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RESEARCH

Visualization of Lubrication Film in Model of Synovial Joint

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A B S T R A C T

Synovial joint is one of the most important parts for human movement system and the right function of it is necessary. When the synovial joint is damaged by illnesses, destroyed natural joint is exchanged for artificial joint. They are commonly used in nowadays, but there are problems with their limited lifetime. Alternative treatment procedures in surgery start appearing in order to postpone acute operation of total endoprosthesis. For proper operation of the alternative treatments lubrication processes have to be understood. The understanding of the lubrication processes can assist in the development of new suitable medical treatments. This study is focused on the visualization of the synovial joint contact and simultaneous measurement of the force effects. Experimental device represents model of synovial joint, which allows pin-on-plate reciprocating tribometer. The goal of this study is to describe the contact area behaviour and to relate it to force effects in the contact.

1. INTRODUCTION

The human movement system is based on joints and muscles. The synovial joints are composed of two bones whose surfaces covers cartilage tissue [1]. The cartilage surfaces are in close contact and the space between them is filled with the synovial fluid. This arrangement, special cartilage structure and synovial fluid, allows movement with very low friction coefficient. Tissue of cartilage is porous material with heterogeneous structure containing very few cells [2]. These specific features cause the unique tribological behaviour. Extracellular matrix is the basis of cartilage structure (ECM). ECM includes type II collagen fibres and proteoglycan [3]. Hyaluronic acid (HA), proteins, decorins, chondrocytes, etc. are also included in the ECM [4]. Cartilaginous bone coating includes three zones. The first one, surface zone, has parallely orientated collagen fibres with respect to the surface. The second one, the middle zone, has randomly orientated fibres and the last one, the deep zone, has fibres perpendicularly orientated to the surface [1]. Each of them has specific composition and properties [5]. Water volume in cartilage tissue is also very important attribute regarding lubricating properties [6,7]. Special lubricating properties are caused by very low elastic module (1 – 20 MPa) of cartilage tissue regarding the position of the cartilage surface and type of the joint [4]. All of the

mentioned specific properties together allow specific lubrication processes in synovial joint with very low coefficient of friction and wear.
Obviously, there is very limited knowledge of lubrication in natural joints in terms of experimental investigation. Only a few studies were published. Topics of these works are frequently focused on visualization of hydrogels, nevertheless, complex visualization of natural cartilage has not been published yet. The majority of the studies is aimed to friction measurement, but studies dealing with visualization of cartilage contact also appear. One of the first works dealing with visualization of hydrogel contact area by fluorescence microscopy [8] was focused on determination of the amount of the fluorescently marked particles contained in the lubricant contact area. Fluorescently marked proteins were used, in each experiment, different type of protein was marked. γ globulin showed the biggest impact on the lubrication processes, therefore, the concentration of it in synovial fluid is very important. The fluorescence microscopy was used for the observation and description of the gel-like layer formation on the cartilage surface. Forsey et al. [9] dealt with impact of the HA in creation of the gel-like layer which is the main component for the formation of the film. The study revealed dependency on size of the HA. The penetration of the cartilage structure by HA was also demonstrated by these experiments. Molecules of HA are bound in the cartilage surface because they are attracted by chondrocytes contained in cartilage structure. Wu et al. [10] showed dependency between the flow of the synovial fluid through cartilage structure and compression of cartilage tissue. The results implied dependency on size of HA molecules, specifically the large molecules of HA were caught on the cartilage surface, while the smaller particles penetrated the cartilage structure. The surface of cartilage is covered by the HA with large molecules, which creates the gel-like layer. This surface layer protects the raw cartilage surface against a damage.

Visualization of joint replacement contact was also carried out with fluorescent microscopy. Number of studies were published at our department. A lot of experience with the use of optical methods was obtained within the mentioned studies. Nečas et al. [11,12] published the papers dealing with visualization of joint replacement contact and soft contact among others.

The vast majority of previous studies mostly dealt with visualization of contact area or with the friction measurements separately. It has never been measured simultaneously yet. This study combinates this two branches of biotribology science. The usage of optical methods used at workplace is described together with classical friction measurements. Specially tailored tribometer was designed for this application. It allows simultaneous visualization of soft contact and friction measurements. Concept like this new designed tribometer, which allows combination of optical methods and friction measurements have never been used yet. The goals of this study are to design the new tribometer, to develop the sampling process and experimental methodology and finally, to perform the pilot experiments.

2. MATERIAL AND METHODS

2.1 Experimental device

The tailored new tribometer allows the measurement of friction forces and insight into the contact area, both simultaneously in real time. The pin on plate configuration of tribometer was used for compliance of this requirements. As an observation method fluorescent microscopy was chosen, therefore the concept of tribometer was adapted to be able to use it. This new design is close to the concept of tribometer which was used in study [13]. The schema of newly designed experimental device is shown in Fig. 1. To allow the visualization of contact area, the cartilage sample is placed under the glass desk. The lubricant flowing through the contact is observed by the fluorescent microscope. Due to the fluorescent microscopy method the high speed camera can record fluorescently marked particles contained in lubricant, which flow through the contact. The mercury lamp was used as a light source. The contact area was flooded by a fluorescently marked lubricant. The floated bath is heated to a human body temperature to achieve comparable conditions to human body joints. The glass desk is mounted to a carriage, which was designed as a moveable part. It performs the reciprocating motion whereas the specimen is stationary.

The new design of the tribometer is outlined in Fig. 1. The essential units of the device are shown in Fig. 2.

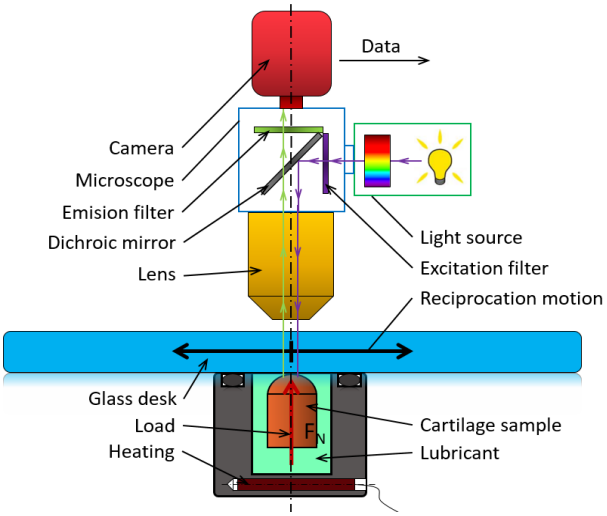

Fig. 1. Schema of the apparatus.

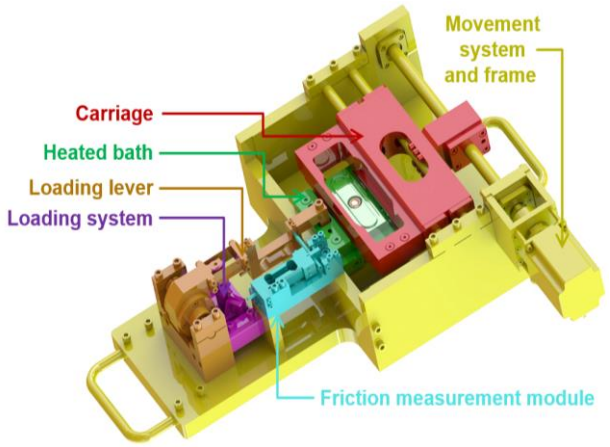

Fig. 2. Real arrangement of apparatus.

Tough frame is a basis of the tribometer, all other components are mounted on it. The carriage as a moveable part, where the glass plate is placed and which performs the reciprocating motion on the guide rods. The accurate guide rods in combination with ball screw and ball bearings in carriage body provide very accurate motion without radial and axial clearances. The heated bath enables flooding and refiling of the contact by a lubricant. The sealing is attached to the glass plate and the bath to avoid the lubricant leakage. Cartilage specimen is mounted on the end of a lever, by which the load is applied. The other end of the lever is placed in two preloaded ball bearings. This arrangement allows rotation around the axis without clearance and radial clearance is precluded too. A strain gauge is connected to the deformation member providing sufficient deformation caused by the low frictional force and great rigidity in the vertical direction (loading direction) at the same time. The first strain gauge is used for measurement of loading, the second one for measurement of friction force. It is connected parallelly to the lever behind the first gauge. Thanks to the deformation member, very low friction forces can be measured. The parallel connection of the second gauge allows its preload to the half of the measuring range, which allows measurement of friction forces during movement of carriage back and forth. The whole tribometer is situated below the fluorescent microscope on an adjustable table. The tailored tribometer is shown in Fig. 3.

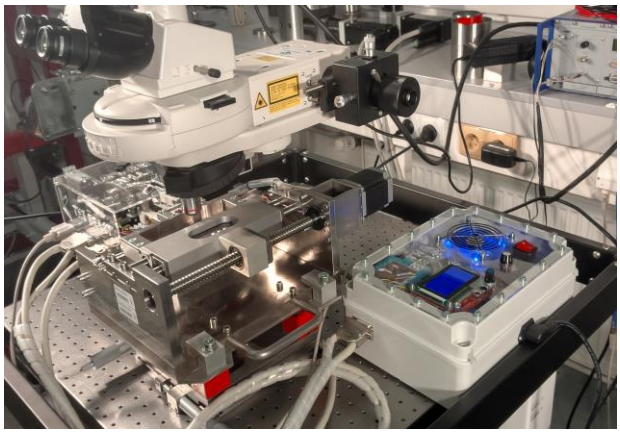

Fig. 3. Complete experimental apparatus.

National Instruments measuring card is a basis of the measuring system. Signal from the gauges is adjusted by the signal amplifier before the measuring card handles them. Data is real-time processed by LabVIEW script through connected PC. Control system is based on Arduino. The motion is ensured by the stepper motor and the load is provided by another linear stepper motor. LCD display and encoder is used as input interface. Both systems (measuring and control) work separately.

Verification and calibration was performed using standard pairs of samples (materials), in order to ensure measurement repeatability among samples. The mentioned material combination was used because the cartilage specimens show variance in results. The pin was made from PTFE-G400 and the plate was made from optical glass B270. The commercial

tribometer Bruker UMT TriboLAB was used to compare with the new tribometer. The compliance of the results obtained using the two simulators was very good.

2.2 Specimens

Specimens from mature pigs were used in the present study. The samples were removed from canopy of the femoral head as soon as possible after the slaughter of animal. The hip joint was chosen for the samples, since it is the most loaded joint, which leads to the best mechanical properties of cartilage tissue [4]. The sampling position was precisely defined through all sample bones. Strict definition assures minimization of deviation in mechanical properties through all samples. Pins of 6 mm diameter were made by the hollow drill bit and the specimens were deeply frozen (-20 ˚C) in PBS immediately after sampling. This sampling process was used in some studies before, e.g. in [14, 15] and the procedure was verified again in [16, 17]. It was proved that the tribological properties did not change. The samples were unfrozen just before testing, otherwise degradation of samples may occur. The sampling process is shown in Fig. 4.

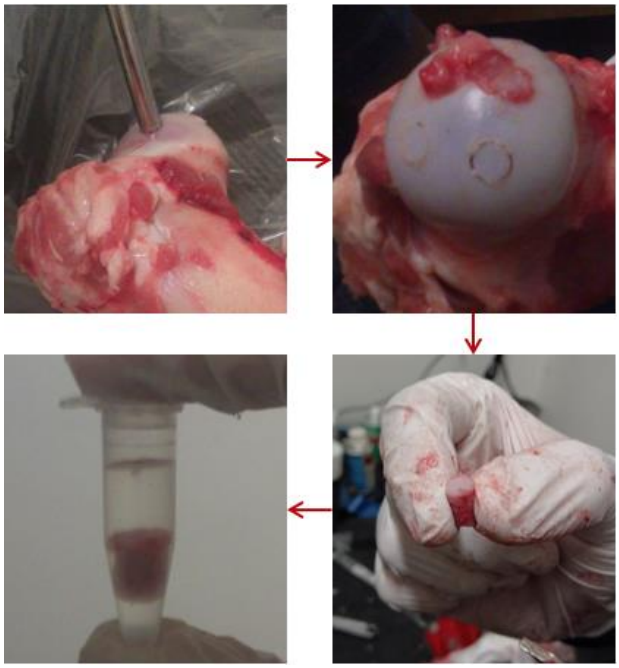

Fig. 4. Specimen preparation process.

Three variants of lubricant were used. The composition of all the used lubricants is shown in Tab. 1.

Table 1. Composition of the used lubricants.

	Concentration [mg/ml]		
	γ-globulin	**HA**	**Albumin**
Lubricant 1	-	-	20
Lubricant 2	3,6	-	-
Lubricant 3	3,6	2,5	20

In the experiments focused on visualization, lubricant 3 was used. The composition of lubricant 3 was constant for all the visualization experiments, despite the fluorescently stained component varied. In the first case, albumin was marked by Rhodamine-B-isothiocyanate (283924, Sigma-Aldrich) and in the second case, γ globulin was marked by Fluorescein-isothiocyanate (F7250, Sigma-Aldrich).

2.3 Experimental methodology

All of the experiments in this study were measured with one sample to be secured comparability of all individual measurements. Although the samples are removed from one rigorously defined place of cartilage surface, there are significant deviations between friction trends of each cartilage samples. This deviation is caused by different mechanical and structural properties among individual animal bones and therefore measurements were performed with one single sample. The validation of one sample measurements was set in this study. 5% deviation in maximum was found comparing the measurements.

The procedure was strictly specified in order to minimize the results deviation and to compare the measurements. Before each measurement, the sample was stored in PBS to rehydrate the cartilage tissue. First, the run-in procedure (20 reciprocating cycles at 10 N load) was performed before each measurement. This procedure suppresses the effect of previous experiments, especially effect of any previously used lubricants.

In the first section, lubricants 1 and 2 were used considering the friction measurements only. In the second section, the fluorescent microscopy was simultaneously combined with friction measurements. The experiments were focused on finding a correlation between visualization of contact area and friction trends. Model synovial fluid with lubricant 3 was used for all measurements in the second section. It had two configurations; the first with fluorescently

marked albumin and the second with stained γ-globulin. The composition was the same in the both cases. The measurements were performed in identic conditions and procedure.

2.4 Experimental condition

The scope of chosen conditions was to simulate human hip joint, therefore the conditions were defined with respects to it. The contact stress was set to1 MPa, which was achieved by 10 N of load. This conditions provides the medium stress comparable with hip joint. Speed of the movement was se to 10 mm/s, which corresponds to slow walking. Stroke of reciprocating motion was taken from the previous studies and it was chosen to be 20 mm. Lubricant bath was heated to 37 ˚C.

3. RESULTS AND DISCUSION

The results of friction measurements are shown in Fig. 5.

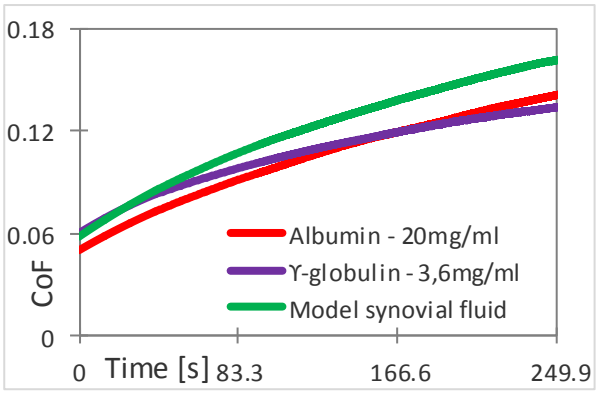

Fig. 5. Friction trends.

Lubricant 1 (albumin 20 mg/ml) exhibits steeper increase of friction than lubricant 2. It can be explained by the presence of higher concentration of proteins and larger size of albumin molecules. This explanation is also supported by images from contact visualization, which show higher light image intensity in the case of measurement with marked albumin. There is only one curve labelled as lubricant 3, which represents both of the performed measurements. In the first case, albumin protein was marked, while in the second case ϒ-globulin protein was visualised. Model synovial lubricant (lubricant 3) shows higher friction than any of the simple protein solutions. The growing global volume of proteins in lubricant leads to apparently higher friction. Very similar lubricant solutions were studied by Murakami et al. [18] who observed higher friction for γ-globulin proteins than for complex synovial fluid. Nevertheless, in the mentioned reference, the authors used different concentration of proteins and different specimens. This can explain the disagreement of the achieved results.

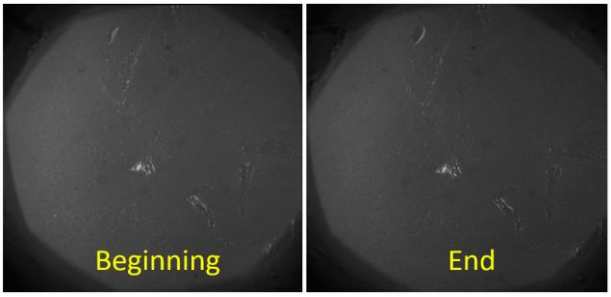

Fig. 6. Contact area visualization – Lubricant 3 with stained albumin at the beginning and at the end of the measurement.

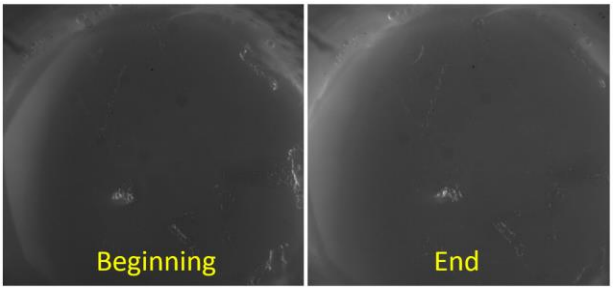

Fig. 7. Contact area visualization – Lubricant 3 with stained ϒ-globulin at the beginning and at the end of the measurement.

In order to visualize the contact area, lubricant 3 was used. The friction measurements were performed simultaneously with the visualization. The visualized contact area shown in Figs. 6 and 7 corresponds to friction curve of lubricant 3 shown in Fig. 5. White spots in in Figs. 6 and 7 are the proteins entrapped within the contact area.

Compared to the Fig. 6, Fig. 7 shows higher global intensity of emitted light. It means, that the thickness of lubricant film formed by lubricant 3 with labelled albumin is higher. Fig. 6 shows more and bigger aggregations of albumin particles. Apparently, the albumin protein is more represented constituent in the contact. The concentration of γ-globulin proteins in lubricant 3 is lower and its molecules are of smaller size at the same time. Comparing the impact of both

marked proteins, we can see that the contribution of albumin is more important in terms of cartilage lubrication.

The proteins are captured in the same location in both experiments (Fig. 6, Fig. 7). It can be assumed, that there are small local damages of the cartilage.

4. CONCLUSION

New specialized reciprocating tribometer for biotribological research was designed. It includes controlling and measuring systems, which allow friction force measurement and contact area visualisation at the same time.

Fluorescence microscopy was chosen as a suitable optical method for visualization of cartilage contact area. It was successfully used for soft contact visualization before and proved again its benefits for such type of visualisations.

The methodology for preparation of the specimens was also demonstrated in the present study.

Calibration and data validation was performed using commercial tribometer Bruker TriboLAB. The capabilities of new device were shown on the set of pilot experiments.

It reveals new unexplored fields in biotribology. Future research may bring a significant contribution, which could be eventually applied in treatment of human joint diseases.

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