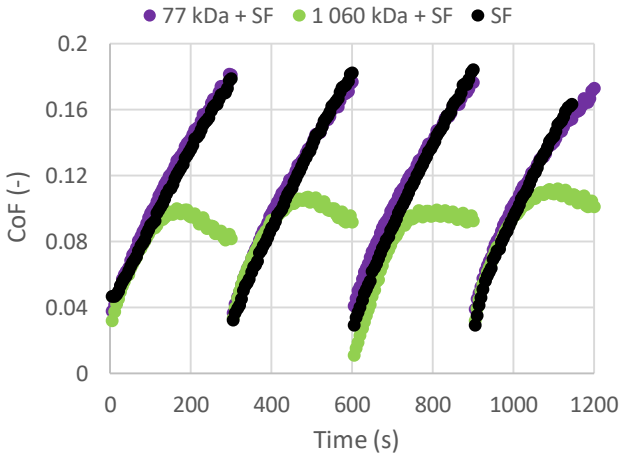

Figure 4. CoF as a function of a time for simple SF and mixtures with HA solutions with various molecular weight

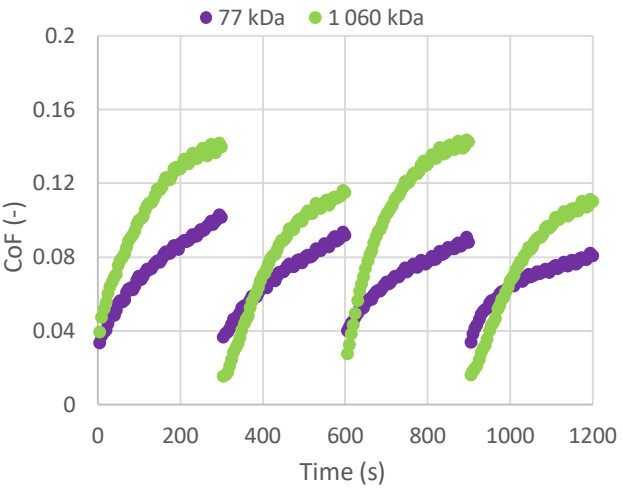

Figure 5. CoF as a function of a time for pure HA solutions with different molecular weight

Comparison of mixed solutions with simple osteoarthritic SF (Fig. 4) shows significant decrease of friction compared to the solutions in Fig. 3. These data support the theory about importance of HA in lowering of friction in osteoarthritic synovial joint. However, following tests with HA mixture solutions with molecular weight of 77 kDa and 1 060 kDa (Fig. 4) showed different results. Especially in the case of 77 kDa , the values of coefficient of friction are very similar to the results of simple SF. This is probably due to the adsorption of proteins on the surface of articular cartilage [14]. All the experiments were measured with one sample of articular cartilage. It is apparent that after the measurement with pure model SF, the structure of cartilage is saturated with proteins. These adsorbed proteins then influence the subsequent experiments. In the case of mixed solution with 1 060 kDa HA, the amount of adsorbed proteins is lower, so the results are more similar to other mixtures in Fig. 3.

This theory was subsequently tested by another experiments with HA solutions with molecular weight of 77 kDa and 1 060 kDa. Results in Fig. 5 show three and five times higher values of coefficient of friction compared to the results in Fig. 2. 77 kDa is lower molecular weight than samples which were tested before, so the friction could be different but molecular weight of 1 060 kDa lies in the range of molecular weight tested before. Measured results showed few times higher values of coefficient of friction at the end of each measurement substep which is attributed to the proteins adsorbed on the cartilage surfaces by the authors.

4. CONCLUSIONS

HA seems to be very effective in lowering of friction in cartilage-on-glass contact. However, the effect of HA molecular weight on friction seems to be insignificant for simple solutions and even for mixtures with osteoarthritic model SF. Results also showed that proteins from SF adsorb on the surface of articular cartilage and affect the results of the following tests. All the tests were performed using one sample of cartilage because repeatability across different cartilage samples is not satisfactory, in general. Therefore, some kind of cleansing process needs to be find for out further studies to remove the adsorbed

proteins. Another way is to substitute cartilage with PVA hydrogel or silica – materials with similar properties but much easier to cleanse from proteins.

ACKNOWLEDGEMENT

This research was carried out under the project LTAUSA17150 with financial support from the Ministry of Education, Youth and Sports of the Czech Republic.

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