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OBSERVATION OF LUBRICATION FILM IN SYNOVIAL JOINT

Pavel ČÍPEK[1,*], David REBENDA[1], Martin VRBKA[1], Martin HARTL[1]

[1] Brno University of Technology, Brno, Czech Republic
* Corresponding author: Pavel.Cipek@vut.cz

Abstract: *Painless movement is very important in active life; therefore, a proper function of our synovial joints is necessary. Synovial joints can be injured during human life in several ways. When a synovial joint is too damaged, it has to be replaced by an artificial joint. There is a common effort to postpone operations of total endoprosthesis as long as possible. The reason is limited lifetime of artificial joints. An operation can be postponed by alternative approaches, for example by viscosuplementation. The issue of lubrication in natural joints is not explored enough. The understanding of lubrication process can assist in the development and understanding of new suitable medical treatments. This study is focused on the observation of a lubrication film in a model of synovial joint. A contact is simulated between a cartilage pin and a glass desk in reciprocating tribometer and the contact area is observed. The goal of this study is to describe the contact area and correlation between friction trends and the formation of lubrication film.*

Keywords: *Biotribology, cartilage, reciprocating tribometer, friction, lubrication*

1. INTRODUCTION

The synovial joint is one of the main components of human motion system. It composes of two bones or more whose surfaces covered by cartilage are in mutual contact [1]. The synovial joints are lubricated by synovial fluid. The combination of special structure of cartilage and synovial fluid allow movement with very low friction coefficient. Cartilage is heterogeneous material with very low cell density and porous structure [2]. These attributes cause the specific tribological behaviour. The main component of the cartilage structure is an extracellular matrix (ECM). ECM is rich in type II collagen fibres and proteoglycan [3]. Hyaluronic acid (HA), proteins, decorins, chondrocytes, etc. are also included in the ECM [4]. The thickness of the cartilage is divided into the three zones, the superficial zone; the middle zone and the deep zone [1]. Each of them has the specific composition and orientation of the collagen fibres [5]. The water volume in cartilage tissue is also very important attribute with regard to lubrication properties [6, 7]. In terms of mechanical properties, the cartilage has very low elastic module (1 – 20 MPa) with respect to the position of the cartilage surface and type of joint [4]. All the mentioned specific properties are basis for lubrication processes in the synovial joint.

Apparently, there is very limited knowledge in terms of experimental investigation of lubrication in natural joints. Nevertheless, some studies focused on visualization of natural cartilage, or hydrogels, were published. All the published studies are focused on

friction measurement or visualization of cartilage contact area separately. One of the first study dealt with visualization of hydrogel contact area by fluorescence microscopy [8]. The aim of the study was to determine the amount of the fluorescently marked particles in the contact area. The measurements were carried out with labelled proteins contained in the synovial fluid. Conclusions of this study were, that the γ-globulin protein has the main influence on the lubrication processes; however, the composition ratio of individual proteins and other components of the synovial fluid is very important. The fluorescence microscopy was used for the description of the gel-like layer formation on the cartilage surface. Forsey, et al. [9], showed that the HA is the main component for the formation of the gel-like layer; however, the process depends on the size of the HA molecules. The penetration of the cartilage structure was demonstrated. Particles of the HA bind with chondrocytes in the cartilage structure. Wu, et al., 2015 [10] studied the flow of the synovial fluid through the cartilage structure depending on the cartilage compression. The results showed that the large molecules of HA were caught on the cartilage surface, while the smaller particles penetrate the cartilage structure. The large molecules create the gel-like layer on the surface that protects the raw cartilage surface against a damage.

The fluorescent microscopy was also used for visualization of contact in joint replacement. Number of studies were carried out and 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 focused on soft contacts, or visualization of joint replacement contact, among others.

The previous studies have always dealt with the friction measurements or with visualization of contact area separately, it has never been measured simultaneously yet. This study combinates this two branches of biotribologic science and uses the optical methods at workplace and classical friction measurements together. The specially tailored tribometer was designed for this application which allows visualization of soft contact and friction measurements at the same time. Concept like the new designed tribometer 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. MATERIALS AND METHODS

2.1 Experimental device

The tailored tribometer allows view into the contact area and it measures friction forces simultaneously in real time. For compliance of this requirements the pin-on-plate configuration of tribometer was used. The concept was adapted to be able to use fluorescent microscopy. This new design is close to concept of tribometer which was used in study [13]. The schema of newly designed experimental device is shown in Fig. 1. The cartilage sample is placed under the glass desk in order to visualize the contact area. The fluorescent microscopy is used for contact observation. The view is obtained by fluorescent microscope and it is record by high-speed camera. The mercury lamp was used as a light source. The contact area was flooded by a lubricant and heated to a human body temperature. The glass desk was designed as a moveable part which performs the reciprocating motion, while the specimen is stationary.

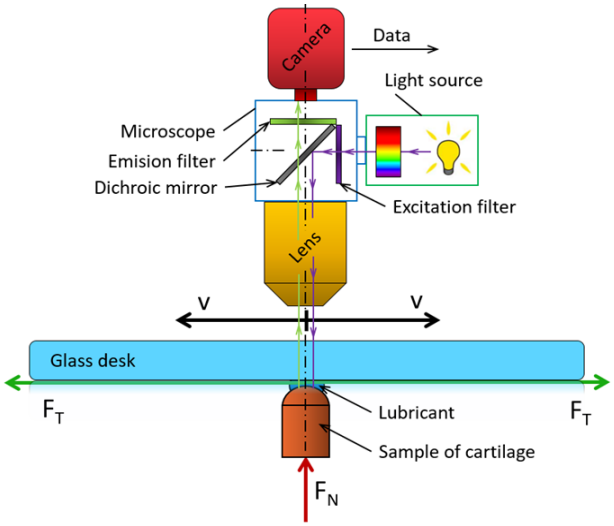

Figure 1. Schematic of the apparatus

According to the schema of function (Fig. 1) a concept of experimental device was designed. The device consists of several main unites, as is shown in Fig. 2.

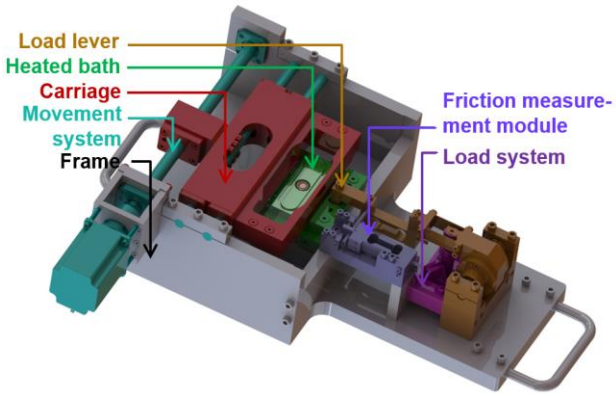

Figure 2. Real arrangement of apparatuses

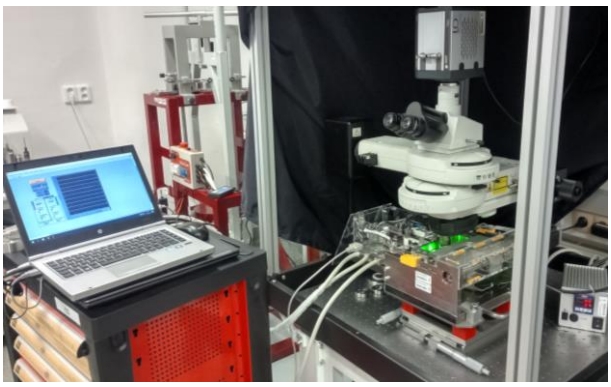

Figure 3. Real arrangement of apparatuses

The basis of the tribometer is a tough frame; all the other components are mounted on it. The moveable part, where the glass plate is mounted, performs the reciprocating motion. The accurate guide rods in combination with ball screw provide very accurate motion without clearances. The lubrication of the contact is provided by a bath. There is a sealing between the glass plate and the bath. Lever is mounted in two preloaded bearings. This arrangement allows the motion without clearance. Lever, on which specimen is attached, exerts the load on the contact. A strain gauge is connected in a serial to the lever and is used for measuring the load. The lever contains a deformable part which allows measuring of very low forces. The other strain gauge allowing friction measurement is connected to the lever in parallel. The whole tribometer is below the fluorescent microscope on an adjustable table. The tailored tribometer is shown in Fig. 3.

Measuring system is based on the National Instruments measuring card, whose data is processed, by LabVIEW script using PC. Control system is based on Arduino. The glass plate motion is ensured by the stepper motor and the load is provided by the linear stepper motor. The input parameters are defined via LCD display and encoder. The measuring system and control system work separately.

The verification and calibration of the device were performed on standard pairs of samples. The pin was made from PTFE-G400 and the plate was made from optical glass B270. The results were compared with commercial tribometer Bruker UMT TriboLAB. The mentioned material combination was used because the cartilage specimens show variance in results. The obtained compliance of the results 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 is the most loaded joint in a human body, which leads to the best mechanical properties [4]. The sampling position was precisely defined through all sample bones, in order to the minimalize deviation of mechanical properties. The hollow drill bit with diameter of 6 mm was used and the specimens were deeply frozen (-20 ˚C) in PBS. This sampling process was used in some studies [14, 15] and it was verified in [16, 17] which proved that the tribological properties did not change. The samples were unfrozen just before testing due to fast degradation of samples. The sampling process is shown in Fig. 4.

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

Tubule 1. Composition of the used lubricants

	Albumin (mg/ml)	γ-globulin (mg/ml)	HA (mg/ml)
Fluid 1	20	-	-
Fluid 2	-	3,6	-
Fluid 3	20	3,6	2,5

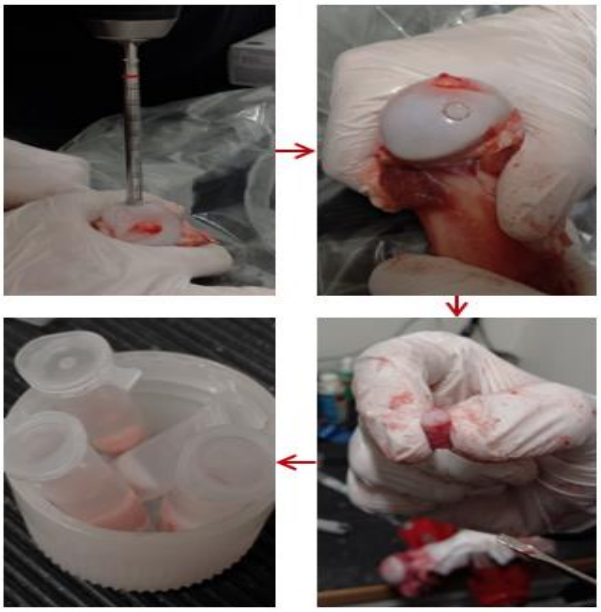

Figure 4. Specimen preparation process

For the experiments focused on visualization fluid 3 was used. The composition was constant for all the visualization experiments, only 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

In order to carry out the individual measurements comparable, all of the experiments in this study were measured with one sample. There are significant deviations between the measurements when they are performed with various samples. This deviation is caused by different mechanical and structural properties among individual animal bones. The comparability of measurements with one sample was validated in this study. The deviation of results was 5% in maximum.

All the experiments were performed according to strict procedure to minimize the results deviation. The sample was stored in PBS between experiments and the run-in procedure consisting of 20 reciprocating cycles at 10 N load was performed before each experiment. This procedure suppresses the effect of previous experiment.

In the first step, the experiments were focused on the friction measurement considering the lubricants 1 and 2. In the second step, the experiments focused on the combination of friction forces measurement and visualisation of the contact area. Lubricant 3 was used in two configurations; the first with stained albumin and the second with stained γ-globulin. The overall composition was always the same.

2.4 Experimental condition

The applied conditions were chosen based on the real conditions in human body in considered the hip joint. The load was 10 N, which provides the medium stress comparable with hip joint stress 1 MPa. Speed was 10 mm/s, which corresponds to slow walk. 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.

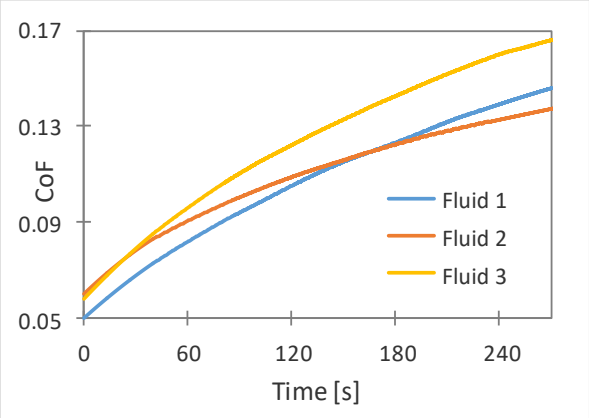

Figure 5. Friction trends, Fluid 1 – albumin, Fluid 2 - γ-globulin, Fluid 3 – model synovial fluid

Fluid 1 exhibits steeper increase of friction than fluid 2, which is the most probably caused by higher concentration of proteins and larger size of albumin molecules. These trends are confirmed by images from contact visualization, which show higher light image intensity in the case of measurement with marked albumin. Although two measurements

were performed with fluid 3 composition, there is only one curve fluid 3, which represents both measurements. This one curve is representative for both measurements. In the first case, albumin protein was marked and in the other case ϒ-globulin protein was marked. Model synovial fluid (Fluid 3) shows higher friction than simple protein solutions; apparently, the grooving global volume of proteins in lubricant causes higher friction. Similar solutions were studied by Murakami, et. al., 2017 [18] who observed higher friction for γ-globulin proteins while complex synovial fluid showed lower friction. However, in the reference, the authors applied different concentration of proteins and different specimens, which explains the disagreement of the achieved results.

Fluid 3 was used for visualization of the contact area. The friction measurement was performed simultaneously with visualization of contact area with both the variations of fluid 3, which corresponds with friction curve fluid 3 in Fig. 5. The visualized contacts are shown in Fig. 6 and 7. White spots in these images are the proteins entrapped within the contact area.

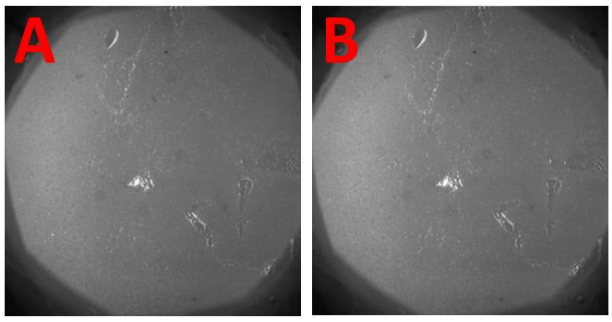

Figure 6. Contact area visualization, A, B – Fluid 3 with stained albumin at the beginning and end of the measurement.

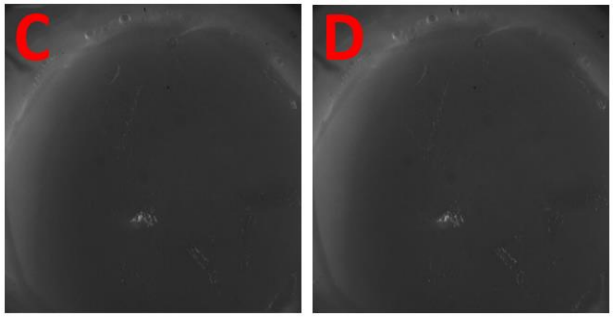

Figure 7. Contact area visualization, C, D – Fluid 3 with stained ϒ-globulin at the beginning and end of the measurement.

It is evident, that figure 6 shows higher global intensity of emitted light than figure 7, which means higher thickness of lubricant film formed by fluid 3 with labelled albumin. Figure 6 shows more and bigger aggregations of albumin particles. This fact means that the albumin protein is more represented constituent in the contact. Fig. 7 shows role of γ-globulin protein in lubrication forming process. Global intensity of emitted light is lower in comparison with Fig. 6 which is caused by lower concentration of γ-globulin proteins in fluid 3 and smaller size of γ-globulin molecules. The comparison of impacts of both marked proteins shows that the contribution of albumin is more important in terms of cartilage lubrication. There is a compliance considering the locations where the proteins are captured in both figures (Fig. 6, Fig. 7). Probably, there are small local damages of the cartilage in these places.

4. CONCLUSION

New specialized reciprocating tribometer in pin-on-plate configuration including measuring and controlling system was developed and designed. This concept connects opportunity of friction force measurement with contact area visualization. Fluorescence microscopy was chosen as a suitable optical method for visualization of cartilage contact area, because it was successfully used for soft contact visualization before. The methodology of specimen preparation and experimental setup were demonstrated in the present study. The new tribometer was validated and calibrated using commercial tribometer Bruker TriboLAB. The first pilot experiments, which were performed in order to demonstrate the possibilities of new the device, are introduced.

The pilot results revealed that the articular cartilage contact is unexplored; therefore, more extensive research is necessary. Future result could bring a significant contribution in the area of cartilage lubrication, which could eventually help in treatment of human joint diseases.

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