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RESEARCH

Tribological Studies in Cartilaginous Tissue of Lamb Synovial Joints Lubricated by Distilled Water and Interstitial-Fluid-Like Solution

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A B S T R A C T

This research work aims to expand the knowledge on how surfaces from human joint behave. The main factor analysed was friction coefficient. Friction coefficient becomes vital as it changes along an individual's life; it is directly connected to tear and wear of cartilage tissue due to aging and diseases. To get an insight on friction coefficient in human joints, experiments were performed in samples obtained from animal models. The experiment consisted in the measurement of the friction coefficient from plateau and condyle portions of bones from synovial joints; controlled temperature was set to be 37ºC as the average body temperature. Setup used was ball-on-three-plates. Two lubrication configurations were set for the experiments: distilled water and a salty solution replicating human's body interstitial fluid. Total joint replacement is a field where tribology plays a vital role; surface interaction in natural motion in joints is characterised for being low-frictional and self-lubricated. The efforts in this study are focused on the pursue of scientific information which leads to improvements for current treatments for diseased joints, before joint replacement to occur, and, moreover, for the cases in which joint replacement is inevitable, to design and construct better prosthetic devices.

1. INTRODUCTION

Mechanics in the human body include a series of motion systems, and therefore, surface interactions between its components. Tribology in biological systems (or bio-tribology) embraces concepts in physics, chemistry, biology and material science [1]. Bio-tribology applications in biomedical engineering are from different nature; related examples are: total joint replacements, footwear tribology, skin tribology, ocular and oral tribology, among others [2].

Total joint replacements combine different materials in terms of surface interactions. Products combining metals, ceramics, UHMWPE, and other materials, have been proposed to recreate human joints; hence, surface interactions have a remarkable importance in the preservation of prosthetic devices.

From a biomechanical point of view, the best artificial replacement for any human natural component is the one that recreates its characteristics as close as possible. The natural surface interactions in joints occur between layers of cartilage that cover the portion of bone structures which participate in motion. In addition, natural lubrication components, such as synovial fluid, are also a relevant part of these mentioned motion systems. Thus, total joint replacement prostheses should aim to replicate a cartilage-cartilage + synovial fluid systems.

1.1 Cartilaginous tissue

Articular cartilage presents remarkable characteristics referring to fluid adhesion; this fluid adhesion helps lubrication in the joint. Lubrication in articular cartilage occur in two different ways: boundary and synovial lubrication [3]. Boundary lubrication can reach a low friction coefficient but lacks a fluid film of synovial fluid to improve its characteristics; this lubrication happens when slow and cyclic motion takes place [3]. In contrast, fluid film lubrication requires a film of synovial fluid between the moving surfaces which reduces even more the friction coefficient; it is common when cyclic and fast motion happens. Also, in this case, thickness of the lubricating fluid must be bigger than of the roughness of the opposing surfaces [3]. Nonetheless, some authors [4, 5], propose that combination of both mechanisms is the reason for low friction in synovial joints.

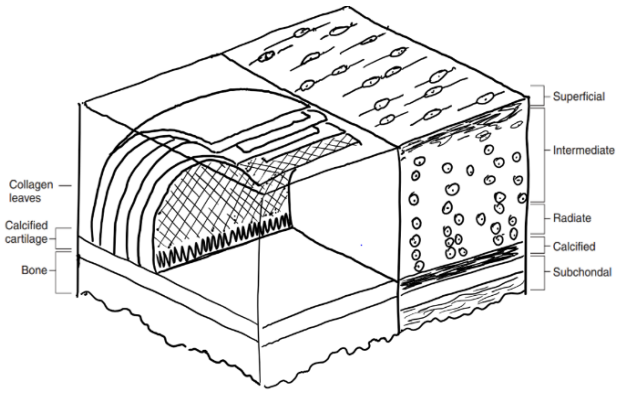


Fig. 1. Cut section cut through the thickness of articular cartilage (adopted from [6]).

Unit cell of cartilage found in articulations are known as chondrocytes. As seen on Fig. 1, they are present in small numbers and composed of several different materials such as proteoglycans, collagen, mainly type II, and others, but specially, 70-80 % water [3]. Chondrocytes density, as well as water content and proteoglycan concentration varies along the tissue; closer to the surface, proteoglycan concentration is relatively low and water is high, while near subchondral bone, water is presence is low and proteoglycan is high [3, 7]. Collagen architecture also differs along the tissue [3].

1.2 Synovial fluid

Synovial fluid is mainly composed of proteoglycan 4 (PRG4), hyaluronic acid (HA) and surface active phospholipids (SAPL); these lubricants are secreted by the chondrocytes in articular cartilage [8]. In a normal synovial joint, the friction coefficient is considered to be low, about 0.001 [9, 3]; to provide a comparison, Teflon has got a 0.04 friction coefficient. Synovial fluid behaviour differs depending on the external conditions, for instance, at high loads it provides poor friction coefficient, whilst, at low loads it has optimal properties [3]. Synovial fluid interaction with articular cartilage is what, specifically, gives joints such optimal motion with low friction coefficient. Studies by James, Fick and Baines, suggest that the hyaluronic acid chains in synovial fluid bind to articular cartilage because of the surface charges existing in the phospholipid layer on top of it [10].

1.3 Synovial joints

Points where two bones connect are known as joints or articulations. Joints are meant to hold together parts of the body; some are moveable, or diarthroidal, and some are fixed [11]; diarthroidal joints are the ones that enable different sort of movements in the human body. Three main types of moveable joints have been defined for the human body: fibrous, cartilaginous and synovial.

Synovial joints allow full range of motion and several degrees of freedom in a single node. Synovial joints consist not only of interactive cartilaginous surfaces at the end of the bones, but also the presence of a natural lubricant known as synovial fluid [12, 11]. The best example for this type of joints is the knee; the knee is the joint created in the connection of the femur and the fibula. Its shape is conserved through the existence of different tendons and ligaments, among other structures. Specifically, the knee is

formed between the condyles from the lower region of the femur and the upper plateau region of the fibula, with an intermediate structure known as the meniscus and protected by the patella, or knee cap [11]. Figure 2 shows the elements of the knee while in motion.

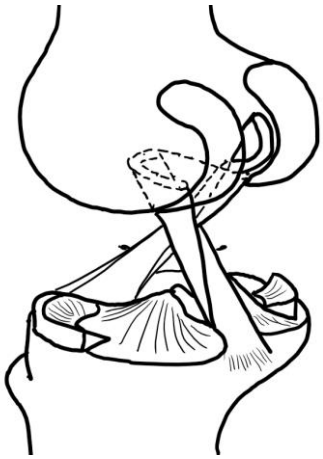


Fig. 2. Elements of the knee in motion (Adopted from [9]).

The knee is the element responsible of providing stability and mobility to the whole body. It must be not only capable of supporting almost half of the body weight all the time (43 % on each knee) [11], but also the most common and cyclic event of human daily activities, walking. The recommended number of steps per day for an adult is ten thousand [13]. These 10.000 steps also mean a minimum of the same number of cycles daily at each knee. The mechanical load existing when walking and standing is fundamental to stimulate osteoclasteogenesis as well as reducing risk of osteoporosis and other joint diseases [14].

2. METHODOLOGY

Methodology proposed for this research work has two main important components: the first one, involves the selection of an adequate animal model; the second one, refers to the study of the surface properties of cartilage, approached from the overview of the interaction of the participant surfaces during motion, thus, friction coefficient is analysed.

These experiments were performed in samples obtained from the selected animal models. These samples were subject to an adequate preservation protocol. The protocol consisted of collecting the models from a local distributor, immediately after slaughtering; this is, having passed less than 24 hours since the animal passed away. This, supported on the fact that cartilaginous samples last up to 72 hours in the appropriate preservation environment [7]. Also, samples were immediately reposed in a cold vacuum container, at approximately 4 ºC, after harvesting; the vacuum container and the cold gel packs used are able to maintain the temperature for as long as two hours, time enough to carry the samples to the laboratory and perform the experiments.

Before the measurement of friction coefficient starts, the samples were reposed in their lubrication medium for. Lubrication mediums to be compared in this research are: distilled water, as a representation of purified water; and, a salty solution, representing interstitial or extracellular-like fluid, with a concentration of 154 mM/L NaCl solved in distilled water [15, 16].

2.1 Models required for sample extraction

Animal models for these experiments had to be selected according to a series of factors. It was found that cartilaginous tissue from human beings is similar to most of bovine and equine models [7], however, two features differed widely: layer thickness and chondrocytes density. Firstly, the thickness layer of human cartilage in a healthy knee is of about 2 mm in thickness, whilst from bovine models it is usually thinner than 1 mm; however, thickness is similar to the tissue found in equine models. Secondly, although cell concentration in cartilaginous tissue, as a non-vascularized tissue, is very low, cell population in human cartilage is broadly higher than what is found in both equine and bovine models. Having said this, results to be obtained from the experiments using samples from animal models for this research work, approximate to what it is expected to be more accurate in studies performed directly in human tissue. From this information, two different scenarios were analysed to select an adequate model for this work.

On one hand, human models' collection for research involves a large amount of ethical concerns and regulatory affairs. Furthermore, availability of these models is short; the type of model required for these experiments must come from a total knee replacement (TKR);

when a TKR is performed, cartilaginous tissue is either unhealthy or has almost completely disappeared. Also, the quantity of samples desired to perform all the experiments is large and may involve more than one patient.

On the other hand, animal models for obtaining samples for these experiments, seem to be only partially adequate as they will show an approximate model. Considering that the analysis to be performed is related to surface characteristics, thickness of the tissue can be omitted. Moreover, as the constitution of the extracellular matrix (ECM) in animal models is largely similar the one found in human tissue, and bearing in mind that ECM is the one playing the most important role in friction coefficient, as it is the largest constituent of the tissue. Then, it is justified to utilize an animal model for the analysis of the matters concerning this investigation. Punctually, young adult lamb models (1-2 years old), acquired from a local butchery, were used to obtain the samples for these experiments. Figure 3 shows the whole forequarter shank of a young adult lamb and the two components fundamental for this research: condyles and plateaus (also, a meniscus is seen at the bottom).

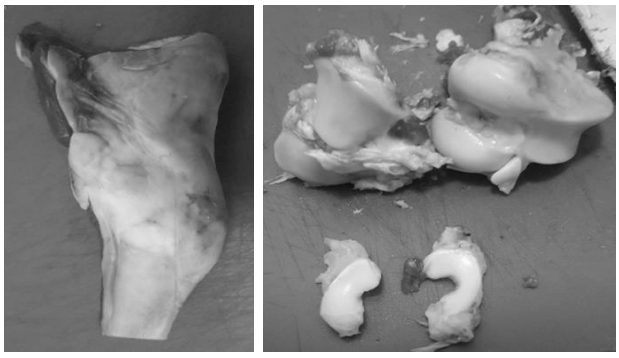


Fig. 3. (left) Lamb forequarter shank; (right) forequarter shank divided in parts.

Fig. 4. Samples extracted from condyle portion of bone.

Cylindrical osteochondral samples were collected; dimensions required were: diameter 6mm, length 6 mm (Fig. 4). Three of these samples were required for each experiment. None of them was reused to guarantee the results of the experiments.

The previously described dimensions were achieved by the construction of a specific tool for harvesting them in such dimensions. Samples were later incubated for 1 hour in the lubrication media to be tested, prior to the experiments.

2.2 Measurement of friction coefficient in cartilaginous tissue from synovial joints

To describe accurately the friction coefficient from cartilaginous tissue from the involved surfaces from synovial joints during motion, the analysis was focused in the two main cartilaginous surfaces from the participant components: plateaus and condyles. Surfaces in these two sections play an active role when movement takes place in a synovial joint.

Experiments consisted in the measurement of the friction coefficient of the samples harvested from plateau and condyle portions of bones from synovial joints. Measurement of the friction coefficient in the samples were carried on using a MCR302 Tribometer, from Anton Paar, equipped with a Peltier heated tribology cell T-PTD200 and a Peltier Hood H-PTD200 for precise temperature control; temperature was set to be 37ºC as the value for the average body temperature. Setup used in the test was a ball-on-three-plates, as shown in Fig. 5. These experiments were partially reproduced with the techniques used and from experiments carried out in [15, 16].

Fig. 5. Illustration: Tribological test setup.

The functional principle of the ball-on-three-plates test involve the application of a normal force in a shaft with a spherical end which presses three faces of a same material. The measuring ball is rotating and the sliding speed of it is calculated. From the torque required to maintain the sliding speed, the frictional force is obtained. Finally, the friction coefficient results from the relation between the normal load and the friction force [17].

The study of the friction coefficient on both, samples harvested from tibia plateaus and femoral condyles, was carried out under the application of two different normal loads: 10N and 5N; these representing a FL on each of the three samples due to the force distribution explained below on Fig. 6.

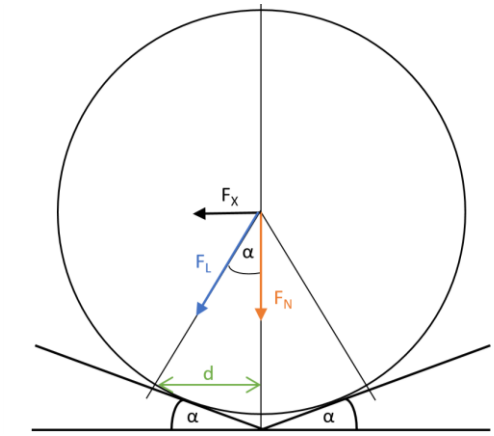


Fig 6. Ball-on-plate friction measurement function principle (adopted from [18]).

Normal force transmitted to the walls of the sample holder, considering it to be solid is:

$$F_L = \frac{F_N}{\cos(\alpha)} \quad (1)$$

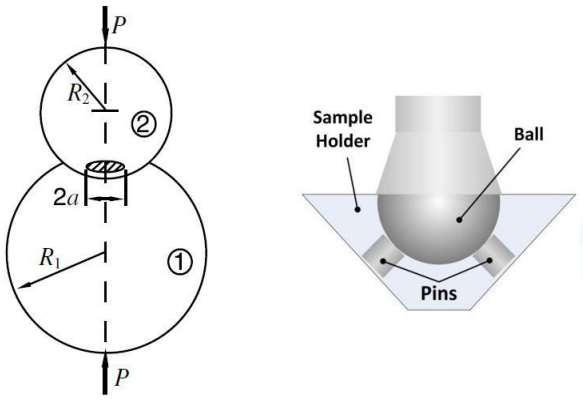
In this case, the force is distributed between three samples, which means:

$$F_L = \frac{F_N}{3 * \cos(\alpha)}$$

and, for the sample holder used, the angle α is equivalent to 45 º.

Forces applied to each of the samples, FL, for each of the main normal forces, FN, are approximately 6.35 N for a normal force of 10 N and 3.18 N for a normal force of 5 N. According to the literature [48, 49], under physiological conditions contact pressures for knees vary between 1 – 5 MPa, with peaks that can reach up to four times these values. In the case of in-vitro frictional tests, a pressure of 0.1 – 1 MPa is suitable and are the values commonly used [15, 19, 20].

A partial validation of this experiment can be affirmed by the fact that the normal forces applied achieve the pressure standardized for in-vitro frictional tests. However, this fact needs to be calculated accurately as the contact area in which the pressure exists, is the contact area between a spherical surface, the measurement device, and a flat surface, each of the samples extracted. Although each of the samples was extracted from a certain surface with a defined curvature (plateau or condyle), it can be considered flat, or, a portion of a sphere with infinite radius [21]. Figure 7 describes this accurately, comparing a general case in (a) and the specific case for this experiment in (b).



(a) (b)

Fig 7. Contact area between two spheres. (a) Standard case (adopted from [21]) and (b) Ball-on-plates case (adopted from [15]).

Contact area between two spheres of radius R1 and R2 which are pressed with a force F (P in Fig. 6), has a resultant contact area radius defined by as a, which results from:

$$a = \left(\frac{3*P*R}{4*E}\right)^{\frac{1}{3}} \quad (2)$$

where E is the contact Young's Modulus and is defined by the Young's modulus (E) and Poisson's ratio (v) of each material (glass and cartilage).

$$\frac{1}{E} = \frac{1-v_1^2}{E_1} + \frac{1-v_2^2}{E_2} \quad (3)$$

Young's modulus for glass is 50 – 90 GPa, while for cartilage it is 0.45 – 0.8 MPa. On the other hand, Poisson's ratio for glass is 0.2 and it is 0.4 for cartilage [3, 22].

$$\frac{1}{R} = \frac{1}{R_1} + \frac{1}{R_2} \quad (4)$$

Where the second sphere has an infinite radius R_2 equivalent to a flat surface. Then:

$$\frac{1}{R} = \frac{1}{R_1} \qquad and\ so, \qquad R = R_1$$

The maximum pressure occurs on the axis of symmetry and it is equivalent to 1.5 times the mean pressure P_m.

$$P_{max} = \frac{3}{2} P_m = \frac{3F}{2\pi a^2} \quad (5)$$

Pressures calculated from forces, which generate a certain radius of contact between the glass ball and cartilage, are within the suggested margin of 0.1 – 1M Pa for in-vitro testing of friction coefficient.

Table 1 describes the pressure generated by the different normal forces intended to be used in the friction coefficient experiments.

Table 1. Maximum pressure for different normal forces.

	Force, N	a, mm	P_{max}, MPa
F_N	10	4.463338	0.240
	5	3.542554	0.190
F_L	6.35	3.836345	0.205
	3.18	3.046507	0.165

For each group of samples different experiments were performed, partially replicating the methodology from [45, 46]. Samples from [15, 16] were tested at incrementing speeds of 0.0001 rad/s to 0.1 rad/s with a salty solution (154 mM/L NaCl in distilled water) acting as lubrication medium replicating extracellular fluid or interstitial fluid. Specific for this work, samples from each group, plateaus and condyles, were reposed for one hour in two different lubricants: distilled water and extracellular-like fluid.

3. RESULTS

The results obtained for the measurement of friction coefficient in cartilaginous tissue from two different surfaces, both in direct contact during motion, are highly dependent on the protocols followed not only to harvest the samples but also to preserve them.

Following the methodology proposed (for each experiment 600 points were collected), different points of view was emphasized in the results. These different approaches were defined to understand how friction coefficient behaves under different conditions. The lines trailed are: the sort of lubrication medium used; the normal load applied; the origin of the harvested sample; and, other combinations of these conditions that the authors considered relevant.

The first parameter to consider for future analysis of the results obtained is the normal force applied when comparing data obtained from experimentation with the two lubricants proposed.

Figure 8 shows the friction coefficient measured versus the sliding speeds. Compilations of the results from the interaction with the two lubricants are presented. It can be observed that the lubrication with distilled water as well as the salty solution is very close, however, a small statistical difference was found for these two means of lubrication ($p < 0.01$).

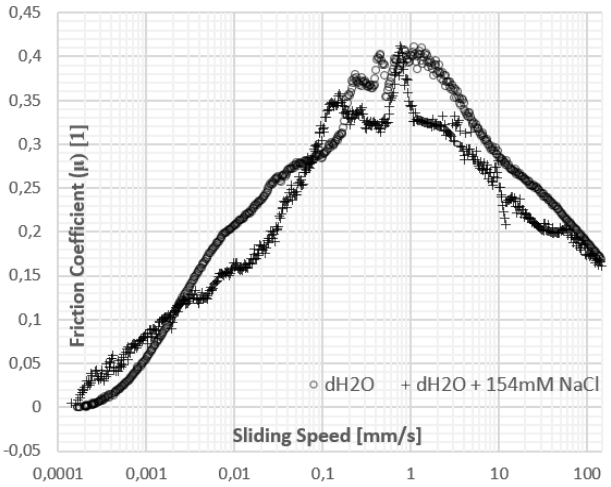


Fig. 8. Compiled result for different lubricants interacting with Plateau samples with a 5 N normal force.

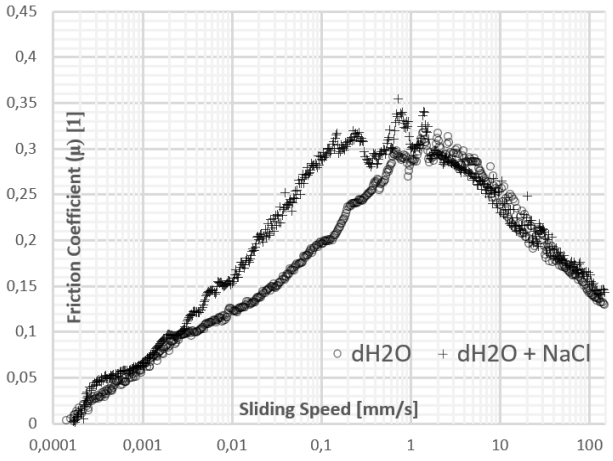


Fig. 9. Compiled result for different lubricants interacting with Plateau samples with a 10 N normal force.

Figure 9 below shows the same characteristics but when the applied force is equal to 10 N. In both cases, the behaviour is similar.

The next two Figs. 10 and 11 illustrates the data collected from the measurements for acting normal forces of 5 and 10 N, respectively, for the two lubricants interacting with harvested samples from condyle sections. In this case first case, 5 N, no significant difference was found between distilled water and salty solution. On the other hand, for 10 N, statistical difference between distilled water and salty solution exists; even though it happens, it stays close to the limits (for $p<0.01$).

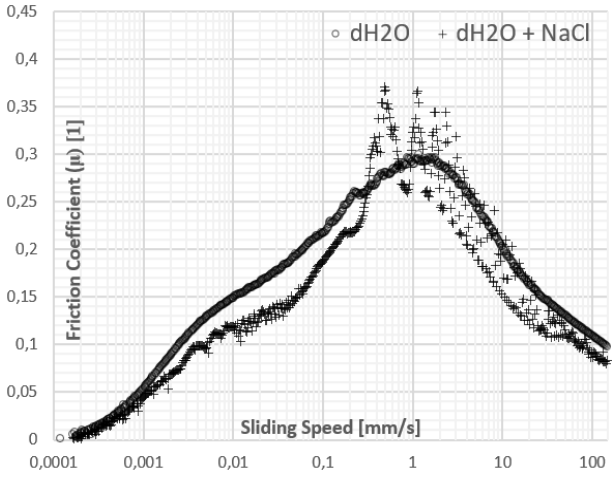


Fig. 10. Compiled result for different lubricants interacting with Condyle samples with a 5 N normal force.

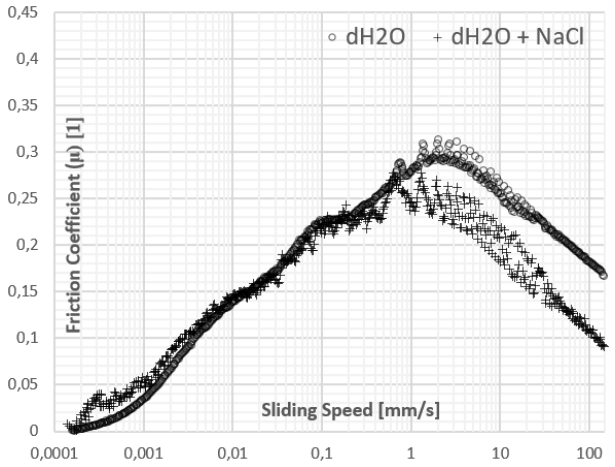


Fig. 11. Compiled result for different lubricants interacting with Condyle samples with a 10 N normal force.

Average friction coefficient collected from the experiments for all the different cases is presented in Table 2 below. Loading at 5 N, as expected, generated lower friction coefficient. Friction coefficient from both, condyle and plateau samples, seem to be very close, however, results obtained from measurements in plateaus appear to be slightly smaller.

Table 2. Overall compilation of friction coefficient results.

μ - Friction Coefficient – 37 ºC				
Normal Force [N]	**Plateau**		**Condyle**	
	dH2O	**dH2O + NaCl**	**dH2O**	**dH2O + NaCl**
5	0.2324	0.2113	0.1670	0.1500
10	0.1724	0.1983	0.1776	0.1578

Table 3. Sliding speeds and average friction coefficients associated to common activities. 5 N normal force.

μ - Friction Coefficient - 5 N – 37 ºC				
Activity	**Plateau**		**Condyle**	
	dH2O	**dH2O +NaCl**	**dH2O**	**dH2O +NaCl**
Steady (0-1 mm/s)	0.2014	0.1897	0.1498	0.1325
Walking (1-50 mm/s)	0.3129	0.2677	0.2215	0.2054
Jogging (51-100 mm/s)	0.2049	0.1923	0.1195	0.1014
Running (101-150 mm/s)	0.1756	0.1683	0.1018	0.0844

Table 4. Sliding speeds and average friction coefficients associated to common activities. 10 N normal force.

μ - Friction Coefficient - 10 N – 37 ºC				
Activity	**Plateau**		**Condyle**	
	dH2O	**dH2O +NaCl**	**dH2O**	**dH2O +NaCl**
Steady (0-1 mm/s)	0.1386	0.1790	0.1381	0.1417
Walking (1-50 mm/s)	0.2541	0.2525	0.2629	0.2072
Jogging (51-100 mm/s)	0.1602	0.1698	0.1940	0.1206
Running (101-150 mm/s)	0.1345	0.1461	0.1730	0.0982

To create a context, data collected from the measurements has been organized in ranges, according to the information available in work by Covert et al. [57], where, sliding speeds in a healthy knee when performing common activities, were defined. Table 3 shows results from 5 N loading conditions and Table 4 results for 10 N loading conditions.

4. DISCUSSION

Comprehension of the tribological behaviour of articular cartilage is important not only for those who suffer osteoarthritis, rheumatoid arthritis or have suffered severe traumatic injuries in joints, it is also important for those who are healthy and can been adequately advised, but, furthermore, it has also outstanding importance for those who have lost the battle to these illnesses and have had to undergo through a total joint replacement.

To obtain good quality results, the protocols established for collection and preservation of all the samples harvested are fundamental; even though cartilaginous tissue lacks vascularization, its preservation, in the adequate environment, would only guarantee the quality of the samples for 72 hours [7]. Another important factor to control the experiments results was having samples from young adult lamb (12 months old) to discard possible existent medical conditions that are not common to occur in young models.

Harvesting the samples has also a great importance in the protocol as, immediate damage could be induced if the selected tool touched the sample surface (surface of analysis); also, heating of the tool can induce dehydration of the sample, reducing its time of life, or even osteonecrosis, which may result in total damage and discard of the sample.

Sample required for the friction coefficient tests, needed to be cylindrical (6 mm in diameter and length), the extraction procedure followed was similar to the one used in osteochondral allograft transplantation, which mainly consists on the extraction of damaged cartilage portions for later replacement of them with the same patient's healthy cartilage extracted from a different location [11]. A tool for extraction of samples on the dimensions required was designed and constructed in stainless steel and samples were extracted by drilling through to areas of the bone: plateaus and condyles.

Different from similar experiments in the area [15], the current work studied samples from two portions of the bone, plateaus and condyles. The reason for this is that they both participate actively in motion, but their geometry and function is different; tissue from cartilage involved in motion should not be considered to be the same in all areas. Similar to [15], lubrication means used to evaluate friction coefficient in the two mentioned bone sections: distilled water (dH2O), for a general friction coefficient approach, and, a salty solution (dH2O+154mM NaCl), mimicking the interstitial fluid found in human body.

Friction coefficient was also evaluated under the application of different normal loads; these loads in accordance to what the literature recommends for in vitro testing of friction coefficient which states that the normal load applied should generate a pressure of 0.1 – 1.5 MPa [15, 20, 19]. The frame of sliding speed at which the friction coefficient was done started at 0.0001 mm/s, or 0.000350 rpm, and was increased up to 150 mm/s, or 350 rpm, in a speed ramp of 1 second. Reasons for the selection of this speed is that, any lower sliding speed, cannot be controlled and would be considered static friction coefficient [15], and, speeds of between 1 and 150 mm/s represent common sliding speeds from walking to fast running [23, 24].

Results from friction coefficient measurements (Table 2) indicated that in plateau portions in average for 5 N was of about 0.2 for distilled water and salty solution, similar to what is observed for 10 N. For condyle portions with a 5 N normal force, friction coefficient was, in average, 0.15 for distilled water and salty solution, similar results were obtained when a 10 N force was applied. No significant difference was found between friction coefficients measured at 5 N and 10 N for each lubrication medium. On the other hand, differences were significant when comparing the results obtained in plateau sections to the ones obtained in condyle sections; condyle sections tend to have a slightly smaller friction coefficient, this can be justified by the fact that condyle has got a thicker layer of cartilage [25].

It is important to remark some important differences in behaviour under diverse experiment conditions. It can be observed in all the figures that the friction coefficient increases up to a certain point and the immediately decreases. Reason for this to happen is that the cartilage starts swelling a certain amount of water in a certain time frame, after it is fully filled, the lubrication is improved; this behaviour

varies depending on the load conditions, as well as, the lubricant.

When the lubrication media was simply distilled water, friction coefficient for a normal load of 5 N was smaller for condyle than for plateau. The reason for this to happen could be, firstly, that distilled water is nothing else than purified water which has a neutral electrostatic charge, and, secondly, that condyle cartilage layer is thicker than the one in plateau portions, and so, it is capable of swelling more water; supporting this fact, when the normal load is increased, the friction coefficient in plateau reduces almost down to the level of condyle, as there is more pressure, the water is forced into the ECM of plateau cartilage. Lubrication, then, occurs in a totally different way when the salty solution (ionized water), as the surface charges from the cartilaginous tissue, negatively charged [12], repel the charges from these lubricants, reducing the swelling ratio, but improving friction coefficient behaviour. Higher swelling ratio was observed in the experiments with distilled water, in some of them, the water applied on top of the sample holder was completely swelled by the tissue and, of course, increased heat and damaged surfaces were observed; when using salty solution swelling was evident but not as noticeable. In summary, surface charges may affect the interaction with different lubrication mediums.

Willing to place friction coefficient in context, Tables 3 and 4, show the predicted friction coefficient for common activities like walking, running and jogging, all these having in consideration the sliding speeds previously described. From these results, it can be stated that, as the synovial fluid is a well-known non-Newtonian fluid [8], its behaviour varies under the application of different loads. Connecting this last to the results from Tables 3 and 4, synovial fluid, does not make a difference in friction coefficient when at low cyclic loads, thus, distilled water and interstitial-like fluid, widely present in the absence of synovial fluid when infection of inflammation occurs. In contrast, for high cyclic loads distilled water and the salty solution used in these experiments, do not prevent cartilage degradation as synovial fluid does. This happens in a similar way under different loads and in both, condyle and plateau, portions.

5. CONCLUSION AND FUTURE WORK

Reliable results from experimentation with biological samples depends on the protocols followed. A cartilage sample, for instance, can only be preserved for 72 hours. Protocols should aim to maintain the natural body environmental conditions to preserve samples. For sample harvesting, heat and stress from the tool should be controlled to avoid affections on the sample structures; heat on tools can lead to dehydration and osteonecrosis of the osteochondral samples.

Friction coefficient measured from different parts of the bone presented different results. Average friction coefficient for plateau portions is of 0.2 for distilled water and interstitial-like fluid. Average friction coefficient for condyle portions was of about 0.16 for both cases. Results did not vary significantly when different loads were applied. Friction coefficient tends to be lower in the condyle portions of bone, perhaps, because of the greater thickness of the cartilage layer, if compared to plateau portions.

Future work should aim to compare these two proposed lubricants with natural synovial fluid or an artificial substitution.

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