BIOTRIBOLOGY OF SYNOVIAL JOINTS AND HIP IMPLANTS: A STUDY BASED ON EVANESCENT WAVES AND FLUORESCENCE

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Abstract. The study of lubrication of synovial joints (articulated joints, such as hip, knee and ankle) has been done from the beginning of last Century till today, without any definitive explanation about the superb mechanism of lubrication of these joints. In terms of tribology, the main characteristics of synovial joints are the low wear associated with the low friction. These characteristics can be easily observed due to the fact that the most of people can live without any damage in their joints during their lifetime. An interesting feature of this lubrication mechanism is the composition of synovial fluid, the natural lubricant of synovial joint, which is water-based. Water is quite accessible, but it has a very low capacity to support loading. However, nature solves this problem with biological additives, such as proteins, glycoproteins, lipids and others. These additives improve the lubrication properties of the synovial fluid. Several researches have been done in order to understand this mechanism of lubrication with a focus on the synovial fluid and the bearing surfaces (articular cartilage). The biomimetics is the major aim of the most of researches of biotribology of synovial joint, i.e., the reproduction of the best characteristics of synovial joints in different applications in the industry. Among these fields of application, the research of functionalized biomaterials for prosthesis, especially hip implants, has increased in the last years. The lifetime of a hip implant is around 20 years, which was quite reasonable 50 years ago, when the firsts prosthesis were developed. However, nowadays, the increase of amount of younger patients has required the increase of lifetime of these prostheses. In this study, techniques based on evanescent waves and fluorescence has been introduced as a tool for the analysis of the surface of articular cartilage and hip implants. Evanescent waves are very sensitive to changes in the refractive index of different mediums. Based on this feature of evanescent waves, a device was developed which allows carrying out measurements of friction with detection of changes in the evanescent waves simultaneously. Tests were carried out with specimens obtained from pigs (6 months of age and about 100kg) and with different test parameters (load, sliding velocity and others). Analysis of the results has shown that articular cartilage can keep a reasonable amount of water on the surface because of the hydrophilic molecules called as proteoglycans. Also, this hydrated layer on the surface could be responsible for the low friction and low wear of cartilage. Based on the results, it has been supposed that the proteoglycans could not be adsorbed onto the articular surface. However, they could be exposed from the internal structure of articular surface, since proteoglycan is one of the components of the solid matrix of articular cartilage. About the experiments with hip implants, techniques based on the fluorescence were used. Some components of synovial fluid (albumin and glycoprotein) were labeled with ATTO Dye 488 and 565 (Sigma Aldrich) in order to evaluate the adsorption of them onto the surface of alumina (Al_2O_3), CoCrMo and UHMWPE (Ultra High Molecular Weight Polyethylene), which are the most common biomaterials used for hip implants. Results have shown a transfer of polymeric film onto the CoCrMo and a continuous exchange of protein during the friction tests for alumina samples. It means that the proteins are not strongly adsorbed onto the alumina surface, and hence, none protective superficial layer of protein is observed. High friction has been observed when albumin is tested isolated from other components of synovial fluid. On the other hand, glycoprotein, which is very hydrophilic, has decreased the friction, which could be an indicative that water plays an important role on the lubrication for both articular cartilage and hip implants. The high friction due to the albumin was not completely understood. One hypothesis is based on the denaturation of albumin due to the hydrophobic surface of polyethylene, which could increase the roughness of the surfaces. These techniques based on evanescent waves and fluoresce has been very useful for the analysis of adsorption during tests of friction and could be useful in new experiments in order to understand the mechanism of lubrication of synovial joints and hip implants. This work was done in Kyoto University - Japan and in Federal Institute of Technology of Switzerland (ETH) - Zurich. The author would like to express his gratitude for these two Universities.

Keywords: lubrication; evanescent waves; fluorescence; articular cartilage; hip implant

1. INTRODUCTION

Synovial joints are involved in the most part of movements undertaken by the human body. Shoulder, elbow, wrist, fingers, hip, knee and ankle are some examples of synovial joints. The term synovial is derived from the Greek word *syn ovia* that means "like the egg" and is associated with the liquid found in the synovial joints, the synovial fluid, which has an appearance and consistency that resembles the egg white (Buchanan and Kean, 2002). This fluid is responsible for the lubrication and transport of nutrients to the joints (Maroudas, 1975). Synovial fluid is a water-based lubricant. Although water has a low capacity to support loading, synovial fluid has some biological additives that improve the lubrication characteristics of this fluid. These additives are proteins (around 80%), glycoproteins, lipids and others components.

One remarkable characteristic of the synovial joints is their superb mode of lubrication, which is responsible for the long durability of these joints. Synovial joints are capable to working with low friction and wear under severe conditions from the lubrication standpoint (Unsworth, 1995). Usually, the lower joints (hip, knee and ankle) work mainly in hydrodynamic lubrication regime, when the sliding speed is high and loading is low. However, in the stance phase of locomotion, loads are quite high and sliding speed becomes low, when the boundary lubrication is dominant. Under these conditions, lubrication is very poor due to the contact of the articular cartilage, the bearing surface of

synovial joints. Several researches have been done in order to understand the mechanism of lubrication of synovial joint, with a special focus on the articular cartilage (bearing surface) and synovial fluid (lubricant). Articular cartilage is a specialized connective tissue consisting of two phases: porous-solid (20 to 30% of wet weight) and liquid (70 to 80% of wet weight) (Woo et al., 1987). In the solid phase, collagen fibers are arranged in a network with proteoglycans and chondrocytes. Proteoglycans are negatively charged under physiological conditions (Wu and Herzog, 2002) and they have hydrophilic properties that are associated with the high water contents of articular cartilage (Laasanen et al., 2003). Electrostatic repulsion forces between these proteoglycan are known to provide above 50% of the equilibrium compressive modulus of cartilage (Buschmann and Grodzinsky, 1995).

In summary, substances present in articular cartilage (Pickard et al., 1998) and synovial fluid (Mabuchi et al., 1999), and mechanical functions of articular cartilage (Wang et al., 1997; Krishnan et al., 2003) are the main factors investigated in order to clarify this mechanism of lubrication. Although articular cartilage has excellent tribological characteristics, some failures can take place in elder people because cartilage has a minimal ability to recovery from damages. The absence of blood vessel and nerves is one of the reasons for this limitation (Hanziker, 2001). Depending on the degree of damage, an implant surgery is unavoidably necessary. This kind of surgery should not be a problem because of the modern implant that can work for about 20 years. However, nowadays, the amount of younger patients has increased and it requires a new superior limit for the lifetime of implants, which must be at least 30 years.

The main challenge is the development of new implants with improved wear resistance and low friction. The biomaterials commonly used for implants are ceramics (alumina: Al_2O_3), polymers (Ultra High Molecular Weight Polyethylene: UHMWPE) and metal alloys (CoCrMo). The understanding about how natural synovial joints work would be very useful to this end. In other words, the identification of which factors would play a crucial role for an efficient lubrication would be helpful. However, there are some limitation for a successful research and analysis of lubrication in artificial and natural joints.

One of these limitations is the surface analysis of articular cartilage and biomaterials. The high contents of water on the surface makes difficult the detection or identification of articular surface and hence, changes hardly can be detected. This is an inherent problem of living tissues. The challenge is to analyze the articular surface, which may assist in the elucidation of the mechanism (or mechanisms) of lubrication of the articular cartilage. Also, the improvement of the tools for surface analysis in hip implants could be very useful in order to understand the effect of components of synovial fluid and the friction mechanism.

In this work, two methods for surface analysis of articular cartilage and biomaterials for hip implants are presented, which are based on evanescent waves and fluorescence, respectively. The results of friction tests were analyzed with the results obtained from the analysis of the surfaces of materials.

1.1. Evanescent waves and fluorescence microscopy

Techniques for the recognition of substances using the evanescent waves have been widely applied on the biological researches (Ksenevich et al., 1996; Challener et al., 2000; Ahmad and Hench, 2005). Evanescent waves are a kind of electromagnetic waves that propagate from an interface where a light undergoes total internal reflection and decay exponentially as the distance from the interface increases (Henkel et al., 1999), as can be seen in Figure 1.
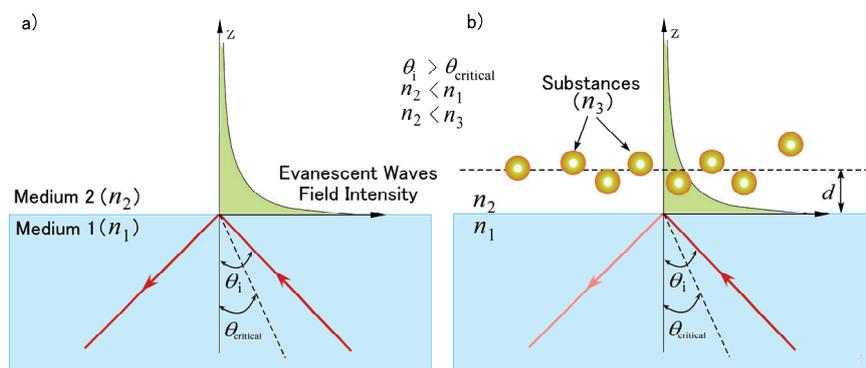

Figure 1. a) Total Internal Reflection and b) Frustrated Total Internal Reflection. n_i (i=1,2) represents the refractive index of medium i, θ_i is the angle of incidence, $\theta_{critical}$ is the critical angle and d is the maximum thickness of the gap between the medium 1 and substances with refractive index n_3 for the occurrence of frustrated total internal reflection

Total internal reflection occurs when the medium where the light is derived (medium 1) has a refractive index (n_1) larger than the other medium (n_2) at the interface, and the angle of incidence (θ_i) must be larger than the critical angle ($\theta_{critical}$). The critical angle is calculated using the following equation Eq. (1):

$$\theta_{critical} = \sin^{-1}\left(\frac{n_2}{n_1}\right) \tag{1}$$

Where n_1 and n_2 correspond to the refractive indices of the medium 1 and 2, respectively.

From this equation (Eq. 1), it is possible to perceive that the refractive index of the medium where the light is derived (n_1) must be larger than that of the other medium (n_2). Even so the parameters, such as refractive indices and the angle of incidence, are adjusted for the total internal reflection, the reflected light can suffer attenuation due to the interaction of the evanescent waves with substances near the interface, as can be observed in Fig. 1.b. These substances must have a refractive index (n_3) higher than the refractive index of the gap (n_2) that separates them from the interface (Stahlhofen, 2000). The thickness of this gap (d) is in degree of incident wavelength light, that is, at nanometer order (Papathanassoglou and Vohnsen, 2003).

This phenomenon is known as frustrated total internal reflection and the attenuation of reflectance depends on the refractive indices of the involved mediums (n_1, n_2) and the additional substances (n_3), the wavelength of light and the angle of incidence of light (Zhu et al., 1999). For the application of this principle in the analysis of the articular surface, it is necessary to define what kinds of substances could be found in articular surface. Articular cartilage is basically divided into two phases: liquid and solid. The water and the collagen fibers are the most abundant components of the liquid and solid phases (Woo et al., 1987) of articular cartilage, respectively.

Therefore, when a specimen of articular cartilage is placed on the interface (in the medium 2 of Fig. 1), the frustrated total internal reflection occurs due to the refractive index of collagen, about 1.46 (Drezek et al., 1999), is higher than that of the water and saline (1.33 and 1.34, respectively), the possible constituents of medium 2.
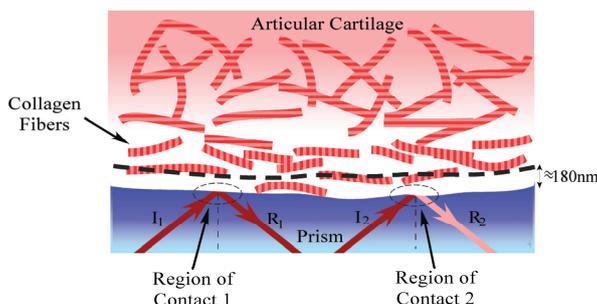

Figure 2. Mechanism of attenuation of the reflectance due to the presence of collagen fibers near the interface. I_i and R_i represents the incident and reflected light at the region i, respectively

Figure 2 illustrates the mechanism of attenuation of the reflectance due to the presence of collagen fibers near the interface between the articular cartilage and a glass (BK-7, $n = 1.51$). Assuming that the wavelength of light is 632.8nm (red color laser light) and the collagen fibers are immersed in a medium of water with saline, the limit of the effect of the evanescent waves is situated around 180 nm from the interface. When the angle of incidence leads to the total internal reflection and a light is reflected at the region 1, the intensity of reflected light is not changed. However, when the light is reflected at the region 2, attenuation in the intensity of laser light can be observed due to the presence of collagen fibers within the region limited by 180 nm from the interface. Thus, the attenuation of reflectance when articular cartilage is placed on the prism surface can be related to the increase of collagen contents near the contact the interface. Hence, it is reasonable to assume that the decrease of water contents is related to the increase of collagen contents.

For the fluorescence microscopy, the technique is based on the fact that protein can be labeled with fluorescent dyes that could be visualized by fluorescence microscopy. In this work, BSA (bovine serum albumin) was labeled with two different colors (red and green) in order to evaluate the exchange and adsorption of BSA during friction tests. The results of fluorescence imaging were correlated with friction results.

2. MATERIALS AND METHODS

2.1. Materials

For test with articular cartilage, the specimens were harvested from the lateral femoral condyles of matured and healthy (about 100 kg) pigs with biopsy punch with 5 mm of diameter and the mean thickness of articular cartilage was about 1.4 mm. The medial femoral condyles of pigs were not used due to the high occurrence of osteochondrosis found in the pigs for consumption (Wardale and Duance, 1994). The specimens were rest in phosphate buffered saline (PBS –

Sigma-Aldrich Co., Saint Louis, USA) solution during 1 hour before the test at 24°C and they were gently shaken in order to eliminate the excess of synovial fluid on the articular surface. The tests were done using PBS as lubricant.

For experiments with biomaterials, pins and discs (Mathys Ltd. Bettlach, Switzerland) were made of CoCrMo, Al_2O_3 and UHMWPE. The geometry of pins was cylindrical with a radius applied on the end. For the pins of UHMWPE, the radius was 35 mm. The radius of pins of CoCrMo was 1200 mm. Different radii were applied in order to keep the contact pressure, which was calculated using Hertzian contact model, at the physiological range (between 8 MPa to 20 MPa (Jin et al., 1999; Yew et al., 2003)), which results in a normal load between 0.5 to 5 N.

Before the tests, the specimens were cleaned in an ultrasonic bath for 15 minutes. Hexane was used for the first bath and isopropanol afterwards. Finally, the specimens were cleaned with deionized water. The procedure was repeated two times for each specimen. Then, the specimens were dried with an N_2 air jet. Three combinations of pins and discs were used: UHMWPE (pin) against CoCrMo (disc), UHMWPE (pin) against Al_2O_3 (disc) and CoCrMo against CoCrMo.

As the fluorescence microcopy imaging was used as a tool for surface analysis, it was necessary to label the lubricant with fluorescent dyes, which were ATTO 488 NHS ester and ATTO 565 NHS ester (Sigma-Aldrich, Germany). A more detailed protocol has been reported in a previous work (Roba et al., 2009).

Three kinds of lubricants were used: PBS as the control; PBS with 20 mg/mL of BSA and 5 mg/mL of ATTO488-BSA and, Bovine synovial fluid (BSF) with ATTO488-BSA. The concentrations of labeled/unlabeled BSA in the BSF solutions were 12.5 and 1.5 mg/mL, respectively. The temperature of the lubricant was kept in physiological range (around 37°C).

2.2. Friction tests of articular cartilage with evanescent waves

The light source was a laser light He-Ne with a wavelength of 632.8 nm (Melles Griot Co.) and 5 mW of power and a beam with 5 mm of diameter with a randomic polarization. Two lenses were mounted in order to expand the laser beam (final diameter of about 100 mm). A schematic illustration of the arrangement of optical components of the apparatus is presented in Fig. 3. As the polarization of light is random, a cube polarizer was mounted in order to p-polarize the incident light.
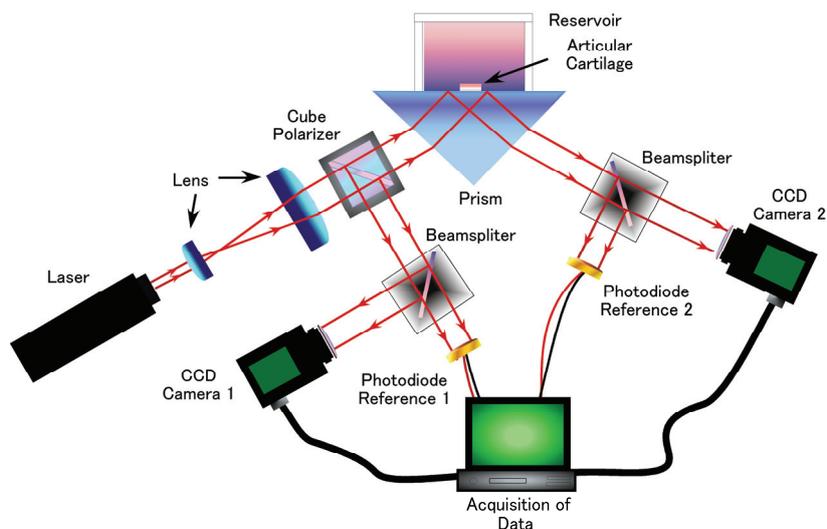

Figure 3. Schematic illustration of arrangement of optical components.

The refractive indices of prism (BK7) and the bath solution, saline, were about 1.51 and 1.34, respectively. The incident angle was set at 64.8^{o} for TIR when the solution environment is physiological saline. Images of the incident beam and reflected beam were captured with CCD cameras (WAT-221S Color Camera, Watec Co. Ltd.). Photodiodes (Industrial Fiber Optics, Inc.) were used for the correction of the reflectance calculated from the image analysis, in order to minimize the effect of noise derived mainly from the thermal and electrical oscillations. The experiment was done in a dark room in order to avoid the capturing of undesired lights.

For the friction test, the specimens of articular cartilage were slid on the prism surface and friction forces were measured. In Fig. 4, a schematic illustration of the device used in the friction tests is shown. The normal load and friction forces were correlated to the deflections of lower and upper parallel leaves springs (0.07 mm of thickness - stainless steel, Shim & Gauge Co., Japan), respectively. The deflections were detected with laser displacement sensors LDS1 and LDS2 (LB-040, Keyence Japan). Micro-stages (SGSP26-100 and 150, Sigma Koki Co., Japan) were used to setting the load (MS1) and sliding displacement (MS2). The effect of intermittent sliding was evaluated with a normal load of 0.1 N and a sliding velocity of 1.0 mm/s. These values were defined for the occurrence of boundary lubrication.
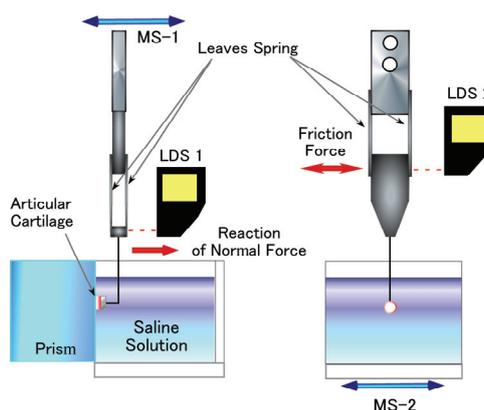
Figure 4. Schematic illustration of the device used for the measurement of normal load and friction force, which are measured due to deflection of leaves springs detected by laser displacement sensors (LDS) 1 and 2, respectively. MS1 and MS2 are the micro-stages used for the setting of normal load and sliding, respectively.

2.3. Friction test of biomaterials and fluorescence micrucopy

The pins and discs were incubated in solutions of 20 mg/mL of BSA with 5 mg/mL of BSA labeled with a red fluorescent dye (ATTO 565 NHS) before the tribological tests. The time of incubation was 1 hour at 24º C. After the incubations, the specimens were washed with PBS and deionized water in order to eliminate the excess of BSA on the surface. The specimens were dried with a weak N_2 air jet.

For the friction measurements, a pin-on-disc apparatus (CSEM S.A., Switzerland) was used. The normal load was 5 N at a sliding velocity of 10 mm/s. The steady-state friction was taken as the average of friction force in the 20th lap. After the friction tests, the samples were carefully washed with PBS and deionized water in order to remove the excess of lubricant (BSF and BSA). A weak flow of N_2 was used for drying. Fluorescence images of the wear tracks of the discs and the contact region of the pins were acquired with fluorescence microscopy (AX10 Imager M1m, Zeiss, Germany). In order to avoid the photobleaching of labeled BSA, the specimens and lubricants were kept in a dark environment during all steps of the experiments.

3. RESULTS

Figure 5 shows the mean values and standard deviations of friction coefficient for an intermittent sliding with short and long interruption for articular cartilage. For short interruption, the first and the second interruption occurred at 330 and 670 s, respectively. After each interruption, a decrease of the friction coefficient is observed. For the long interruption, the decrease of the friction coefficient was higher than that of the short interruption. For the long interruption, the first and the second interruption occurred at 330 and 690 s, respectively. A gradual increase of friction as the time evolves was observed in each interval of the short and the long interruptions.
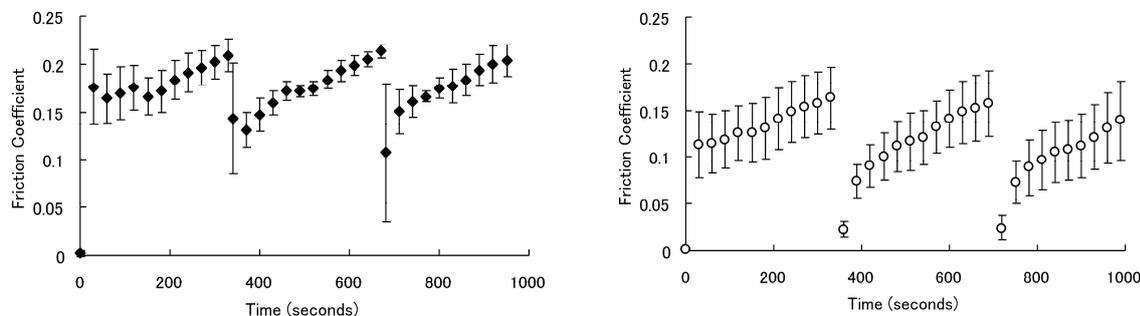

Figure 5. Mean values and standard deviations (n = 4) of the friction coefficient for intermittent sliding and loading with 2 interruptions of 10 s (left hand) and 30 s (right hand).

In Figure 6, the mean values and standard deviations of the attenuation of reflectance of articular surface are presented for the short and long time of interruption, respectively. The reduction in the attenuation of reflectance for the long interruption was higher than that of the short interruption after the both interruptions.
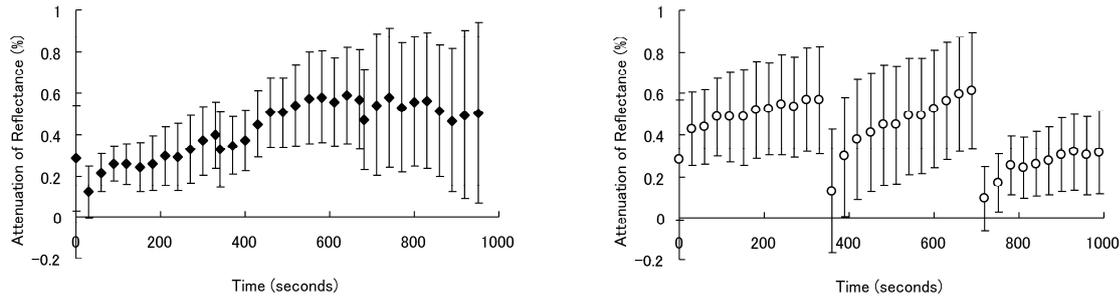
Figure 6. Mean values and standard deviations (*n* = 4) of the attenuation of reflectance for intermittent sliding and loading with 2 interruptions of 10 s (left hand) and 40 s (right hand).

In the test of biomaterials, the highest values of friction were observed for the pair CoCrMo-CoCrMo in all kinds of lubricants (Fig. 7). For this pair, PBS was the lubricant that presented the highest friction. On the other hand, the lowest friction was observed for BSF and BSA with HA and there was no significant difference between these two lubricants.
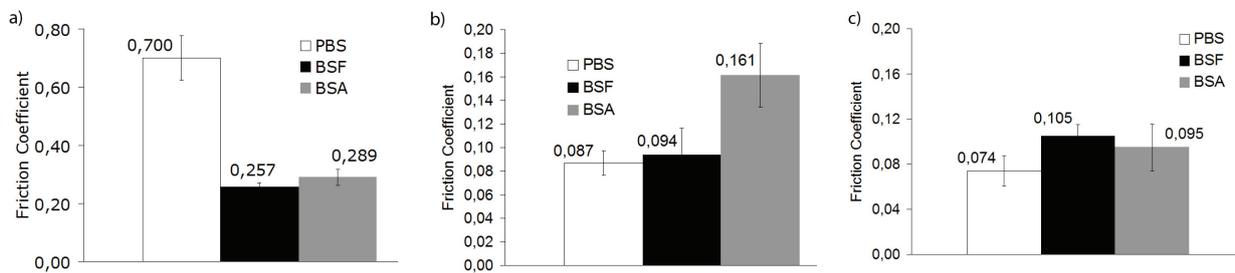

Figure 7. Friction coefficient for biomaterials tested in pin-on-disc. a) CoCrMo disc against CoCrMo pin, b) CoCrMo disc against UHMWPE pin, c) Al$_2$O$_3$ disc against UHMWPE pin. Scales of y axis are not the same for all the graphs.
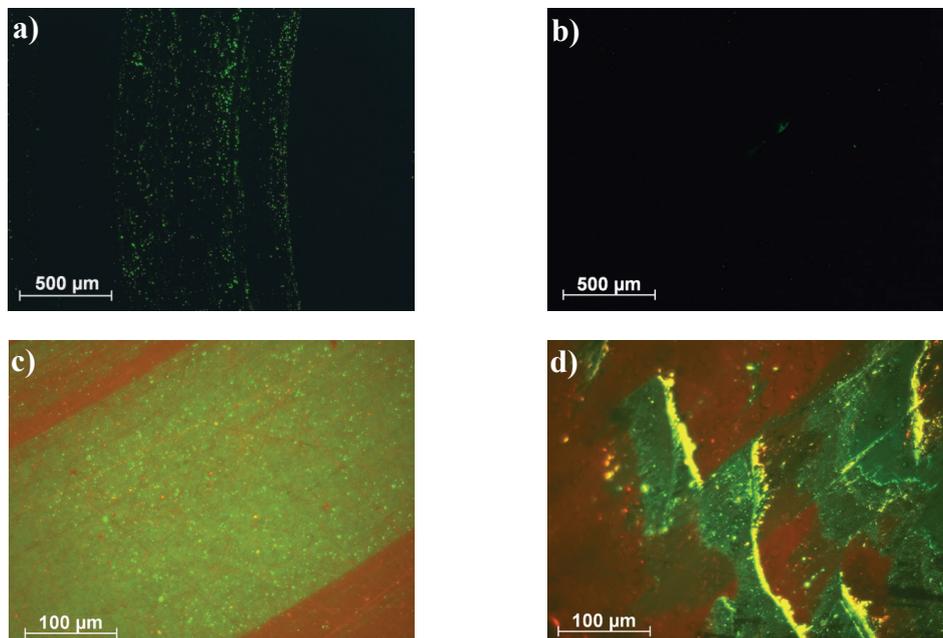

Figure 8. Fluorescent images obtained after friction tests with red albumin previously adsorbed onto the surfaces and green albumin used in the lubricant. a) disc of CoCrMo after test against UHMWPE pin (Load: 2 N). b) CoCrMo disc after test against CoCrMo pin (Load: 2 N). c) disc of Al$_2$O$_3$ after test against UHMWPE pin (Load: 1 N). d) pin of UHMWPE after test with Al$_2$O$_3$ disc (Load: 1 N).

The lowest values of friction coefficients were observed for the CoCrMo-UHMWPE tribopair . These values of friction were very close to the other literatures (Chandrasekaran et al., 1998; Nassutt et al., 2003). Moreover, the friction was lower when the CoCrMo pins were used in all lubricants, except for the BSF, where no significant difference was

observed. For the lubricants, the lowest values of both steady-state and start-up friction were observed when PBS was used, which is a similar result obtained by Mazzuco and Spector (2004). However, when the UHMWPE was used as a pin, no significant difference was observed between PBS and BSF. These results demonstrate an opposite tendency in comparison to the CoCrMo-CoCrMo pair, where the friction values were highest for PBS.

Images acquired after pin-on-disc measurements showed different results in comparison to the incubated specimens. When the UHMWPE was used as a pin with a disc of CoCrMo, it was possible to observe some traces of fluorescence on the surface of CoCrMo (Fig. 8.a), which are characterized as a set of small spots arranged in the sliding direction. Exchange of protein on the Al_2O_3 discs was clearly observed (Fig. 8.c), where the red intensity means the presence of previously adsorbed albumin (ATTO 565-BSA) and green intensity means the albumin from the lubricant (ATTO 488-BSA), which was adsorbed only on the wear tracks. No fluorescence traces could be observed on the CoCrMo disc used against a CoCrMo pin (Fig. 8.b). Although only fluorescence images for unlabeled BSA with labeled BSA have been shown, similar results were observed when different lubricants were used for all tribological pairs.

Figure 9 shows the fluorescence traces on a CoCrMo disc when PBS was used as lubricant for a pair of CoCrMo disc and UHMWPE pin. As the lubricant did not contain labeled BSA, the discs were incubated after the friction tests during 60 minutes in a similar solution used for the lubricant, that is PBS and ATTO 488-BSA. It was possible to observe some wear tracks as spots arranged in the sliding direction, which resemble those on CoCrMo when ATTO 488-BSA was used as lubricant (Fig. 8.a).
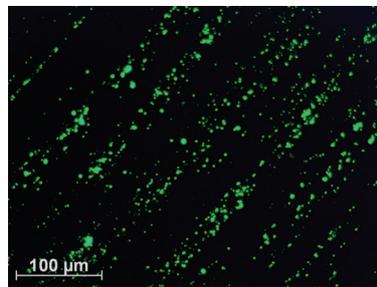

Figure 9. Wear tracks on the CoCrMo disc after friction tests against UHMWPE pin using PBS as lubricant. The disc was post-incubated in ATTO 488 BSA solution after the test (Load: 1 N).

4. DISCUSSION

4.1. Articular Cartilage

The tendency of the increase of the friction coefficient as the sliding time evolves (Fig. 5) is in good accord with the results of the other researchers (Mabuchi and Fujie, 1996). Furthermore, the decrease of the friction coefficient after the unloading is in good agreement with the experiments carried out by Forster and Fisher (1998), McCutchen (1962) and the previous research (Naka et al., 2005). In each interval, the attenuation of reflectance presented the same tendency of the friction coefficient, that is, its intensity increases as the time evolves and decreases after the unloading. As the attenuation of reflectance is associated with the contact or existence of collagen near the interface, it is reasonable to assume that the decrease of water contents is related to the increase of the friction coefficient.

It is interesting to perceive that the variation of the attenuation of reflectance was smaller for the short interruption than that for the long interruption, as can be seen in Fig. 6. Furthermore, the decrease of the friction coefficient was also smaller in the short than that of the long interruption (Fig. 5). The results of the attenuation of reflectance seem to indicate that the hydration of cartilage has a dependence on the time; such as it occurs for the exudation of the water in the articular cartilage when it undergoes a sustained loading. In any case, 10s seems to be sufficient for a decrease of the friction coefficient, however not at same level of 60s of interruption; and 10s is not sufficient for an adequate recovery of the water content at the articular surface in comparison to the 60s. Other interesting aspect is that the level of reduction does not change significantly between the first and second interruption for both friction coefficient and attenuation of reflectance. This result can mean that the recovery of water and reduction of friction have a homogeneous behavior in despite of different levels of friction and attenuation of reflectance due to the non-homogeneity of specimens. A similar result has been found in a previous work (Naka et al., 2007), which had focused the role of preloading and exudation of water on the friction coefficient of articular cartilage. Moreover, Naka et al. (2005) have detected proteoglycans on the articular surface when the friction is quite low using surface plasmon resonance. These proteoglycans would be responsible for the high contents of water on the articular surface, which means that an hydrophilic surface can assist the lubrication of synovial joints.

In the physiological conditions, articular cartilage hardly undergoes a process of continuous sliding and loading during 5.5 minutes as occurred in these tests. Hasler and Herzog (1998) demonstrated that the patellofemoral contact forces reach values nearly to zero in a cycle time of about 1 s. In this situation, the friction coefficient can be kept in the

low levels because of the short time of loading and sliding. In this work, the time of sliding and loading of 5.5 minutes corresponds to a severe condition in physiological terms. However, whether a short time of sliding and loading could be used in the experimental tests, probably the decrease of the friction and the recovery of the water after the unloading could not be observable.

4.2. Biomaterials for hip implants

For the pin-on-disc friction measurements with biomaterials, the main aim was the evaluation of the role of proteins and their exchange during friction tests. Thus, it was important that the experiments had been carried out in the boundary lubrication regime. To this end, the sliding velocity was low (around 1 mm/s) and the friction was taken as the average of friction force in the 20th lap. The short sliding distance was preferred because long distances can gradually increase the temperature at the interface between the pin and the disc. Moreover, long distances mean long duration of tests, thus increasing the possibility of contamination of the lubricant and denaturation of proteins.

An interesting result was the fluorescent images for CoCrMo-UHMWPE and CoCrMo-CoCrMo pairs because fluorescence emission could only be observed on the surface of CoCrMo tested against UHMWPE (Fig. 8.a) and not on that was tested with CoCrMo pin (Figs. 8.b). This phenomenon can be associated with the quenching of fluorescence, which occurs when the energy derived from the emission waves that should excite the fluorophores (that is, induce the fluorescence emission) is transferred to metallic surfaces (Pérez-Luna et al., 2002; Holmes et al., 1993) and hence, no fluorescence emission can be detected.

A reasonable explanation is that UHMWPE was being transferred onto the CoCrMo, enabling fluorescence detection. In order to support this hypothesis of UHMWPE transfer, further experiments were carried out using UHMWPE and CoCrMo as tribopair with PBS as lubricant. After friction test, CoCrMo discs were incubated with ATTO 488-BSA. Fluorescence traces were observed on the CoCrMo disc (Fig. 9) corresponding to the wear tracks, indicating that there was a transfer of UHMWPE taking place during the friction test.

This UHMWPE transfer did not occur in the usual form, that is, like a lamellar transfer (Zhang, 1998), as can be seen in Figs. 8.a and 9. Marcus and Allen (1994) have observed that the transfer of UHMWPE to a metal counterface usually involves interlamellar shear and results in the development of a highly oriented transfer film when water is used as a lubricant, which could be considered as a similar lubricant to PBS. In this work, the wear debris particles were produced and stuck onto the surface, which corresponds to the fluorescence spots onto the wear tracks. Probably, a similar mechanism to the one occurring in the third body wear between two surfaces in relative displacement, could be taking place. During third body wear, hard particles create wear tracks onto the bearing surfaces. In this case, relatively soft UHMWPE particles are pressed against the CoCrMo, thus being adhered onto the surface. This result is similar to the one found by Barret et al. (1992). In their work, the wear and friction of UHMWPE pins against stainless steel discs were measured using a pin-on-disc apparatus under dry conditions. They observed that the transfer film on the disc surface was limited to isolated deposits of wear particles.

The friction results showed that the BSF and BSA worked as a lubricant for the CoCrMo-CoCrMo tribopair. However, for the CoCrMo-UHMWPE pairs, the friction coefficient increases for the same lubricants. As it was observed before in the fluorescence images, a transfer of UHWMPE occurs when it is slid against the CoCrMo surface. This transfer can affect the friction in different ways. One is related to how homogenous this transfer is, because a more homogeneous transfer corresponds to low frictions due to the decrease of the shear stress (ploughing effect) between the transferred UHMWPE and the UHMWPE pin. The presence of albumin adsorbed on the CoCrMo surface can make difficult a homogeneous transfer of UHMWPE, since albumin can work as a protective layer that would avoid the occurrence of this transfer. On the other hand, as no adsorption occurs when the PBS is used as the lubricant, the transfer should be more homogenous and hence, friction could be lower in comparison to BSA and BSF.

Another cause for the increase of friction may be the effect of denaturation of proteins. BSA could be kept in the native state on CoCrMo because the CoCrMo surfaces are more hydrophilic than the UHMWPE (Gispert et al., 2006). Widmer et al. (2001) has shown that for hydrophilic surfaces of functionalized UHMWPE with oxygen plasma, BSA improved the lubrication. On the other hand, for the hydrophobic surface (UHMWPE), an increase of friction was observed. Chandrasekaram and Loh (2001) have also observed that the proteins in the lubricant resulted in severe denaturation and reaction with polymer structure, degrading the surface properties of UHMWPE.

Before this work, it was supposed that this transfer was not taking place due to the formation of a protective layer of adsorbed protein on the CoCrMo surface (Serro et al., 2006; Saikko, 2006). As it can be seen in the fluorescence images, this protective layer does not work effectively. These results indicate that the increase of friction between CoCrMo and UHMWPE when proteins were present in the lubricant, which was observed in this work and others researches (Sawae et al., 1998; Scholes et al., 2000), could be due to the UHMWPE transfer.

5. CONCLUSION

The use of evanescent waves to analyze the contents of water in biological tissues (articular cartilage) has provided very useful information about the friction behavior of articular cartilage. In turn, the novel approach based on

fluorescence labeling of BSA for friction tests of biomaterials introduced in this work was efficient in enabling the evaluation of protein adsorption and exchange on UHMWPE and CoCrMo surfaces. It also allowed coming across an unexpected result, which is the transfer of UHMWPE onto CoCrMo surfaces during friction tests when using UHMWPE pin and CoCrMo disc as tribopair. In summary, fluorescence labeling of BSA applied to friction tests can be considered a very useful tool to study the lubrication of materials used in hip implants, and could be applied to future studies using different combinations of proteins and biomaterials.

6. ACKNOWLEDGEMENTS

7. REFERENCES

Ahmad, M., Hench, L.L., 2005, "Effect of taper geometries and launch angle on evanescent wave penetration depth in optical fibers", Biosensors Bioelectronics, Vol.20, pp. 1312-1319.

Barret, T.S., Stachowiak, G.W., Batchelor, A.W, 1992, "Effect of roughness and sliding speed on the wear and friction of ultra-high molecular weight polyethylene", Wear, Vol.153, pp. 331-350.

Buschmann, M.D., Grodzinsky, A.J., 1995, "A molecular model of proteoglycan-associated electrostatic forces in cartilage mechanics", Journal of Biomechical Enginnering, Vol.117, pp.179-191.

Buchanan, W.W., Kean, W.F., 2002, "Osteoarthritis II: Pathology and pathogenesis", Inflammopharmacology, Vol.10, pp. 23-25.

Challener, W.A., Edwards, J.D., McGowan, R.W., Skorjanec, J., Yang, Y., 2000, "A multiplayer grating-based evanescent wave sensing technique", Sensors and Actuators B, Vol.71, pp. 42-46.

Chandrasekaran, M., Wei, L.Y., Venkateshwaran, K.K., Batchelor, A.W., Loh, N.L, 1998, "Tribology of UHMWPE tested against a stainless steel counterface in unidirectional sliding in presence of model synovial fluids: part 1", Wear, Vol.223, pp. 13-21.

Chandrasekaran, M., Loh, N.L., 2001, "Effect of counterface on the tribology of UHMWPE in the presence of proteins", Wear, Vol.250, pp. 237-241.

Drezek, R., Dunn, A., Richards-Kortum, R., 1999, "Light scattering from cells: finite-difference time-domain simulations and goniometric measurements", Applied Optics, Vol.38 (16), pp. 3651-3661.

Forster, H., Fisher, J., 1999, "The influence of continuous sliding and subsequent surface wear on the friction of articular cartilage", Proceedings of the Institution of Mechanical Engineers, Vol.213 (H), pp. 329-345.

Gispert, M.P., Serro, A.P., Colaço, R., Saramago, B., 2006, "Friction and wear mechanism in hip prosthesis: Comparison of joint materials behaviour in several lubricants", Wear, Vol.260, pp. 149-158.

Hasler, E.M., Herzog, W., 1998, "Quantification of *in vivo* patellofemoral contact forces before and after ACL transaction", Journal of Biomechanics, Vol.31, pp. 37-44.

Henkel, C., Wallis, H., Westbrook, N., Aspect, A., Sengstock, K., Ertmer, W., 1999, "Theory of atomic diffraction from evanescent waves", Applied Physics B Laser and Optics, Vol.69, pp. 277-289.

Hunziker, E. B., 2001, "Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects", Osteoarthritis and Cartilage, Vol. 10, pp. 432–463.

Jin, Z.M., Heng, S.M., Ng, H.W., Auger, D.D., 1999, "An axisymmetric contact model of ultra high molecular weight polyethylene cups against metallic femoral heads for artificial hip joint replacements", Proceedings of the Institution of Mechanical Engineers, Vol.213(H), pp. 317-327.

Krishnan, R., Park, S., Eckstein, F., Ateshian, G.A., 2003, "Inhomogeneous cartilage properties enhance superficial interstitial fluid support and frictional properties, but do not provide a homogeneous state of stress", Journal of Biomechanical Engineering, Vol.125, pp. 569-577.

Ksenevich, T.I., Beloglazov, A.A., Nikitin, P.I., Kalabina, N.A., Zaitsev, S.Y., 1996, "Study of biochemical reaction in thin organic films by means of evanescent optical wave", Applied Surface Sciences, Vol.92, pp. 426-430.

Laasanen, M.S., Toyras J., Korhonen R.K., Rieppo, J., Saarakkala S., Nieminen M.T., Hirvonen J. and Jurvelin, J.S., 2003, "Biomechanical properties of knee articular cartilage", Biorheology, Vol.40, pp. 133-140.

Mabuchi, K., Obara, T., Ikegami, K., Yamaguchi, T., Kanayama, T., 1999, "Molecular weight independence of the effect of additive hyaluronic acid on the lubricating characteristics in synovial joints with experimental deterioration", Clinical Biomechanics, Vol.14, pp. 352-356.

Marcus, K., Allen, C., 1994, "The sliding wear of ultrahigh molecular-weight-polyethylene in an aqueous environment.", Wear, Vol.178, pp. 17-28.

Maroudas, A., 1975, "Biophysical chemistry of cartilaginous tissues with special reference to solute and fluid transport", Biorheology, Vol.12, pp. 233-248.

Mazzucco, D., Spector, M, 2004, "The role of joint fluid in the tribology of total joint arthroplasty", Clinical Orthopaedics and Related Research, Vol. 429, pp. 17-32.

McCutchen, C.W., 1962, "The frictional properties of animal joints", Wear, Vol. 5, pp. 1-17.

Naka, M.H., Arima, Y., Iwata, H., Hasuo, M., Fuwa, Y., Morita, Y., Ikeuchi, K., 2005, "A novel technique for evaluation of articular cartilage lubrication based on the surface plasmon resonance", In: D. Dowson; M. Priest; G. Dalmaz; A. Lubrecht. (Org.). Life Cycle Tribology, Tribology and Interface Engineering Series. Amsterdam: Elsevier; Vol.48, pp. 389–397.

Naka, M.H., Morita, Y., Ikeuchi, K., 2005, "Influence of proteoglycan contents and of tissue hydration on the frictional characteristics of articular cartilage", Proceedings of the Institution of Mechanical Engineers, Vol. 219 (H), pp. 175-182.

Naka, M.H., Hasuo, M., Fuwa, Y., Ikeuchi, K., 2007, "Correlation between friction of articular cartilage and reflectance intensity from superficial images", Tribology International, Vol.40 (2), pp. 200-207.

Nassutt, R., Wimmer, M.A., Schneider, E., Morlock, M.M, 2003, "The influence of resting periods on friction in the artificial hip", Clinical Orthopaedics and Related Research, Vol. 407, pp. 127-138.

Papathanassoglou, D.A., Vohnsen, B., 2003, "Direct visualization of evanescent optical waves", The American Journal of Physics, Vol. 71, pp. 670-677.

Pérez-Luna, V.H., Yang, S., Rabinovich, E.M., Buranda, T., Sklar, L.A., Hampton, P.D., López, G.P.F, 2002, "Fluorescence biosensing strategy based on energy transfer between fluorescently labeled receptors and a metallic surface", Biosensors and Bioelectronics, Vol. 17, pp. 71-78.

Pickard, J.E., Fisher J., Ingham E., Egan J., 1998, "Investigation into the effects of proteins and lipids on the frictional properties of articular cartilage", Biomaterials, Vol. 19, pp. 1807-1812.

Roba, M., Naka, M., Gautier, E., Spencer, N.D., Crockett, R., 2009, "The adsorption and lubrication behavior of synovial fluid proteins and glycoproteins on the bearing-surface materials of hip replacements", Biomaterials, Vol. 30, pp. 2072–2078.

Saikko, V. 2006. "Effect of contact pressure on wear and friction of ultra-high molecular weight polyethylene in multidirectional sliding", Proceedings of the Institution of Mechanical Engineers, Vol. 211 (H), pp. 723-731.

Sawae, Y., Murakami, T., Chen, J., 1998. "Effect of synovia constituents and wear of ultra-high molecular weight polyethylene sliding against prosthetic joint materials", Wear, Vol. 216, pp. 213-219.

Scholes, S.C., Unsworth, A., Goldsmith, A.A.J, 2000, "A frictional study of total hip joint replacements", Physics and Medicine in Biology, Vol. 45, pp. 3721-3735.

Serro, A.P., Gispert, M.P., Martins, M.C.L., Brogueira, P., Colaço, R., Saramago, B., 2006, "Adsorption of albumin on prosthetic materials: Implication for tribological behavior", Journal of Biomedical Materials Research, Vol.78A, pp. 581-589.

Stahlhofen, A.A., 2000, "Photonic tunneling time in frustrated total internal reflection", Physical Review A, Vol.62, pp. 012112-1.

Unsworth, A., 1995, "Recent developments in the tribology of artificial joints", Tribology International, Vol.28, pp. 485-495.

Wang, H., Ateshian, G.A., 1997, "The normal stress effect and equilibrium friction coefficient of articular cartilage under steady frictional shear", Journal of Biomechanics, Vol.30, pp. 771-776.

Wardale, R.J., Duance, V.C., 1994, "Characterization of articular and growth plate cartilage collagens in porcine osteochondrosis", Journal of Cell Science, Vol.107, pp. 47-59.

Widmer, M.R., Heuberger, M., Voros, J., Spencer, N.D., 2001, "Influence of polymer surface chemistry on frictional properties under protein-lubrication conditions: implications for hip-implant design", Tribology Letters, Vol.10, pp. 111-116.

Woo, S.L-Y., Mow, V.C. and Lai, W.M., 1987, "Biomechanical properties of articular cartilage", In: Handbook of Bioengineering (Eds R. Skalak et al. (Eds.), pp. 4.1-4.44 (McGraw-Hill, USA).

Wu, J.Z. and Herzog, W., 2002, "Elastic anisotropy of articular cartilage is associated with the microstructures of collagen fibers and chondrocytes", Journal of Biomechanics, Vol.35, pp. 931-942.

Yew, A., Jagatia, M., Ensaff, H., Jin, Z.M., 2003, "Analysis of contact mechanism in McKee-Farrar metal-on-metal hip implants", Proceedings of the Institution of Mechanical Engineers, Vol.217(H), pp.333-340.

Zhang, S.W., 1998, "State-of-the-art of polymer tribology", Tribology International, Vol.31, pp. 49-60.

Zhu, Y., Yao, C., Chen, J., Zhu, R., 1999, "Frustrated total internal reflection evanescent switching", Optics & Laser Technology, Vol.31, pp. 539-542.

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