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The role of lamellate phospholipid bilayers in lubrication of joints

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This study aims to determine the effect of progressive loss of the surface active phospholipids on the characteristics, and hence tribological function of articular cartilage. In accordance to Hill's hypothesis, 3–7 lipid bilayers at pH 7.4 operate as the solid lubricant in the cartilage–cartilage interface during physiological function. These bilayers are known to be depleted during cartilage degeneration. This study models this loss of phospholipid bilayers, studying experimentally both wet and dry cartilage surfaces, measuring surface wettability, and friction coefficient under a constant stress of 1.2 MPa. The results demonstrate that the friction coefficient increases gradually with loss of the phospholipid bilayers, and gains in value with decrease in wettability.

Key words: *articular cartilage, lipids, wettability, friction coefficient*

1. Introduction

The articular cartilage in adults consists of layers, i.e., phospholipid, superficial or tangential, middle transitional, radial and calcified layers (figure 1) (HILLS and CRAWFORD [11]; KOBAYASHI et al. [14]; GUERRA et al. [4]; JURVELIN et al. [13]). The amorphous lipid layer is composed of proteoglycans, glycoproteins, hyaluronic acid, liposomes and lamellar phases, and is bound to phospholipid zone cartilage (GUERRA et al. [4]; KUMAR et al. [15]; SIVAN et al. [27]). Current electron microscopy and biochemical studies have proven that phospholipid lamellate solid bilayers (LSB) are present in joints with articular cartilage (SIVAN et al. [27]; RICHTER et al. [26]; HILLS [7]). In Hills model, phospholipids (PLs) are stacked in three to seven bilayers at pH 7.4 and are hydrophilic, are hydrated and act as solid lubricant at the boundary of the ultra-low friction interface between contacting cartilage surfaces (HILLS [7], [8], [10]; RABINOWITZ et al. [25]). Both *in vivo* and *in vitro* studies to determine the effects of the number of lipid bilayers adsorbed to the surface of cartilage on the friction coefficient between the surfaces of contacting cartilages, including potential biotribological applications, have been conducted (HIGAKI et al. [5]; TRUNFIO-SFARGHIU et al. [28]).

In laboratory experiment, the friction coefficient (denoted by f) decreased about 40 times with two bilayers DPPC, compared to single-bilayer MARRA [17]), also f decreased significantly when the number of PLs bilayers increased from one to five (HIGAKI et al. [5]). As PLs bilayers are part of the AC matrix, their decrease or removal would arguably lead to changes in fluid hydration, and the properties of the layers formed around the surface (HILLS [6]). An in-

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sight into the hydrophobic matching between proteins, such as hyaluronan and PLs bilayers may shed some light onto the microscale mechanism underlying joint ultra-low friction coefficient.

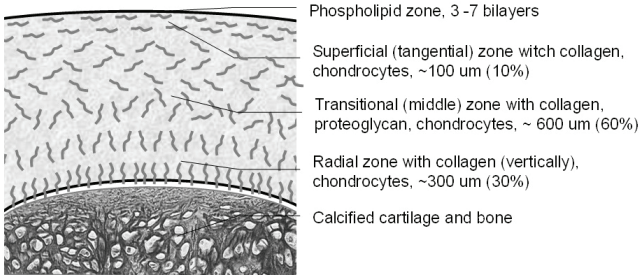

Fig. 1. Diagram of articular cartilage surface showing normal cartilage zones; traditionally, three zones are identified. Importantly, we included the uppermost pthospholipid multi bilayer, with a remarkable hydrophobic property (when the surface is dry), and the vital biological lubricant which is in direct contact with the synovial fluid (containing lamellar spheres, liposomes and macromolecules) in the joint

the role of phospholipid bilayers in supporting friction between biological surfaces of articular cartilage. In this study, our objective is to extract lipids gradually from cartilage surface, determine surface wettability and measure the friction coefficient of cartilage-on-cartilage tribopairs. The response of the altered samples will then be compared to those of the normal intact samples.

2. Materials and methods

The articular cartilage specimens were collected from bovine knees aged between 15–20 month old. Osteochondral plugs of two sizes, 5 and 10 mm diameter, were harvested from the lateral and medial femoral condyles using a circular stainless steel cutter. The 3 mm diameter cartilage-on-bone discs were prepared

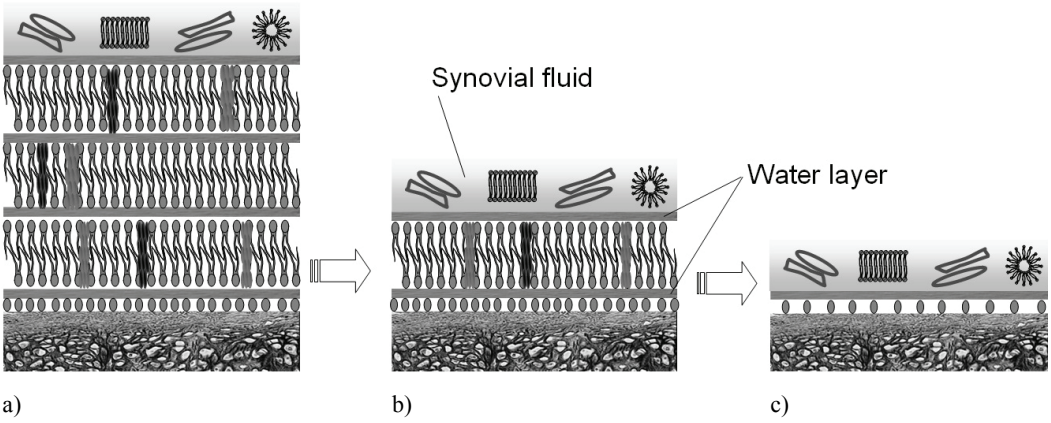

Fig. 2. Schematic representation of normal articular cartilage surface with three lamellate solid bilayers and surface amorphous layer on the top (a), partially osteoarthritic cartilage surface (less hydrophobic) with one bilayer (b) and osteoarthritic cartilage surface without PLs bilayers (c) not in place. On the top the lamellar spheres, liposomes and macromolecules act as if they had to follow a roller-bearing mechanism between two cartilage surfaces

Delipidization process is comparable to naturally depleted bilayers in osteoarthritis. The delipidization of cartilage surface conducted was geared at gradual removal of the lipid bilayer structure covering the cartilage surface (figure 2). Previous studies on AC degeneration have examined the friction coefficient, *f*, of the tissue following lipid extraction from cartilage surface (HILLS [10]; PAWLAK et al. [23]) and the relationship between surface hydrophobicity and this parameter (PAWLAK et al. [20], [21], [22]). The present study extends this further into the evaluation of the roles of cartilage lipids and their correlates with wettability, thus the role of water in the AC system, instead of quantity of the lipids. This work investigates from these osteochondral blocks. Two types of samples were tested: untreated bovine cartilage and bovine cartilage rinsed with enzyme to remove the lipids from the surface of the cartilage. After preparation, the specimens were stored at –20 °C in saline of 0.15 M NaCl (pH = 6.3), and fully defrosted prior to testing. The cartilage-on-bone samples were then glued to the disc and pin stainless surfaces in order to perform the lubrication test.

The contact angle between saline and cartilage surface was measured using a KSV CAM100 tensiometer with multiple contact angles measured by placing a droplet of saline on the cartilage surface at five different locations on each sample. The measurements of

contact angle as a function of time were carried out over 105 min for both the normal and delipidized cartilage samples. The tests were conducted at ambient laboratory temperature of $T \sim 22$ °C and relative humidity (HR ~ 45%). A total number of five tests were performed using fresh samples for each experimental specimen and set up.

The coefficient of friction (f) was measured at room temperature using a sliding friction tester pin-on-disc tribotester manufactured by ITeR, Poland. The friction between two discs of cartilage soaked in saline (as the lubricating fluid) and subjected to a load, sliding velocities and time was measured. The tests were performed at a very low speed of 1 mm/sec during 10 min and a load of 15 N (1.2 MPa), which corresponds to lubrication under physiological condition. The cartilage samples were left for one hour in saline, before the test. Friction tests on delipidized samples were performed under the same conditions as described above. A total number of five tests were performed using fresh samples of each experimental specimen and set up.

The delipidization procedure was carried out as described elsewhere (HILLS [7]). Briefly, the cartilage samples were immersed in a fat solvent (2:1 chloroform/ methanol, v/v) for 5, 13 and 23 min, taking care to maintain the same meniscus. Immersion for up to 15 min was sufficient to remove phospholipid from SPL (LITTLE et al. [16]; OLOYEDE et al. [18]). After each extraction the sample was placed in saline solution for 60 min for rehydration and to remove organic solvent left on the surface of the cartilage. These samples were used in our experiments for determining surface wettability and lubrication characteristics (coefficient of friction).

3. Results

3.1. Wettability of normal and extracted cartilage samples

The surfaces of the cartilage covered with synovial fluid can be seen to be quite hydrophilic with a contact angle of zero. Exposure of a normal and delipidized cartilage surface to the atmosphere changes wettability with increasing time. The contact angle values were plotted as a function of air-drying time (see figure 3). From figure 3 it can be concluded that the wettability changed in the course of extraction time in a way reminiscent of how the friction coefficient would change against the loading (thus, hydrophobization) time (WIERZCHOLSKI and MISZCZAK [29]; GADOMSKI et al. [2]). Thus, a correlation between the two quantities mentioned has to be anticipated.

3.2. Friction of normal and delipidized cartilage/cartilage tribopairs

Figure 4 shows friction coefficients between AC-AC pairs in the delipidized conditions relative to those of the normal AC-AC pair. It is sufficient to note that the friction coefficients of the delipidized (AC-AC) pairs are significantly higher than those of the intact normal (AC/AC) pairs. Note first that the most delipidized tribopairs from figure 4 express the highest friction coefficient values, and second, that the increase-in-time

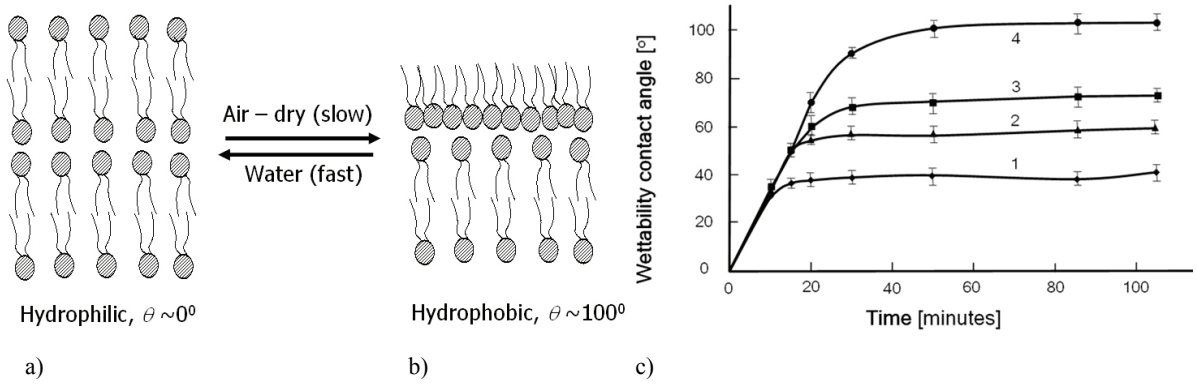

Fig. 3. A change in surface energy leads to conformational changes (flip-flop) of phospholipid molecules in the surface of cartilage dispersed in water (a) and air-dry (b) conditions from bilayer (superhydrophilic ~0° contact angle wettability) to monolayer (superhydrophobic ~100° contact angle wettability). The wet-contact angle of saline, expressing the wettability, drops on cartilage samples as a function of air-drying time (c). Cartilage sample surfaces that were delipidized (5, 13 and 23 min): (curves 1, 2, 3) and normal untreated cartilage (curve 4). Samples were dabbed in saline solution for 60 min and air-dried (n = 5, error bars = 95% confidence limit)

tendencies of the curves from figure 3 and figure 4 justify correlations of both principal quantities plotted on the ordinates of the figures – a finding of the subsequent study that is worth examining further, e.g., the time coordinate has to be adjusted or rescaled for both quantities of interest (a future task of special interest, too).

The ratio of the coefficient of friction for the delipidized (AC-AC) pair/normal (AC-AC) pair values after 6 min increased by 1.7, 2.0 and 2.6 times for the delipidized AC-AC pairs, curves 2, 3 and 4, respectively.

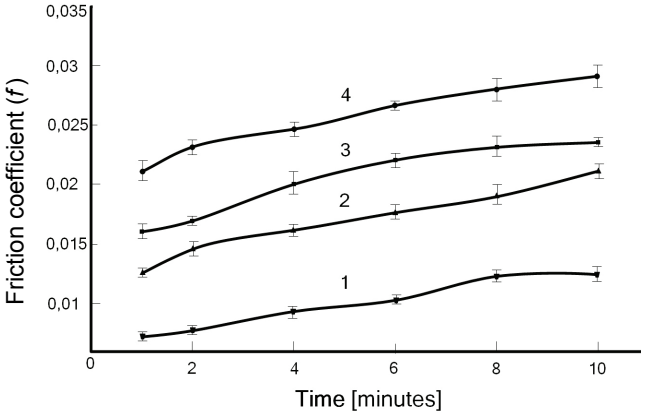

Fig. 4. Friction coefficient vs. time for the cartilage/cartilage tribopairs measured in saline solution; normal pair (curve 1) and delipidized surface pairs (5, 13 and 25 min): curves 2, 3 and 4, respectively; (see text for details) (n = 5, error bars = 95% confidence limit)

3.3. Relation between friction coefficient and wettability

Figure 5 represents the plot of coefficient of friction (f) vs. hydrophobicity (wettability) for normal and delipidized cartilage surface samples.

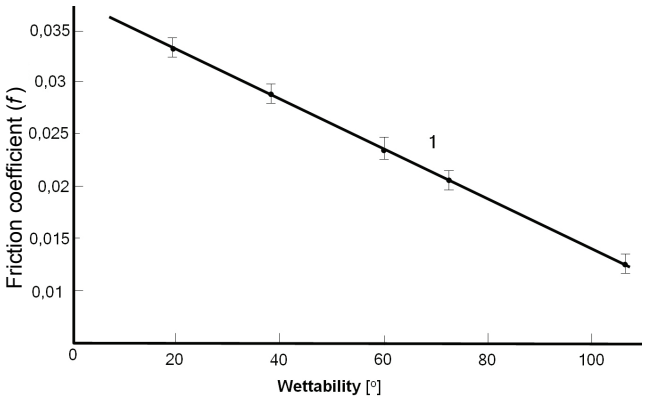

Fig. 5. Coefficient of friction (f) vs. hydrophobicity (wettability) of normal cartilage and gradually delipidized cartilage surfaces measured in saline solution (see text for details); n = 5, error bars = 95% confidence limit

4. Discussion

We have examined natural surface of articular cartilage covered by PLs bilayers and shown the relation between wettability and friction coefficient. It has been demonstrated that the "smart surface" of cartilage is highly hydrophilic when wet and hydrophobic when air-dry. The former provides the condition facilitating lubrication in the joint system, in a similar manner to how floor tiles provide hydrophobic surface on which water can impart wetting to lower frictional resistance. The friction coefficient f of the delipidized cartilage specimens increased about 2 to 3 times when compared to their normal counterparts. It can be argued from our results that the number of PLs bilayers controls the wettability and surface friction of articular cartilage.

Solvent rinsing of AC reduced the hydrophobicity of the surface, elevating its friction coefficient. In joints afflicted with osteoarthritis, the outermost layer is deficient of PLs along with other minor components such as proteolipid (HILLS and MONDS [9]). In this study, we have conducted experiments leading to the mechanical characterization of the phospholipid bilayer structure relative to its effect on surface and lubrication (virtually, facilitated) properties. Our results show that the friction test that we employed provides a suitable measure of the lubrication properties of cartilage surfaces with and without the presence of lipids.

We showed a very low friction coefficient between normal cartilage surfaces; in degenerative cartilage the number of PLs bilayers, also due to diminishing role of PLs engaged water deficiency, is decreased when friction coefficient increases markedly.

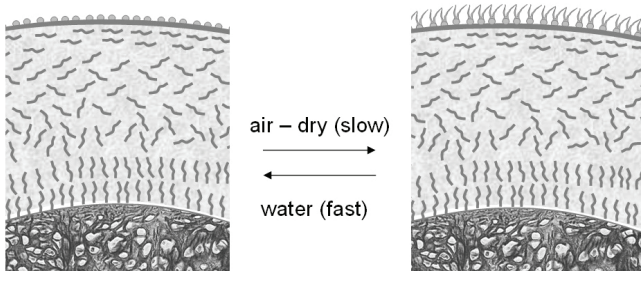

Fig. 6. Schematic representation of articular cartilage, showing wet superhydrophilic surface (a) with wettability ~ 0°, and the remarkable hydrophobic surface (when air-dry) with wettability 100° (b). The analogy of biological surfaces with very small contact angles and low coefficients of friction looks this way more convincing

The hydrophilic surface by air-drying time manifested itself by a slowly increasing wettability contact angle indicating conformational changes in the surface (*flip-flop*) phospholipid molecules, where it can be argued that by activating hydrophobic groups, as an aqueous fluid film, were preferentially established due to the drying (figure 6) (PAWLAK et al. [23]; CHEN et al. [1]; PESIKA et al. [24]). These friction tests thus confirm our hypothesis on the relation between the number of phospholipid bilayers (or hydrophobicity) and friction (LITTLE et al. [16]; HIGAKI et al. [5]; OZTURK et al. [19]; TRUNFIO-SFARGHIU et al. [28]; PAWLAK et al. [23]). This hypothesis can also be extended to the molecular scale to study, for instance, the role played by the hydrophobic protein-lipid, and water-mediated, matching (JENSEN and MOURITSEN [12]; GADOMSKI et al. [2]). The frictional characteristics of this extracted cartilage were greatly increased by ~200% compared with the un-extracted cartilage surface. Furthermore, it has been shown that phospholipid involved in AC as bilayers is, indeed, a lubricant and supports lubrication mechanism by lowering the friction of biosurfaces (GREENE et al. [3]).

Acknowledgements

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