Articular cartilage: Hydrophilic and boundary layered lubrication mechanism with phospholipid – (lubricin, hyaluronan) participation

Zenon Pawlak*, Tomasz J Kaldonski1, Krzysztof Gocman3, Tadeusz Kaldonski2 and Kehinde Yusuf3

1University of Economy, Biotribology Laboratory, 85-229 Bydgoszcz, Poland
2Military University of Technology, Faculty of Mechanical Engineering, Warsaw, Poland
3College of Medical Rehabilitation Sciences, Department of Prosthetics and Orthotics, Taibah University, PO Box 344, Madinah, Kingdom of Saudi Arabia

Abstract

Objective: We investigated the phospholipid (PL) bilayers on articular cartilage surface with respect to lubricant role. Atomic force microscopy and electron microscopy studies proved bilayers adsorbed to the articular cartilage surfaces.

Design: The decreased number of bilayers changed the wettability and increased friction. Thus, the cartilage surfaces of the phosphate groups were negatively charged.

Results: Phospholipid (PL) bilayers coated cartilage surfaces play lubricant role making the slippage frictionless. Bilayers of PLs widely named "lamellar bodies" (LB) with weak van der Waals forces between bilayers allow them to slide easily at their spacing 4.5 nm

Conclusion: Hydrophilic model of natural boundary lubrication supported by lamellar slippage boundary layered mechanism was demonstrated. The decreased number of bilayers changed the wettability and increased friction. Typical cartilage wettability contact angles 100°-103° (surface in air-dry condition).

Introduction

The search for mechanism of joints lubrication and full understanding of most is still expected. The concept that natural boundary lubrication is capable to act at interfaces by a surface coating deposited onto the articular surface from synovial fluid, SF was demonstrated by Jones in 1934 [1]. The phospholipid (PLs) content of cartilage amounts ~10% on a dry – weight basis [2] was suggested by Little et al. [3] that could be playing a role in joint lubrication. Surface-active phospholipid (SAPL) names by Hills [4] appear to have the highly desirable properties that their adsorption can contribute to negatively charged or hydrophilic surfaces [4-6]. The PLs molecules act in two ways by the strong adsorption and strong cohesion after forming a monolayer, and continue to form bilayers, Figure 1. The multilamellar lipid structures reduce friction, also reduce wear and act as barriers against invasion by microorganisms [4,6]. The lipid bilayers on the cartilage surface (surface active phospholipid, SAL) of the healthy joint cartilage contain mainly phosphatidylcholine (41%), phosphatidylethanolamine (27%), and sphingomyelin (32%) of total phospholipids [7].

The cartilage models with hydrophobic and hydrophilic surfaces

The phosphatidylcholine quaternary ammonium (QA) ion (Me3N+-PO43-) offers strong electrostatic bonding to negative charged proteoglycan surface (carboxyl and sulphonyl ions) [11]. In both models mobile cations Ca(II) between negatively charged phosphate ions (-PO43-) can enhance cohesion by pulling adsorbed phosphatidylcholine (PC) molecules together by making the close-packed hydrophobic solid layer (Figure 1) or bilayer is formed and the surface is negatively charged (Figure 1). The multilamellar structure of phospholipids, namely the surface amorphous layer (SAL), covers the natural surface of articular cartilage. A very high porosity (70%-85%) is a critical factor in providing excellent hydration lubrication properties of articular cartilage. Hills hydrophobic surface model of AC has no support in all experimental facts presented in the current literature which actually supports the concept that AC is amphoteric, hydrophilic with the negatively charged surface (-PO43-) [9,12]

In this paper we studied the multilamellar structure of cartilage surface namely the surface amorphous layer (SAL), which covers the

*Correspondence to: Zenon Pawlak, Tribochemistry Consulting, Salt Lake City, Utah 84117, USA, Email: zpawlak@xmission.com

Key words: articular cartilage, hydrophilic and hydrophobic surface, phospholipids (PLs) bilayers, friction boundary layered mechanism, atomic force microscopy (AFM)

Received: December 02, 2019; Accepted: December 17, 2019; Published: December 27, 2019
The natural surface of articular cartilage. We investigated the phospholipid (PL) bilayers on articular cartilage surface with respect to lubricant role. Electron microscope and atomic force microscopy (AFM) studies proved multi bilayer adsorbed to the articular cartilage surfaces. Hydrophilic model of natural boundary lubrication supported by lamellar slippage boundary layered mechanism was demonstrated.

Material and methods

Atomic force microscopy (AFM) imaging

The articular cartilage samples used in this study were obtained from the patellae of 3-4 year old bovine animals harvested from the local abattoir and stored at -20°C until required for testing. The samples were thawed out in continuous running water at room temperature and kept in saline solution (0.15M sodium chloride) prior to testing. A stainless steel punch was used to cut osteochondral plugs (n = 20), containing full thickness articular cartilage-bone laminate and trimmed into specimens of 5 mm by 5 mm. The bony layer underlying the cartilage was dabbed with a paper towel and immediately glued onto a Petri dish using fast-drying Loctite® 454 glue (Henkel Australia PTY Ltd, Victoria, Australia). The Petri dish was mounted onto the AFM sample holder, ready for AFM measurements. During gluing, the articular surface was moistened repeatedly with drops of saline solution to keep surface intact. The glued sample was submerged in saline solution ready for AFM imaging using the SMENA® head of the NT-MDT P47 Solver scanning probe microscope (SPM) (NT-MDT, Moscow, Russia). The surface imaging was done using methods described elsewhere in the literature.

After imaging, lipids were selectively removed from the articular surface in accordance with the delipidization procedure described elsewhere in the literature using Folch reagent (i.e. a mixture of chloroform/methanol (2:1) v/v). The delipidized samples were placed in saline solution for 30 min for rehydration and to remove the lipid rinsing agent and any organic solvent left on the surface of the tissue. Each sample was then mounted on the AFM for imaging.

Results and discussion

The present results demonstrate that the biological lubrication mechanism needs three or more bilayers to achieve effective layered lubrication.

Morphological evidence showing the presence of phospholipid bilayers as the outermost lubricating lining of the joint surface is shown in Figure 2. Electron microscopic studies of the cartilage surface showed lamellar structure and bilayers visible on the image 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 [4,5]. 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 2. In our 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 [4,5] (Figure 2). Wiping of the articular surface with a lipid rinsing reagent resulted in a drastic removal of the SAL (Figure 2). In mammals, the intact lipid layer of cartilage is lost during degeneration, thus showing the need for efficient lubrication of the joint [5].

Figure 3 is showing 3D topographical image of normal healthy articular cartilage and Figure 2 the surface of depleted articular cartilage after image processing, showing the nano structural arrangement of the surface amorphous layer with several peaks and troughs (length (X) and breadth (Y) of the scanned area, and average peak height of SAL (Z)). The depleted surface of articular cartilage after image processing, shows the loss of the membranous overlay (surface amorphous layer) of the articular surface.

Boundary layered lubrication of phospholipid bilayers is capable of lower friction and the wear of interacting surfaces in relative motion under load in a better way than the classic boundary lubrication. Hydrated hyaluronan and lubricin are free to adsorb active phospholipid and losing the ability to adsorb cartilage surface are playing supportive the role in electrostatic lubrication [12-15].
Bilayers structures exhibited thickness of about 0.2um with spacing of about 4.5 nm. Hydrated hyaluronan and lubricin without presence of active phospholipid is disabled to play role of lubricants similar to phospholipid bilayers.

Phospholipid in synovial fluid strongly adsorbed by hydrated macromolecules ==> Support boundary layered lubrication on cartilage surfaces lubricin and hyaluronan.

Friction in water environment medium with presence of phospholipids has ability of self-organized interfaces [12]. Interface of bilayers is negatively charged and surrounded by water such hydrated layers have supper lubricating properties. Sliding hydrated charged surfaces under load can sustain a large load, which has been termed the hydration lubrication [13]. Especially hydrated PLs bilayers exposed with high density charges (-PO ) create lamellar- repulsion mechanism of joints lubrication [16,17]. Insoluble PLs molecules are in permanent movement with ability to attach to polar or charged part of lubricin and hyaluronan (A ). Once PLs molecules are electrostatically attached to negative part of molecules the lubricin and (A ) form complex are trapped between charged cartilage surfaces to be implicated in lubrication. Lubricin and hyaluronan (A ) by forming complex with PLs appear highly inefficient to adsorb to cartilage surface. Such molecules are weakly attached to sliding interfaces; this has consequences for frictional effects in living system.

Conclusion

We investigated surface conditions of an articular cartilage with respect of some properties: the phospholipid (PL) layers formation, wettability changes and friction. The surface of articular cartilage is hydrophilic and negatively charged. Hydrophilic model of natural boundary lubrication is supported by lamellar slippage of the bilayers mechanism. As the PL bilayers were gradually removed to model deterioration, the hydration repulsion mechanism of the joint became less effective as friction increased. The decreased number of bilayers changed the wettability and lowered the PL lubricant properties. Finally, we suggest for a given phospholipid multi bilayers and hydrated macromolecules complex with PLs are leading to a lamellar-repulsive mechanism under highly negatively charged conditions.

Acknowledgements

We wish to express our gratitude to Miss Joanna Pawlak for editing the paper.

Ethical approval

This article does not contain any studies with human participants performed by any of the authors.

Funding

This research received funding from Tribochemistry Consulting.

Conflicts of interest

The authors declare no conflict of interest.

References

1. Jones ES (1934) Joint lubrication. Lancet 1: 1426-1427.
2. Hills BA, Crawford RW (2003) Normal and prosthetic synovial joints are lubricated by surface-active phospholipid: A hypothesis. J Arthroplasty 18: 499-505. [Crossref]
3. Little T, Freeman MAR, Svanson SAV (1969) Experiments on friction in the human hip joint. In Wright V (Ed.) Lubrication and Wear in Joints. Sector, London.
4. Hills BA (1988) The Biology of Surfactant. Cambridge, Cambridge University Press.
5. Hills BA (1989) Oligolamellar lubrication of joints by surface active phospholipid. J Rheumatol 16: 82-91.
6. Hills BA (2002) Surface-active phospholipid: a Pandora’s box of clinical applications, Part II Barrier and lubricating properties. Int Med Journ 2: 242-251.
7. Sarma JAV, Powell, GL, LaBerge M (1991) Phospholipid composition of articular cartilage boundary lubricant. J Orthop Research 19: 671-676.
8. Hills BA, Butler BD, Barrow RE (1982) Boundary lubrication imparted by pleural surfactants and their identification. J Appl Physiol 53: 463-469.
9. Schmidt G, Muller G (1991) Structure and function of lamellar bodies, lipid-protein complexes involved in storage and secretion of cellular lipids. J Lipid Res 32: 1539-1570.
10. Hills BA (1992) Graphite-like lubrication of mesothelium by oligolamellar pleural surfactant. J Appl Physiol (1985) 73: 1034-1039. [Crossref]
11. Hills BA (2000) Boundary lubrication in vivo. Proc Inst Mech Eng H 214: 83-94. [Crossref]
12. Pawlak Z (2018) Articular Cartilage: Lamellar- Repulsive Lubrication of Natural Joints. KDP pp: 161.
13. Jahn S, Klein J (2018) Lubrication of articular cartilage. Physics Today 71: 4-48.
14. Williams PF, Powell G, LaBerge M (1993) Sliding friction analysis of phosphatidylcholine as a boundary lubricant for articular cartilage. Proc. Institution of Mechanical Engineers, Part H. J Eng Medicine 207: 59-66.
15. Pawlak Z, Urbaniak W, Hagner-Derenowska M, Hagner W (2015) The probable explanation for the low friction of natural joints. Cell Biochem Biophys 71: 1615-1621.
16. Yusuf KO, Motta N, Pawlak Z, Oloyede A (2012) A microanalytical study of the surfaces of normal, delipidized, and artificially “resurfaced” articular cartilage. Connect Tissue Res 53: 236-245. [Crossref]
17. Oloyede A, Guadimeto P, Crawford R, Hills BA (2004) Biomechanical responses of normal and delipidized articular cartilage subjected to varying rates of loading. Connect Tissue Res 45: 86-93. [Crossref]