Natural Joints: Biological boundary layered lubrication of phospholipid bilayers

Zenon Pawlak1,2; Raghuvir Pai3
1Tribochemistry Consulting, Salt Lake City, Utah 84117, USA and University of Economy, Biotribology Laboratory, 85-229 Bydgoszcz, Poland
2Department of Mechanical and Manufacturing Engineering, Manipal University, Manipal, 576 104, India

Abstract

In this article, we examined the surface topography and frictional characteristics (cartilage/cartilage) pair normal and osteoarthritic bovine cartilage. The amount of structured synovial fluid hydrated (lubricin-hyaluronan) complex is not enough to resist the effects of loading and friction. Thus, we conclude that lamellar phospholipid bilayers slippage, as well as the short-range repulsion forces between the interfaces of the negatively charged (–PO4-) cartilage surfaces is a primary determinant of the low frictional properties of the joint. A decrease in the number of bilayers on the cartilage surface indicates abnormal joint degeneration. This study showed that the tissue surface is ruined under inflammation where participation of (β2-Glycoprotein I, (β2-GPI) is most important and this is a novelty of our study.

Keywords: Boundary layered lubrication; Charged amphoteric surface; (cartilage/cartilage) pair friction; Interfacial energy; Spherical lipid bilayer; Lamellar bilayers slippage

Introduction

Phospholipids are molecules present in various tissues and body fluids are also names surfactants substances which lower surface energy [1,2]. The chemistry of the articular cartilage surface depends on liposomes, phospholipid lamellar phases and bilayer formation, the adsorption rate, hydration macromolecules [3-6]. Hydrated (lubricin – hyaluronan) form complexes and provide support lubrication of synovial joints [4-6]. These multilayer phospholipid membranes are usually categorized as lamellar bodies [1-3]. According to the Hills’ natural lubrication model [3] and our lamellar-repulsive mechanism [4], phospholipids are the main solid phase components in the diarthrodial articulated joint system.

The hydrated surfaces (pH~7.4) of the phospholipid membranes are negatively charged (–PO4-) [4]. Poor lubrication in animal joints can be attributed to deterioration of the bilayers where the wettability or contact angle (θ) changes from 104° to less than 70° [3,4,7].

Figure 1: (A) Electron microscopic images of the multilamellar lining of adsorbed bilayers of phospholipid of the articular surface of a human knee [3,4]. 3D topographical image from atomic force microscopy of (B) normal healthy and phospholipid depleted (C) unhealthy cartilage surface.

Figure 1 represents the self-assemble of phospholipid showing the presence of phospholipid bilayers as the outermost lubricating lining of the joint surface. Electron microscopic studies of the cartilage surface showed lamellar structure and bilayers visible on the image like similar to natural membranes. The number of lamellae on the cartilage surface can be determined by rinsing with a lipid extraction solvent (2:1 chloroform: methanol) and then identifying and quantifying the phospholipids. Once quantity and the areas of the articular surface are derived, the number of monolayers could be calculated [3,10]. Since phospholipids are highly osmophilic, their presence on the articular cartilage is visualized as a highly electron-dense multilamellar structure of phospholipids as seen Figure 1A.

Surface-active phospholipids (SAPL) have been experimentally proved to be present in the synovial fluid and cartilage surface [3]. They play an essential role on the surface of articular cartilage by providing an excellent lubrication. However, when the total amount of phospholipids increases during osteoporosis, the content of adsorbed surface-active phospholipids was reduced, and joint performance was deteriorated [11,12]. This is one of consequences of osteoarthritis, a degenerative joint disease [3,9]. In a previous study, we established using a high-resolution nano-imaging technique, atomic force microscopy (AFM), that a well-organized surface-active phospholipid covers the surface of the cartilage (SAPL) in a lamella-like arrangement, as previously described by Hills [3,10]. In mammals, the intact lipid layer of cartilage is lost during degeneration, thus showing the need for efficient lubrication of the joint [3,4,10].

In this study of surface parameters, interfacial energy of spherical phospholipid bilayers and friction coefficient of (cartilage/cartilage) pair led to a model PLs membrane based on a “bell-shaped curve” (amphoteric character). Friction curve of osteoporotic (cartilage/cartilage) pair has lost amphoteric character and the SAL on surface is not present. A decreased number of bilayers on the cartilage surface indicate abnormal joint degeneration. The tissue surface is ruined under inflammation where participation of β2-Glycoprotein I (β2-GPI) is most important. The basic hypothesis of this research is that restoration (resurfacing) of the surface amorphous layer can be achieved by re-introducing synthetic surface-active phospholipids (SAPL) into the joint space.

**Materials and methods**

The samples of articular cartilage were taken from the knees of an aged, one to two-years-old ox. Osteochondral plugs, which diameters were 5 and 10 mm long, were collected from lateral and medial femoral condyles with the use of a circular stainless-steel cutter. The articular cartilage discs were then cut in such a way to form 3-mm plugs with the underlying bone. The samples were stored at 253 K in 0.15 M NaCl solution (pH = 6.9) and, before testing, they were fully defrosted. To prepare the buffer solutions, 0.2 M sodium hydroxide was added to 100 mL of a solution made of 0.04 M acids: acetic (80% of the solution), phosphoric and boric acids. A sodium hydroxide solution was used at 22ºC to adjust to a suitable buffer pH [8 a,b]. The electrolyte pH was controlled using a pH-meter in the process of the measurements.

Measurement the interfacial energy (γ), of spherical lipid bilayers procedure is described in our previous work [4]. The results of interfacial energy as a function of pH are shown in Figure 3.

Figure 2: Schematic diagram of the friction test apparatus

![Friction Test Apparatus](image-url)
The apparatus was designed to provide a reciprocating sliding motion between two samples of cartilage immersed in a buffer solution (Figure 2). The friction coefficient (f) was measured using of the sliding friction tester pin-on-disc tribotester T-11 manufactured by NISTR, Radom, Poland. The discs of articular cartilage were glued to the holders, using adhesive glue. Finally, friction tests were carried out and diagram of friction coefficient as a function of test time was generated. The tribotester measured the friction between two samples of articular cartilage which were equilibrated with each buffer under a load for 5 minutes, at room temperature under a 15N load and (1 mm/s) sliding velocity during 300 seconds run for each pH buffer solution. The charge density of the cartilage surface was changed from positive to negative by varying the pH of a buffer solution. Positively (-NH\textsubscript{+}/-NH\textsubscript{3}+) and negatively (-PO\textsubscript{4}/-PO\textsubscript{3}) charged tribological pairs showed lower values of friction coefficient than the cartilage surface at the isoelectric point (a maximum on the curve) (electric neutrality conditions), IEP, pH ~4.5, Fig. 3(B). The low friction between two cartilage surfaces demonstrates that the friction is mostly associated with their charge density by electrostatic interaction between the two cartilage surfaces. The same charge of cartilage surface should be attributed to the surface electrostatic repulsion of charges fixed on the AC. Joint lubrication in the lamellar-repulsive mechanism described in the literature [4,15,16] is provided by phospholipids, which form a multilayered structure on the articular surface of cartilage, known as surface-active phospholipids. Klein et al. proposed a lubrication mechanism based on lubricin and hyaluronan complexes with phosphatidylcholines to provide a remarkable lubrication of synovial joints via hydration-lubrication mechanism [5,6].

The lubrication mechanism in the joints takes place: (i) through lamellar lubrication which occurs when the bilayers are sliding against each other, and (ii) in the structured synovial fluid that occurs when the lamellar spheres, liposomes, and macromolecules play a role of a roller-bearing between two cartilage surfaces in an effective biological lubrication [4,17,18].

Friction and lubrication are surface processes, only strongly adsorbed moieties to the surface are a primary lubricant and have an important roles in friction (charged macromolecules in synovial fluid) have supportive role and weakly adsorbed (Figure 4). Osteoporosis is teaching us about the importance of phospholipids which are deactivated (we call this a phospholipid silage surface should be attributed to the surface electrostatic repulsion of charges fixed on the AC. Joint lubrication in the lamellar-repulsive mechanism described in the literature [4,15,16] is provided by phospholipids, which form a multilayered structure on the articular surface of cartilage, known as surface-active phospholipids. Klein et al. proposed a lubrication mechanism based on lubricin and hyaluronan complexes with phosphatidylcholines to provide a remarkable lubrication of synovial joints via hydration-lubrication mechanism [5,6].

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Boundary layered lubrication of phospholipid bilayers

The deformation of the AC surface is discussed because it is related to several aspects of the lubrication of joints. The relation between deformability (or softening) and lower friction is observed at pH below 3, Figure 3(B) (curve 2). Swelling of articular cartilage in the presence of acid, a distinct softening, i.e., an increase in deformation per unit load was observed below pH 5 [8a]. Above this value, it was constant. Under most conditions, softening was associated with an enhanced hydration with reduction in friction. In synovial fluid free buffers, the pH of the lubricant bath altered both the friction and deformability.
of the cartilage. Since these experiments were carried out in the absence of synovial fluid, no conclusions could be drawn about this phenomenon under conditions of synovial fluid lubrication.

Figure 4: Biological boundary layered lubrication of phospholipid bilayers

Schematic illustration of lamellar bilayer slippage friction mechanism in (cartilage/cartilage) pair is presented in Figure 4. As a lubricant, it may be either a multilayer solid (e.g. MoS₂, h-BN) or in biological lubrication via phospholipid bilayers, a boundary layer or else, a boundary lamellar lubrication. Boundary layered lubrication is capable of lowering friction and wear of interacting surfaces in relative motion under load in a better way than the classical boundary lubrication [1,2,9]. Our hypothesis of lamellar lubrication relies on a supporting mechanism of lamellar-repulsive phenomena in natural joints [4].

As illustrated in Figure 5 when the multibilamellar PL lubricant present on sliding surfaces slide over one another with relative ease to provide very low friction, the interlayer share mechanism is believed to be responsible for the low friction of most lamellar solid lubricants. As far as the excellent solid-lubricating capacities of bilayers are concerned, a region of negative electrical charge is contained within the layers. Thus, the surfaces of the phosphate groups are negatively charged, and through creating an electrostatic repulsion between the layers and them make the interlayer slippage much easier. The relatively larger interlayer separation in PL bilayers is thought to result from electrostatic repulsion between the successive atomic layers of these lipid bilayers. If these lamellae are able to slide over one another at relatively low shear stress, then the multibilayer becomes a self-lubricating bearing [20,21]. The planes of low resistance allow relative movement between lamellae. The PLs molecule adheres strongly to the worn surface and the lamellar structure deforms at very low-stress levels. When the PLs multibilayer surfaces are brought into contact, there is an electrostatic attraction between the corresponding interfaces [10,21]. Thus, the cartilage surfaces of the phosphate groups are negatively charged, creating electrostatic hydration repulsion between the interfaces and making the slippage frictionless [22,23].

The tissue surface under inflammation and activation of (β²-Glycoprotein I, (β²-GPI)

The mechanism of osteoarthritis (OA) is still not fully understood, but it has been established that this debilitating disease is often accompanied by a change in the synovial fluid composition, reduction in viscosity and deterioration of cartilage surface. Well-defined outermost bilayers were clearly visible on healthy cartilage surface but OA may involve in the depletion of important joint molecules and SAPLs on the articular surface [11]. Further, evidence for SAPL lining depletion was demonstrated by the cartilage wettability contact angle change from 103 to 65 degrees [4]. This insight led to the hypothesis that the SAPL is deactivated in the pathologic state of OA and remains present in synovial fluid but in an inactive state.

Figure 6: (A) Phospholipid bilayer of articular cartilage in wet (hydrophilic ~ 0°) and air-dry condition (hydrophobic 104°) Book cover [4], and conversion of β²-Glycoprotein I of the (B) circular conformation into (C) an open hockey-stick-like conformation, each molecule has five domains (1-5).

Deactivation of phospholipid molecules and transformation β²-Glycoprotein I (β²-GPI)

The pathological synovial fluid contains three times more phospholipids (PL) [11] but the cartilage structure changes and its ability to lubricate, is remarkably poor. During normal functioning, the SAPL serves as a sacrificial perturbation bilayer, whereby it can improve by self-assembly mechanisms. Additionally, phospholipid molecules lose their surface active properties to form vesicles, lamellar phases, and bilayers spontaneously. The active role played by PLs in OA and RA SF as compared with that of control SF and their functions in cartilage boundary lubrication remains still poorly understood.
Cartilage destruction in most rheumatic diseases and osteoarthritis has generally been accepted as a mechanism of deactivation of phospholipid bilayers [11]. An acid-base interaction occurs between protonated amino acid group (-NH$_3^+$) of β$_2$-Glycoprotein I and the phospholipid (-PO$_4^-$) group: (-NH$_3^+$) + (-PO$_4^-$) → (-NH$_3^+$PO$_4^-$) that is strong enough to deactivate the PLs bilayer surface. β$_2$-Glycoprotein I (β$_2$-GP I) is a protein that circulates in blood at variable levels (50–500 μg mL$^{-1}$) with a molecular weight of 50 kDa. β$_2$-Glycoprotein I (β$_2$-GP I) can exist in (a) closed conformation and (b) open hockey stick-like conformation, Figure 6. β$_2$-GP I in its hockey stick-like conformation is a strongly adhesive protein and binds to different receptors on cells. Binding of β$_2$-GP I to anionic charged phospholipid (–PO$_4^-$) groups at pH ~ 7.4, results in a change in conformation and exposure of the epitope for the autoantibodies, Figure 7. Softening of the cartilage is the first phase of cartilage deterioration. The classic morphological changes of osteoarthritic articular cartilage begin with fibrillation and a local surface disorganization involving splitting of the superficial layers of the cartilage, Figure 5. The early splitting is tangential with the cartilage surface, following the axes of the predominant collagen bundles. Continued deterioration of articular cartilage leads to an exposure of the subchondral bone and more generalized synovial change.

**Conclusion**

A layered structure with weak inter-layer forces (van der Waals) that ensure an easy and low-strength shearing characterizes solid phospholipid lubricants. Interfacial energy of spherical lipid bilayers serve as a membrane model displaying the amphoteric character of bilayers and is helpful to understand the (cartilage/cartilage) friction expressed as a “bell-shaped” curve. This experimental fact has not been highlighted in the literature of natural lubrication. Finally, we suggest that the friction coefficient for a given phospholipid multibilayers and hydrated macromolecules is leading to a lamellar-repulsive mechanism under highly negatively charged conditions. The (β2-GPI) participates in the antiphospholipid antibody syndrome (APS) through binding of (β2-GP I) to the anionic charged phospholipid (–PO$_4^-$) group. At a pH around 7, β$_2$-GP I - amino acids (arginine, lysine and tryptophan) are positively charged (-NH$_3^+$); an acid-base interaction occurs between the protonated amino acid group (-NH$_3^+$) and the phosphate (–PO$_4^-$) membrane group: (β$_2$-GPI-NH$_3^+$) + (PLs–PO$_4^-$) → (-NH$_3^+$PO$_4^-$) interaction and electrostatic attractions is strong enough to destroy the PLs bilayer on cartilage surface and deactivate all phospholipids in SF. All deactivated molecules of phospholipids lost ability to form bilayers, lamellar phases and vesicles. The basic hypothesis of this research is that restoration (resurfacing) of the surface amorphous layer can be achieved by reintroducing synthetic surface-active phospholipids (SAPL) into the joint space.

**References**


